Enhanced solubility and dissolution rate of simvastatin by solid dispersions and inclusion complex

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ชื่อวิทยานิพนธ์	การเพิ่มการละลายและอัตราการละลายของซิมวาสแตติน โดยการเตรียมในรูปโซลิดดิสเพอร์สชันและสารเชิงซ้อนอินคลูชัน
ผู้เขียน	นางสาวชมพูนุท เพชรนิรมล
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บทคัดย่อ

ซิมวาสแตตินเป็นยาที่ใช้ลดระดับคลอเลสเตอรอลในเลือด เนื่องจากซิมวาสแตตินละลายน้ำได้น้อยมาก ส่งผลให้ตัวยามีอัตราการละลายต่ำ ดังนั้นในงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อเพิ่มการละลายและอัตราการละลายของซิมวาสแตตินโดยการเตรียมในรูปโซลิดดิสเพอร์สชัน โดยใช้โพลีไวนิลไพโรลิโดน K-30 เป็นตัวพา และเตรียมในรูปสารเชิงซ้อนอินคลูชัน โดยใช้เมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตริน โซลิดดิสเพอร์สชันของยาซิมวาสแตตินและโพลีไวนิลไพโรลิโดน K-30 เตรียมโดยวิธี solvent method ในอัตราส่วนของตัวยาต่อตัวพาเป็น 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 และ 1:10 โดยน้ำหนัก ส่วนสารเชิงซ้อนอินคลูชันของยาซิมวาสแตตินและเมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตริน เตรียมโดย 3 วิธี ได้แก่ kneading method, co-evaporation method และ freeze-drying method ในอัตราส่วน 1:1 โดยโมล ศึกษาค่าการละลาย การละลายและคุณสมบัติทางเคมีฟิสิกส์ของโซลิดดิสเพอร์สชันและสารเชิงซ้อนอินคลูชัน เปรียบเทียบกับสารผสมทางกายภาพและตัวยาเดี่ยว โดย Differential scanning calorimetry, Fourier-transform infrared spectroscopy และ Powder X-ray diffractometry

จาก phase-solubility study พบว่าค่าการละลายของยาซิมวาสแตตินในเมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตริน ที่อุณหภูมิ 37°C เพิ่มขึ้น เมื่อความเข้มข้นของเมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตรินเพิ่มขึ้น Phase-solubility diagram ที่ได้เป็น A_L-type บ่งชี้การเกิดสารเชิงซ้อนอินคลูชันในอัตราส่วน 1:1 โดยโมล และค่า apparent stability constant ($K_{1:1}$) เท่ากับ 1,240 M^{-1} ค่าการละลายและอัตราการละลายของยาซิมวาสแตตินเพิ่มขึ้นในสารเชิงซ้อนอินคลูชันเมื่อเปรียบเทียบกับสารผสมทางกายภาพและยาเดี่ยว โดยอัตราละลายของตัวยาซิมวาสแตตินที่เวลาต่างๆ มีค่าสูงกว่าสารผสมทางกายภาพและยาเดี่ยว จากการศึกษาคุณสมบัติทางเคมีฟิสิกส์ พบว่าตัวยาซิมวาสแตตินในรูปสารเชิงซ้อนอินคลูชันเตรียมโดยวิธี co-evaporation method จะอยู่ในรูปอสัณฐาน และอันตรกิริยาที่เกิดขึ้นระหว่างตัวยากับเมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตรินแข็งแรงกว่าสารเชิงซ้อนอินคลูชันที่เตรียมโดย kneading method และ freeze-drying method

เมื่อศึกษาค่าการละลายและอัตราการละลายของโซลิดดิสเพอร์สชัน พบว่าโซลิดดิสเพอร์สชันให้ค่าการละลายและอัตราการละลายของตัวยาซิมวาสแตตินสูงกว่าสารผสมทางกายภาพและยาเดี่ยว โซลิดดิสเพอร์สชันในอัตราส่วน 1:4 โดยน้ำหนัก ให้อัตราการละลายที่เร็วกว่าสารผสมทางกายภาพ ตัวยาเดี่ยว และโซลิดดิสเพอร์สชันในอัตราส่วน 1:1, 1:2 และ 1:3 จากคุณสมบัติทางเคมีฟิสิกส์ พบว่าตัวยาซิมวาสแตตินในรูปโซลิดดิสเพอร์สชันจะอยู่ในรูปอสัณฐานและเกิดอันตรกิริยาระหว่างโมเลกุลของยาและโพลีไวนิลไพโรลิโดน K-30 โดยเกิดพันธะไฮโดรเจนระหว่างตัวยาซิมวาสแตตินกับโพลีไวนิลไพโรลิโดน K-30

เมื่อศึกษาความคงตัวของโซลิดดิสเพอร์สชัน ในอัตราส่วน 1:4 โดยน้ำหนัก และสารเชิงซ้อนอินคลูชันในอัตราส่วน 1:1 โดยโมล ที่เตรียมโดยวิธี co-evaporation method ซึ่งให้

อัตราการละลายของตัวยาซิมวาสแตตินสูงสุด หลังจากนำมาเก็บไว้ที่อุณหภูมิห้องและที่อุณหภูมิ 45°C เป็นเวลา 3 เดือน จากผลการทดลองพบว่า สารตัวอย่างที่ผ่านการเก็บไว้ที่อุณหภูมิห้องยังคงให้อัตราการละลายใกล้เคียงกับสารตัวอย่างที่เตรียมเสร็จใหม่ๆ ส่วนสารตัวอย่างที่เก็บไว้ที่อุณหภูมิ 45°C พบว่าการละลายของตัวยาลดลงเมื่อเปรียบเทียบกับสารตัวอย่างที่เตรียมเสร็จใหม่ๆ เมื่อศึกษาคุณสมบัติทางเคมีฟิสิกส์ของโซลิดดิสเพอร์สชันและสารเชิงซ้อนอินคลูชันที่เก็บไว้เป็นเวลา 3 เดือน พบว่าตัวยาซิมวาสแตตินในรูปโซลิดดิสเพอร์สชันและสารเชิงซ้อนอินคลูชันยังคงอยู่ในรูปอสัณฐาน และยังคงเกิดอันตรกิริยาระหว่างซิมวาสแตตินและโพลีไวนิลไพโรลิโดน K-30 และระหว่างซิมวาสแตตินและเมทิลเลเตต-เบต้า-ไซโคลเดกซ์ตริน

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ABSTRACT

Simvastatin is used for lowering high levels of cholesterol in the blood. It is a poorly water soluble drug and slow dissolution. Therefore, this research is aimed to enhance the solubility and dissolution rate of simvastatin by solid dispersion with polyvinylpyrrolidone K-30 as carrier and by inclusion complex with methylated-β-cyclodextrin. Solid dispersions of simvastatin-polyvinylpyrrolidone K-30 in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10 were prepared by solvent method. Inclusion complexes of simvastatin-methylated-β-cyclodextrin in molar ratio of 1:1 were prepared by kneading, co-evaporation and freeze-drying method. The physicochemical characteristics of solid dispersions and inclusion complexes were investigated by differential scanning calorimetry (DSC), fourier-transform infrared spectroscopy (FT-IR) and powder X-ray diffractometry (PXRD).

From the phase-solubility study of simvastatin in methylated-β-cyclodextrin at 37°C, the solubility of simvastatin was increased linearly with the concentration of methylated-β-cyclodextrin. A_L-type phase-solubility diagram was obtained, indicating the formation of 1:1 stoichiometric inclusion complex with the apparent stability constant ($K_{1:1}$) of 1,240 M^{-1}. The solubility and the dissolution of simvastatin were increased in all inclusion complexes compared with physical mixture and pure drug. Dissolution profiles of simvastatin from all inclusion complexes were higher than physical mixture and pure drug. Physicochemical characterization of inclusion complexes compared with physical mixture and pure drug showed that the inclusion complex prepared by co-evaporation method gave drug amorphilization, stronger complex formation than those of inclusion complexes prepared by kneading and freeze-drying method.

From the solubility study and the dissolution study of solid dispersions, all solid dispersions gave higher solubility and dissolution of simvastatin than physical mixtures and pure drug. Solid dispersion in weight ratio of 1:4 showed faster dissolution rate than its physical mixture, pure drug and solid dispersions in weight ratios of 1:1, 1:2 and 1:3. Physicochemical characterization of solid dispersions compared with physical mixtures and pure drug found that solid dispersion gave drug amorphilization and showed the intermolecular hydrogen bonding between drug and polyvinylpyrrolidone K-30.

Solid dispersion in weight ratio of 1:4 and inclusion complex in molar ratio of 1:1 prepared by co-evaporation method which gave highest dissolution rate were stored at room temperature and at 45°C for 3 months. The percentage of drug dissolved at 5 and 10 minutes of both aged samples stored at room temperature in simulated gastric fluid without pepsin and in simulated intestinal fluid without pancreatin was not significantly difference compared with that of freshly prepared samples. But the percentage of drug dissolved at 5 and 10 minutes of both aged samples stored at 45°C in simulated gastric fluid without pepsin and in simulated intestinal fluid without pancreatin was significantly decreased compared with that of freshly prepared samples. Physicochemical characterization of aged solid dispersion showed that aged solid dispersion still gave drug amorphilization and still showed the intermolecular hydrogen bonding between drug and polyvinylpyrrolidone K-30. Physicochemical characterization of aged inclusion complex showed that aged inclusion complex still gave drug amorphilization and still gave strong complex formation.

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LISTS OF ABBREVIATIONS AND SYMBOLS

α	=	alpha
°A	=	angstrom
β	=	beta
°C	=	degree Celcius
CD	=	cyclodextrin
cm	=	centimeter
Conc.	=	concentration
DSC	=	differential scanning calorimetry
et al.	=	et all
°F	=	Fahrenheit
FT-IR	=	fourier-transform infrared spectroscopy
g	=	gram
HMG-CoA	=	3-hydroxy-3-methylglutaryl-coenzyme A
γ	=	gamma
i.e.	=	idest
IR	=	infrared
KBr	=	potassium bromide
µg	=	microgram
µm	=	micrometer
mg	=	milligram
M	=	molar
mM	=	millimolar
ml	=	milliliter
MW	=	molecular weight
N	=	normality
n	=	number of sample
nm	=	nanometer

LISTS OF ABBREVIATIONS AND SYMBOLS (CONT.)

%	=	percentage
PVP	=	polyvinylpyrrolidone
PXRD	=	powder X-ray diffractometry
R^2	=	correlation coefficient
rpm	=	round per minute
RT	=	room temperature
sd	=	standard deviation
SD	=	solid dispersion
USP	=	The United States Pharmacopeia
UV	=	ultraviolet
V	=	volt
λ	=	wavelength
w/w	=	weight by weight

CHAPTER 1

INTRODUCTION

1.1 Background

Simvastatin is 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetra-hydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1*S*-[1α,3α,7β,8β (2*S**,4*S**),-8aβ]]. The empirical formula of simvastatin is $C_{25}H_{38}O_5$, its molecular weight and melting point are 418.57 and 135-138°C, respectively. Molecular structure of simvastatin is shown in Figure 1.1. Simvastatin was derived synthetically by Merck Sharp and Dohme Research Laboratories from lovastatin. It is a pharmacologically inactive prodrug for several active metabolites. After oral ingestion, it is absorbed from the gastrointestinal tract. Then, it undergoes rapid enzymic hydrolysis of the lactone ring and converted to the simvastatin β-hydroxyacid form. Molecular structure of simvastatin β-hydroxyacid is shown in Figure 1.2.

The simvastatin β-hydroxyacid is a potent, reversible, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. Inhibition of HMG-CoA reductase leads to reduced cholesterol synthesis in the liver and lower intracellular cholesterol concentrations. HMG-CoA reductase inhibitors (also called statins) reduce total cholesterol, low-density lipoprotein cholesterol, and very low-density lipoprotein-cholesterol concentration in plasma. It also tend to reduce triglycerides and to increase high-density lipoprotein-cholesterol concentration.

Simvastatin is used to reduce low-density lipoprotein-cholesterol, apoliprotein B, triglycerides and increase high-density lipoprotein-cholesterol in the treatment of primary hyperlipidaemias. Simvastatin is a substrate for the cytochrome P450 isoenzyme CYP3A4 and undergoes extensive first-pass metabolism in the liver, its primary site of action. Less than 5% of the oral dose has been reported to reach the circulation as active metabolites. Both simvastatin and its β-hydroxyacid

metabolite are about 95% bound to plasma proteins. Simvastatin is mainly excreted in the faeces by the bile as metabolites. About 10 to 15% is recovered in the urine, mainly in active form. The half-life of the active β-hydroxyacid metabolite is 1.9 hours.

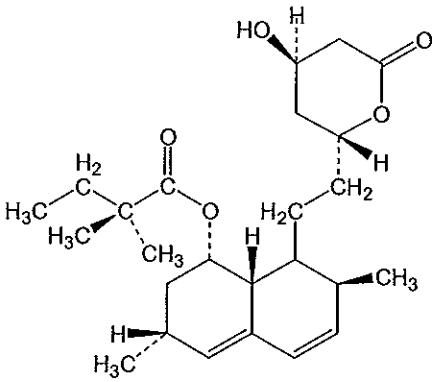


Figure 1.1 Molecular structure of simvastatin (Ellison *et al.*, 1993)

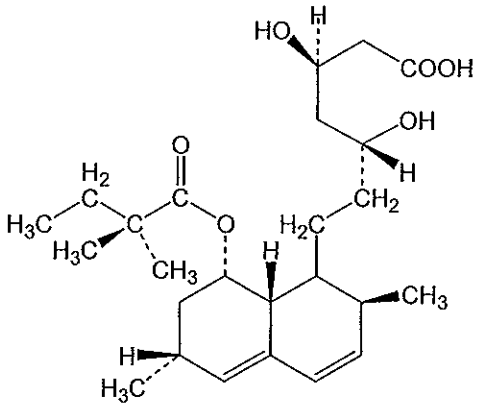


Figure 1.2 Molecular structure of simvastatin β-hydroxyacid (Ellison *et al.*, 1993)

Simvastatin is a white, non-hygroscopic, crystalline powder. It is practically insoluble in water, freely soluble in polar organic solvents. The solubility of simvastatin in water and organic solvents is shown in Table 1.1. The solubility class and solubility value are shown in Table 1.2.

Table 1.1 Solubility of simvastatin in water and organic solvents (Ellison *et al.*, 1993)

Solvent	Solubility (mg/ml)
Chloroform	610
Dimethyl sulfoxide	540
Methanol	200
Ethanol	160
Water	0.03

Table 1.2 Solubility class and solubility value (Stegemann *et al.*, 2007)

Solubility class	Solubility value (mg/ml)
Very soluble	> 1,000
Freely soluble	100-1,000
Soluble	33-100
Sparingly soluble	10-33
Slightly soluble	1-10
Very slightly soluble	0.1-1
Practically insoluble	< 0.1

Commercial dosage forms of simvastatin are typically available in tablets, capsules for oral administration. Examples of trade name of simvastatin are Bestatin® (Berlin Pharm), Eucor® (Greater Pharma), Lipex® (MSD), Lochol® (Siam Pharmaceutical), Simvor® (Ranbaxy Unichem), Simvotin® (Ranbaxy), Torio® (Unison), Vascor® (Biolab), Zimmex® (Silom Medical), Zimva® (GPO), Zocor® (MSD). One of the commercial simvastatin oral tablet, (Zocor®), contains either 5 mg, 10 mg, 20 mg, 40 mg or 80 mg of simvastatin and the following inactive ingredients: hydroxypropylcellulose, hydroxypropyl-methylcellulose, cellulose, iron oxides, lactose, magnesium stearate, starch, talcum, titanium dioxide and butylated hydroxyanisole (Hitti, 2008).

Simvastatin is given by mouth in a usual initial dose of 10 to 20 mg in the evening and an initial dose of 40 mg may be used in patients who are at high cardiovascular risk. The dose may be adjusted at intervals of not less than 4 weeks up to a maximum of 80 mg once daily in the evening. Children aged 10 to 17 years may be given an initial dose of 10 mg once daily, increased according to response, to a maximum dose of 40 mg once daily.

According to the biopharmaceutical classification system (BCS) of drugs that shown in Table 1.3, simvastatin is a drug in BCS class II (Graeser *et al.*, 2007) which shows high permeability but low solubility.

Table 1.3 Biopharmaceutical classification system (BCS) of drugs (Spencer, 2009)

Class	Solubility	Permeability
I	high	high
II	low	high
III	high	low
IV	low	low

BCS class I:

Drugs exhibit high solubility and high permeability. The drug is good absorbed (though its systemic availability may be low due to first pass metabolism). The rate limiting step of drug absorption is drug dissolution or gastric emptying if dissolution is very rapid. The dissolution profile must be good defined and reproducible to insure the bioavailability. For immediately release dosage forms that dissolve very rapidly, the absorption rate will be controlled by the gastric emptying rate.

BCS class II:

Drugs exhibit low solubility and high permeability but the absorption of drugs in this class is slower than that of drugs in BCS class I. Good *in vitro-in vivo* correlation can be obtained if *in vitro* dissolution rate is similarly to *in vivo*

dissolution rate, *in vitro* can estimate *in vivo*. The absorption is limited by its solubility. Drugs in this class are expected to have variable absorption due to many formulations.

BCS class III:

Drugs exhibit high solubility and low permeability. The permeability of drugs is the rate controlling step in drug absorption. The absorption may be highly variable in this class. The absorption is controlled by the physiological factors and biopharmaceutical properties such as gastrointestinal mobility, permeability, metabolism, dissolution and the interaction or binding of drugs with excipients. If the dissolution is fast i.e. drugs rapidly dissolve to be 85% in less than 15 minute, this variation will be due to physiological factors more than dosage form factors.

BCS class IV:

Drugs exhibit low solubility and low permeability. No *in vitro-in vivo* correlation can be expected. This class of drugs presents significant problems for effective oral delivery (Amidon *et al.*, 1995).

Simvastatin is practically insoluble in water that comes to poorly absorbed from gastro-intestinal tract (Kang *et al.*, 2004 and Ambike *et al.*, 2005). Because simvastatin is a drug in BCS class II, therefore improving its solubility as well as dissolution will lead to enhance drug absorption or bioavailability (Ventura *et al.*, 2005 and Prabagar *et al.*, 2007). Several methods have been developed for BCS class II drugs which are based on improving the efficiency of drug by enhancing the solubility and increasing the dissolution such as particle size reduction, micronisation (Varshosaz *et al.*, 2008), lyophilization (Betageri *et al.*, 1995), solid dispersion (Serajuddin,1999), addition of surfactants such as sodium lauryl sulfate (Patel and Patel, 2007), Tween 20 (Veiga and Ahsan, 1998), formulation as emulsions and microemulsions systems (Kang *et al.*, 2004), inclusion complex with cyclodextrin (Ruan *et al.*, 2005). Among these, solid dispersion and complexation with cyclodextrin were methods of the most promising strategies.

Solid dispersion was one of the successful methods in improving drug dissolution (Okonogi *et al.*, 1997 and Franco *et al.*, 2001) and to obtain better bioavailability (Pan *et al.*, 2000 and Kohri *et al.*, 1999). Solid dispersion is defined as the dispersion of one or more active ingredients in an inert hydrophilic carrier or matrix at solid state prepared by the fusion, solvent or solvent-fusion method (Chiou *et al.*, 1971). This system allows a particle size reduction of drug to nearly a molecular level or a molecular level. As this system exposed to aqueous media, the carrier is dissolved and the drug is released as very fine particles for quick dissolution and absorption (Serajuddin, 1999). Hydrophilic synthetic polymers such as polymethacrylate, polyethylene glycols and polyvinylpyrrolidone have been widely investigated as carrier substances for solid dispersion. Polyvinylpyrrolidone K-30 (PVP K-30) is amongst the most frequently used as the carrier. It can improve the solubility and the dissolution rate of many poorly water soluble drugs (Marin *et al.*, 2002 and Tantishaiyakul *et al.*, 1999).

Cyclodextrins are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity which can accommodate a variety of lipophilic drugs (Del Valle, 2004 and Brewster *et al.*, 2007). Complexation with cyclodextrins has been widely used to enhance the oral absorption of poorly water soluble drugs by increasing the solubility and the dissolution rate. Natural cyclodextrins such as β-cyclodextrin is limited in its pharmaceutical applications due to its limited water solubility. Therefore, chemically modified cyclodextrins such as methylated-β-cyclodextrin, amorphous derivative, is introduced to overcome this problem. Methylated-β-cyclodextrin was reported to improve water solubility, dissolution rate and complexing power than the parent cyclodextrin (Figueiras *et al.*, 2007 and Mura *et al.*, 1999).

There are several previous studies about enhancing of the solubility and the disolution rate of simvastatin and other drugs by using solid dispersion method and inclusion complex with cyclodextrin. These include:

- Jun *et al.* (2007) reported the supercritical antisolvent processed simvastatin/hydroxypropyl-β-cyclodextrin inclusion complex improved

drug solubility from 24.4 ± 1.5 μg/ml to 296.9 ± 13.0 μg/ml and enhanced the dissolution rate to 34 fold when compared with pure drug. Moreover, the inclusion complex reduced total cholesterol better than pure simvastatin.

- Patel and Patel (2007) reported the complexation of simvastatin with hydroxypropyl-β-cyclodextrin prepared by kneading and co-evaporation method improved the solubility 11.3 folds and 10.1 folds, respectively when compared with pure drug. The improvement in wettability of simvastatin by complexation with hydroxypropyl-β-cyclodextrin prepared by kneading method was higher when compared with pure drug. The drug content of the inclusion complex prepared by co-evaporation method when heating at 45-50°C and kneading method when drying in an oven at 45-50°C for 24 hours were found to be 100% and 99%, respectively. These results indicated the chemical stability of simvastatin.

- Sethia and Squillante (2004) found that solid dispersion of carbamazepine in PVP K-30 showed increased the dissolution rate 4 folds higher than pure drug.

- Ruan *et al.* (2005) found that the solubility and the dissolution rate of ampelopsin were increased by solid dispersion with PVP K-30.

- Kim *et al.* (2006) found that solid dispersion of felodipine with the high amount of PVP K-30 could increase the solubility and the dissolution rate.

- Ventura *et al.* (2005) found that the inclusion complex of celecoxib with dimethyl-β-cyclodextrin by kneading and freeze-drying methods could enhance the drug solubility.

The objective of this study was to increase the solubility and the dissolution rate of simvastatin by solid dispersion using PVP K-30 as a hydrophilic carrier and by inclusion complexation with a cyclodextrin derivative, methylated-β-cyclodextrin.

The solubility, the dissolution rate and the physicochemical characterization based on differential scanning calorimetry, powder X-ray diffractometry, and fourier-transform infared spectroscopy were evaluated. Drug content, dissolution rate and physicochemical properties of aged solid dispersion and aged inclusion complex were studied.

CHAPTER 2

REVIEW OF LITERATURES

2.1 Introduction

Prior to enhance a drug absorption, it must be first dissolved in the fluid at the absorption site. For instance, a drug administered orally in tablet or capsule cannot be absorbed until the drug particles are dissolved by the fluids at some point within the gastrointestinal tract. The solubility of a drug is dependent upon either an acidic or basic medium, therefore, the drug would be dissolved in the stomach or intestine respectively. The process by which a drug particle dissolves is termed dissolution.

As a drug particle undergoes dissolution, the drug molecules on the surface are the first to enter into solution creating a saturated layer of drug-solution which envelops the surface of the solid drug particle. This layer is referred to as the diffusion layer. From this diffusion layer, the drug molecules pass through the dissolving fluid and make contact with the biologic membranes and absorption ensues. As the molecules of drug continue to leave the diffusion layer, the layer is replenished with dissolved drug from the surface of the drug particle and the process of absorption continues. Diffusion layer model is shown in Figure 2.1. A stagnant layer h is formed around the solid particle. Diffusion occurred from this layer at the boundary to the bulk of solvent (Abdou, 1990).

If the process of dissolution for a drug particle is rapid, the rate at which the drug becomes absorbed would be dependent upon its ability to traverse the membrane barrier. However, if the rate of dissolution for a drug particle is slow, as may be due to the physical characteristics of the drug substance or the dosage form, the dissolution process itself would be a rate-limiting step in the absorption process. Thus, poorly soluble drugs or poor formulated drug products may result in an incomplete absorption of drug and its passage, unchanged, out of the system via the feces.

The dissolution of a substance may be described by the modified Noyes-Whitney as the following equation (Ansel and Popovich, 1990):

$$\frac{dc}{dt} = kS(C_s - C_t) \qquad (2.1)$$

Where; dc/dt : the rate of dissolution

k : the dissolution rate constant

S : the surface area of the dissolving solid

C_s : the saturation concentration of drug in the diffusion layer (which may be approximated by the maximum solubility of the drug in the solvent since the diffusion layer is considered saturated)

C_t : the concentration of the drug in the dissolution medium at time t

C_s-C_t : the concentration gradient

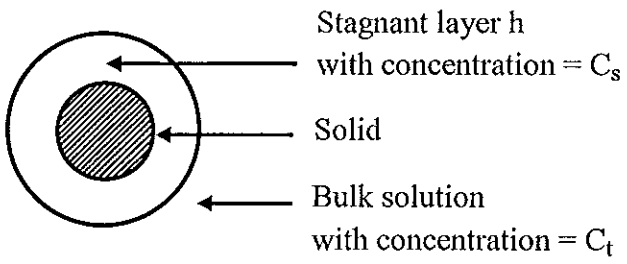


Figure 2.1 Diffusion layer model (Abdou, 1990)

The rate of dissolution is governed by the rate of diffusion of solute molecules through the diffusion layer into the body of the solution. The equation reveals that the dissolution rate of a drug may be increased by increasing the surface area (reducing the particle size) of the drug, by increasing the solubility of the drug in the diffusion layer, and by factors embodies in the dissolution rate constant, k, including the intensity of agitation of the solvent and the diffusion coefficient of the dissolving drug. For a given drug, the diffusion coefficient and the concentration of the drug in the diffusion layer will increase with increasing temperature. In addition, increasing the rate of agitation of the dissolving medium will increase the rate of dissolution.

A reduction in the viscosity of the solvent employed is another means which may be used to enhance the dissolution rate of a drug. Changes in the pH or the nature of the solvent which influence the solubility of the drug may be used to advantage in increasing the dissolution rate.

There are various methods available to improve the solubility and the dissolution rate of poorly soluble drugs. Reduced particle size by solid dispersion and preparation by inclusion complex with cyclodextrins are most frequently used (Ruan *et al.*, 2005). These methods are widely used in pharmaceutical field due to the ability to improve the solubility of poorly water soluble drugs, enhance the dissolution rate, increase drug stability and lead to increase the bioavailability of drug.

2.2 Solid dispersion

2.2.1 Definition of solid dispersion

Solid dispersion is defined as the dispersion of one or more active ingredients in an inert hydrophilic carrier or matrix at solid state prepared by the fusion, solvent or solvent-fusion method (Chiou and Riegelman, 1971). This system allows a particle size reduction of drug. The drug can be dispersed molecularly, in amorphous or in crystalline particles. The solid dispersion can enhance the solubility, the dissolution rate, the stability and improved the bioavailability of poorly water soluble drugs (Shanbhag *et al.*, 2008 and Leuner *et al.*, 2000). For examples, Moneghini *et al.* (1998) reported the solubility and the dissolution rate of atenolol by PVP K-30 were improved. Sethia and Squillante (2004) reported that an increasing in solubility of carbamazepine by PVP K-30 may be due to the formation of soluble complexes between water-soluble polymeric carrier and poorly soluble drug and the dissolution rate of carbamazepine prepared by sold dispersion with PVP K-30 as inert carrier was enhanced about 4 folds when compared with pure drug. Similar result was reported by Ruan *et. al.* (2005) which revealed that the dissolution rates of ampelopsin were significantly increased by solid dispersion with the amount of ampelopsin dissolved was 93% while the amount of pure ampelopsin dissolved was 53% within 10 minutes. In addition, Kim *et al.* (2006) presented that the dissolution rate of felodipine solid dispersion with high amount of PVP K-30 as carrier was

significantly increased when compared with pure drug and physical mixture. Leuner *et al.* (2000) revealed that the drug prepared with PVP improved the release and bioavailability of the drug more than pure drug.

2.2.2 Preparation of solid dispersion

Solid dispersion may be prepared by the melting, solvent and melting-solvent method.

2.2.2.1 Melting method

Sekiguchi and Obi (1961) were the first to use a melting method to prepare solid dispersion that consisted of a drug, sulphathiazole and urea, a water soluble carrier. The physical mixture of a drug and a water soluble carrier was heated until melted together at a temperature of the eutectic point, followed by cooling in an ice bath. The obtained solid dispersion was milled, pulverized and sieved to reduce particle size. The dispersed drug was trapped within the carrier matrix due to solidification. A molecular dispersion can be achieved depends on the degree of supersaturation and rate of cooling attained in the process. The process has an effect on the obtained dispersion and can be varied to optimize the product. The quenching the melt mixture rapidly (when the solute molecules are arrested in the solvent matrix by instantaneous solidification) gave a supersaturation of the drug that leads to the popularity in the fast solidification. Chiou and Riegelman (1969) accerelated the cooling rate by the melt mixture was snap cooled when spread in thin layer form on stainless steel plates. Kanig (1964) introduced a modification of the process of spray-congealing form a spray-drier onto cold metal surfaces.

Advantages:

- Toxic solvents are not use.
- Simplicity and economy
- Small crystallites may be obtained by quench cooling (Collett *et al.*, 1976).

Disadvantages:

- The solidification temperature will affect crystallization rate and may alter both the crystallite size and the hardness of the dispersion.

- The solidified may be tacky and unhandable.

- Either drugs or carriers may decompose or evaporate during the fusion process at high temperature.

- Immiscibility between drug and carrier may occur during fusion process.

2.2.2.2 Solvent method

Tachibana and Nakumara (1965) were the first to use a solvent method to produce a solid dispersion of the highly lipophilic β-carotene in the highly water soluble carrier; PVP. This method consisted of the solubilization of the drug and the carrier in a common solvent, an organic solvent, after that the solvent was evaporated under vacuum to produce a solid dispersion. Bates (1969) introduced the term coprecipitates to describe solid dispersion that is produced by the solvent evaporation method. Simonelli *et al.* (1969) used the term coprecipitates to more correctly to describe a solid dispersion of sulphathiazole and PVP that had been precipitated from a solution in sodium chloride by the addition of hydrochloric acid. Solid dispersion that is produced by the solvent evaporation method should be called coevaporates and not coprecipitates. A basic process of preparing solid dispersion by using a solvent method consisted of dissolving the drug and the polymeric carrier in a solvent such as ethanol, chloroform or a mixture of solvents. Then, the solvent was removed. Finally, the obtained product was pulverized and milled. An important prerequisite for the manufacture of a solid dispersion using the solvent method is that both the drug and the carrier are sufficiently soluble in the solvent. The solvent can be removed by various methods such as by spray-drying (Ambike *et al.*, 2005), freeze-drying (Abdul-Fattah and Bhargava, 2002), vacuum drying (Kim *et al.*, 2006), slow evaporation of the solvent at low temperature (Ruan *et al.*, 2005), the use of supercritical fluids (Sethia and Squillante, 2004), a stream of nitrogen (Prabhu *et al*, 2005) and a use of a rotary evaporator (Ceballos *et al*, 2005). Temperatures used for solvent evaporation generally lie in the range of 23-65°C. The solvent evaporation method is one of the most commonly used in the production of solid dispersion. One of examples of

solid dispersion that prepared by solvent method was griseofulvin-PVP K-30 solid dispersion that presented by Mayersohn and Gibaldi (1966). Griseofulvin and PVP K-30 were dissolved in chloroform and then the solvent was evaporated. The release rate of griseofulvin from the solid dispersion was 5 to 11 times higher than that of micronized drug, depending on the drug-carrier ratios.

Advantages:

- The thermal decomposition of drugs or carriers was prevented due to the low temperature required for the evaporation of organic solvents.
- High melting point carrier can be used.
- The immiscibility between drug and carrier can be used.

Disadvantages:

- The high cost of preparation
- The difficulty in completely removing liquid solvent
- The toxicity of organic solvents
- The volumes of solvents used may be excessive. For example, 500 ml of ethanol was used to prepare only 5 g. of the 10% griseofulvin dispersion in polyethylene glycol 6000 (Ford, 1986).
- Since the chosen carriers are generally hydrophilic and the drugs are hydrophobic, the selection of a common solvent is difficult.
- The difficulty of reproducing crystal forms

2.2.2.3 Melting-solvent method

This method is prepare by dissolving a drug in a suitable liquid solvent and then this solution incorporate into the melt of carrier, polyethylene glycol (Chiou and Riegelman, 1971), obtainable below 70°C without removing the liquid solvent. The selected solvent or dissolved drug may not be miscible with the melt of the polyethylene glycol. In this method, polyethylene glycol is used as carrier due to 5-10% w/w of liquid components could be incorporated into polyethylene glycol without significant loss of its solid property. The polymorphic form of the drug precipitated in the solid dispersion may be affected by the liquid solvent used.

Advantages:

- Small quantities of organic solvents are used.
- It is suitable for thermolabile drugs.

Disadvantages:

- It is possible that the selected solvent or dissolved drug may not be miscible with the melt of polyethylene glycol.
- The polymorphic form of the drug precipitated in the solid dispersion may be affected by the liquid solvent used.
- It is limited to drugs with a low therapeutic dose e.g. below 50 mg

2.2.3 Carriers of solid dispersion

The most commonly used hydrophilic carriers for solid dispersion include PVP, polyethylene glycols. Surfactants such as Tweens, sodium lauryl sulphate may be used in the formation of solid dispersion. Chemical class and examples of carriers are shown in Table 2.1.

A carrier chosen for solid dispersion designed to increase the dissolution rates of drugs should meet the following criteria:

- It should be freely water soluble with intrinsic rapid dissolution properties.
- It should be non-toxic.
- Any carrier that used in melting method should be chemically, physically, and thermally stable with a low melting point to avoid the use of excessive heat during dispersion preparation. The use of the melting method requires thermal stability and chemical interaction should not occur between the drug and the carrier. Ideally the carrier should solidify into a stable solid by rapid and complete crystallization which should maintain the drug as a fine crystalline dispersion. Alternatively, the carrier may solidify through a viscous state which would maintain the drug in a near molecularly dispersed state. The carrier and drug should be miscible in the liquid state otherwise subsequent irregular crystallization may occur on cooling.
- Any carrier that intended for solvent method should be soluble in a variety of organic solvents and it should be capable of passing through a vitreous state when the

carrier should retard crystallization of the drug and maintain it at or near the molecularly dispersed state. It is essential that the drug and carrier cocrystallize, otherwise a solid dispersion will not be produced.

- The carrier should preferably increase the aqueous solubility of the drug. This is not absolute criterion since the sulphathiazole-urea dispersion (Sekiguchi and Obi, 1961) increased in sulphathiazole dissolution rate despite urea reducing the aqueous solubility of the drug.

- The chosen carrier should be chemically compatible with the drug and in the solid state should not form strongly-bonded complex with a strong association constant which may reduce dissolution rate.

- The carrier should be pharmacologically inert.

Table 2.1 Chemical class and examples of carriers (Shinde, 2007)

Chemical Class	**Examples**
Acids	Citric acid, Tartaric acid, Succinic acid
Sugars	Dextrose, Sorbitol, Sucrose, Maltose, Galactose, Xylitol
Polymeric Materials	PVP, Polyethylene glycol, Carboxymethyl cellulose, Hydroxypropyl cellulose, Methyl cellulose, Hydroxypropyl methyl cellulose, Guar gum, Xanthan gum, Sodium alginate
Surfactants	Polyoxyethylene stearate, Poloxamer, Deoxycholic acid, Tweens (Polysorbates), Spans (Sorbitan esters)
Miscellaneous	Pentaerythritol, Urea, Urethane, Hydroxyalkyl xanthines

2.2.4. Classification of solid dispersion

The structures of solid dispersion play an important role in controlling the drug release. It is considered to classify solid dispersion on the basic of their release mechanisms as following:

1. Simple eutectic mixtures
2. Solid solutions
3. Glass solutions and glass suspensions
4. Amorphous precipitations in a crystalline carrier
5. Compound or complex formations
6. Combinations

2.2.4.1. Simple eutectic mixtures

The simple eutectic mixture is usually prepared from the rapid solidification of the fused liquid of two compounds which are completely miscible in the liquid state and negligible solid-solid solubility (Chiou and Riegelman, 1971).

Phase diagram for a eutectic system is shown in Figure 2.1. When a mixture of A and B with composition E is cooled, A and B crystallize out simultaneously, whereas when other compositions are cooled, one of the components starts to crystallize out before the other. Solid eutectic mixtures are usually prepared by rapid cooling of a comelt of the two compounds in order to obtain a mixture of very fine crystals of the two components. When a mixture with composition E, consisting of a poorly soluble drug and an inert, highly water soluble carrier, is dissolved in an aqueous medium, the carrier will dissolve rapidly, releasing very fine crystals of the drug (Sekiguchi and Obi, 1961). The large surface area of the resulting suspension should result in an enhanced the dissolution rate and improved the bioavailability.

The examples of simple eutectic mixture include itraconazole-poloxamer 188 which increased the itraconazole solubility and the itraconazole dissolution rate (Liu *et al.*, 2006), solid dispersion of fenofibrate-polyethylene glycol which enhanced the fenofibrate dissolution rate (Law *et al.*, 2003) and solid dispersion of flunarizine-PVP which increased the flunarizine solubility (Marin *et al.*, 2002).

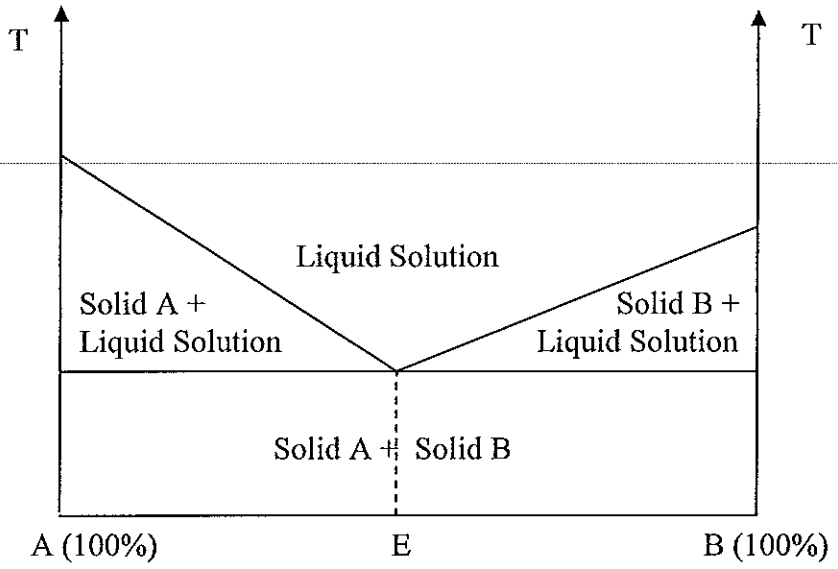


Figure 2.2 Phase diagram for a eutectic system (Leuner and Dressman, 2000)

2.2.4.2. Solid solutions

Solid solutions are comparable to liquid solutions, consisting of one phase irrespective of the number of components. Solid solutions of a poorly water soluble drug dissolved in a carrier with relatively good aqueous solubility are of particular interest as a means of improving oral bioavailability. In the case of solid solutions, the particle size of drug has been reduced to its absolute minimum (Goldberg *et al.*, 1965) and the dissolution rate is determined by the dissolution rate of the carrier. By judicious selection of a carrier, the dissolution rate of the drug can be increased by up to several orders of magnitude. Solid solutions can be classified according to two methods. First, they can be classified according to their miscibility (continuous, discontinuous solid solutions) or second, according to the way in which the solvate molecules are distributed in the solvent (substitutional, interstitial and amorphous).

I. Continuous and discontinuous solid solutions

a) Continuous solid solutions

In a continuous solid solution, the components are miscible in all proportions. Theoretically, this means that the bonding strength between the two components is stronger than the bonding strength between the molecules of each of the individual

components. Solid solutions of this type have not been reported in the pharmaceutical literature to date.

b) Discontinuous solid solutions

In the case of discontinuous solid solutions, the solubility of each of the components in the other component is limited. Phase diagram for a discontinuous solid solution is shown in Figure 2.2. α and β show the regions of true solid solutions. In these regions, one of the solid components is completely dissolved in the other solid component.

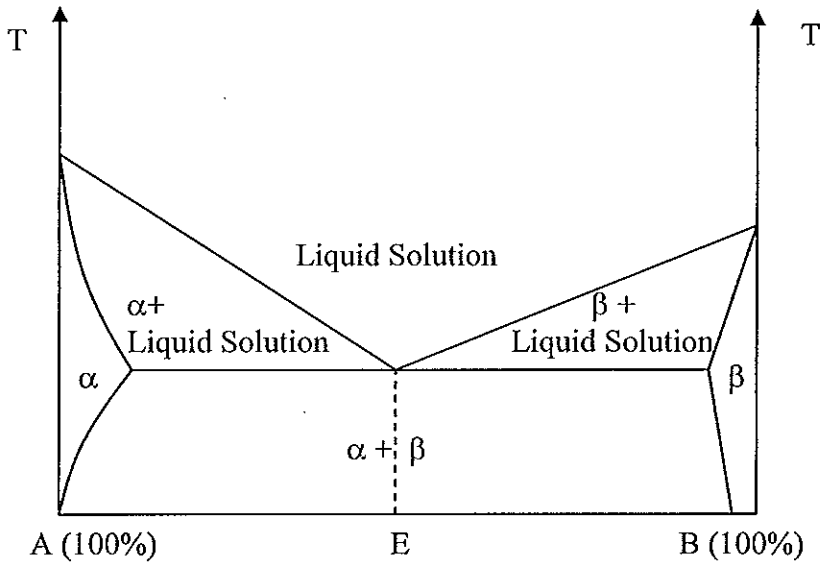


Figure 2.3 Phase diagram for a discontinuous solid solution (Leuner and Dressman, 2000)

II. Substitutional crystalline, interstitial crystalline and amorphous solid solutions

a) Substitutional crystalline solid solutions

Classical solid solutions have a crystalline structure, in which the solute molecules can either substitute for solvent molecules in the crystal lattice or fit into the interstices between the solvent molecules. The formation of the substitutional crystalline solid solution is shown in Figure 2.3. Substitution is only possible when

the size of the solute molecules differs by less than 15% from that of the solvent molecules (Leuner and Dressman, 2000).

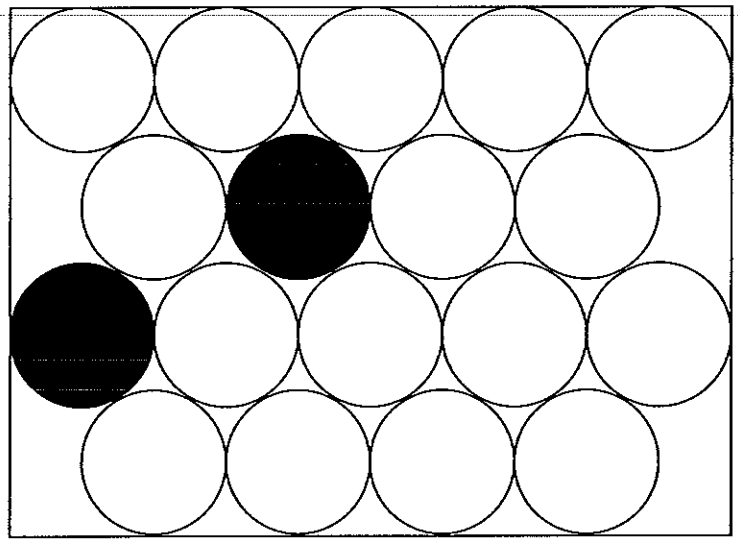


Figure 2.4 The formation of the substitutional crystalline solid solution (Leuner and Dressman, 2000)

b) Interstitial crystalline solid solutions

In interstitial crystalline solid solutions, the dissolved molecules occupy the interstitial spaces between the solvent molecules in the crystal lattice. The formation of the interstitial crystalline solid solution is shown in Figure 2.4 and the formation of the interstitial crystalline solid solution in a polymer is shown in Figure 2.5. In the case of interstitial crystalline solid solutions, the solute molecules should have a molecular diameter that is no greater than 0.59 of molecular diameter of solvent molecule. Furthermore, the volume of the solute molecules should be less than 20% of the solvent.

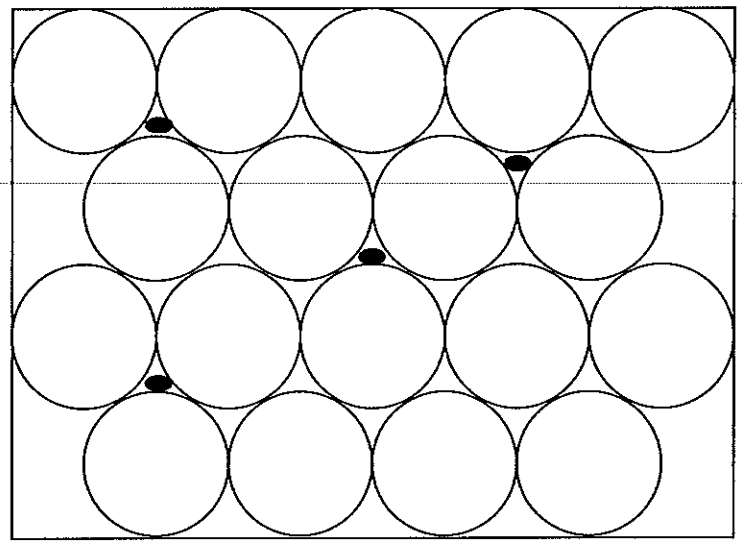


Figure 2.5 The formation of the interstitial crystalline solid solution (Leuner and Dressman, 2000)

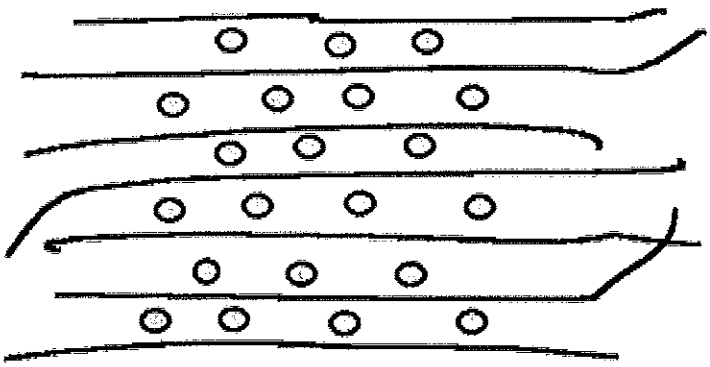


Figure 2.6 The formation of the interstitial crystalline solid solution in a polymer (Leuner and Dressman, 2000)

c) Amorphous solid solutions

In amorphous solid solutions, the solute molecules are dispersed molecularly but irregularly within the amorphous solvent. The formation of the amorphous solid solution is shown in Figure 2.6. Using griseofulvin in citric acid, Chiou and

Riegelman (1969) were the first to report the formation of an amorphous solid solution to improve drug dissolution. Other carriers that were used in early studies included urea and sugars such as sucrose, dextrose and galactose. More recently, organic polymers such as PVP, polyethylene glycol and various cellulose derivatives have been utilized for this purpose. Polymer carriers are particularly likely to form amorphous solid solutions as the polymer itself is often present in the form of an amorphous polymer chain network.

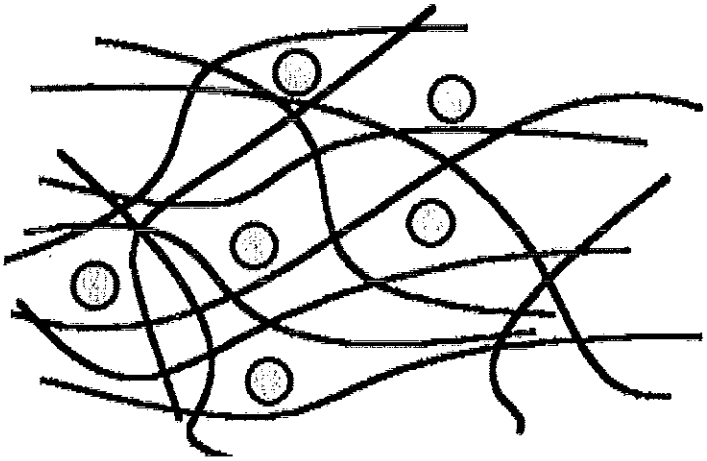


Figure 2.7 The formation of the amorphous solid solution (Leuner and Dressman, 2000)

2.2.4.3. Glass solutions and glass suspensions

A glass solution is a homogeneous glassy system in which a solute dissolves in a glassy solvent. A glass suspension refers to a mixture in which precipitated particles are suspended in a glassy solvent. The glassy state is characterized by transparency and brittleness. The glass softens progressively on heating and continuously without a sharp melting point. A glass produced weak and diffuse diffraction effects, while crystallites gave strong and sharp diffraction effects. If a water-insoluble drug forms a glass solution with a water-soluble, glass-forming carrier, then the in situ dissolved drug is released into the aqueous medium rapidly because the carrier quickly dissolves upon exposure to the aqueous medium. Glassy solutions possess the advantage over that of solid solutions, solid solutions have

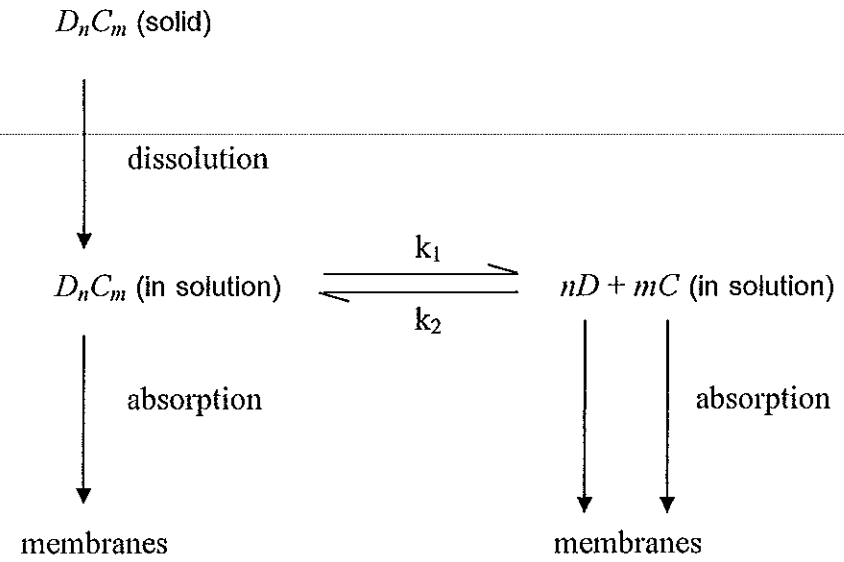
usually a relative strong chemical binding between the solute and the solvent, while the lattice energy in the glass solution is expected to be much less because of its similarity with the liquid solution. The lattice energy represents a barrier to rapid dissolution. The dissolution rate of drugs in the glass solution should be theoretically faster than that of the solid solution. Examples of carriers that favored glass formation are citric acid and sugars such as dextrose, sucrose and galactose. Other carriers that favored glass solutions are urea, polyethylene glycol and PVP.

2.2.4.4. Amorphous precipitations in a crystalline carrier

The drug may precipitate out in an amorphous form in the crystalline carrier. The amorphous form is the highest energy form of a pure drug. For example, amorphous form of novobiocin has faster dissolution and absorption rates than the crystalline form (Chiou and Riegelman, 1971).

2.2.4.5. Compound or complex formations

The modification of a dosage form by a compound or complex formation (D_nC_m) between a drug (D) and an inert soluble carrier (C) should not be classified under the applications of solid dispersion systems. Nevertheless, it frequent occurred during preparation of solid dispersion. The dissolution and absorption of a drug into the body from a compound or a complex are shown in Scheme 2.1. The bioavailability of a drug depends on the solubility, the dissociation constant and the intrinsic absorption rate of the complex.



Scheme 2.1 The dissolution and absorption of a drug into the body from a compound or a complex (Chiou and Riegelman, 1971).

2.2.4.6. Combinations

Phase interactions between drugs and carriers are difficult to quantify because dispersions may combinations. Quantification is made difficult by the structure of the dispersions. It depends on the method of preparation and age of the dispersion. Increasing in dissolution and absorption rates may be the contribution by different mechanism.

2.2.5. Mechanisms of increased dissolution rate

There are many factors which increased drug dissolution form solid dispersion:

2.2.5.1. Particle size reduction

Molecular dispersions, as solid dispersion, represent the last state on particle size reduction, and after carrier dissolution, the drug is molecularly dispersed in the dissolution medium. Solid dispersion apply this principle to drug release by creating a mixture of a poorly water soluble drug and highly soluble carriers

(Leuner and Dressman, 2000). High surface area is formed, resulting in an increased dissolution rate and, consequently, improved bioavailability (Kang *et al.*, 2004).

2.2.5.2. Improved wettability

A strong contribution to the enhancement of drug solubility is related to the drug wettability improvement in solid dispersion (Karavas *et al.*, 2006). It was observed that even carriers without any surface activity such as urea improved drug wettability (Sekiguchi *et al.*, 1964). Carriers with surface activity, such as cholic acid and bile salts can significantly increase the wettability properties of drugs. Moreover, carriers can influence the drug dissolution profile by direct dissolution or co-solvent effects (Pouton, 2006). Recently, the inclusion of surfactants in the third generation solid dispersion reinforced the importance of this property (Ghebremeskel *et al.*, 2007).

2.2.5.3. High porosity

Particles in solid dispersion have been found to have higher degree of porosity (Vasconcelos *et al.*, 2007). The increase in porosity also depends on the carrier properties, for instance, solid dispersion containing linear polymers produce larger and more porous particles than those containing reticular polymers and, therefore, result in a higher dissolution rate. The increased porosity of solid dispersion particles also hastens the drug release profile (Ghaderi *et al.*, 1999).

2.2.5.4. Drugs in amorphous state

Poorly water soluble crystalline drugs, when in the amorphous state tend to have higher solubility (Pokharkar *et al.*, 2006). The enhancement of drug release can usually be achieved by using the drug in its amorphous state because no crystal lattice in amorphous state, therefore no energy is required to break up the crystal lattice during the dissolution process (Taylor and Zografi, 1997). In solid dispersion, drugs are presented as supersaturated solutions after dissolution, and it is speculated that, if drugs precipitate, it is as a metastable polymorphic form with higher solubility than the most stable crystal form (Leuner and Dressman, 2000).

2.2.5.5. Avoid drug recrystallization

The carrier that used in solid dispersion can inhibit or prevent drug crystallization. For examples, solid dispersion of loperamide with PVP K-30 was prepared to avoid the crystallization of drug (Weuts *et al.*, 2005). The ability of three different polymers such as PVP, hydroxypropyl methylcellulose and hydroxypropyl methylcellulose acetate succinate stabilize amorphous and against crystallization of felodipine (Konno and Taylor, 2006). Each polymer inhibited crystallization of felodipine by reducing the nucleation rate. These polymers affect nucleation kinetics by increasing their kinetic barrier to nucleation.

2.2.6. Characterization of solid dispersion

There are various methods that analyzed the physicochemical properties of solid dispersion. The most important methods are thermal analysis, X-ray diffraction, infrared spectroscopy and measurement of the release rate of the drug. In addition to characterized the solid dispersion, these methods can be used to differentiate between solid solutions (molecularly dispersed drug), solid dispersion in which drug is only partly molecularly dispersed and physical mixtures of drug and carrier. Due to the complex composition of these preparations, it is often difficult to delineate precisely between molecularly dispersed and not molecularly dispersed systems and different analytical methods may yield disparate results. It is usually assumed that dispersions in which no crystallinity can be detected are molecularly dispersed and the absence of crystallinity is used as a criterion to differentiate between solid solutions and solid dispersion.

Thermal analysis method:

Thermal analysis method includes all that examine a characteristic of the system as a function of temperature. Of these, differential scanning calorimetry (DSC) is the most highly regarded method. DSC enables the quantitative detection of all processes in which energy is required or produced (endothermic and exothermic phase transformations). The usual method of measurement is to heat the reference and test samples in such a way that the temperature of the two is kept identical. If an energy-requiring phase transition occurs in the test sample, extra heat is applied to this

sample so that its temperature climbs at the same rate as in the reference. The additional heat required is recorded and used to quantitate the energy of the phase transition. Exothermic transitions, such as conversion of one polymorph to a more stable polymorph, can also be detected. Lack of a melting peak in the DSC of a solid dispersion indicates that the drug is present in an amorphous rather than a crystalline form. Since the method is quantitative in nature, the degree of crystallinity can also be calculated for systems in which the drug is partly amorphous and partly crystalline. However, crystallinity of less than 2% cannot generally be detected with DSC (Leuner and Dressman, 2000).

X-ray diffraction method:

The principle of X-ray diffraction is that when an X-ray beam is applied to the sample, interference bands can be detected. The angle at which the interference bands can be detected depends on the wavelength applied and the geometry of the sample with respect to periodicities in the structure. Crystallinity in the sample is reflected by a characteristic fingerprint region in the diffraction pattern. Owing to the specificity of the fingerprint, crystallinity in the drug can be separately identified from crystallinity in the carrier. Therefore, it is possible with X-ray diffraction to differentiate between solid solutions, in which it is molecularly dispersed of drug in carrier, and solid dispersion, in which it is at least partly present in the crystalline form, regardless of whether the carrier is amorphous or crystalline. However, crystallinity of under 5-10% cannot generally be detected with X-ray diffraction (Leuner and Dressman, 2000).

Spectroscopic method:

Infrared (IR) spectroscopy is one of these methods that analyzed the interaction of drug and carrier. Structural changes and lack of a crystal structure can lead to change in bonding between functional groups which can be detected by infrared spectroscopy (Leuner and Dressman, 2000).

Dissolution rate method:

Release rate experiments cannot be used on a stand-alone basis to classify the solid dispersion. However, in conjunction with other physicochemical data, they provide strong evidence for the formation of a molecularly dispersed or nearly molecularly dispersed system. When the goal of preparing a solid dispersion is to improve the dissolution characteristics of the drug, the results of the dissolution study are obviously of prime importance in assessing the success of the approach. A well-designed release experiment will show whether the solubility of the drug and its dissolution rate has been enhanced, and also whether the resulting supersaturated solution is stable or tends to precipitate quickly. Comparison of results with those for pure drug powder and physical mixtures of the drug and carrier can help to indicate the mechanism by which the carrier improves dissolution: via solubilization and wetting effects which could be affected by a simple mixture of the components, or by formation of a solid dispersion/solution (Leuner and Dressman, 2000).

2.3 Inclusion complexes with cyclodextrin

2.3.1. Definition of inclusion complex

The class of inclusion complexes such as channel lattice type, clathrate and monomolecular complex results more from the architecture of molecules than from their chemical affinity. One of inclusion complexes, monomolecular inclusion complex involves the entrapment of a single guest molecule in the cavity of the one host molecule. Monomolecular host structures are represented by the cyclodextrins (Martin, 1993).

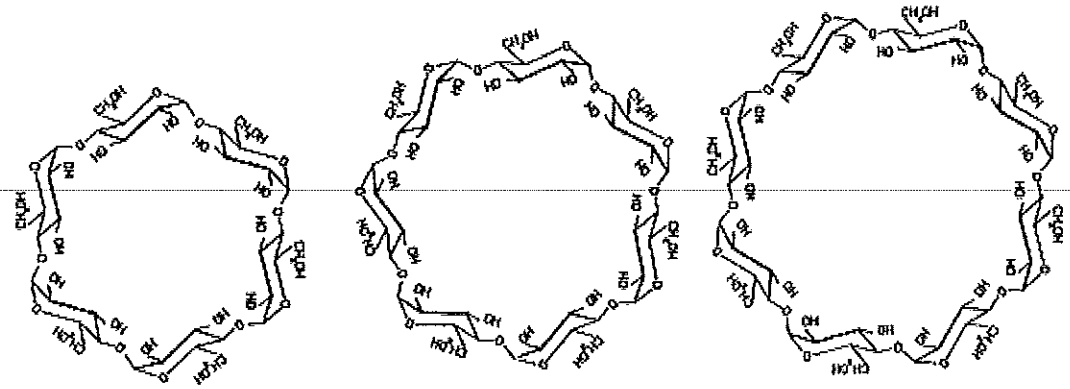
It is generally accepted that in aqueous solution cyclodextrin form what is called "inclusion complex" where water molecules located within the lipophilic central cavity are replaced by a lipophilic guest molecule. However, the hydroxy groups on the outer surface of the cyclodextrin molecule are able to form hydrogen bonds with other molecules and cyclodextrin can, like non-cyclic oligosaccharides and polysaccharides, form water soluble complexes with lipophilic water-insoluble compounds (Loftsson and Duchene, 2007).

2.3.2. History of cyclodextrins

The first record of cyclodextrins was published in 1891 by Villiers, the report was described that the substance in his experiment was a dextrin had isolated from starch by a bacterial digest. He named it that "cellulosine". Later, Schardinger founded two compounds are that of α-dextrin and β-dextrin. These compounds were isolated from bacterial digest of potato starch, so he identified β-dextrin as Villiers' "cellulosine". Now these compounds are commonly called cyclodextrins or less cyclomaltodextrins (i.e. cyclomaltohexaose and cyclomaltoheptaose) or cycloamyloses (i.e. cyclohexaamylose and cycloheptaamylose). In 1935, γ-cyclodextrin was first recorded by Freudenberg and Jacobi.

2.3.3. The chemical structure of cyclodextrins

Cyclodextrins are cyclic oligosaccharides (Jun *et al.*, 2007) derived from starch. It composes of (α-1,4)-linked α-D-glucopyranose units. The most commonly known natural cyclodextrins are made up of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin) (α-1,4)-linked α-D-glucopyranose units. Molecular structure of the natural cyclodextrins is shown in Figure 2.7. The cyclodextrins take the shape of a truncated cone or torus rather than a perfect cylinder due to the conformation of the glucopyranose units. The shape of a truncated cone of cyclodextrin is shown in Figure 2.8. The hydroxyl functions are orientated to the cone exterior with the primary hydroxyl groups at the narrow edge of the cone and the secondary hydroxyl groups at the wider edge. The cavities have different diameters depend on the number of glucopyranose units. Cavity diameter of the natural cyclodextrins is shown in Figure 2.9. The central cavity of the cyclodextrin molecule is lined with skeletal carbons and ethereal oxygens, which gives a lipophilic character and in aqueous solutions, the hydroxyl groups form hydrogen bonds with the water molecules resulting in a hydration shell around the dissolved cyclodextrin molecule, so that the inclusion complex can enhanced the solubility (Miyake *et al.*, 2000), increased the dissolution rate (Savolainen *et al.*, 1998) and improved the bioavailability of poorly soluble drugs (Prabagar *et al.*, 2007).



α-cyclodextrin β-cyclodextrin γ-cyclodextrin

Figure 2.8 Molecular structure of the natural cyclodextrins (Szejtli, 2004)

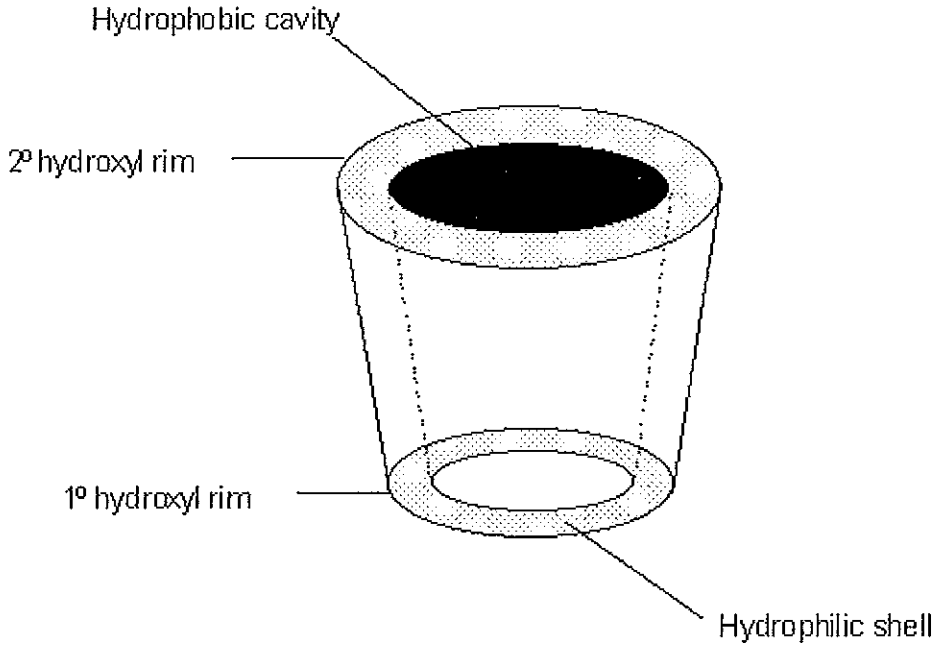


Figure 2.9 The shape of a truncated cone of cyclodextrin (Marshell *et al.*, 2000)

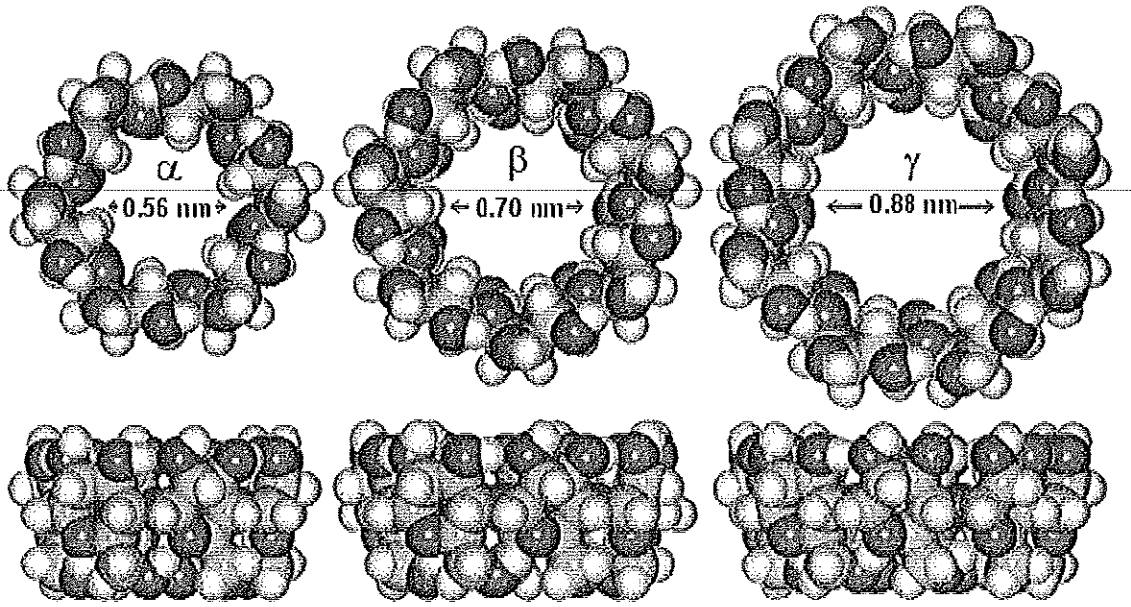


Figure 2.10 Cavity diameter of the natural cyclodextrins (Chaplin, 2008)

2.3.4. Cyclodextrin derivatives

The natural cyclodextrins, in particular β-cyclodextrin, are limited in aqueous solubility (18.5 g/L at 25 °C in water) resulting in poorly soluble in water and other aqueous systems of the complexes obtained. In fact, the aqueous solubility of the natural cyclodextrins is much lower than that of the comparable acyclic dextrin, due to relatively with strong intramolecular hydrogen bonding in the crystal lattice that is shown in Figure 2.10. Substitution of some of the hydroxyl groups in cyclodextrin lead to improvement in the aqueous solubility. Cyclodextrin derivatives are interested in the pharmaceutical field. Examples of cyclodextrin derivatives are hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, dimethyl-β-cyclodextrin, sulfobutyl ether-β-cyclodextrin and maltosyl-β-cyclodextrin. The molecular structure of examples of cyclodextrin derivatives is shown in Figure 2.11 and the solubility of natural cyclodextrins and their derivatives is shown in Table 2.2.

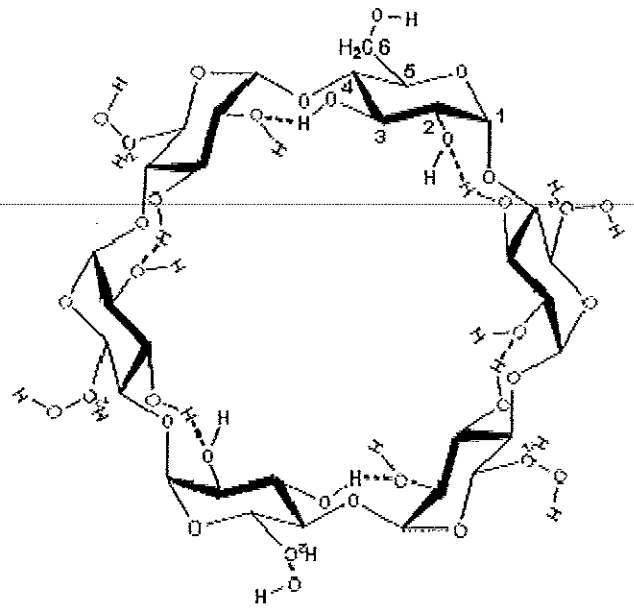
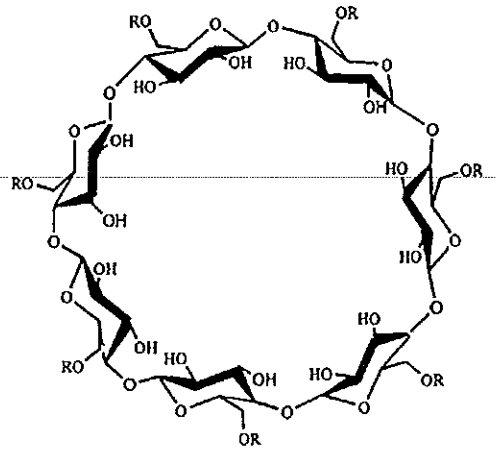


Figure 2.11 Intramolecular hydrogen bonding in the crystal lattice of cyclodextrin (Chaplin, 2008)

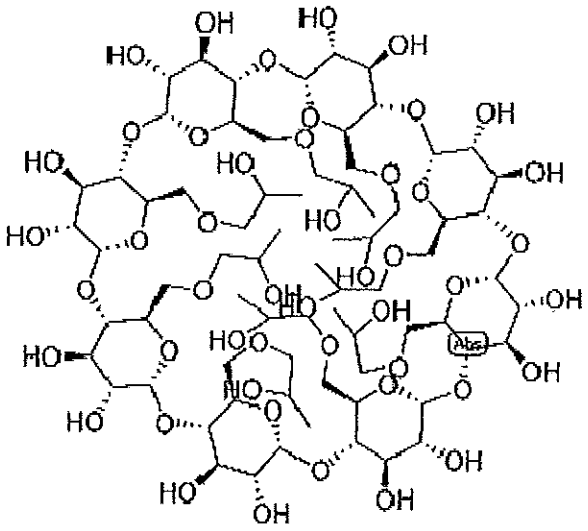
Table 2.2 Solubility of natural cyclodextrins and examples of cyclodextrin derivatives (Brewster and Loftsson, 2007)

Cyclodextrin	Solubility in water (g/L)
α-cyclodextrin	145
β-cyclodextrin	18.5
γ-cyclodextrin	232
Hydroxypropyl-β-cyclodextrin	>600
Hydroxypropyl-γ-cyclodextrin	>500
Dimethyl-β-cyclodextrin	>500
Sulfobutylether-β-cyclodextrin	>500
Randomly methylated-β-cyclodextrin	>500
Maltosyl-β-cyclodextrin	>1500



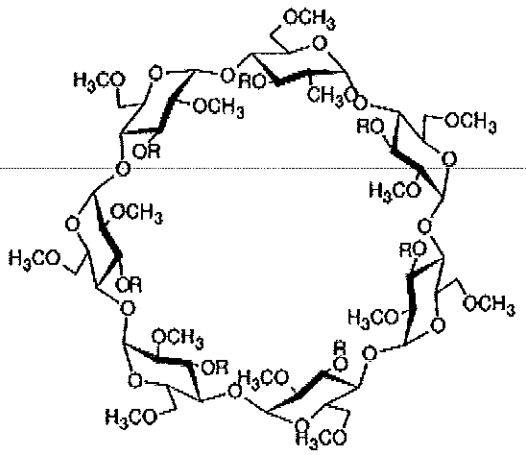
R = C_3H_7OH

Hydroxypropyl-β-cyclodextrin



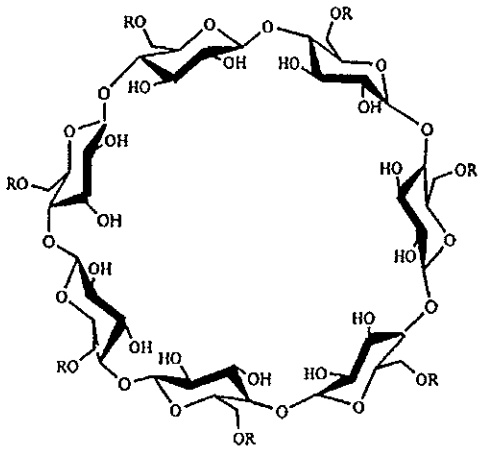
Hydroxypropyl-γ-cyclodextrin

Figure 2.12 The molecular structures of examples of cyclodextrin derivatives (Fried, 2010)



R = H group

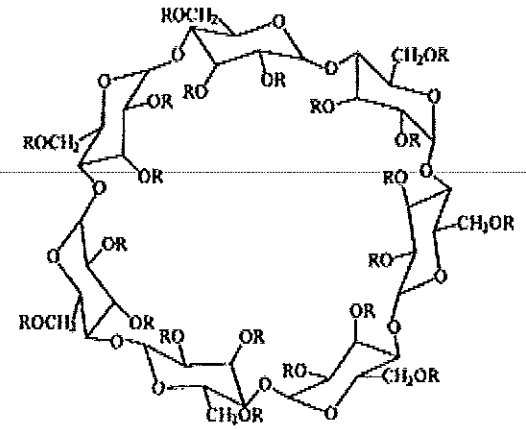
Dimethyl-β-cyclodextrin



R = $(CH_2)_4SO_3Na$

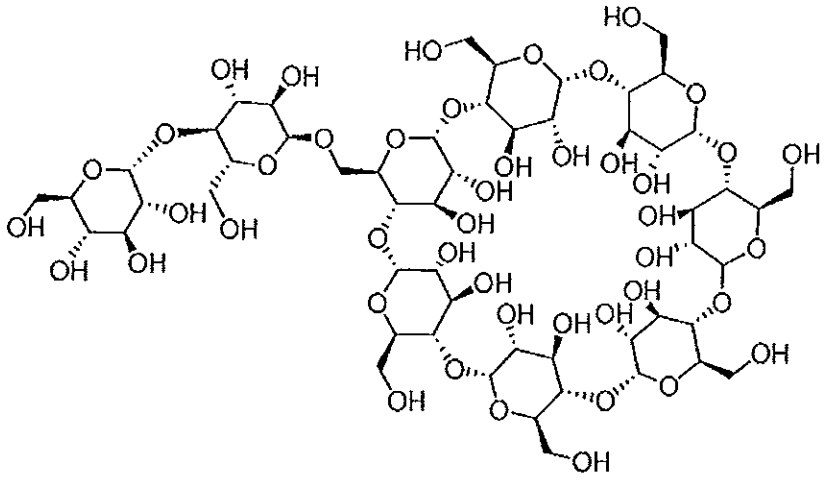
Sulfobutylether-β-cyclodextrin (SBE-β-cyclodextrin)

Figure 2.12 (Continued)



R = CH_3 or H group

Randomly methylated-β-cyclodextrin



Maltosyl-β-cyclodextrin

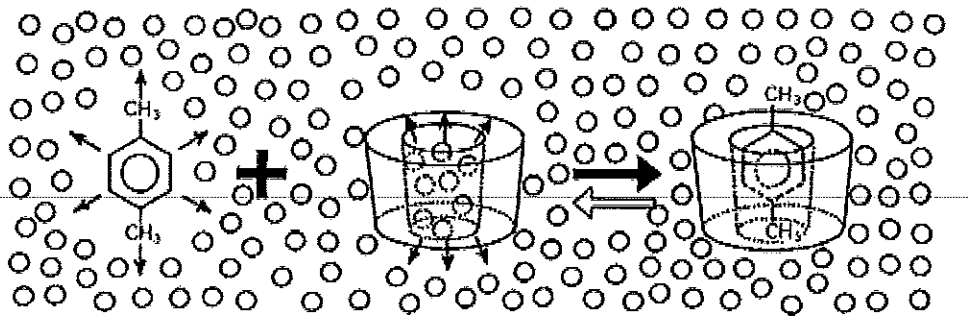
Figure 2.12 (Continued)

Poorly soluble drugs can be prepared in the inclusion complex form with cyclodextrins to increase the solubility, the dissolution rate and improved the bioavailability. For examples, the preparation of ursodeoxycholic acid and chenodeoxycholic acid by inclusion complexes with dimethyl-β-cyclodextrin can improved the solubility and the dissolution rate. The dissolution rate of

ursodeoxycholic acid increased to 100 percentage of drug released and chenodeoxycholic acid-dimethyl-β-cyclodextrin inclusion complexes increased to 85 percentage of drug released (Ventura, *et al.*, 1997). Ventura *et al.* (2005) reported that the solubility of celecoxib was 0.004 mg/ml. The solubility of celecoxib-dimethyl-β-cyclodextrin inclusion complexes prepared by kneading and freeze-drying method was increased to 0.328 and 0.5 mg/ml, respectively. Figueiras *et al.* (2007) found the inclusion complex of omeprazole-methylated-β-cyclodextrin enhanced the solubility and the dissolution rate with 90 percentage of drug released after 6 minutes.

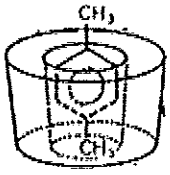
2.3.5. Formation of inclusion complex

The formation of inclusion complex between cyclodextrin and guest is shown in Figure 2.12 In an aqueous solution, the slightly apolar cyclodextrin cavity is occupied by water molecules that are energetically unfavored (polar-apolar interaction), and therefore can be readily substituted by appropriate "guest molecules", which are less polar than water. One, two, or three cyclodextrin molecules contain one or more entrapped "guest" molecules. Most frequently, the host:guest ratio is 1:1. This is the essence of "molecular encapsulation" (Szejtli, 2004).



O : Aqueous molecule

: Guest molecule



: Guest molecule and cyclodextrin

Figure 2.13 The formed inclusion complex of the cyclodextrin and guest (Szejtli, 2004)

Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of guest molecule into the cavity of host molecule. The major structural requirement for inclusion complexation is a fit of the guest into the cavity of host molecule. The cavity of host must be large enough to suit the guest and small enough to eliminate water. No covalent bonds are formed between the drug and cyclodextrin complex formation and in aqueous solutions, the complexes are readily dissociated. Cyclodextrin complex formation usually results from a combination of electrostatic interactions, van der Waals forces, hydrogen bonding and charge-transfer interaction (Brewster and Loftsson, 2007).

2.3.6. Phase-solubility analysis

The phase-solubility profile is obtained by assessing the effect of the cyclodextrin on the apparent solubility of the drug that phase-solubility analysis was developed (Higuchi and Connors, 1965). Phase-solubility diagram is shown in Figure 2.13. It was classified to two major types as A-type and B-type.

2.3.6.1. A-type profiles

The apparent solubility of the substrate increased as a function of cyclodextrin concentration. These three subtypes have been defined as A_L, A_P and A_N-types. A_L-type indicates that a linear increase in solubility as a function of cyclodextrin concentration, A_P-type indicates that an isotherm wherein the curve deviates in a positive direction from linearity (the cyclodextrin is proportionally more effect at high concentrations) and A_N-type indicates that an isotherm wherein the curve deviates in a negative deviation from linearity (the cyclodextrin is proportionally less effect at high concentrations). These isotherms indicate that water soluble complexes are being formed with solubilities higher than that of the uncomplexed substrate. A_L-type is first order with respect to the cyclodextrin (CD) and may be higher order with respect to the drug (D) (i.e. D•CD, D_2•CD, D_3•CD, etc). If the slope of the A_L isotherm is more than unity, so higher order complexes are presented in the solubilization. If the slope of the A_L isotherm is less than one, so a one-to-one complex is presented in the solubilization.

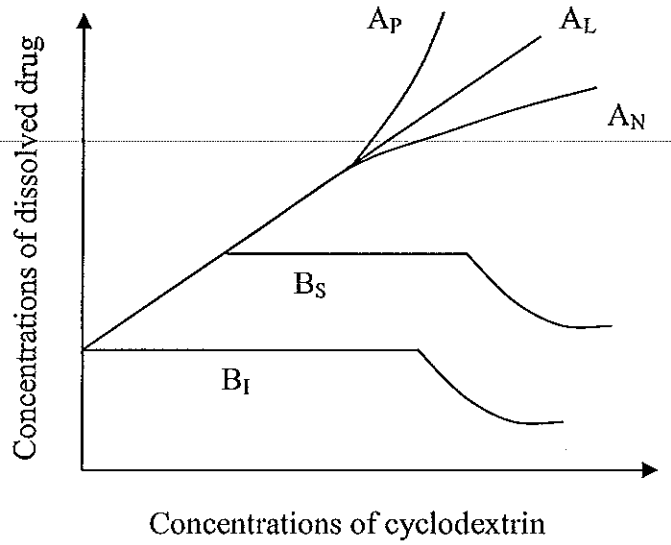


Figure 2.14 Phase-solubility diagram (Brewster and Loftsson, 2007)

Equilibrium constants can derive from A-type phase-solubility profiles. The equilibrium constant for a complexation or the equilibrium constant ($K_{m:n}$) can carried out as follows:

$$m\text{CD} + n\text{D} \xleftrightarrow{K_{m:n}} \text{CD}_m \bullet \text{D}_n \qquad (2.2)$$

$$[a - mx][b - nx] \qquad [x] \qquad (2.3)$$

$$K_{m:n} = \frac{[x]}{[a - mx]^m [b - nx]^n} \qquad (2.4)$$

a : the total concentration of the cyclodextrin

b : the total concentration of the drug (the sum of the complexed and uncomplexed forms)

In the current review, the substrate is termed, D (drug), and the ligand, CD (cyclodextrin). To this point, intrinsic drug solubility is given as D_o and a formed complex is represented by D • CD.

$$[D] = D_o \quad (2.5)$$

$$D_t = D_o + m[D_m \bullet CD_n] \quad (2.6)$$

$$CD_t = CD + n[D_m \bullet CD_n] \quad (2.7)$$

D_o : the equilibrium solubility of the drug in the absence of the cyclodextrin

D_t : the total concentration of the drug (the sum of the complexed and uncomplexed forms)

CD_t : the total concentration of the cyclodextrin

The values for $[D_m \bullet CD_n]$, [D] and [CD] can be derived as:

$$[D_m \bullet CD_n] = \frac{D_t - D_o}{m} \quad (2.8)$$

$$[CD] = CD_t - n[D_m \bullet CD_n] \quad (2.9)$$

D_o is the equilibrium solubility of the drug in the absence of the cyclodextrin, D_t is the total concentration of the drug (the sum of the complexed and uncomplexed forms) and CD_t is the total concentration of the cyclodextrin. For equilibria that are first order with respect to the solubilizer (n=1), the following equation can obtained:

$$D_t = \frac{mKD_o^m CD_t + D_o}{1 + KD_o^m} \quad (2.10)$$

A plot of D_t versus CD_t for the formation of D_m•CD should, therefore, give a straight line with the y-intercept representing D_o and the slope defined as:

$$\text{Slope} = \frac{mKD_o^m}{1 + KD_o^m} \quad (2.11)$$

Therefore, if m is known, the K can be calculated meaning that for one-to-one complexation, m = 1, the following graphical approach can be applied:

$$K_{1:1} = \frac{\text{slope}}{D_o\,(1 - \text{slope})} \quad (2.12)$$

2.3.6.2. B-type profiles

B-type profiles indicate the formation of complexes with limited water solubility and are observed with natural cyclodextrins, especially β-cyclodextrin. These two subclasses are B_S and B_I. B_S-type isotherm indicates the cyclodextrin concentration increases as a soluble complex forms which the total solubility of the substrate was increased. At a particular point in this solubilization process, the maximum solubility of the drug is achieved. Additional cyclodextrin forms additional complex which precipitates but so long. B_I-type is similar to B_S-type except that the complexes are so insoluble that they do not give rise to the initial ascending component of the isotherm.

2.3.7. Preparation of inclusion complex

The inclusion complex can be prepared in several methods such as co-precipitation, slurry complexation, grinding method, spray-drying method, kneading method, evaporation method, freeze-drying method and etc.

2.3.7.1. Co-precipitation

Cyclodextrin is dissolved in water and the guest is added while stirring the cyclodextrin solution. The solution of cyclodextrin and guest is cooled while stirring before a precipitate is formed. The precipitate is separated by decanting,

centrifugation or filtration. The precipitate may be washed with a small amount of water or other water-miscible solvent such as ethyl alcohol, methanol or acetone. Then the precipitate is dried. The example of inclusion complexes prepared by co-precipitation was reported by Liu and Zhu (2005), Tayade and Vavia (2006) and Sapkal *et al.* (2007).

Advantages:

- Treatment and disposal of the mother liquor obtained after collecting the complex may also be a concern. This can be diminished in many cases by recycling the mother liquor (Pitha and Hoshino, 1992).

Disadvantages:

- Solvent washing may be detrimental with some complexes, so this should be tested before scaling up.

- Tank capacity, time and energy for heating and cooling may become important cost factors.

2.3.7.2. Slurry complexation

A slurry is a thick suspension of solids in a liquid. It is not necessary to dissolve the cyclodextrin completely to form a complex. Cyclodextrin can be added to water and stirred. The aqueous phase will be saturated with cyclodextrin in solution. Guest molecules will complex with the cyclodextrin in solution and the complex will precipitate out of the aqueous phase. The inclusion complex is separated and dried to give the product.

Advantages:

- Generally, slurry complexation is performed at ambient temperatures.

- The reduction of the amount of water needed.

Disadvantages:

- The amount of time required to complete the complexation is variable, and depends on the type, molecular size of guest. Assays must be done to determine

the amount of time required.

- With many guests, some heat may be applied to increase the rate of complexation, but care must be applied since too much heat can destabilise the complex and the complexation reaction may not be able to take place completely.

2.3.7.3. Grinding method

Inclusion complexes can be prepared by simply grinding the guest with cyclodextrin. The guest and cyclodextrin are mixed in the solid state or with a minimal amount of water to form a paste. A mixture is ground and placed in a sealed container. After that, it is heated and dried. The examples of inclusion complexes prepared by this method were prazosin hydrochloride-β-cyclodextrin and prazosin hydrochloride-hydroxypropyl-β-cyclodextrin (Liu and Zhu, 2005).

Advantages:

- This method uses little or no added water. If the drug is poorly water-soluble, the grinding method can be employed.

Disadvantages:

- The length of time is required.
- Degree of complexation achieved is very low.
- The amount of water added, the degree of mixing and the heating time have to be optimised for each guest.
- The resulting complex may be cake and lead to incomplete complexation.

2.3.7.4. Spray-drying

The guest is added to a solution containing an excess amount of cyclodextrin. It is also possible to add an excess of the drug to an aqueous cyclodextrin solution. The mixture is agitated and may optionally be heated until the equilibrium is reached, which may take several hours or several days. The equilibrated solution is filtered or centrifuged to give a clear solution of the drug-cyclodextrin complex. The clear solution was spray dried. A solid complex can be obtained by removal of the water by evaporation. The examples of inclusion complexes prepared by this method are

omeprazole-β-cyclodextrin and omeprazole-methylated-β-cyclodextrin (Figueiras *et al*, 2007).

Advantages:

- The inclusion complex resembles very fine amorphous particles.
- This method can produce high yield.

Disadvantages:

- Precipitation must be controlled in order to avoid the particles becoming too large and blocking the spray nozzle.
- With volatile guests, some optimisation of drying conditions is required.
- Spray drying is not a suitable means for drying highly volatile and heat-labile guests.

2.3.7.5. Kneading method

The kneading method is similar to the slurry complexation. The cyclodextrin is dissolved with a minimal amount of water to form a paste. The guest is added and mixing them together. The resulting complex was dried and milled to obtain a powdered form. The example of inclusion complex prepared by this method is nimesulide-β-cyclodextrin (Nalluri *et al*, 2003).

Advantages:

- Simplicity and not high cost.
- The resulting complex can be dried directly.
- If the drug is poorly water-soluble, the kneading methods can be employed
- Not using of organic solvents.

Disadvantages:

- Pastes will sometimes dry forming a hard mass instead of a fine powder. Generally, the hard mass can be dried and milled to obtain a powdered form of the complex.
- The amount of time required is depended on the guest.

2.3.7.6. Evaporation method

For preparation of complexes using the evaporation method, the required quantities of guest molecules and cyclodextrin were dissolved in the solvent. Both solutions were mixed and the solvent was evaporated by heating. The resulting complex was pulverized and sieved. The examples of inclusion complexes prepared by this method are simvastatin-β-cyclodextrin and simvastatin-hydroxypropyl-β-cyclodextrin (Patel and Patel, 2007).

Advantages:

- Degree of mixing, amount of heating and time can be controlled.
- Not all guests are readily solubilised in water, making complexation either very slow or impossible. The use of an organic solvent to dissolve the guest is desirable.
- The guest and cyclodextrin were completely and easily dissolved.

Disadvantages:

- Use of organic solvent.
- The solvent must be selected to suitable for the guest and cyclodextrin.

2.3.7.7. Freeze-drying method

The cyclodextrin was dissolved in aqueous solution. The guest was added to this solution under stirring. The clear solution was frozen and then frozen solution was lyophilized in a freeze dryer. The example of inclusion complex prepared by this method is celecoxib-dimethyl-β-cyclodextrin (Ventura *et al*, 2005).

Advantages:

- Low temperature reduces the loss of extremely volatile guests.
- This method is especially useful for heat labile guests

Disadvantages:

- The solution was controlled by freezing in order to avoid degradation.
- The process is time consuming.

CHAPTER 3

MATERIALS AND METHOD

Materials

1. Simvastatin (Batch number M070505, Crosschem, Switzerland)
2. Methylated-β-cyclodextrin (CAVASOL® W7 M Pharma, Wacker-Chemie GmbH, Germany)
3. PVP K-30 (Lot no. 03300103183, P.C. Drug Center Co., Ltd., Bangkok, Thailand)
4. Sodium hydroxide (Merck®, Darmstadt, Germany)
5. Hydrochloric acid (Baker Analyzed®, Mallinckrodt Baker Inc., Phillipsburg, USA)
6. Potassium phosphate (Univar®, Batch number AF705005, Ajax Finechem Pty Ltd., Australia)
7. Sodium chloride (Batch number 1256, P.C. Drug Center Co., Ltd., Bangkok, Thailand)
8. Ethanol (Lot no. L023600, AnalaR®, BDH Laboratory Supplies, England)
9. Methanol (Batch number 08121117, LAB-SCAN®, Analytical Sciences, RCI Labscan Ltd., Bangkok, Thailand)
10. Potassium bromide (SIGMA-ALDRICH Chemie GmbH, Germany)

Equipments

1. Rotary evaporator (EYELA® N-1000 Series, Tokyo Rikakikai Co., Ltd., Japan)
2. Dissolution apparatus (HANSON Model SR2, USA)
3. Water bath shaker (WB-14, Memmert, Germany)
4. UV-spectrophotometer (Spectronic® Genesys 5™, USA)
5. Freeze dryer (Flexi-Dry™ μP Microprocessor Control, USA)
6. Differential scanning calorimeter (Perkin Elmer DSC7, USA)
7. Powder X-ray diffractometer (Philips X'Pert MPD, Netherland)
8. FT-IR spectrometer (Spectrum One, Perkin-Elmer Ltd., United Kingdom)
9. Analytical balance (Sartorius®, Germany)
10. Incubator (Memmert, Germany)
11. Sieves (Fritsch® Analysette, Germany)
12. Sonicator (Elma®, Transonic 310/H, Germany)

Methods

3.1. Standard curve of simvastatin

The stock solution I of simvastatin was prepared by dissolving accurately weight 20 mg of simvastatin with methanol and adjusted to 50-ml with methanol. The stock solution II of simvastatin were prepared by pipetting 2 ml of the stock solution I, transferring to 50-ml volumetric flask, and adjusting to volume with methanol.

The standard solutions of simvastatin were prepared by pipetting the stock solution II of simvastatin 2, 3, 4, 5, 6 and 7 ml, transferring to 10-ml volumetric flask and adjusting to volume with distilled water, simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin. The absorbance of each standard solution of simvastatin was analyzed by using a UV-spectrophotometer at 238 nm without the interference of methylated-β-cyclodextrin. The standard curve was constructed by plotting the absorbance value of simvastatin concentrations against the concentration of simvastatin. The equation with linear regression of the line was used to determine the concentration of simvastatin from the samples.

3.2. Phase-solubility study

Phase-solubility study was modified from the method described by Jun *et al.* (2007). Briefly, excess amount of simvastatin (100 mg) was added to 10 ml of aqueous solution containing various concentrations of methylated-β-cyclodextrin (0, 2, 4, 6, 8 and 10 mM). The contents were shaken at 37 ± 0.5 °C until equilibrium was reached (48 hours). Then, the samples were withdrawn, filtered through a 0.45 μm membrane filter and suitably diluted with distilled water. Drug concentration was determined by spectrophotometer at 238 nm without the interference of methylated-β-cyclodextrin. Each experiment was carried out in triplicate. The apparent stability constant (K_S) was calculated from the phase-solubility diagram with the assumption of 1:1 stoichiometry according to the following equation:

$$K_S = \text{slope} / \text{Intercept}\ (1 - \text{slope}) \qquad (3.1)$$

Slope is obtained from the initial straight-line portion of the plot of simvastatin concentration against methylated-β-cyclodextrin concentrations.

Intercept is the intrinsic solubility of simvastatin in the absence of methylated-β-cyclodextrin.

3.3. Preparation of simvastatin-methylated-β-cyclodextrin inclusion complexes

Inclusion complexes of simvastatin and methylated-β-cyclodextrin were prepared in the molar ratio of one to one by 1) kneading method 2) co-evaporation method and 3) freeze-drying method.

3.3.1. Preparation of inclusion complex by kneading method

Dissolved methylated-β-cyclodextrin (3.13 g) in small amount (5 ml) of distilled water. Simvastatin (1 g) was added slowly. The mixture was ground for 1 hour. The paste was dried at 40°C for 12 hours. The dried complex was pulverized and sieved through a 40-160 μm sieve for 5 minutes.

3.3.2. Preparation of inclusion complex by co-evaporation method

The simvastatin (1 g) and methylated-β-cyclodextrin (3.13 g) were dissolved in 40 ml of ethanol. The solvent was evaporated by a rotary evaporator at 40-50°C. The dried complex was pulverized and sieved through a 40-160 μm sieve for 5 minutes.

3.3.3. Preparation of inclusion complex by freeze-drying method

The methylated-β-cyclodextrin (3.13 g) was dissolved in 200 ml of water. Simvastatin (1 g) was added to the solution. The suspension was sonicated at room temperature for 1 hour and the suspension was shaken at room temperature until equilibrium was reached (48 hours). The suspension was freeze-dried. Finally, the dried complex was pulverized and sieved through a 40-160 μm sieve for 5 minutes.

3.4. Preparation of simvastatin-methylated-β-cyclodextrin physical mixture

The powder of both components was sieved through a 40-160 μm sieve. Physical mixture of simvastatin and methylated-β-cyclodextrin was prepared by simply mixing until homogeneous.

3.5. Preparation of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10 by solvent method

Simvastatin and PVP K-30 was dissolved in 40 ml of ethanol. The obtained solution was evaporated by rotary evaporator at 40-50°C. The dried complex was pulverized and sieved through a 40-160 μm sieve for 5 minutes.

3.6. Preparation of simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10

The powder of both components was sieved through a 40-160 μm sieve. Physical mixture of simvastatin and PVP K-30 was prepared by simply mixing until homogeneous.

3.7. Drug content

The drug content of simvastatin-PVP K-30 solid dispersions, simvastatin-methylated-β-cyclodextrin inclusion complexes and physical mixtures was determined by accurately weighed of sample (simvastatin was about 5 mg in all cases) and transferred to a 50-ml volumetric flask, dissolved with methanol and adjusted to 50 ml. The 1 ml of this solution was transferred into 10-ml volumetric flask and adjusted to 10 ml with distilled water. The simvastatin content was analyzed by spectrophotometer at 238 nm. Each experiment was carried out in triplicate.

3.8. Solubility studies

The solubility of simvastatin-methylated-β-cyclodextrin inclusion complexes and simvastatin-PVP K-30 solid dispersions compared with physical mixtures and pure drug was studied.

The excess amount of samples (100 mg) was added to test tubes containing 10 ml of distilled water. The solution was shaken at 37 ± 0.5°C until equilibrium was reached (48 hours). Then, the samples were withdrawn, filtered through a 0.45 μm membrane filter and suitably diluted with distilled water. Drug concentration was determined by UV-spectrophotometer at 238 nm. Each experiment was carried out in triplicate.

3.9. Dissolution studies

The dissolution of simvastatin-methylated-β-cyclodextrin inclusion complexes and simvastatin-PVP K-30 solid dispersions compared with physical mixtures and pure drug was determined.

Dissolution studies were modified from USP 27 using dissolution apparatus type II (the paddle method). Each flask contained 900 ml of simulated gastric fluid without pepsin pH 1.2 or simulated intestinal fluid without pancreatin pH 6.8 as dissolution medium and maintained at 37 ± 0.5°C. The paddles were rotated at 100 rpm. Powdered samples were tested with the dispersed amount method (Liu and Zhu, 2005) by adding 5 mg of simvastatin or its equivalent on the surface of dissolution medium. At 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes, the 5 ml aliquot was withdrawn from the dissolution medium, filtered through a 0.45 μm membrane filter and replaced with a 5 ml of fresh dissolution medium after each sampling. The amount of simvastatin was determined spectrophotometrically at 238 nm without the interference from methylated-β-cyclodextrin. Concentration of simvastatin was calculated using the regression equation of a standard curve developed in the same medium and expressed as percentage of drug dissolved from the mean of six determinations.

3.10. Physicochemical characterization

Physicochemical characterizations of simvastatin-methylated-β-cyclodextrin inclusion complexes and simvastatin-PVP K-30 solid dispersions compared as physical mixtures and pure drug were studied.

3.10.1. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to determined melting point characterization of the simvastatin, methylated-β-cyclodextrin, PVP K-30, simvastatin-PVP K-30 physical mixtures, simvastatin-methylated-β-cyclodextrin physical mixture, simvastatin-PVP K-30 solid dispersions and simvastatin-methylated-β-cyclodextrin inclusion complexes. Temperature and enthalpy were calibrated with the standard materials. The samples were accurately weighed in crimped aluminum pans, using an empty pan sealed as reference and heated from 50-200°C at scan rate of 10°C/minute under the nitrogen stream with the flow rate of 50 ml/minute.

3.10.2. Powder X-ray diffractometry (PXRD)

Powder X-ray diffraction patterns of simvastatin, methylated-β-cyclodextrin, PVP K-30, simvastatin-PVP K-30 physical mixtures, simvastatin-methylated-β-cyclodextrin physical mixture, simvastatin-PVP K-30 solid dispersions and simvastatin-methylated-β-cyclodextrin inclusion complexes were analyzed by using Powder X-ray diffractometer with Ni filtered Cu K_α (λ = 1.54 °A) line as the source of radiation. This was operated at the voltage of 40 kV and the current of 45 mA. Each sample was weighed in a cavity of an aluminum sample holder, smoothed with a glass slide and inserted into the sample holder. The sample and detector were moved in a circular path to determine the angles of scattered radiation. The sample was analyzed in the 2θ angle range between 0° and 60° with a scan speed of 3θ/min and a step size of 0.01°.

3.10.3. Fourier-transform infrared (FT-IR) spectroscopy

FT-IR spectra of simvastatin, methylated-β-cyclodextrin, PVP K-30, simvastatin-PVP K-30 physical mixtures, simvastatin-methylated-β-cyclodextrin physical mixture, simvastatin-PVP K-30 solid dispersions and simvastatin-methylated-β-cyclodextrin inclusion complexes were analyzed by FT-IR

spectroscopy. Each spectrum was recorded in the frequency range of 4,000-450 cm^{-1} and 16 scans were obtained at 4 cm^{-1} resolution. Potassium bromide pellet method was used for determination. The sample (10 mg) was mixed with potassium bromide (100 mg). The KBr disk was prepared by compressing the powder, under force in a hydraulic press.

3.11. Stability studies

Simvastatin-methylated-β-cyclodextrin inclusion complex and simvastatin-PVP K-30 solid dispersion which exhibited the highest dissolution rate were stored in a desiccator and protected from light at room temperature and at 45°C for 3 months. The dissolution rate, drug content and physicochemical characterizations were evaluated.

3.12. Statistical analysis

The statistical significance was performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p<0.05$ and $p<0.01$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Standard curve of simvastatin

UV absorbance of various concentrations of simvastatin in distilled water, simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin are shown in Table 4.1, Table 4.2 and Table 4.3, respectively. Standard curves of simvastatin in distilled water, simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin are shown in Figure 4.1, Figure 4.2 and Figure 4.3, respectively. Standard curves of simvastatin in distilled water, simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin were linear over the range of simvastatin concentrations used. The correlation coefficient (R^2) of straight line were 0.9997, 0.9993 and 0.9995, respectively.

Table 4.1 UV absorbance of various concentrations of simvastatin in distilled water

Concentration (μg/ml)	Absorbance[a]
3.20	0.131 (0.006)
4.80	0.217 (0.006)
6.40	0.305 (0.008)
8.00	0.392 (0.017)
9.60	0.471 (0.012)
11.20	0.564 (0.008)

a : Means (s.d.) of triplicate results

Table 4.2 UV absorbance of various concentrations of simvastatin in simulated gastric fluid without pepsin

Concentration (μg/ml)	Absorbance[a]
3.20	0.187 (0.004)
4.80	0.273 (0.009)
6.40	0.363 (0.006)
8.00	0.453 (0.013)
9.60	0.555 (0.012)
11.20	0.648 (0.006)

a : Means (s.d.) of triplicate results

Table 4.3 UV absorbance of various concentrations of simvastatin in simulated intestinal fluid without pancreatin

Concentration (μg/ml)	Absorbance[a]
3.20	0.184 (0.008)
4.80	0.274 (0.006)
6.40	0.364 (0.004)
8.00	0.460 (0.012)
9.60	0.543 (0.011)
11.20	0.645 (0.005)

a : Means (s.d.) of triplicate results

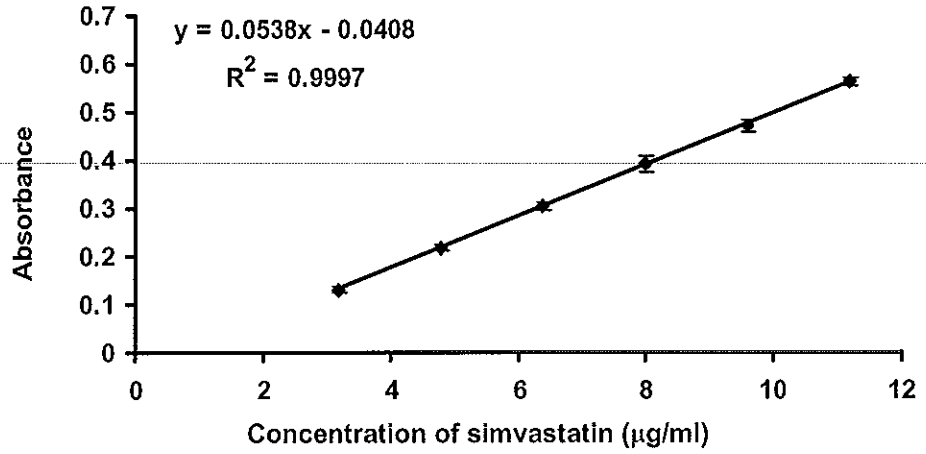


Figure 4.1 Standard curve of simvastatin in distilled water at 238 nm. The plotted data are means ± SD (n = 3).

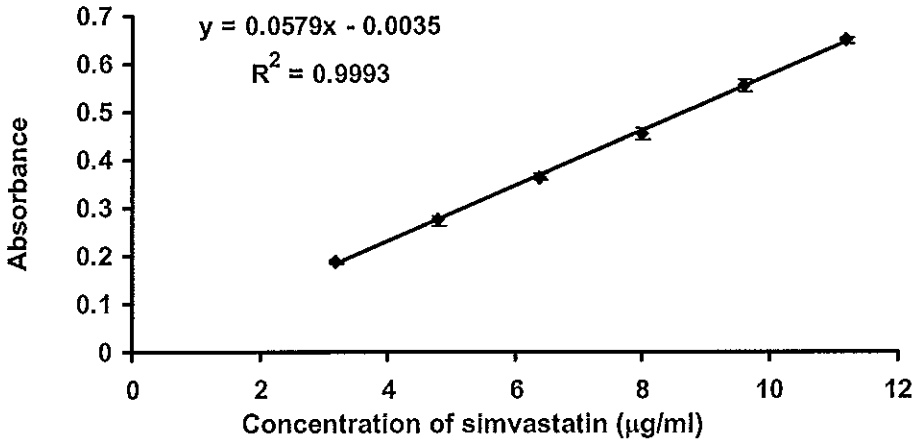


Figure 4.2 Standard curve of simvastatin in simulated gastric fluid without pepsin at 238 nm. The plotted data are means ± SD (n = 3).

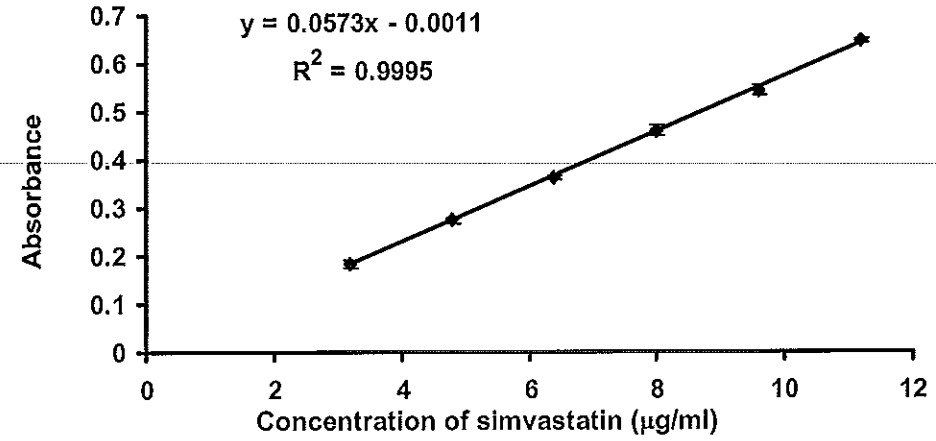


Figure 4.3 Standard curve of simvastatin in simulated intestinal fluid without pancreatin at 238 nm. The plotted data are means ± SD (n = 3).

4.2. Phase-solubility study

The phase-solubility diagram of simvastatin in the presence of various methylated-β-cyclodextrin concentrations (0-10 mM) at 37 ± 0.5°C is shown in Figure 4.4. From this curve, it can be seen that the solubility of simvastatin was increased linearly as the function of methylated-β-cyclodextrin concentrations. The increase in simvastatin solubility may be due to the formation of inclusion complex between simvastatin and methylated-β-cyclodextrin during process of solubility study. The water solubility of simvastatin was 0.013 mM. The solubility of simvastatin was increased by 12 folds at 10 mM of methylated-β-cyclodextrin. Similar result was reported by Patel and Patel (2007) which found that the solubility of simvastatin was increased by 5.84 folds and 10.14 folds at 10 mM concentration of β-cyclodextrin and hydroxypropyl-β-cyclodextrin, respectively. Cunha-Filho *et al.* (2007) reported that methylated-β-cyclodextrin had higher solubilizing effect on the β-lapachone, antitumor drug, than β-cyclodextrin and hydroxypropyl-β-cyclodextrin. The solubilization performance of methylated-β-cyclodextrin could be derived from the presence of the methyl groups, which can extend the hydrophobic region of the cyclodextrin cavity favoring and stabilizing the inclusion complexation

of the included molecule.

The solubility of simvastatin was increased linearly with the increasing concentration of methylated-β-cyclodextrin over the range of concentration used. The solubility curve showed an A_L type as described by Higuchi and Connors (1964) with the slope value, 0.0137, was lower than one indicating that 1:1 molar ratio of simvastatin-methylated-β-cyclodextrin inclusion complex was formed. The stability constant can be calculated from the equation as K_s = slope/intercept (1-slope), intercept is the intrinsic solubility of simvastatin in the absence of methylated-β-cyclodextrin. The stability constant of the inclusion complex between simvastatin and methylated-β-cyclodextrin was 1,240 M^{-1} which indicated a suitable and stable complex formation. It is supported by Patel and Patel (2007) which suggested that the inclusion complexes of drug with cyclodextrin with the values of K_s in the range of 200-5,000 M^{-1} enhanced dissolution properties and increased better bioavailability.

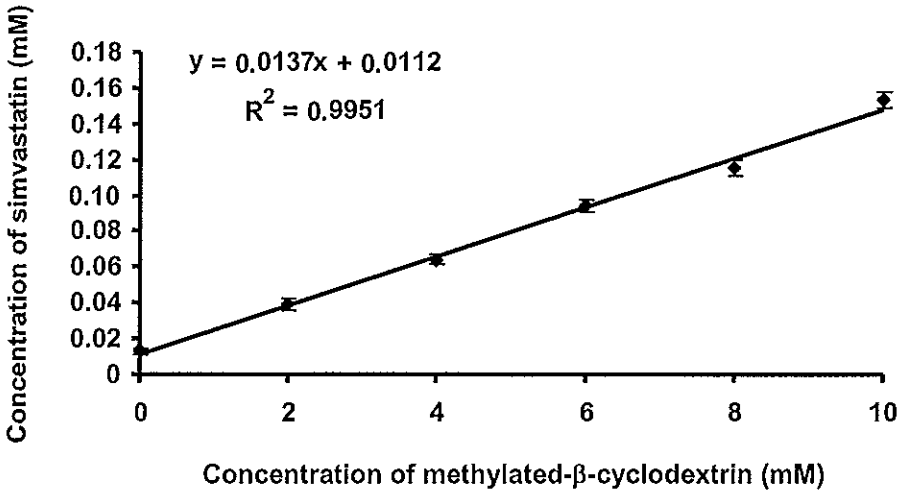


Figure 4.4 Phase-solubility diagram of simvastatin as a function of methylated-β-cyclodextrin concentrations at 37°C. The plotted data are means ± SD (n = 3).

4.3. Product of simvastatin-methylated-β-cyclodextrin inclusion complexes

The kneaded, co-evaporated and freeze-dried samples were obtained as white, odorless and free-flowing powders.

4.4. Product of simvastatin-methylated-β-cyclodextrin physical mixture

The physical mixture was obtained as white, odorless, free-flowing powder.

4.5. Product of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10 by solvent method

The samples in weight ratios of 1:1, 1:2, 1:3 and 1:4 were obtained as creamy white, odorless powders. The samples in weight ratios of 1:6, 1:8 and 1:10 were obtained as very sticky powders and difficult to manipulate. Therefore, the samples in weight ratios of 1:1, 1:2, 1:3 and 1:4 were selected for further studies.

4.6. Product of simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10

The physical mixtures of all weight ratios were obtained as creamy white, odorless powders.

4.7. Drug content

The drug content of all samples analyzed by UV-spectrophotometer is shown in Table 4.4 and Table 4.5. The content of drug was between 95.09 and 104.45%.

Table 4.4 Drug content of simvastatin-methylated-β-cyclodextrin physical mixture and simvastatin-methylated-β-cyclodextrin inclusion complexes prepared by kneading, co-evaporation and freeze-drying methods.

Simvastatin-methylated-β-cyclodextrin	**%Drug content [a] (SD)**
Physical mixture	104.25 (0.236)
Kneaded sample	102.21 (0.131)
Co-evaporated sample	96.27 (0.143)
Freeze-dried sample	97.61 (0.310)

a : Means of triplicate results

Table 4.5 Drug content of simvastatin-PVP K-30 physical mixtures and simvastatin-PVP K-30 solid dispersions prepared by solvent method.

Simvastatin-PVP K-30	**%Drug content [a] (SD)**
Physical Mixtures	
1:1 Simvastatin:PVP K-30	103.24 (0.219)
1:2 Simvastatin:PVP K-30	103.34 (0.242)
1:3 Simvastatin:PVP K-30	104.45 (0.203)
1:4 Simvastatin:PVP K-30	104.23 (0.186)
Solid dispersions	
1:1 Simvastatin:PVP K-30	95.46 (0.474)
1:2 Simvastatin:PVP K-30	95.09 (0.398)
1:3 Simvastatin:PVP K-30	97.20 (0.041)
1:4 Simvastatin:PVP K-30	97.36 (0.372)

a : Means of triplicate results

4.8. Solubility studies

The solubility of simvastatin, simvastatin-methylated-β-cyclodextrin physical mixture and simvastatin-methylated-β-cyclodextrin inclusion complexes

at 37 ± 0.5°C are shown in Table 4.6 and Figure 4.5.

Table 4.6 Solubility of simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples at 37 ± 0.5°C

Samples	**Solubility ± SD (μg/ml)[a]**
Simvastatin	2.08 ± 0.15
Physical mixture	5.10 ± 0.17
Kneaded	5.84 ± 0.57
Co-evaporated	13.62 ± 0.38
Freeze-dried	6.32 ± 0.20

a : Means (s.d.) of triplicate results

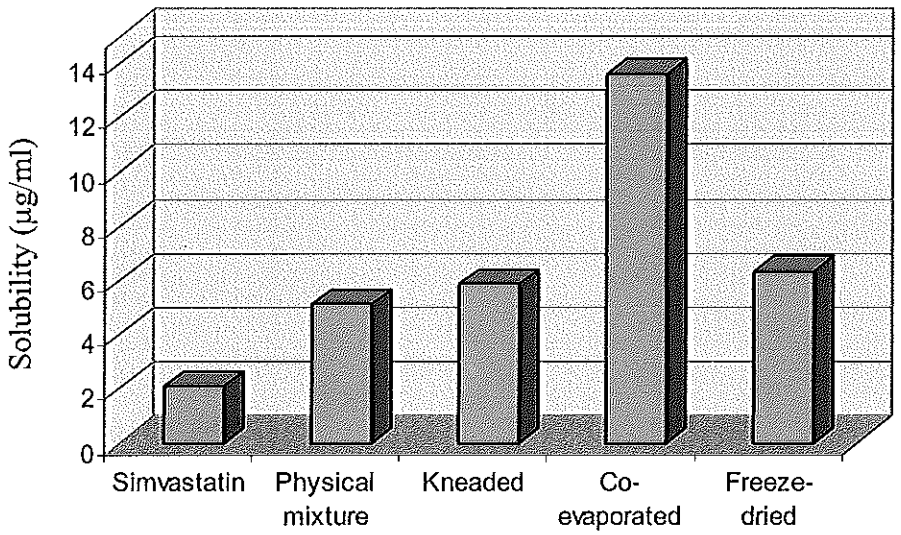


Figure 4.5 The solubility of simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples in distilled water at 37 ± 0.5°C

The solubility of pure simvastatin was found to be 2.08 ± 0.15 μg/ml. The solubility of simvastatin from physical mixture and all inclusion complexes are significantly ($p<0.05$) higher than that of pure drug. The solubility of simvastatin-

methylated-β-cyclodextrin physical mixture was 5.10 ± 0.17 μg/ml. The 2-folds increased in drug solubility with respect to pure drug may be due to the improvement in drug wettability and a lower interfacial tension between simvastatin and the distilled water by cyclodextrin (Ventura *et al.*, 2005 and Arias *et al.*, 2000), lowers the advancing contact angle and aids in displacing an air phase at the surface and replacing it with a liquid phase (Martin *et al.*, 1969).

Kneaded, co-evaporated and freeze-dried samples enhanced solubility of simvastatin to 5.84 ± 0.57, 13.62 ± 0.38 and 6.32 ± 0.20 μg/ml respectively. The physical mixture, kneaded, co-evaporated and freeze-dried samples improved water solubility of simvastatin by 2.4 folds, 2.8 folds, 6.6 folds and 3 folds, respectively. Simvastatin-methylated-β-cyclodextrin inclusion complex prepared by co-evaporation method showed the highest solubility. This may be due to the improvement of wettability, hydrophilicity and the lowering of interfacial tension between drug and water. Additionally, the formation of higher energetic amorphous state of simvastatin was confirmed by PXRD study (Fernandes *et al.*, 2002). The slightly increased in drug solubility of kneaded and freeze-dried samples compared to pure drug may be due to the increasing in drug wettability, hydrophilicity and a reduction in the degree of drug crystallinity (Veiga *et al.*, 1998). The solubility of drug from inclusion complexes prepared by kneading and freeze-drying method was not much more different compare with that of physical mixture because of the physicochemical properties of kneaded and freeze-dried samples were similarly to physicochemical properties of physical mixture.

The solubilities of simvastatin, simvastatin-PVP K-30 physical mixtures and solid dispersions at 37 ± 0.5°C are shown in Table 4.7 and Figure 4.6. Simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 increased simvastatin solubility to 3.55 ± 0.12, 5.13 ± 0.28, 5.79 ± 0.35 and 6.56 ± 0.39 μg/ml, respectively. The physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 enhanced simvastatin solubility by 1.7 folds, 2.5 folds, 2.8 folds and 3.2 folds, respectively. This may be due to the improvement in drug wettability and the reduction in a surface tension.

Simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 improved water solubility of simvastatin to 24.6± 0.32, 27.53 ± 0.36, 28.69 ± 0.44 and 29.75 ± 0.30 μg/ml, respectively.

The solubility of simvastatin-PVP K-30 solid dispersions in weight ratio of 1:2 was significantly ($p<0.01$) higher than that of simvastatin-PVP K-30 solid dispersions in weight ratio of 1:1.

The solubility of simvastatin-PVP K-30 solid dispersions in weight ratio of 1:3 was significantly ($p<0.01$) higher than that of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1 and 1:2.

The solubility of simvastatin-PVP K-30 solid dispersions in weight ratio of 1:4 was significantly ($p<0.01$) higher than that of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2 and 1:3.

All solid dispersions gave the significantly ($p<0.05$) higher solubility of simvastatin compared with physical mixtures and pure drug. The solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 enhanced simvastatin solubility of by 11.8 folds, 13.2 folds, 13.8 folds and 14.3 folds, respectively. This may be due to the improvement in drug wettability, the reduction in a surface tension, the intermolecular hydrogen bonding between drug and PVP K-30 which confirmed by FT-IR study and the higher energetic amorphous state of simvastatin which confirmed by PXRD study. This similarly results were reported by Ruan *et al.* (2005), Paradkar *et al.* (2004) and Tantishaiyakul *et al.* (1996).

Table 4.7 Solubility of simvastatin, simvastatin-PVP K-30 physical mixtures and simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 37 ± 0.5°C

Samples	Water solubility ± SD (µg/ml)[a]
Simvastatin	2.08 ± 0.15
Physical mixture 1:1	3.55 ± 0.12
Physical mixture 1:2	5.13 ± 0.28
Physical mixture 1:3	5.79 ± 0.35
Physical mixture 1:4	6.56 ± 0.39
Solid dispersion 1:1	24.56 ± 0.32
Solid dispersion 1:2	27.53 ± 0.36
Solid dispersion 1:3	28.69 ± 0.44
Solid dispersion 1:4	29.75 ± 0.30

a : Means of triplicate results

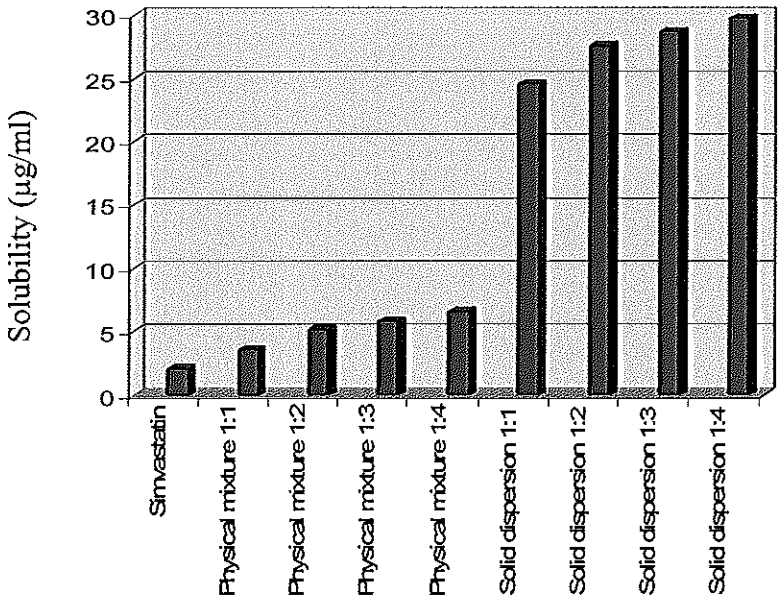


Figure 4.6 The solubility of simvastatin, simvastatin-PVP K-30 physical mixtures and simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 37 ± 0.5°C

4.9. Dissolution studies

The percentage of drug dissolved of pure simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin are presented in Table 4.8 and Table 4.9, respectively. The dissolution profiles of simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin are presented in Figure 4.7 and Figure 4.8, respectively. The dissolution profiles were plotted as the percentage simvastatin dissolved versus time. Each point is the mean of six determinations.

From Figure 4.7, the percentage of drug dissolved of pure drug, physical mixture and all inclusion complexes reached a peak after 5 minutes then decreased because of drug supersaturation in the initial stage of dissolution (Janssens *et al.*, 2008). The simvastatin gave the slowest dissolution property because the hydrophobic properties of the drug resulted in drug floated on the surface of the dissolution medium for a long period. The simvastatin-methylated-β-cyclodextrin physical mixture showed slow dissolution rate. The percentage of drug dissolved in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin after 10 minutes were 9.04% and 14.84% and after 2 hours were 22.72% and 25.42%, respectively.

The physical mixture gave slightly higher dissolution rate than pure drug. The improvement of dissolution obtained from physical mixture could be attributed to both the improvement in drug wettability and the formation of readily soluble inclusion complex in the dissolution medium (Ruan *et al.*, 2005).

The dissolution profiles of all inclusion complexes demonstrated higher drug dissolution than pure drug and physical mixture. The increment in dissolution from inclusion complexes have been explained on the increase of wettability, the basis of a reduction of crystallinity, a lower interfacial tension between simvastatin and the dissolution medium induced by methylated-β-cyclodextrin and a greater water solubility of drug from the inclusion complex (Figueiras *et al.*, 2007, Ventura *et al.*, 1997 and Ventura *et al.*, 2005).

The percentage of drug dissolved of kneaded sample in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin after 10 minutes were 17.64% and 20.92% and after 2 hours were 38.28% and 36.84%, respectively. The percentage of drug dissolved of co-evaporated sample in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin after 10 minutes were 31.37% and 34.01% and after 2 hours were 36.56% and 43.96%, respectively. The percentage of drug dissolved of freeze-dried sample dissolved in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin after 10 minutes were 18.49% and 21.08 and after 2 hours were 35.37% and 27.88%, respectively.

The co-evaporated samples exhibited the fastest dissolution. The percentage of drug dissolved in simulated gastric fluid without pepsin after 10 minutes were about 4 folds, 3.5 folds higher than that of pure drug and physical mixture, respectively. The percentage of drug dissolved in simulated intestinal fluid without pancreatin after 10 minutes were about 3.1 folds, 2.3 folds higher than that of pure drug and physical mixture, respectively. The enhanced in drug dissolved may be due to the higher solubility and the higher energetic amorphous state of simvastatin obtained from co-evaporated sample (Reddy *et al.*, 2004 and Ruan *et al.*, 2005).

Table 4.8 The percentage of drug dissolved from simvastatin, simvastatin-methylated-β-cyclodextrin physical mixture, kneaded, co-evaporated and freeze-dried samples of simvastatin-methylated-β-cyclodextrin inclusion complexes in molar ratio of 1:1 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated gastric fluid without pepsin

Times	% Drug dissolved ± SD				
	Simvastatin	Physical mixture	Kneaded	Co-evaporated	Freeze-dried
5	13.98 ± 0.58	14.82 ± 1.52	22.77 ± 1.18	43.62 ± 1.27	20.84 ± 0.64
10	7.49 ± 1.27	9.04 ± 1.27	17.64 ± 1.28	31.37 ± 1.40	18.49 ± 1.02
15	7.23 ± 0.60	8.33 ± 0.97	19.38 ± 1.21	31.73 ± 1.02	19.80 ± 0.92
20	7.86 ± 1.06	10.78 ± 1.14	23.10 ± 0.72	32.64 ± 1.13	22.02 ± 1.02
30	10.64 ± 1.41	11.67 ± 1.38	26.39 ± 0.90	33.45 ± 0.98	24.73 ± 1.16
45	12.33 ± 1.17	14.07 ± 1.11	30.81 ± 0.79	37.03 ± 1.07	27.37 ± 1.51
60	14.21 ± 1.05	17.50 ± 0.99	31.42 ± 0.64	35.42 ± 1.16	30.28 ± 1.36
90	19.57 ± 1.46	19.99 ±0 .63	36.31 ± 0.94	36.30 ± 1.34	31.58 ± 1.20
120	21.97 ± 0.97	22.72 ± 1.15	38.28 ± 1.25	36.56 ± 1.20	35.37 ± 1.07

Table 4.9 The percentage of drug dissolved from simvastatin, simvastatin-methylated-β-cyclodextrin physical mixture, kneaded, co-evaporated and freeze-dried samples of simvastatin-methylated-β-cyclodextrin inclusion complexes in molar ratio of 1:1 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated intestinal fluid without pancreatin

Times	**% Drug dissolved ± SD**				
	Simvastatin	Physical mixture	Kneaded	Co-evaporated	Freeze-dried
5	16.26 ± 0.88	22.75 ± 1.12	22.81 ± 1.20	44.38 ± 0.83	23.85 ± 0.83
10	11.03 ± 1.12	14.84 ± 0.67	20.92 ± 0.95	34.01 ± 1.11	21.08 ± 1.30
15	9.98 ± 1.25	15.11 ± 1.12	24.32 ± 0.88	37.73 ± 0.66	22.20 ± 0.81
20	10.34 ± 1.18	15.06 ± 0.90	27.46 ± 1.22	38.04 ± 1.16	23.64 ± 1.42
30	12.54 ± 1.25	16.89 ± 1.12	30.50 ± 1.45	40.29 ± 0.85	20.87 ± 0.86
45	15.90 ± 0.88	18.93 ± 1.40	33.17 ± 1.08	41.39 ± 0.58	24.74 ± 1.33
60	16.68 ± 1.26	20.97 ± 1.36	34.69 ± 0.86	42.75 ± 0.99	25.42 ± 1.18
90	19.72 ± 0.97	22.70 ± 0.78	35.74 ± 0.99	43.28 ± 1.51	26.78 ± 0.78
120	21.55 ± 0.91	25.42 ± 1.20	36.84 ± 1.11	43.96 ± 0.75	27.88 ± 0.92

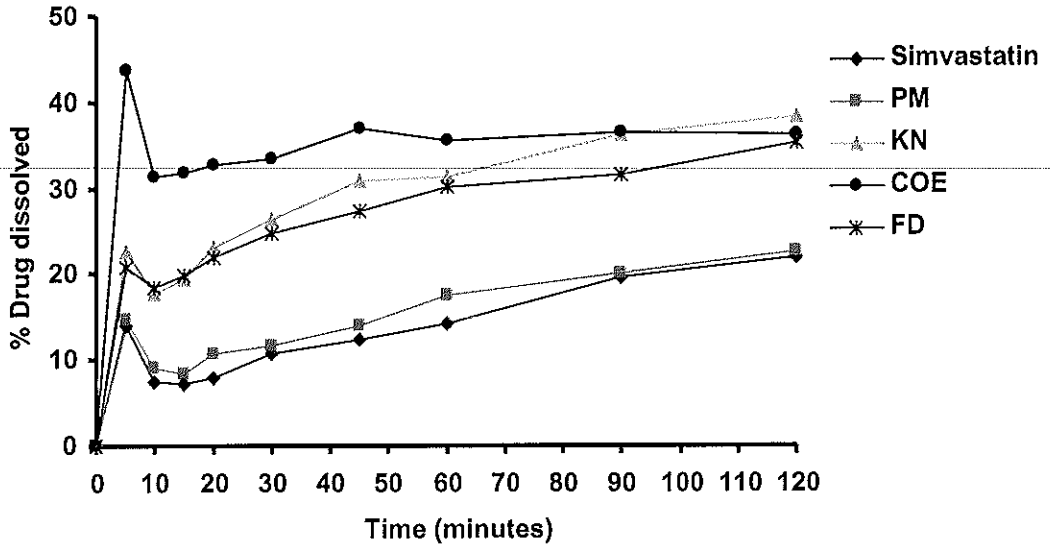


Figure 4.7 Dissolution profiles of simvastatin, physical mixture (PM), kneaded (KN), co-evaporated (COE) and freeze-dried (FD) samples in simulated gastric fluid without pepsin

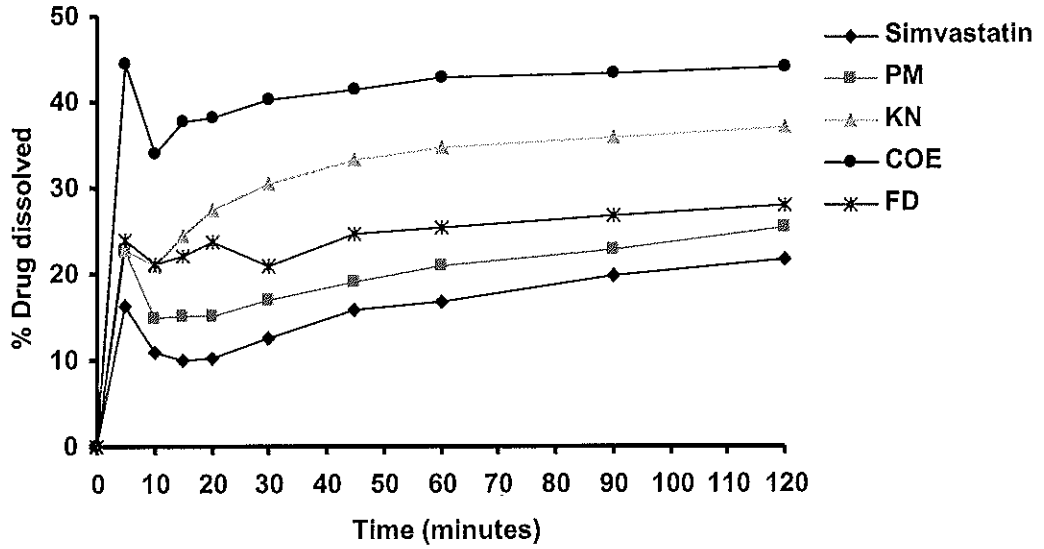


Figure 4.8 Dissolution profiles of simvastatin, physical mixture (PM), kneaded (KN), co-evaporated (COE) and freeze-dried (FD) samples in simulated intestinal fluid without pancreatin

The results from the dissolution studies of simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 are presented in Table 4.10, Table 4.11, Figure 4.9 and Figure 4.10.

The percentage of drug dissolved of simvastatin-PVP K-30 physical mixtures in simulated gastric fluid without pepsin was increased compared with pure drug. The percentage of drug dissolved of simvastatin from all physical mixtures in simulated gastric fluid without pepsin at 5 minutes was significantly difference ($p<0.01$) when compared with that of pure drug. The increased in drug dissolution observed for physical mixtures might be mainly attributed to the hydrophilic effect of the carrier which can reduce the interfacial surface tension between drug and the dissolution medium (Ruan *et al.*, 2005) whereas the pure drug float on the surface of the dissolution medium.

The results of the dissolution of simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 are presented in Table 4.12, Table 4.13, Figure 4.11 and Figure 4.12. The dissolution profiles of all simvastatin-PVP K-30 solid dispersions in two dissolution media were higher than physical mixtures and pure drug. The dissolution profiles of all solid dispersions in simulated intestinal fluid did not show the supersaturation in the first 5 minutes of the period of study. This is probably due to the higher solubility of simvastatin obtained from the solid dispersions than that of inclusion complexes.

The percentage of drug dissolved of the solid dispersions in weight ratio of 1:1 in simulated gastric fluid without pepsin at 10 minutes was significantly ($p<0.05$) lower than that of solid dispersions in weight ratios of 1:3 and 1:4. The percentage of drug dissolved of the solid dispersion in weight ratio of 1:1 in simulated intestinal fluid without pancreatin at 10 minutes was significantly ($p<0.01$) lower than that of solid dispersion in weight ratios of 1:3 and 1:4.

The percentage of drug dissolved of the solid dispersion in weight ratio of 1:4 in simulated gastric fluid without pepsin at 10 minutes was significantly ($p<0.01$) higher than that of solid dispersions in weight ratios of 1:1 and 1:2. The percentage of drug dissolved of the solid dispersions in weight ratio of 1:4 in simulated intestinal fluid without pancreatin at 10 minutes was significantly ($p<0.01$) higher than that of

solid dispersion in weight ratios of 1:1, 1:2 and 1:3. These results demonstrated that the amount of PVP K-30 used in preparation of solid dispersion may have an effect on drug dissolution rate. This finding is similar to the results reported by Ruan *et al.* (2005) which revealed that the dissolution rate of ampelopsin from solid dispersion with PVP K-30 in weight ratio of 1:5 was increased when compared with physical mixture and pure drug and it almost released completely in weight ratios of 1:10 and 1:15.

The solid dispersion in weight ratio of 1:4 exhibited the fastest dissolution rate. The percentage of drug dissolved in simulated gastric fluid without pepsin after 10 minutes were about 2.6 folds and 1.4 folds higher than that of pure drug and physical mixture, respectively. The percentage of drug dissolved in simulated intestinal fluid without pancreatin after 10 minutes were about 2.6 folds and 2.4 folds higher than that of pure drug and physical mixture, respectively. The enhanced in drug dissolved may be due to the higher solubility of simvastatin obtained from solid dispersion in weight ratio of 1:4.

Table 4.10 The percentage of drug dissolved from simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated gastric fluid without pepsin

Times	% Drug dissolved ± SD				
	Simvastatin	Physical mixture 1:1	Physical mixture 1:2	Physical mixture 1:3	Physical mixture 1:4
5	13.98 ± 0.58	16.99± 0.82	17.82 ± 0.64	18.24± 0.87	19.59 ± 1.63
10	7.49 ± 1.27	9.67 ± 0.85	12.01 ± 0.72	12.58 ± 0.88	14.40 ± 1.07
15	7.23 ± 0.60	8.79 ± 0.57	10.92 ± 0.42	11.85 ± 0.47	13.26 ± 0.87
20	7.86 ± 1.06	11.18 ± 1.09	13.26 ± 0.91	14.09 ± 0.60	15.23 ± 0.58
30	10.64 ± 1.41	13.46 ± 0.89	14.03 ± 1.04	15.18 ± 1.22	16.37 ± 0.95
45	12.33 ± 1.17	14.66 ± 0.42	15.75 ± 1.25	16.84 ± 0.51	18.50 ± 0.88
60	14.21 ± 1.05	17.93 ± 0.91	18.50 ± 0.56	19.59 ± 0.85	20.42 ± 0.46
90	19.57 ± 1.46	20.11 ± 0.46	20.42 ± 0.46	21.25 ± 0.80	22.49 ± 0.77
120	21.97 ± 0.97	22.96 ± 1.39	23.17 ± 0.96	23.74 ± 0.91	24.93 ± 0.75

Table 4.11 The percentage of drug dissolved from simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated intestinal fluid without pancreatin

Times	% Drug dissolved ± SD				
	Simvastatin	Physical mixture 1:1	Physical mixture 1:2	Physical mixture 1:3	Physical mixture 1:4
5	16.26 ± 0.88	16.30 ± 0.91	16.59 ± 0.95	16.73 ± 0.50	17.07 ± 0.26
10	11.03 ± 1.12	11.17 ± 1.02	11.22 ± 0.89	11.31 ± 0.65	11.46 ± 0.68
15	9.98 ± 1.25	10.05 ± 0.43	10.25 ± 0.66	10.30 ± 0.44	10.49 ± 0.60
20	10.34 ± 1.18	10.59 ± 0.47	10.88 ± 0.70	10.83 ± 1.16	11.31 ± 0.92
30	12.54 ± 1.25	12.81 ± 0.95	13.44 ± 0.68	13.83 ± 0.56	14.60 ± 0.84
45	15.90 ± 0.88	16.15 ± 0.85	16.49 ± 0.41	16.73 ± 0.34	17.12 ± 1.00
60	16.68 ± 1.26	17.17 ± 0.63	18.23 ± 1.28	18.67 ± 1.50	19.59 ± 0.79
90	19.72 ± 0.97	20.07 ± 0.47	20.60 ± 1.01	21.09 ± 0.85	21.62 ±1.05
120	21.55 ± 0.91	21.72 ± 0.55	22.25 ± 0.62	22.68 ± 0.79	23.12 ± 1.15

Table 4.12 The percentage of drug dissolved from simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated gastric fluid without pepsin

Times	% Drug dissolved ± SD				
	Simvastatin	Solid dispersion 1:1	Solid dispersion 1:2	Solid dispersion 1:3	Solid dispersion 1:4
5	13.98 ± 0.58	18.71 ± 0.73	20.16 ± 0.96	21.30 ± 0.56	22.39 ± 0.43
10	7.49 ± 1.27	15.64 ± 0.41	16.68 ± 0.66	17.82 ± 0.84	19.59 ± 0.68
15	7.23 ± 0.60	16.53 ± 0.54	17.20 ± 0.41	19.43 ± 1.08	20.21 ± 0.85
20	7.86 ± 1.06	17.25 ± 0.52	18.03 ± 0.83	19.80 ± 0.99	20.57 ± 0.70
30	10.64 ± 1.41	18.76 ± 1.03	19.43 ± 0.59	21.92 ± 1.32	23.12 ± 0.66
45	12.33 ± 1.17	19.22 ± 0.75	20.94 ± 0.46	25.97 ± 1.21	28.88 ± 0.90
60	14.21 ± 1.05	19.64 ± 0.80	22.08 ± 1.29	27.68 ± 0.76	30.38 ± 1.03
90	19.57 ± 1.46	21.04 ± 0.60	23.43 ± 1.01	31.37 ± 0.98	34.54 ± 0.88
120	21.97 ± 0.97	24.21 ± 1.00	25.40 ± 0.82	34.02 ± 1.33	37.60 ± 0.64

Table 4.13 The percentage of drug dissolved from simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in simulated intestinal fluid without pancreatin

Times	% Drug dissolved ± SD				
	Simvastatin	Solid dispersion 1:1	Solid dispersion 1:2	Solid dispersion 1:3	Solid dispersion 1:4
5	16.26 ± 0.88	20.87 ± 1.31	21.55 ± 1.14	23.96 ± 0.98	25.53 ± 0.88
10	11.03 ± 1.12	22.60 ± 0.31	23.38 ± 0.26	25.90 ± 0.68	28.15 ± 1.97
15	9.98 ± 1.25	24.27 ± 0.67	25.58 ± 0.43	28.15 ± 0.52	30.82 ± 1.70
20	10.34 ± 1.18	26.42 ± 0.60	27.05 ± 1.12	29.14 ± 0.58	31.34 ± 0.68
30	12.54 ± 1.25	27.46 ± 0.38	28.09 ± 0.62	30.08 ± 1.19	32.12 ± 0.83
45	15.90 ± 0.88	29.04 ± 0.79	30.40 ± 0.58	31.97 ± 0.43	35.58 ± 0.73
60	16.68 ± 1.26	31.44 ± 1.27	33.02 ± 0.74	34.90 ± 0.89	37.83 ± 0.92
90	19.72 ± 0.97	35.21 ± 1.45	36.73 ± 0.70	38.56 ± 0.32	42.28 ±1.17
120	21.55 ± 0.91	38.51 ± 1.03	40.03 ± 0.68	43.38 ± 0.63	46.58 ± 1.35

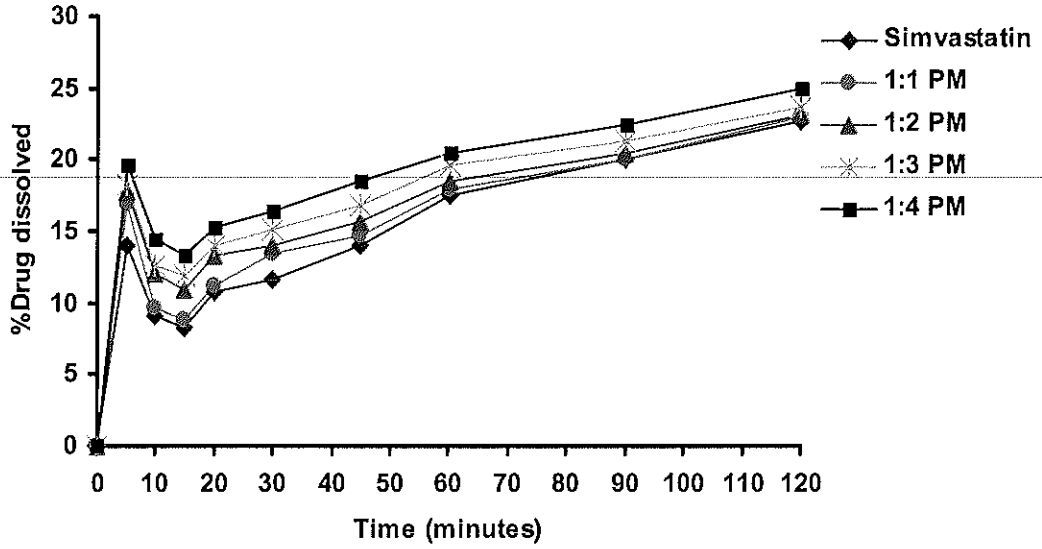


Figure 4.9 Dissolution profiles of simvastatin, simvastatin-PVP K-30 physical mixtures (PM) in weight ratios of 1:1, 1:2, 1:3 and 1:4 in simulated gastric fluid without pepsin

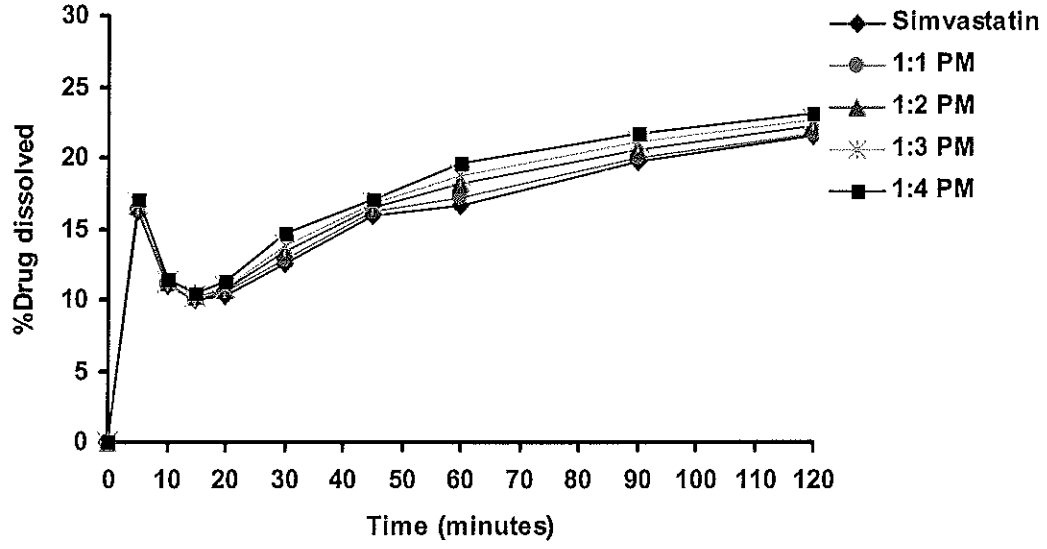


Figure 4.10 Dissolution profiles of simvastatin, simvastatin-PVP K-30 physical mixtures (PM) in weight ratios of 1:1, 1:2, 1:3 and 1:4 in simulated intestinal fluid without pancreatin

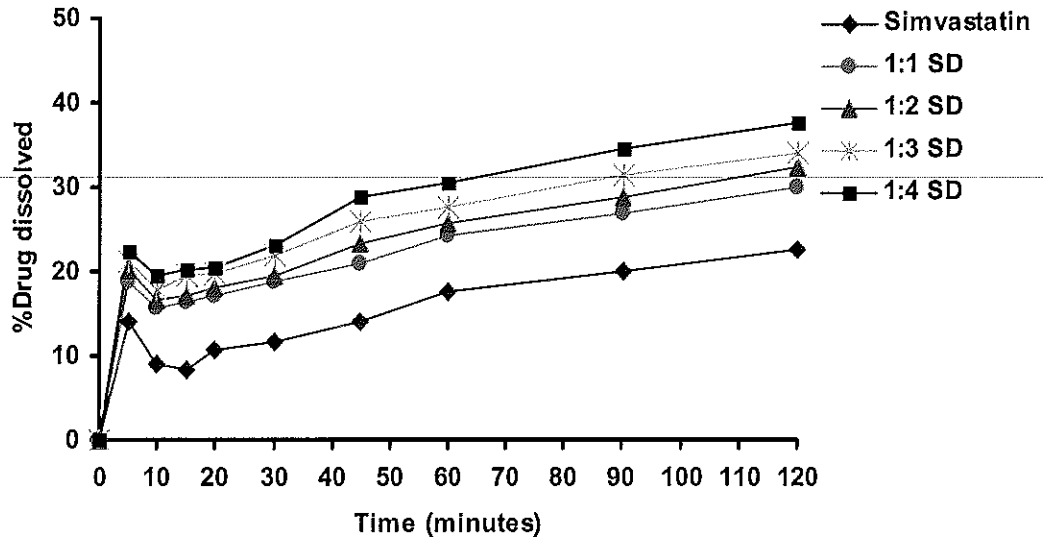


Figure 4.11 Dissolution profiles of simvastatin, simvastatin-PVP K-30 solid dispersion (SD) in weight ratios of 1:1, 1:2, 1:3 and 1:4 in simulated gastric fluid without pepsin

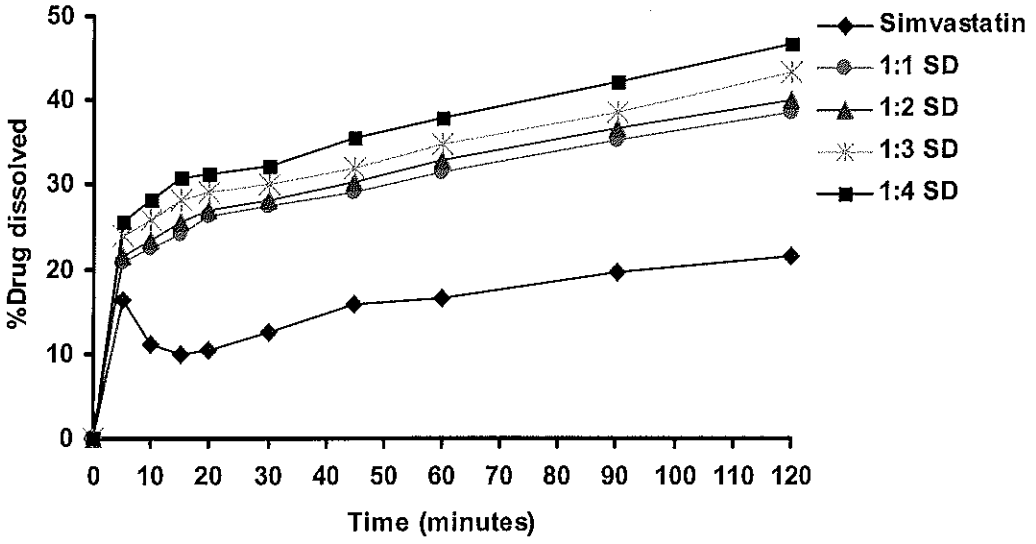


Figure 4.12 Dissolution profiles of simvastatin, simvastatin-PVP K-30 solid dispersion (SD) in weight ratios of 1:1, 1:2, 1:3 and 1:4 in simulated intestinal fluid without pancreatin

4.10. Characterization of inclusion complexes

4.10.1 Differential scanning calorimetry (DSC)

The DSC study is a useful technique to determine the interaction and the complex formation between drug molecules and cyclodextrins in form of endothermic or exothermic reaction. If the DSC curves of complex represent a sum of DSC thermograms of drug and cyclodextrin, this suggests that no interaction occur between guest and host molecules. The absence of dehydration endotherms in DSC curves of complexes may be an additional indication of inclusion of drug in the cyclodextrin cavity (Reddy *et al.*, 2004).

The DSC thermograms of methylated-β-cyclodextrin, simvastatin, physical mixture and inclusion complexes are shown in Figure 4.13. The DSC thermogram of methylated-β-cyclodextrin showed a very broad endotherm.

The DSC thermogram of simvastatin was typical of a highly crystalline compound, showing a single sharp fusion endothermic peak at 136°C corresponding to its melting point.

The DSC thermograms of physical mixture, kneaded and freeze-dried samples showed the drug melting endothermic peak, which shifted to lower temperature, more broadened and reduced in intensity, indicating the interaction of simvastatin and methylated-β-cyclodextrin (Patel and Patel, 2007).

The complete disappearance of endothermic peak corresponding to simvastatin was observed for co-evaporated sample. This indicated the formation of an amorphous inclusion complex and/or trapping of simvastatin inside the methylated-β-cyclodextrin cavity (Patel and Patel, 2007).

The DSC thermograms of PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 are shown in Figure 4.14 and theirs corresponding solid dispersions are shown in Figure 4.15.

The DSC thermogram of PVP K-30 showed a very broad endotherm in the temperature range of 50-120°C corresponding to the evaporation of water from the hygroscopic nature of PVP polymer (Sethia and Squillante, 2004).

The DSC thermograms of simvastatin-PVP K-30 physical mixtures in

weight ratios of 1:1, 1:2, 1:3 and 1:4 presented very broad endotherm of PVP K-30 and the melting endotherm of the drug. This demonstrated the loss of water due to hygroscopic nature of PVP polymer and simvastatin remained in the crystalline form.

The DSC thermograms of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 showed very broad endotherm of PVP K-30 but the melting endotherm of the drug was not observed. This indicated that simvastatin was no longer present as a crystalline material, but was converted into the amorphous state (Ruan *et al.*, 2005).

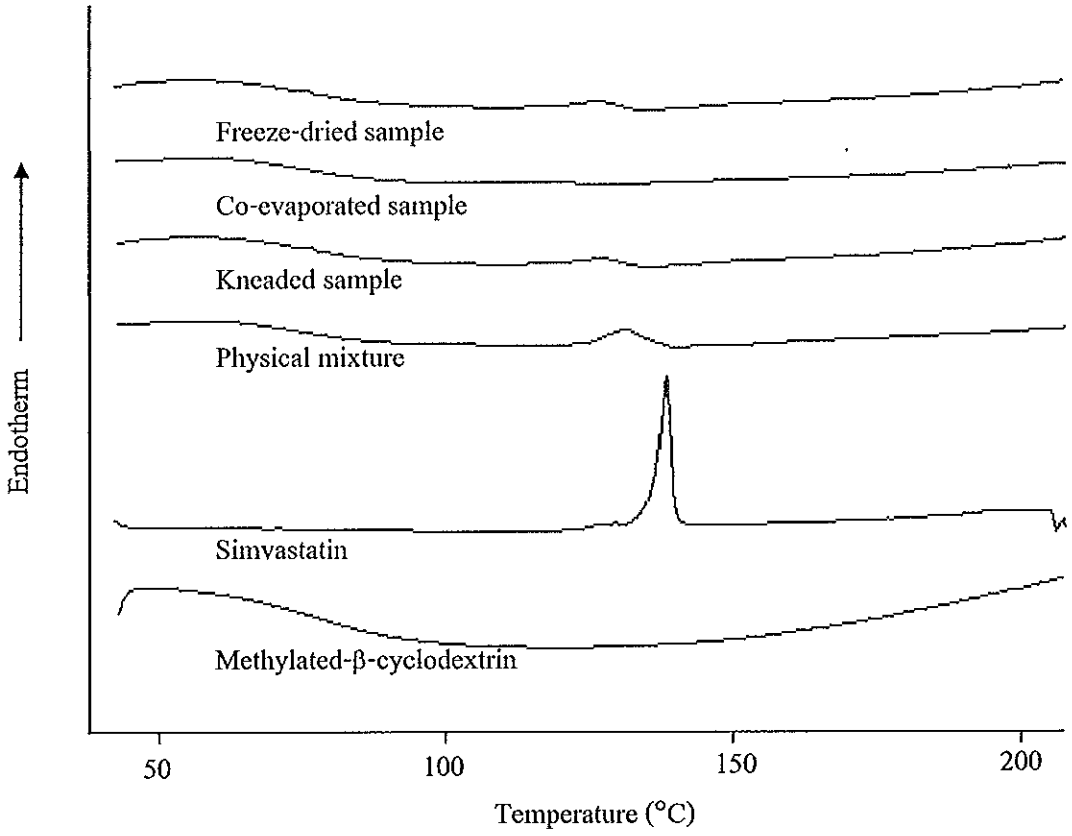


Figure 4.13 DSC thermograms of methylated-β-cyclodextrin, simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples

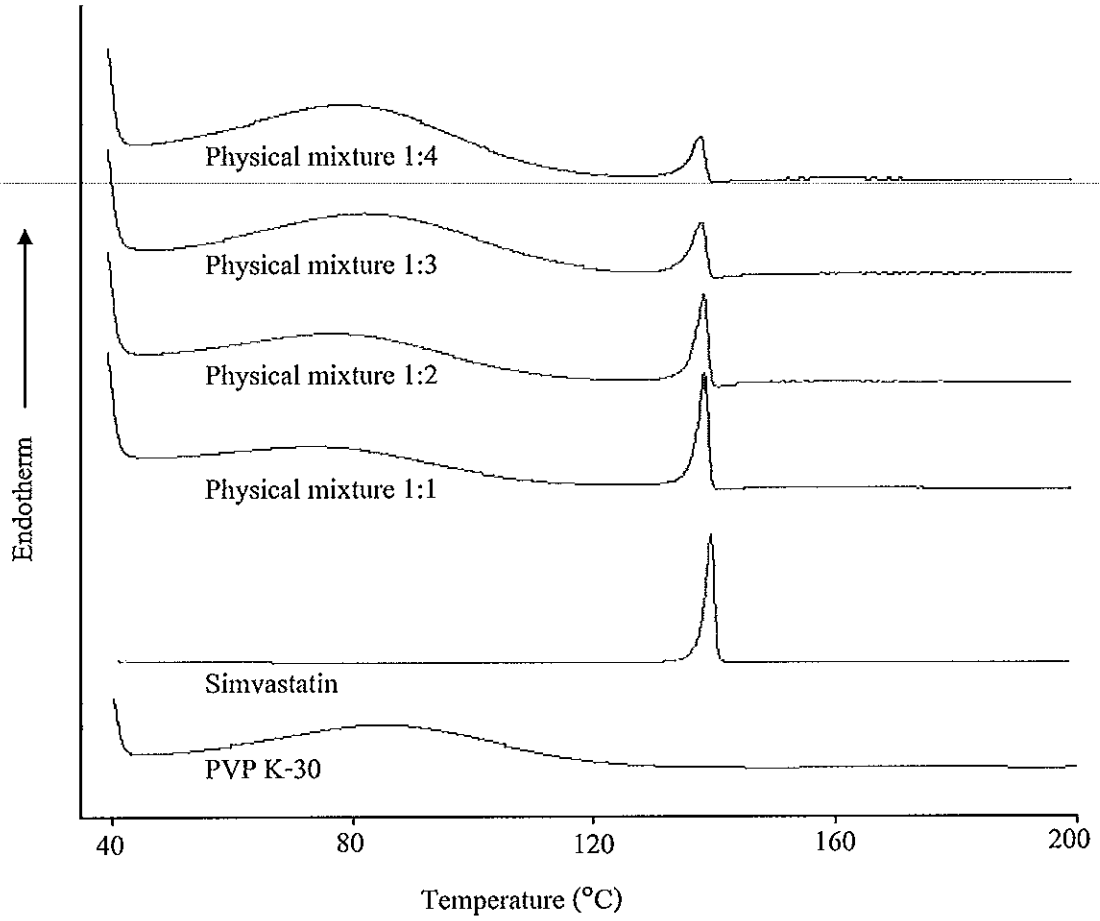


Figure 4.14 DSC thermograms of PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4

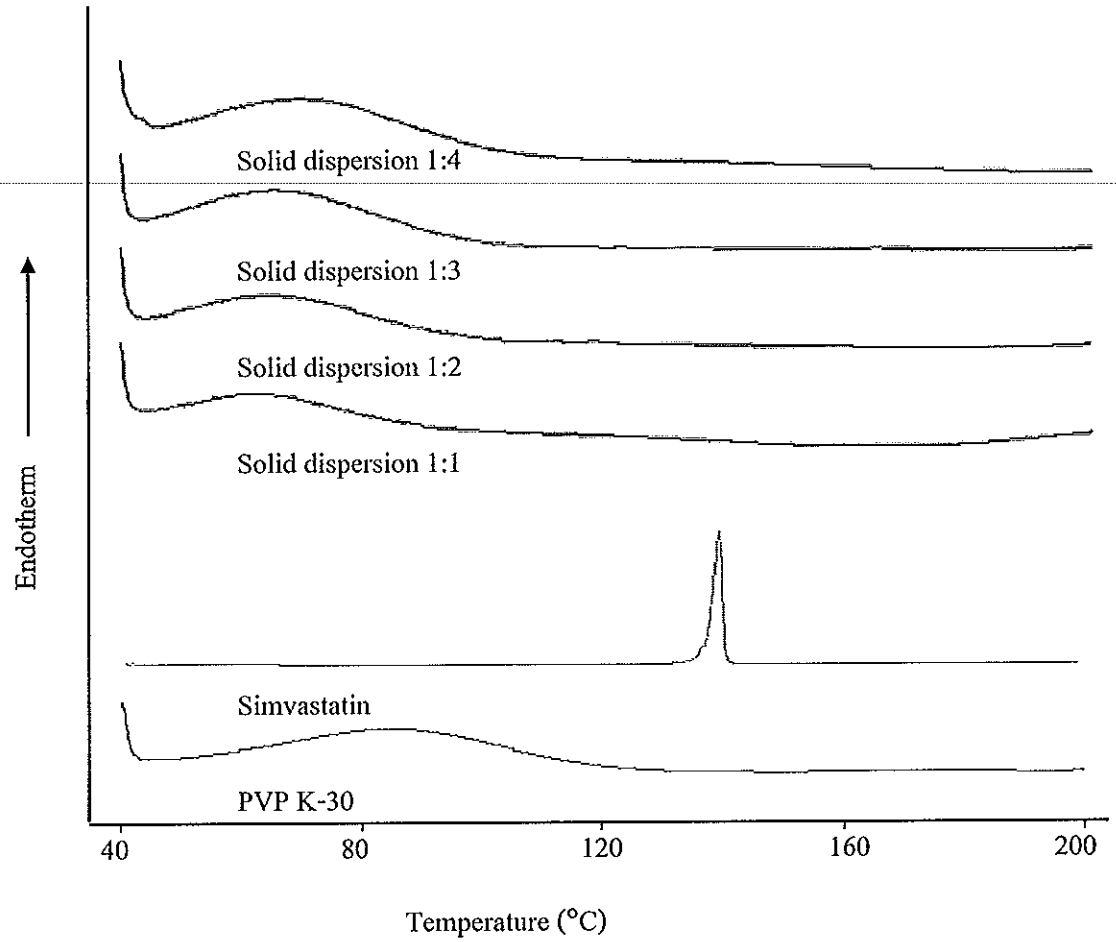


Figure 4.15 DSC thermograms of PVP K-30, simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4

4.10.2 Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR study is a useful technique to determine the interaction and the complex formation between drug molecules and cyclodextrins in the solid state. Shifts or intensity changes in the characteristic bands of pure substance are considered as evidence of the complex existence (Cirri *et al.*, 2005). However, some of the changes are very subtle, requiring careful interpretation of the spectrum (Patel and Patel, 2007).

The FT-IR spectra of methylated-β-cyclodextrin, simvastatin, physical mixture and inclusion complexes are presented in Figure 4.16. The FT-IR spectrum of methylated-β-cyclodextrin showed absorption bands at 3,429 cm^{-1} for O-H stretching vibration, 2,935 cm^{-1} for C-H stretching vibration and 1,044 cm^{-1} for C-O stretching vibration. The FT-IR spectrum of simvastatin showed the presence of the following peaks at 3,549 cm^{-1} for alcohol O-H stretching vibration, 3,011 cm^{-1} for olefinic C-H stretching vibration, 2,955 and 2,871 cm^{-1} for methyl C-H stretching vibration, 1,709 and 1,697 cm^{-1} for stretching vibration of lactone and ester carbonyl functional group, 1,267 cm^{-1} for lactone -C-O-C stretching vibration and 1,166 cm^{-1} for ester -C-O-C stretching vibration.

The FT-IR spectrum of physical mixture did not show any significant differences from the spectra of the simvastatin and methylated-β-cyclodextrin. This indicated that physical mixture spectrum was only the summation of simvastatin and methylated-β-cyclodextrin spectra and reflected that there was no interaction between simvastatin and methylated-β-cyclodextrin. The similar results were reported by Jun *et al.* (2007) which revealed that the FT-IR spectrum of simvastatin/hydroxypropyl-β-cyclodextrin physical mixture did not show any significant differences from the respective spectra of the simvastatin and hydroxypropyl-β-cyclodextrin. Figueiras *et al.* (2007) demonstrated that spectra of physical mixtures of omeprazole with native and chemically modified β-cyclodextrin did not show new peaks indicating that no chemical bonds were created in the formed compounds.

The FT-IR spectra of kneaded and freeze-dried samples gave similar pattern

to the FT-IR spectrum of methylated-β-cyclodextrin than the spectrum of simvastatin. The presented FT-IR spectra of kneaded and freeze-dried samples were similarly to the FT-IR spectrum of physical mixture indicating that minor simvastatin-methylated-β-cyclodextrin interactions.

On the other hand, co-evaporated sample showed complete disappearance of the absorption band at 3,549 cm^{-1} for alcohol O-H stretching vibration of simvastatin and the disappeared absorption bands at 1,724, 1,709 and 1,697 cm^{-1} was presented together with an absorption band at 1,718 cm^{-1}. These results indicated strong interactions between simvastatin and methylated-β-cyclodextrin as compared to kneaded and freeze-dried samples. The assumption for this is part of simvastatin; lactone and ester carbonyl functional group; may fit inside the cavity of methylated-β-cyclodextrin, whereas the other part may remain outside the methylated-β-cyclodextrin. These findings were confirmed by the experiment of Wen *et al.* (2005) reported that the lactone ring of simvastatin was fully trapped into the cavity of β-cyclodextrin which was not the case for simvastatin-α-cyclodextrin inclusion complex due to the larger internal diameter of β-cyclodextrin (6.5 Å) relative to that of α-cyclodextrin cavity (5.3 Å). In addition, Jun *et al.* (2007) reported that the FT-IR spectrum of the simvastatin-hydroxypropyl-β-cyclodextrin inclusion complex exhibited some significant differences compared with simvastatin-hydroxypropyl-β-cyclodextrin physical mixture. The characteristic absorption peaks of the carbonyl group of simvastatin in the range 1,600-1,800 cm^{-1} have disappeared from the spectrum of the inclusion complex. This is probably due to the inclusion complexation of simvastatin into the hydroxypropyl-β-cyclodextrin cavity with the carbonyl group of lactone ring of simvastatin might be involved in the inclusion complexation. This finding is similarly to the experiment reported by Wen *et al.* (2005) which revealed that besides the hydroxyl of simvastatin, the oxygen atoms of C=O and bridging oxygen of lactone ring could form intermolecular hydrogen bonding with secondary hydroxyl groups of glucopyranose units of β-cyclodextrin.

FT-IR spectroscopy was carried out to further elucidate the interaction between simvastatin and PVP K-30 in the solid state. The FT-IR spectra of

PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures and solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 are showed in Figure 4.17 and Figure 4.18. The FT-IR spectrum of PVP K-30 showed important bands at 2,956 cm^{-1} for C–H stretching vibration and 1,657 cm^{-1} for C=O stretching vibration and a very broad band was presented at about 3,050-3,720 cm^{-1} due to the presence of water which was confirmed by the broad endotherm occurred in the DSC thermogram (Van den Mooter *et al.*, 1998). The FT-IR spectra of simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 were similar to the spectra of the simvastatin and PVP K-30. These results suggested that there was no interaction between drug and PVP K-30 in physical mixtures and still presented the crystallinity of drug that confirmed by the DSC and PXRD results. The FT-IR spectra of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 are represented in Figure 4.18. The FT-IR spectra of simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 showed some differences compared with their corresponding physical mixtures. The O-H stretching vibration at 3,549 cm^{-1} of simvastatin in all solid dispersions was disappeared. Therefore, the solid dispersions showed an interaction such as the intermolecular hydrogen bonding between simvastatin and PVP K-30. This may resulting in changing simvastatin crystalline structure to amorphous form (Tantishaiyakul *et al.*, 1999, Kaewnopparat *et al.*, 2009).

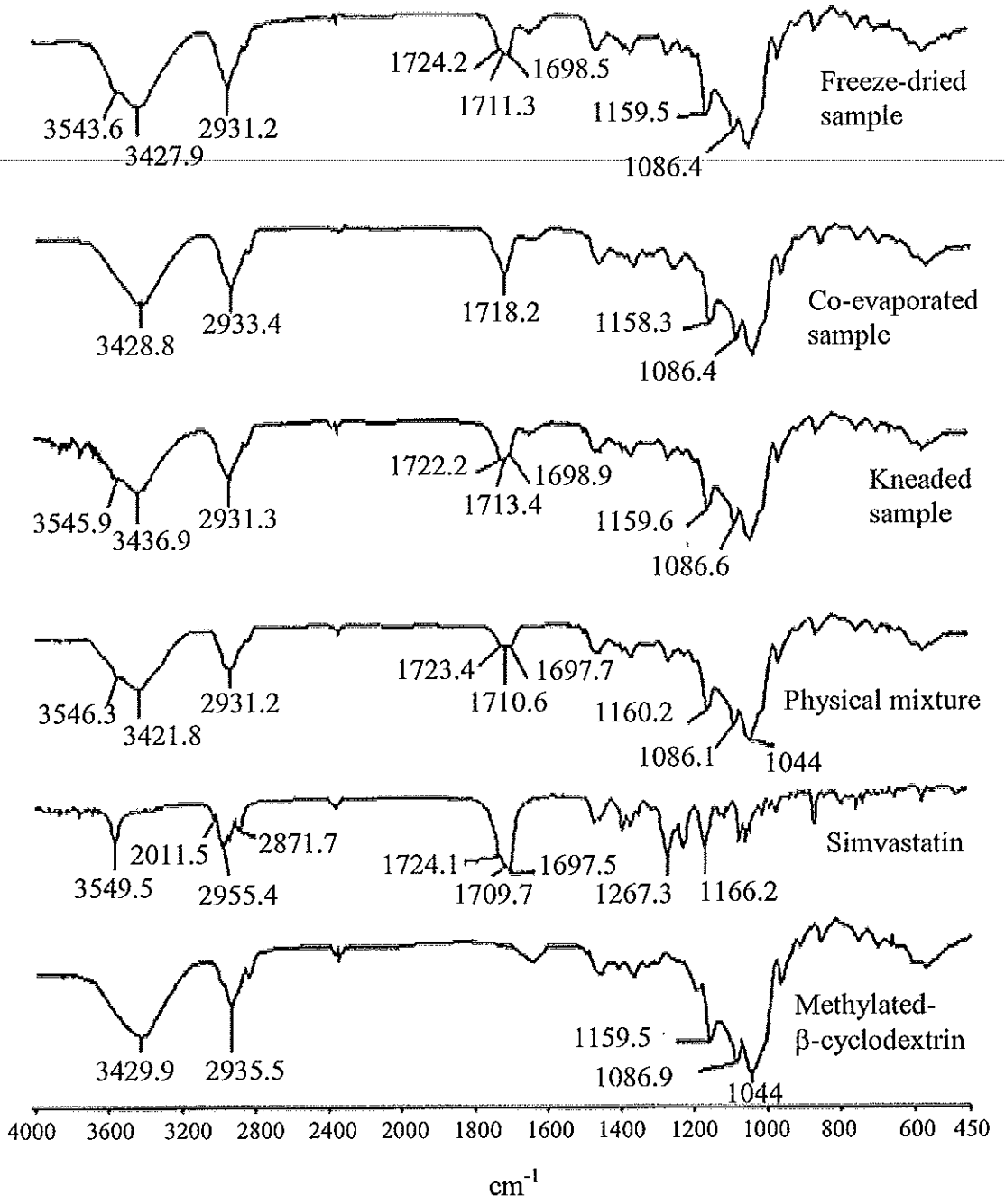


Figure 4.16 FT-IR spectra of methylated-β-cyclodextrin, simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples

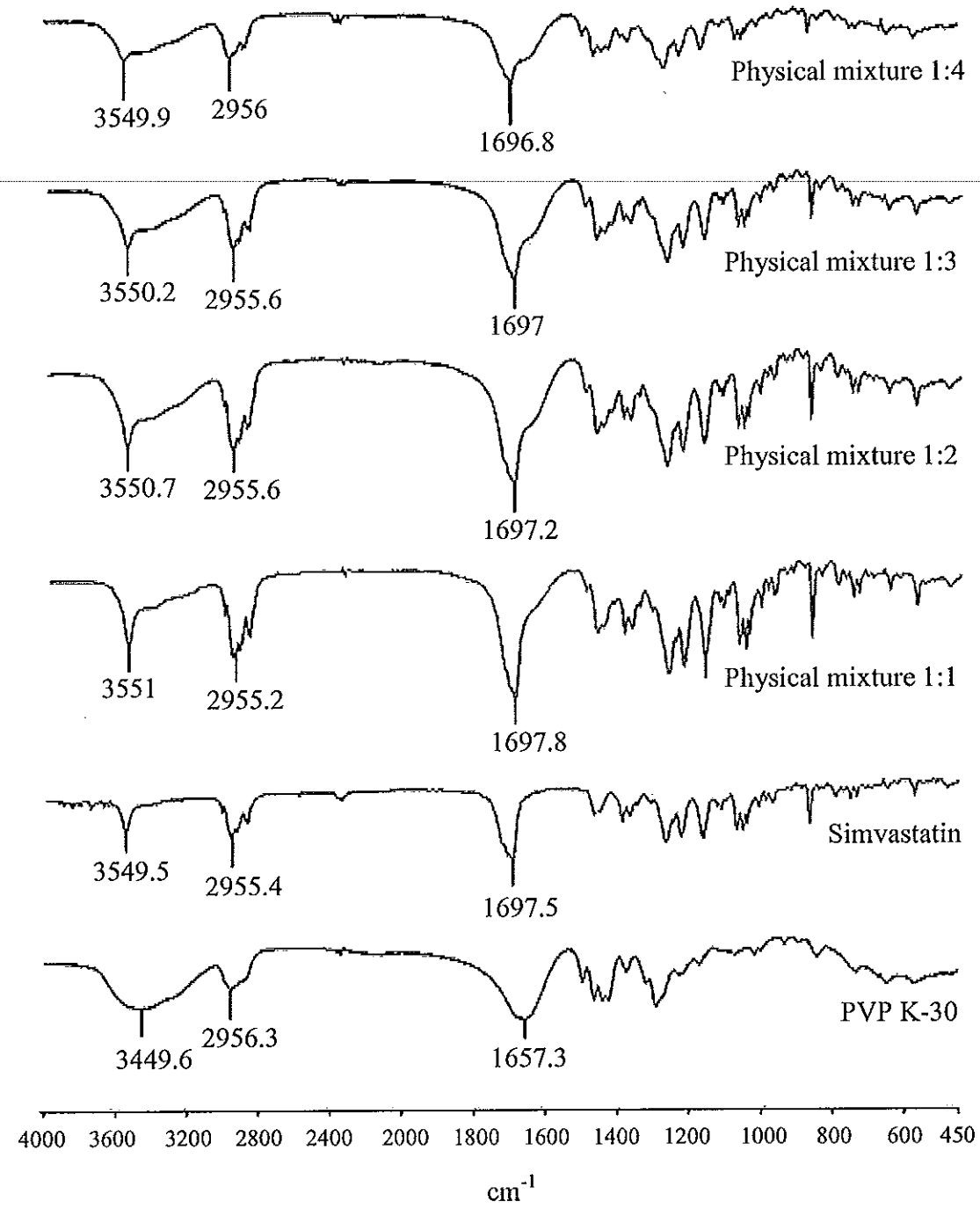


Figure 4.17 FT-IR spectra of PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4

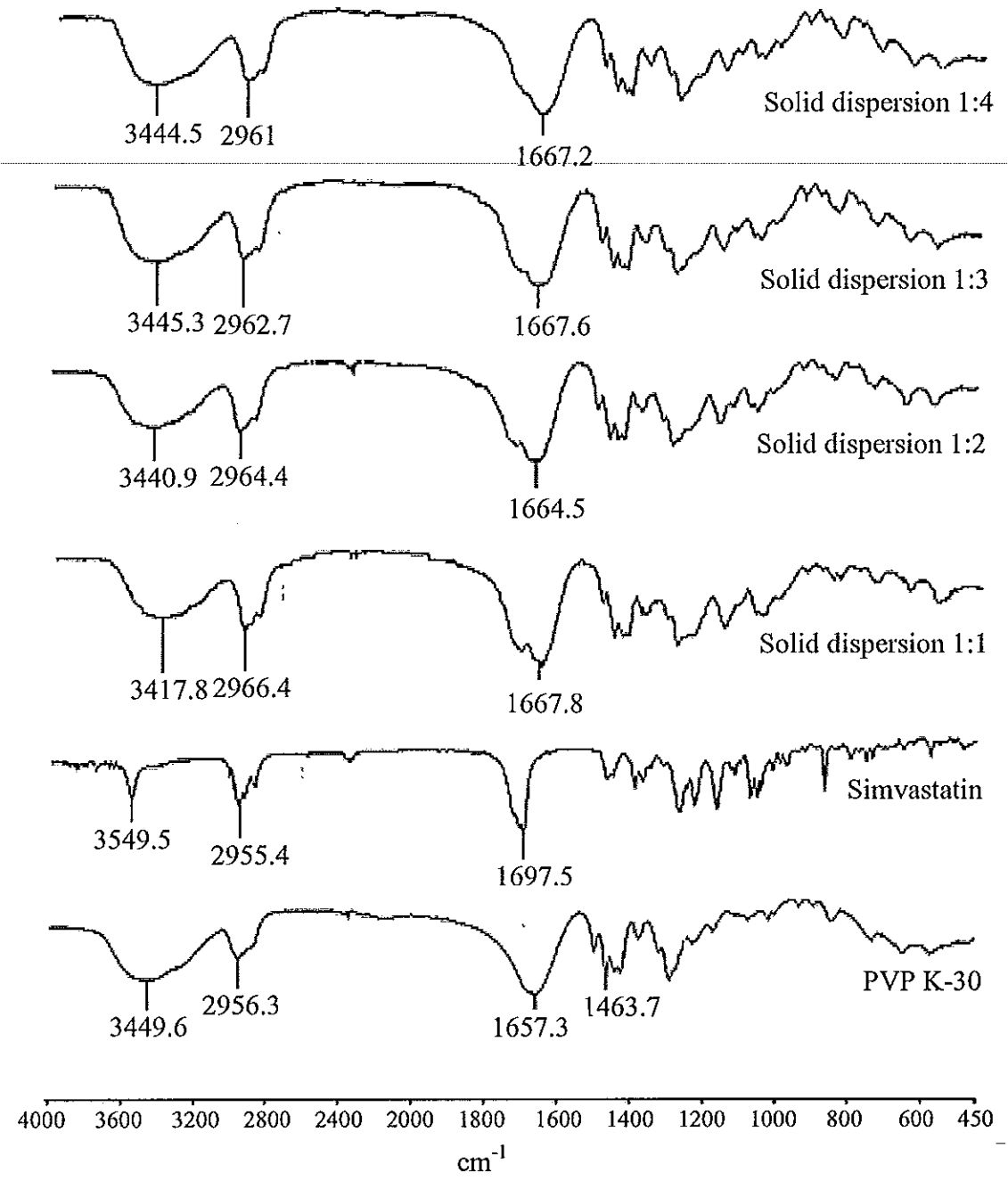


Figure 4.18 FT-IR spectra of PVP K-30, simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4

4.10.3 Powder X-ray diffractometry (PXRD)

Powder X-ray diffraction patterns of methylated-β-cyclodextrin, simvastatin, physical mixture and inclusion complexes are represented in Figure 4.19. The absence of any peaks in methylated-β-cyclodextrin diffractogram revealed the amorphous nature of this compound. The presence of several sharp peaks at 7.82, 9.34, 10.90, 15.58, 16.54, 17.19, 18.77, 19.35, 21.99, 22.50, 25.84, 28.31, 31.87 (2θ) of simvastatin suggested that the drug is in a crystalline form.

The diffraction pattern of physical mixture correspond to the superposition of those of the pure components, indicated that the drug was still in a crystalline form. Kneaded and freeze-dried samples showed less lower intense peaks compared with corresponding physical mixture, indicating the reduction in crystallinity of simvastatin. The similar observation have been reported by Figueiras *et al.* (2007) which stated that the diffraction patterns of kneaded products displayed lower crystallinity than that of physical mixtures. The presence of crystals of simvastatin in freeze-dried samples is probably due to the lyophilization of drug and methylated-β-cyclodextrin suspension during preparation process. The similar result was reported by Ribeiro *et al.* (2003). According to the results from DSC thermograms, FT-IR spectra and powder X-ray diffractograms, the kneaded and freeze-dried samples are probably a mixture of simvastatin-methylated-β-cyclodextrin inclusion complex and fine crystals of free simvastatin dispersed onto methylated-β-cyclodextrin (Redenti *et al.*, 1996). On the other hand, no diffraction peaks of simvastatin in co-evaporated sample were observed. This indicated that the drug was in an amorphous state. Since the amorphilization of drug can be a result from the co-evaporation process, it is possible that the X-ray diffraction data cannot discriminate whether the drug-cyclodextrin co-evaporated system obtained are true inclusion complex or homogeneous dispersed mixtures of the amorphous components (Badr-Eldin *et al.*, 2008). However, these results can be interpreted on the basis of the formation of amorphous phase, possibly an amorphous inclusion complex formation which confirmed by FT-IR and DSC analysis.

Powder X-ray diffraction patterns of PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 are shown in Figure 4.20. The absence of any peak in the PVP K-30 diffractogram revealed the amorphous nature of this compound. The diffraction patterns of simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4 were similar to the diffraction peaks of pure simvastatin indicating that the crystallinity of the drug was not changed. The simvastatin-PVP K-30 physical mixture in weight ratio of 1:4 was observed the lowest in peak intensity due to the most quantitative of PVP K-30 in sample.

Powder X-ray diffraction patterns of PVP K-30, simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4 are shown in Figure 4.21. The absence of crystalline peaks attributable to pure drug in all solid dispersion systems revealed that drug crystals were transformed to an amorphous state. This is probably due to the effect of PVP K-30 in inhibiting the recrystallization of the drug during preparation process. In addition, the hydrogen bonding between drug and PVP K-30, verified by FT-IR analysis, would inhibit drug crystallization and causing drug precipitated out in the amorphous form (Tantishaiyakul *et al.*, 1996). A similar behavior was previously observed for ketoconazole (Van den Mooter *et al.*, 2001) and flunarizine (Marin *et al.*, 2002).

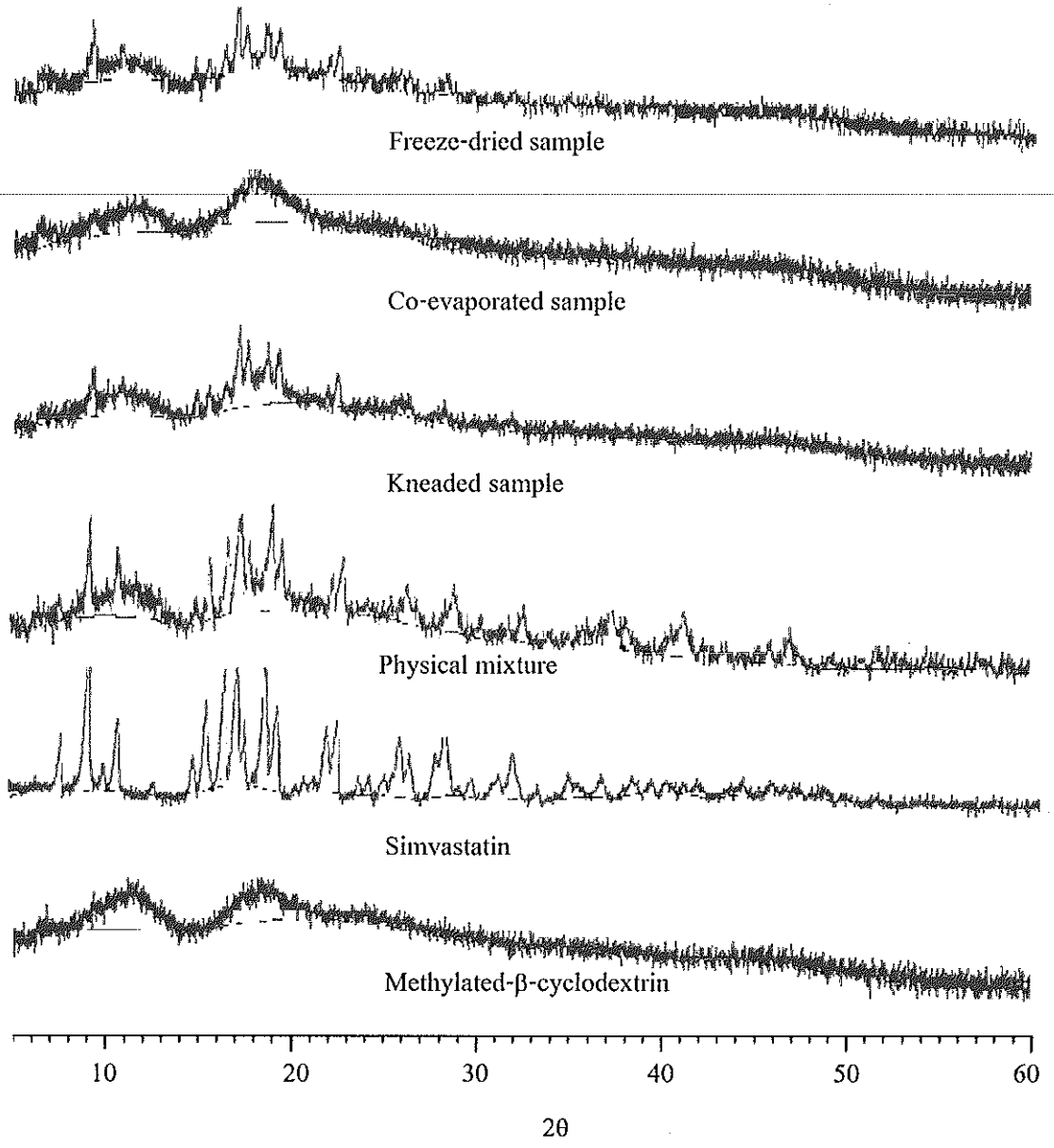


Figure 4.19 Powder x-ray diffraction patterns of methylated-β-cyclodextrin, simvastatin, physical mixture, kneaded, co-evaporated and freeze-dried samples

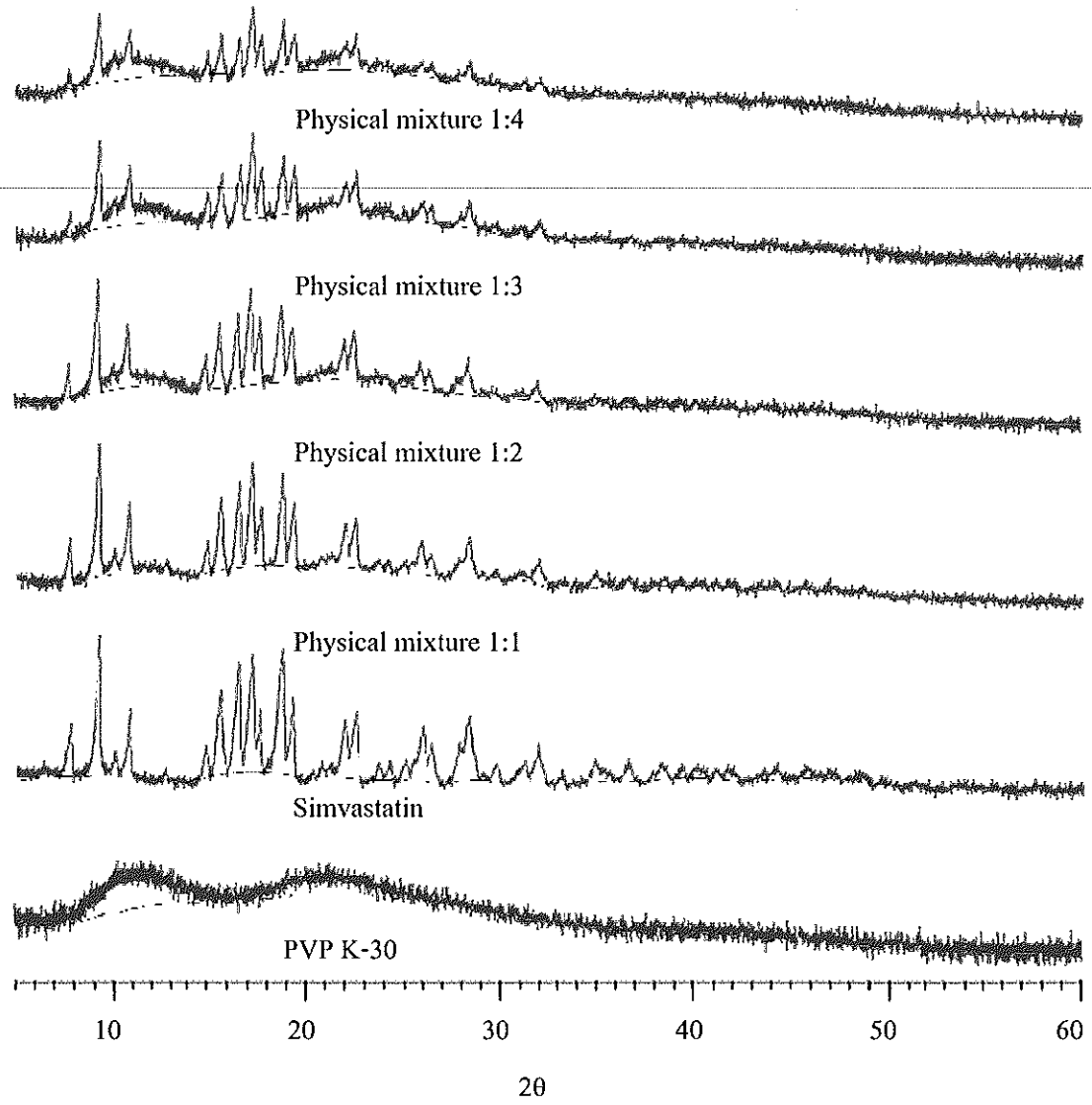


Figure 4.20 Powder X-ray diffraction patterns of PVP K-30, simvastatin, simvastatin-PVP K-30 physical mixtures in weight ratios of 1:1, 1:2, 1:3 and 1:4

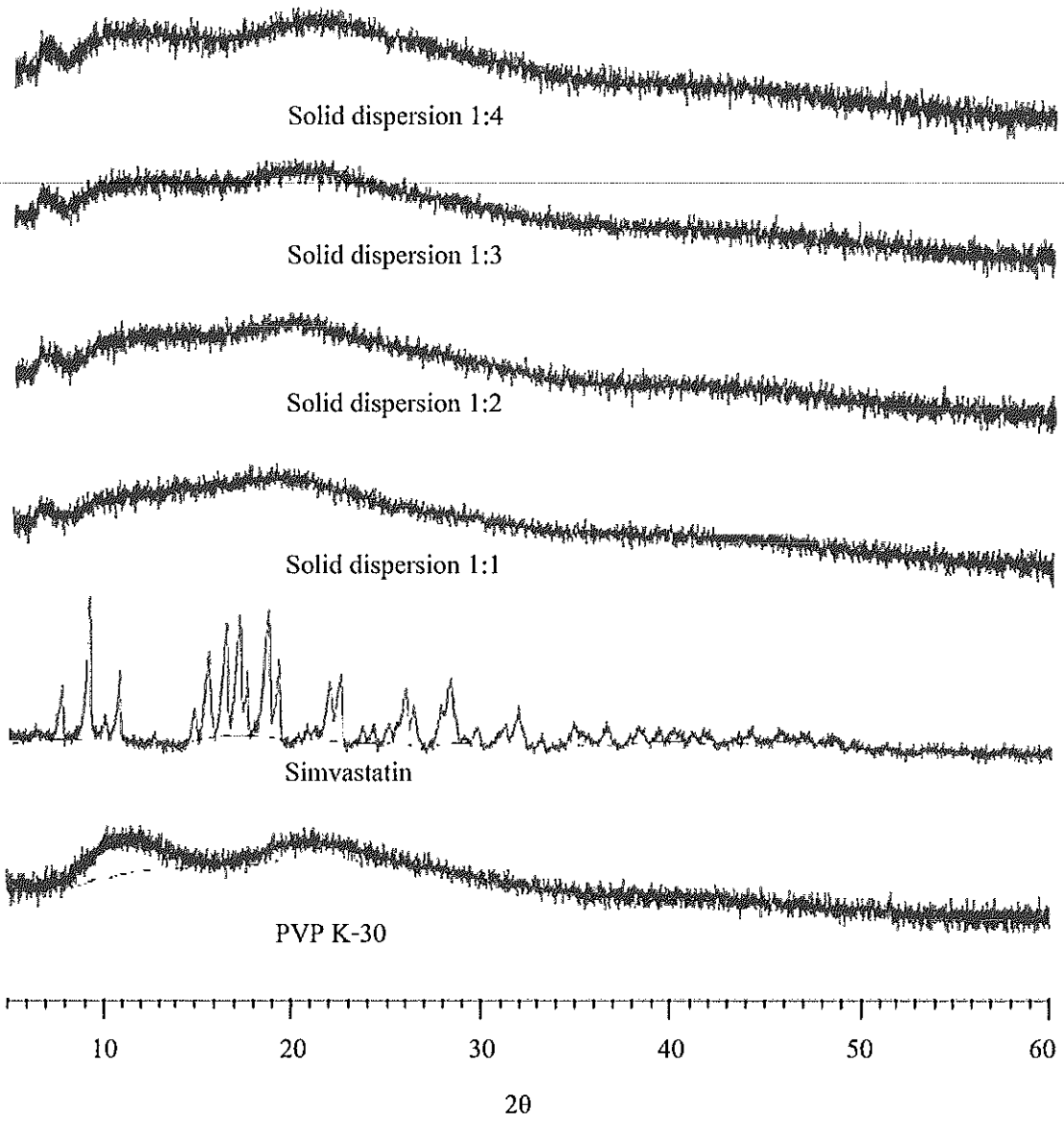


Figure 4.21 Powder X-ray diffraction patterns of PVP K-30, simvastatin, simvastatin-PVP K-30 solid dispersions in weight ratios of 1:1, 1:2, 1:3 and 1:4

4.11. Stability studies

Co-evaporated and solid dispersion in weight ratio of 1:4 samples were selected for this study since both samples showed the highest dissolution rate. The samples were kept at room temperature and at 45°C for 3 months. Drug content, dissolution, DSC thermogram, FT-IR spectra and X-ray diffractogram were examined.

4.11.1 Drug content

The drug content of aged co-evaporated and aged solid dispersion in weight ratio of 1:4 samples are shown in Table 4.14. Selected samples were stored at room temperature and at 45°C for 3 months. This result indicated inclusion complex and solid dispersion samples were stable during storage at room temperature and at 45°C for 3 months.

Table 4.14 Drug content of aged co-evaporated and aged solid dispersion in weight ratio of 1:4 samples after storing at room temperature and at 45°C for 3 months

Aged sample	%Drug remaining [a] (SD)
Co-evaporated at room temperature	99.62 ± 0.360
Co-evaporated at 45°C	99.74 ± 0.652
1:4 Solid dispersion at room temperature	97.38 ± 0.454
1:4 Solid dispersion at 45°C	97.57 ± 0.276

a : Means of triplicate results

Initial drug content (before aging) = 100%

4.11.2 Dissolution studies

The dissolution profiles of freshly prepared co-evaporated sample and aged sample stored at room temperature and at 45°C for 3 months in simulated gastric fluid without pepsin and in simulated intestinal fluid without pancreatin are shown in Figure 4.22 and Figure 4.23, respectively. The aged samples stored at room

temperature gave 43%, 31% and 31% of drug dissolved in simulated gastric fluid without pepsin and 44%, 33% and 37% of drug dissolved in simulated intestinal fluid without pancreatin at 5, 10 and 15 minutes, respectively. This result is similar to that of freshly prepared co-evaporated sample. Therefore, storage at room temperature for 3 months did not markedly effect on drug dissolution property. On the other hand, the percentage of drug dissolved at 5, 10 and 15 minutes of co-evaporated sample stored at 45°C for 3 months in simulated gastric fluid without pepsin was 35%, 28% and 29%, respectively. The percentage of drug dissolved at 5,10 and 15 minutes of co-evaporated sample stored at 45°C for 3 months in simulated intestinal fluid without pancreatin was 35%, 28% and 29%, respectively. The dissolution of simvastatin after storage was significantly ($p<0.05$) lower than that of freshly prepared co-evaporated sample and aged sample stored at room temperature. This result indicated that storage at 45°C had markedly effected on drug dissolution.

The dissolution profiles of freshly prepared simvastatin-PVP K-30 solid dispersion and aged samples stored at room temperature and at 45°C for 3 months in simulated gastric fluid without pepsin and simulated intestinal fluid without pancreatin are shown in Figure 4.24 and Figure 4.25, respectively. The percentage of drug dissolved at 5 and 10 minutes of aged sample stored at room temperature for 3 months in simulated gastric fluid was 22% and 19%, respectively. The percentage of drug dissolved at 5 and 10 minutes of aged sample stored at room temperature for 3 months in simulated intestinal fluid without pancreatin was 25% and 27%, respectively. However, the drug dissolved of aged sample was not significantly ($p>0.05$) difference from freshly prepared sample in both media. This indicated that storage at room temperature did not markedly effect on drug dissolution. In contrast, the percentage of drug dissolved at 5 and 10 minutes of aged sample stored at 45°C for 3 months in both media was significantly ($p<0.01$) lower than that of freshly prepared sample. This indicated that storage at 45°C had markedly effected on drug dissolution. This result was similar to results of Abdul-Fattah and Bhargava (2002) which reported that the dissolution of halofantrine in the solid dispersion with PVP K-30 as a carrier was decreased after the sample was stored at 45°C for

3 months. This was probably due to a physical instability. Most likely, physical loss may be due to precipitation of drug from solid dispersion.

4.11.3 Differential scanning calorimetry (DSC)

DSC thermograms of methylated-β-cyclodextrin, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and at 45°C for 3 months are shown in Figure 4.26. The DSC thermogram of methylated-β-cyclodextrin showed a very broad endothermic peak at 70-190°C. The simvastatin presented a single, sharp melting endothermic peak at 136°C. The DSC thermograms of aged samples showed a very broad endothermic peak at 70-190°C which similar to the DSC thermogram of freshly prepared co-evaporated sample. These results demonstrated that storage at room temperature and at 45°C for 3 months did not markedly effect on drug crystallinity.

DSC thermograms of PVP K-30, simvastatin, freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and at 45°C for 3 months are shown in Figure 4.27. The DSC thermogram of aged samples showed a very broad endothermic peak of PVP K-30 at 50-120°C due to the loss of water of PVP K-30 but a single, sharp melting endothermic peak of simvastatin was disappeared. Therefore, storage at room temperature and at 45°C for 3 months did not markedly effect on drug crystallinity.

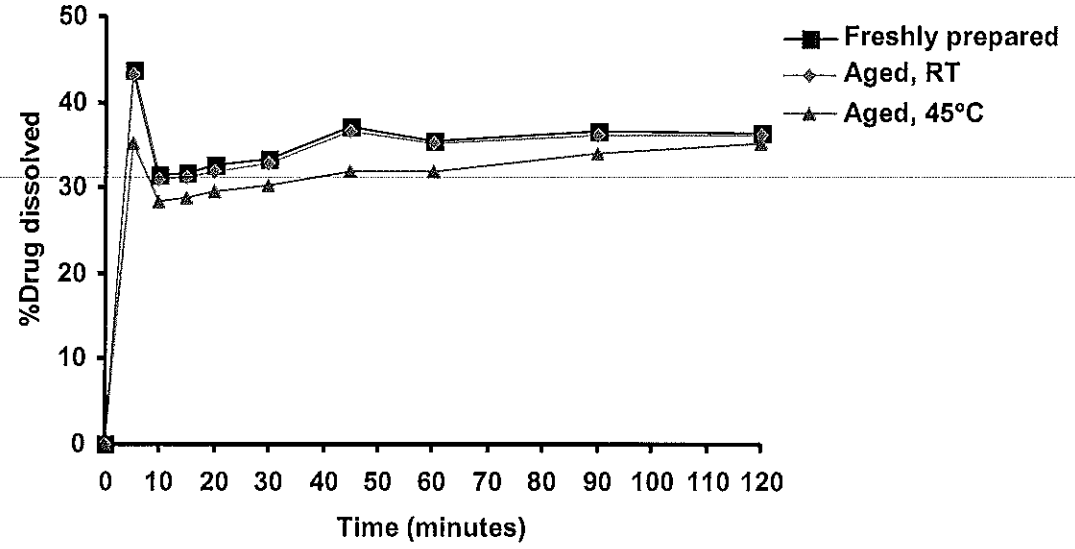


Figure 4.22 Dissolution profiles of freshly prepared co-evaporated sample (Freshly prepared), co-evaporated sample stored at room temperature (Aged, RT) and 45°C (Aged, 45°C) for 3 months in simulated gastric fluid without pepsin.

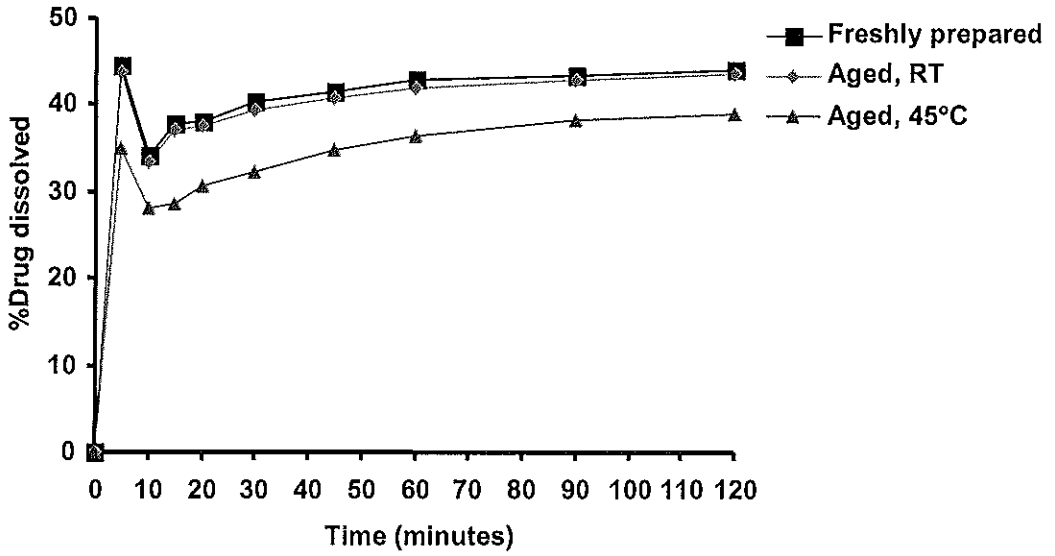


Figure 4.23 Dissolution profiles of freshly prepared co-evaporated sample (Freshly prepared), co-evaporated sample stored at room temperature (Aged, RT) and 45°C (Aged, 45°C) for 3 months in simulated intestinal fluid without pancreatin.

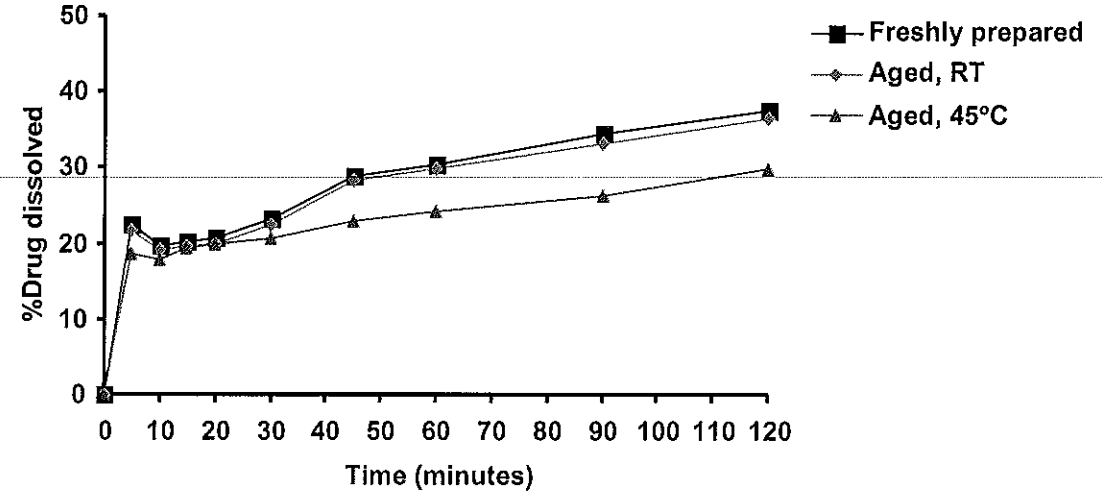


Figure 4.24 Dissolution profiles of freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 (Freshly prepared), simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature (Aged, RT) and 45°C (Aged, 45°C) for 3 months in simulated gastric fluid without pepsin.

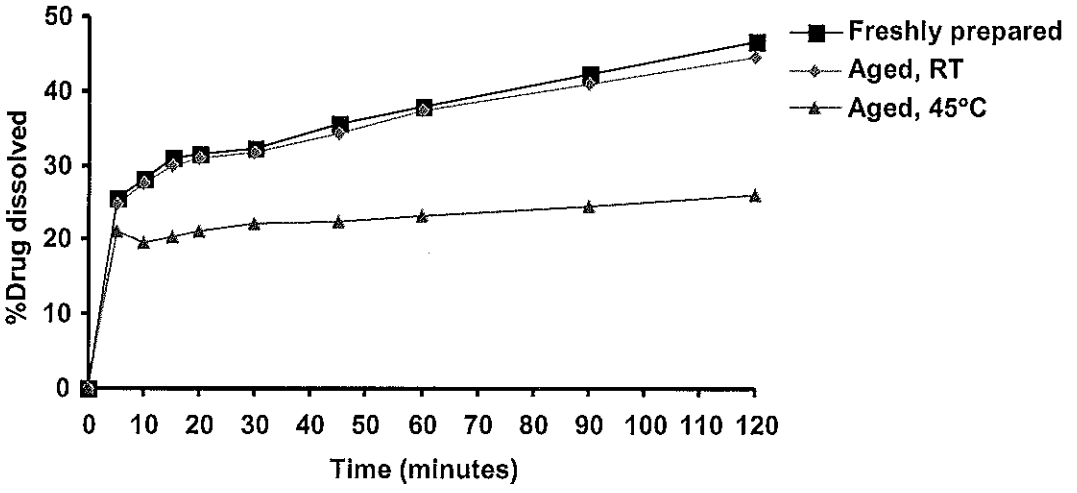


Figure 4.25 Dissolution profiles of freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 (Freshly prepared), simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature (Aged, RT) and 45°C (Aged, 45°C) for 3 months in simulated intestinal fluid without pancreatin

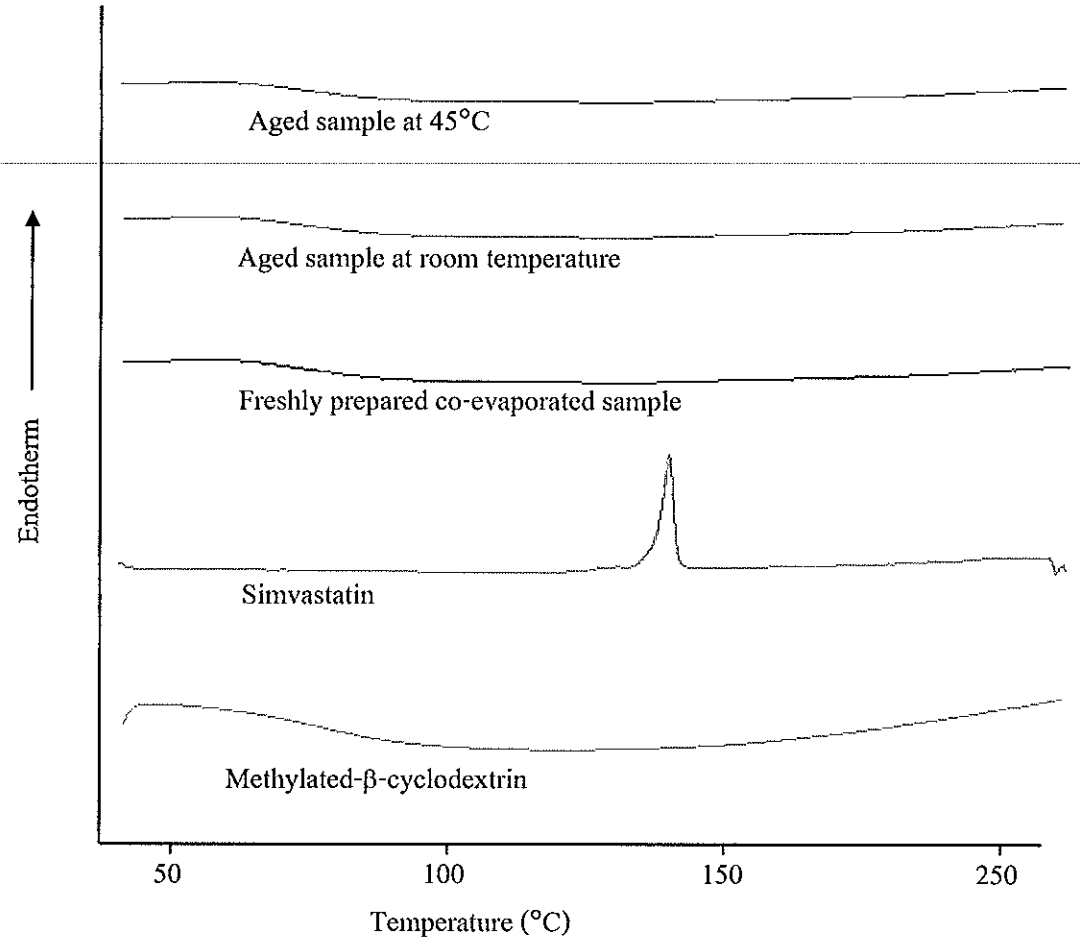


Figure 4.26 DSC thermograms of methylated-β-cyclodextrin, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and 45°C for 3 months.

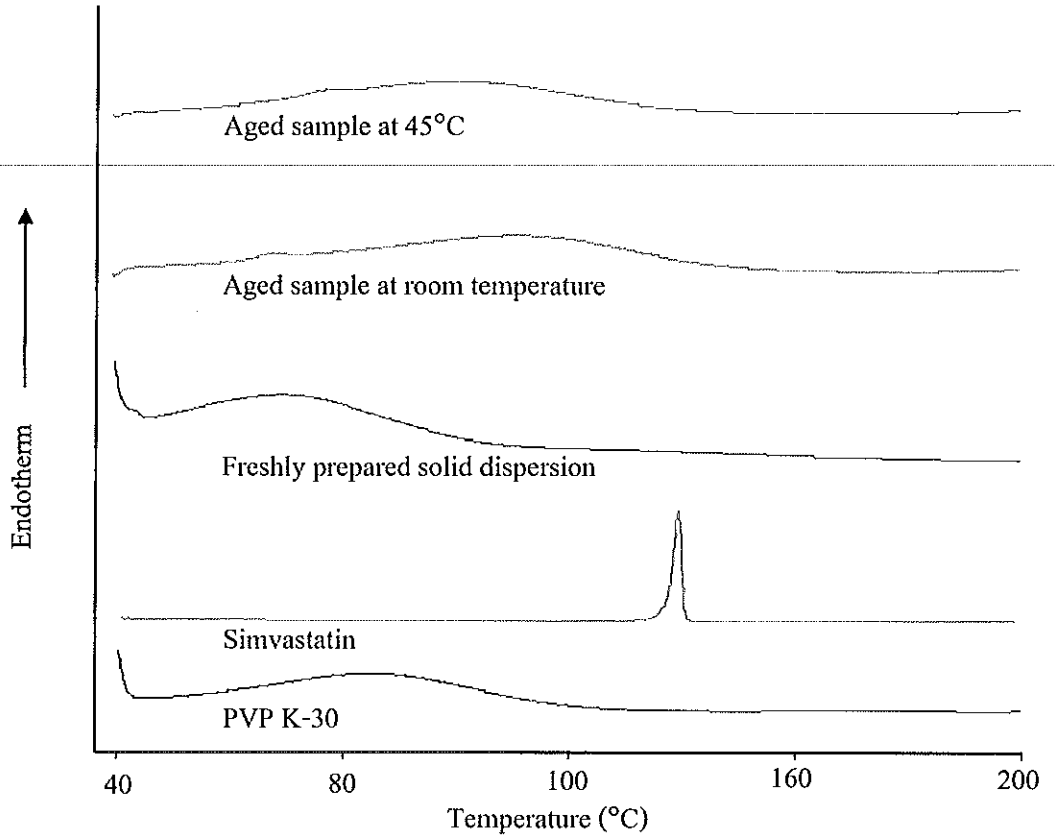


Figure 4.27 DSC thermograms of PVP K-30, simvastatin, freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and 45°C for 3 months.

4.11.4 Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of methylated-β-CD, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and at 45°C for 3 months are shown in Figure 4.28. The FT-IR spectrum of methylated-β-cyclodextrin showed absorption bands at 3,429 cm^{-1} for O-H stretching vibration, 2,935 cm^{-1} for C-H stretching vibration and 1,044 cm^{-1} for C-O stretching vibration. The FT-IR spectrum of simvastatin showed the presence of the following peaks at 3,549 cm^{-1} for alcohol O-H stretching vibration, 3,011 cm^{-1} for olefinic C-H

stretching vibration, 2,955 and 2,871 cm^{-1} for methyl C-H stretching vibration, and 1,697 cm^{-1} for stretching vibration of ester and lactone carbonyl functional group. The FT-IR spectrum of co-evaporated sample when stored at room temperature for 3 months exhibited some significant differences. Most of characteristic peaks similar to that of methylated-β-cyclodextrin, except one peak at 1,718 to 1,721 cm^{-1} for ester and lactone carbonyl functional groups which is the characteristic of simvastatin. The assumption for this is part of simvastatin may remain outside the methylated-β-cyclodextrin, whereas the remaining part fit inside the cavity of cyclodextrin. The FT-IR spectrum of aged sample when stored at room temperature and at 45°C for 3 months did not show any difference from the FT-IR spectrum of freshly prepared co-evaporated sample. This indicated that the drug and methylated-β-cyclodextrin were still interacted.

The FT-IR spectra of PVP K-30, simvastatin, freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and at 45°C for 3 months is shown in Figure 4.29. The FT-IR spectrum of aged samples when stored at room temperature and at 45°C for 3 months did not show any difference from the FT-IR spectrum of freshly prepared solid dispersion. This indicated that the drug and PVP K-30 were still interacted.

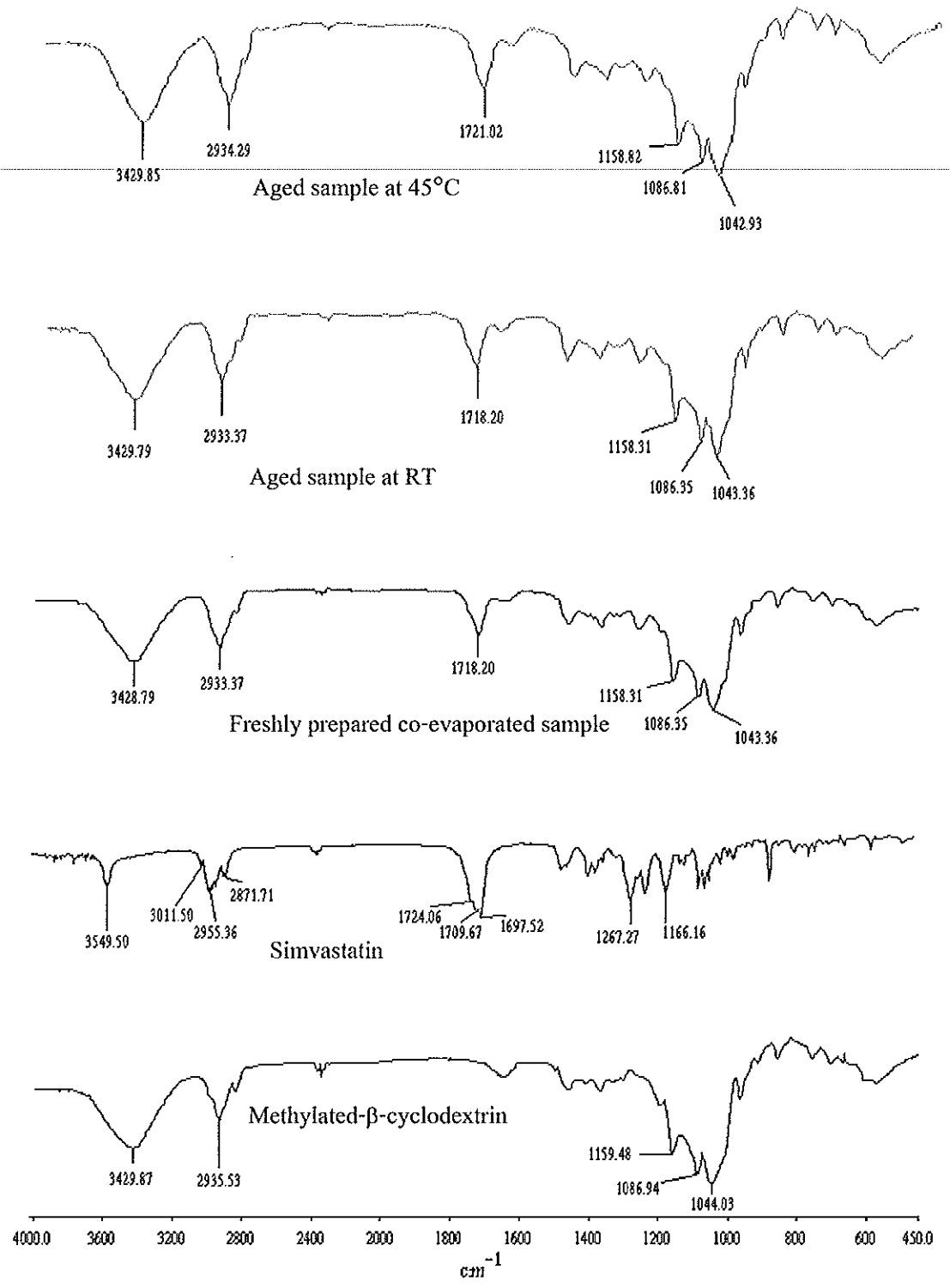


Figure 4.28 The FT-IR spectra of methylated-β-cyclodextrin, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and 45°C for 3 months.

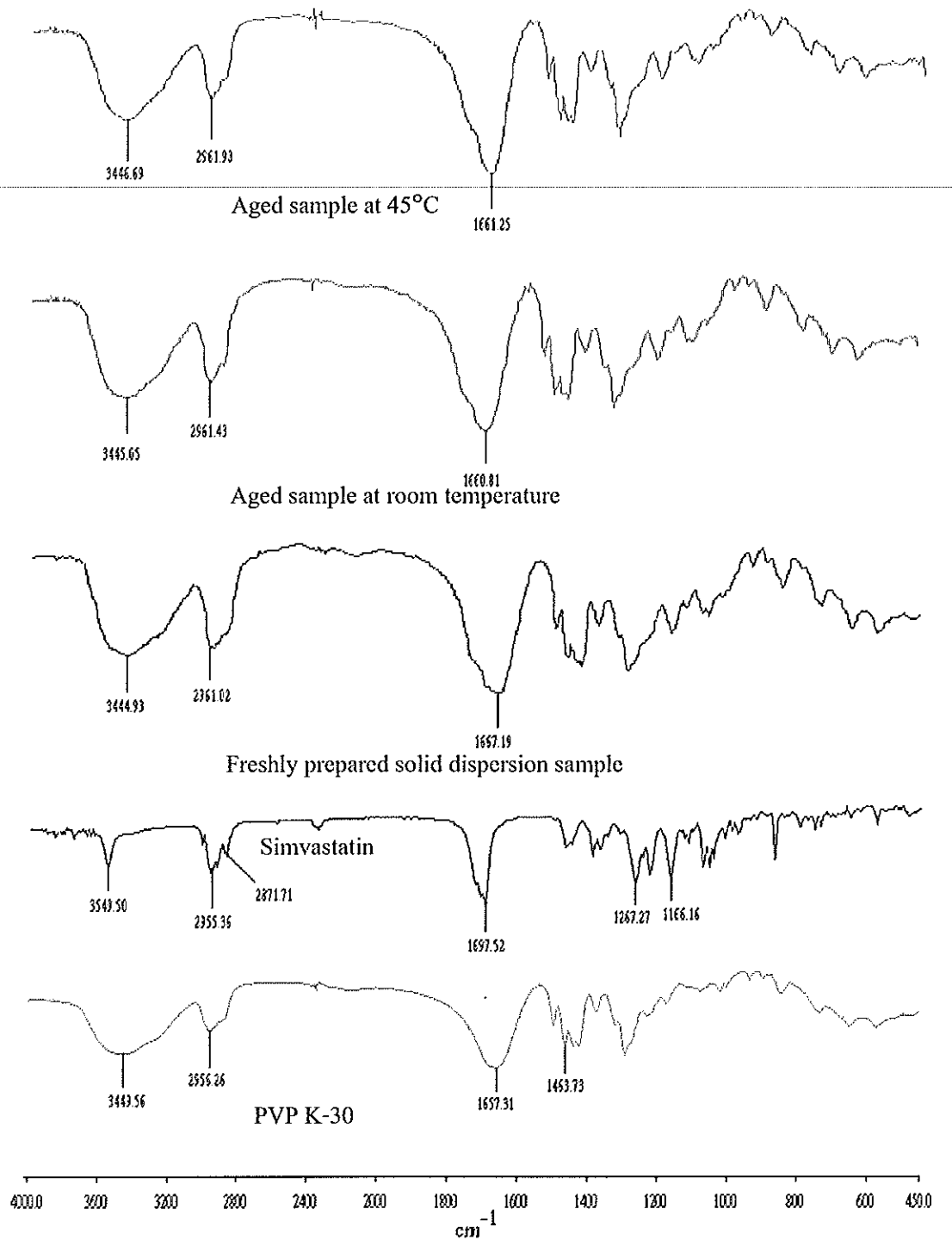


Figure 4.29 The FT-IR spectra of PVP K-30, simvastatin, fresh simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and 45°C for 3 months.

4.11.5 Powder X-ray diffractometry (PXRD)

Powder X-ray diffraction patterns of methylated-β-cyclodextrin, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and 45°C for 3 months are presented in Figure 4.30. The absence of any peaks in powder X-ray diffraction patterns of methylated-β-cyclodextrin exhibited the amorphous nature of methylated-β-cyclodextrin. The presence of several sharp peaks of simvastatin confirmed the crystalline form of drug. The diffraction peaks of co-evaporated sample stored at room temperature and at 45°C for 3 months were not observed. These results indicated that the drug still in an amorphous form. Therefore, storage of this sample at room temperature and at 45°C for 3 months did not have effect on the amorphous nature of drug.

Powder X-ray diffraction patterns of PVP K-30, simvastatin, freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and at 45°C for 3 months are shown in Figure 4.31. The diffraction peak of PVP K-30 disappeared due to this compound is in an amorphous state. Several sharp peaks of simvastatin were observed because of the drug is in a crystalline form. The hollow patterns of simvastatin-PVP K-30 solid dispersion stored at room temperature and 45°C for 3 months were obtained. This demonstrated that the drug still remain in an amorphous state.

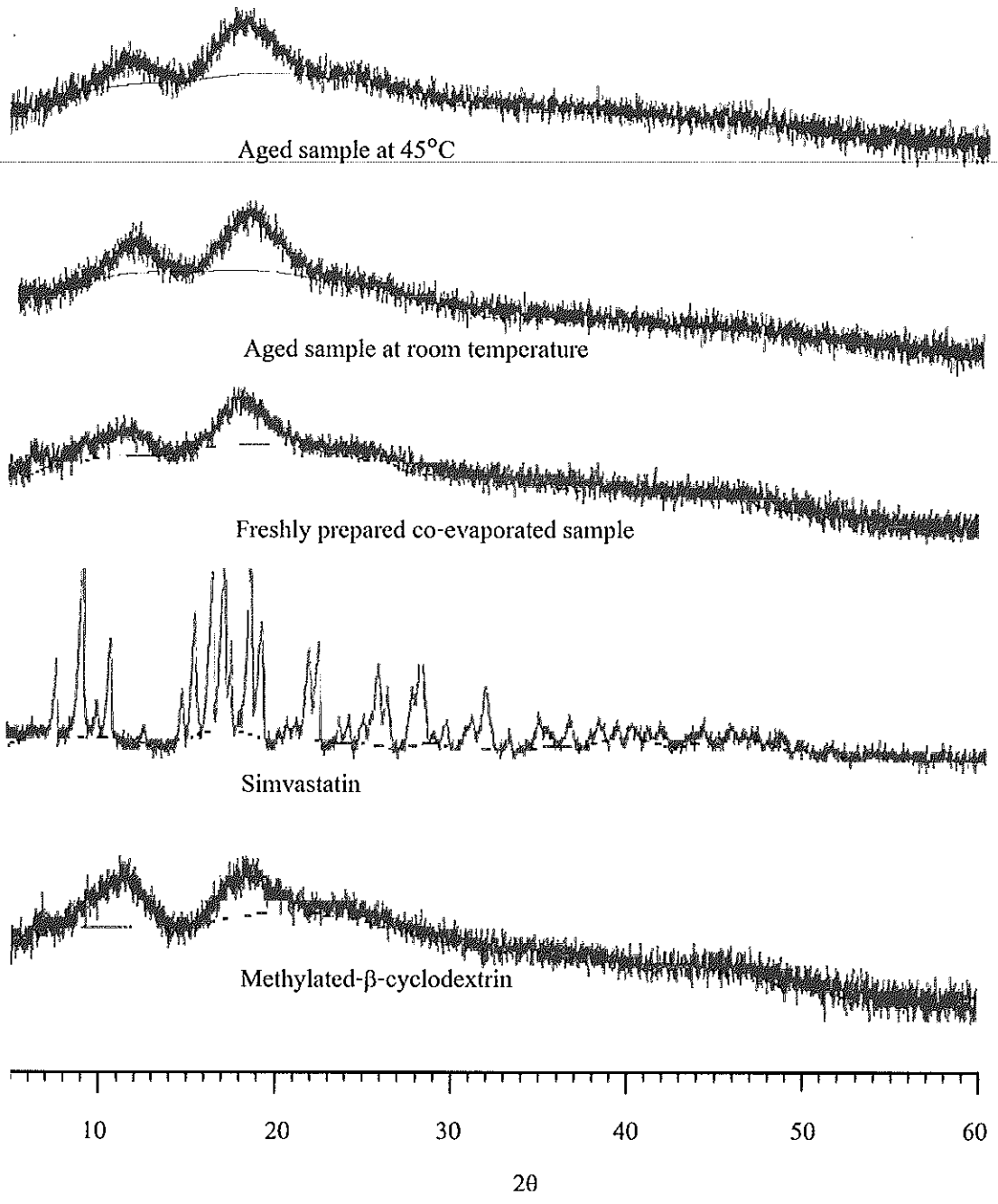


Figure 4.30 Powder X-ray diffraction patterns of methylated-β-cyclodextrin, simvastatin, freshly prepared co-evaporated sample, co-evaporated sample stored at room temperature and 45°C for 3 months.

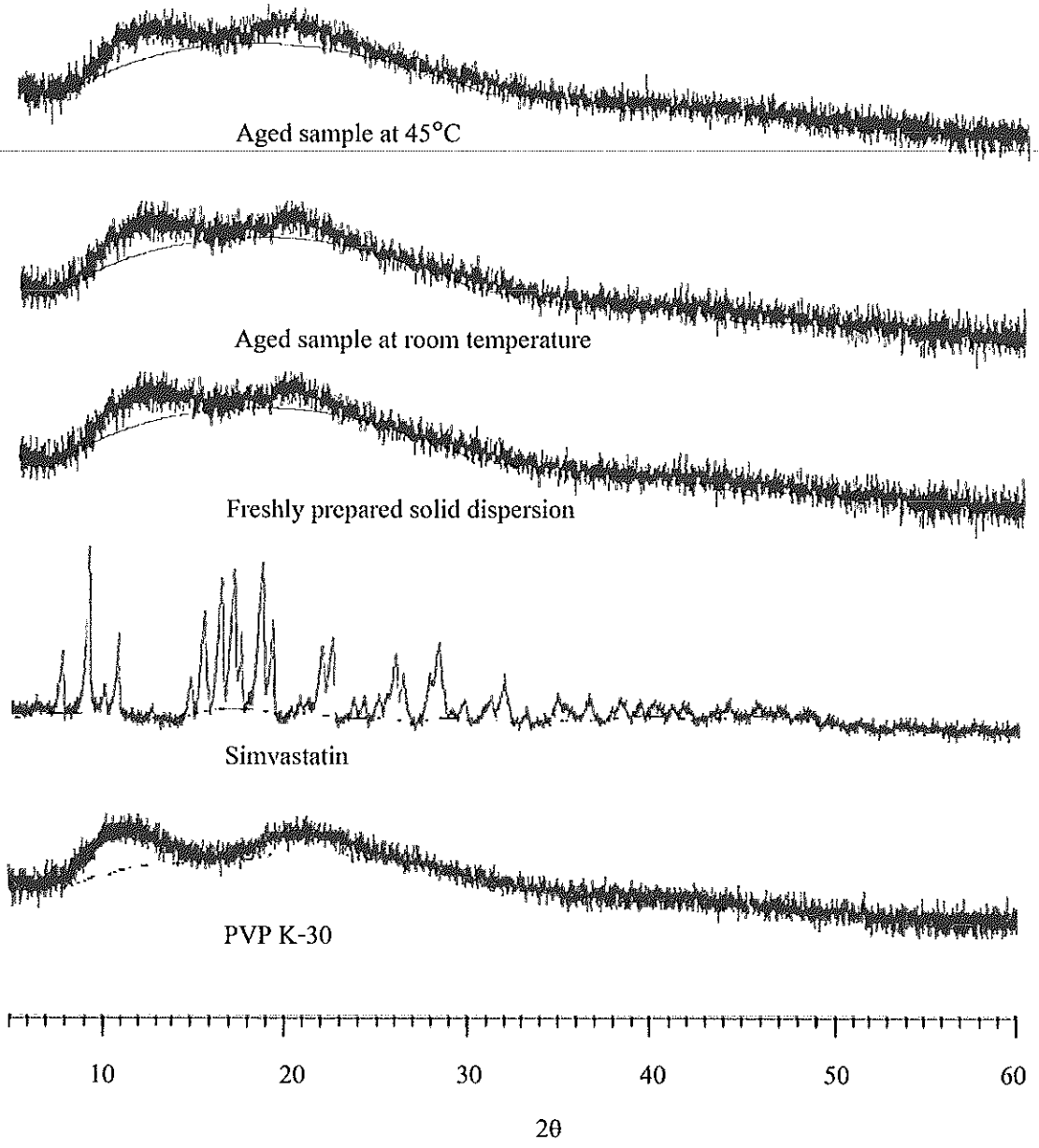


Figure 4.31 Powder X-ray diffraction patterns of PVP K-30, simvastatin, freshly prepared simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4, simvastatin-PVP K-30 solid dispersion in weight ratio of 1:4 stored at room temperature and 45°C for 3 months.

CHAPTER 5

CONCLUSIONS

Inclusion complexes of simvastatin-methylated-β-cyclodextrin were prepared by kneading, co-evaporation and freeze-drying methods in order to increase drug solubility and dissolution property. The solubility of simvastatin was increased linearly with the concentration of methylated-β-cyclodextrin. A_L-type phase-solubility diagram was obtained, indicating the formation of 1:1 stoichiometric inclusion complex with the apparent stability constant ($K_{1:1}$) of 1,240 M^{-1} at 37°C.

The solubility and the dissolution of simvastatin were increased in all inclusion complexes, compared with physical mixture and pure drug. From DSC, FT-IR and PXRD studies, the inclusion complex prepared by co-evaporation method gave drug in amorphous state, stronger complex formation than those of inclusion complexes prepared by kneading and freeze-drying method. The co-evaporated sample gave the highest dissolution. (Therefore, co-evaporated sample was selected for stability study.)

Solid dispersion of simvastatin-polyvinylpyrrolidone K-30 in weight ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8 and 1:10 were prepared by solvent method in order to increase drug solubility and dissolution. The solubility and the dissolution of all simvastatin-polyvinylpyrrolidone K-30 solid dispersions were increased compared with their corresponding simvastatin physical mixtures and pure drug. From DSC, FT-IR and PXRD studies, all simvastatin-polyvinylpyrrolidone K-30 solid dispersions showed an intermolecular hydrogen bonding between drug and polyvinylpyrrolidone K-30 and exhibited amorphous state. Simvastatin-polyvinylpyrrolidone K-30 solid dispersion in weight ratio of 1:4 gave the highest solubility and dissolution. Therefore, simvastatin-polyvinylpyrrolidone K-30 solid dispersion in weight ratio of 1:4 was selected for stability study.

Aged simvastatin-polyvinylpyrrolidone K-30 solid dispersion in weight ratio of 1:4 kept at room temperature for 3 months did not give significantly difference in drug dissolution when compared with that of freshly prepared sample. This indicated

that storage at room temperature for 3 months did not markedly effect on drug dissolution.

On the other hand, aged simvastatin-polyvinylpyrrolidone K-30 solid dispersion in weight ratio of 1:4 kept at 45 °C for 3 months gave difference in drug dissolution rate when compared with that of freshly prepared sample. This indicated that storage at 45 °C for 3 months markedly effect on drug dissolution property. The reduce in drug dissolution of aged sample may be due to precipitation of drug from the solid dispersion (Abdul-Fattah and Bhargava, 2002) and coarsening of drug particles because the interfacial energy of system was reduced by the reduction in interfacial area (Kaewnopparat *et al.*, 2001).

Aged co-evaporated sample kept at room temperature for 3 months did not gave difference in drug dissolution when was compared with that of freshly prepared sample. This indicated that storage at room temperature for 3 months did not markedly effect on drug dissolution.

On the contrary, aged co-evaporated sample kept at 45 °C for 3 months gave difference in drug dissolution property when compared with that of freshly prepared sample. This indicated that storage at 45 °C for 3 months markedly effect on drug dissolution. The dissolution of aged sample was changed may be due to agglomeration of the fine amorphous powder that could have been formed (Betageri and Makarla 1995).

The DSC, FT-IR and PXRD studies indicated that storage at room temperature and 45°C for 3 months did not markedly effect on drug crystallinity with drug still present in an amorphous state. Aged simvastatin-polyvinylpyrrolidone K-30 solid dispersion still showed the intermolecular hydrogen bonding between drug and polyvinylpyrrolidone K-30. Aged co-evaporated sample still gave strong complex formation between drug and methylated-β-cyclodextrin.

However, storage of these samples at room temperature and at 45°C for a longer period may effect on drug crystallinity.

SUGGESTIONS FOR THE FUTURE WORK

Future research should be performed according to these findings:

- from this study, solid dispersion in weight ratio of 1:4 should be selected for further study, since at this ratio the dissolution property of simvastatin was enhanced in term of effectiveness, ease to prepare, concern on stability, low cost and less time
- long term stability study of simvastatin in inclusion complexes and solid dispersions and the dissolution rate of the stored samples
- determination of simvastatin content during stability study should be analyzed by stability indicating HPLC method
- bioavailability study of simvastatin in inclusion complexes and solid dispersions compared with physical mixture and pure drug
- preparation of trial tablet formulation or capsule formulation and study in drug release from these dosage forms
- development of tablets or capsules in large scale, bioequivalence and clinical studies

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APPENDIX

APPENDIX

CHEMICAL COMPOUNDS

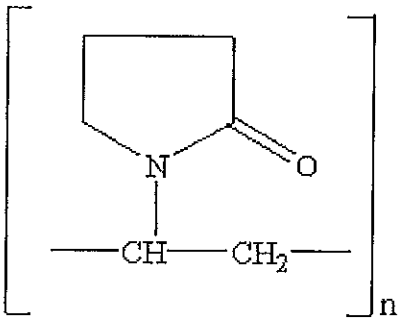
1. Polyvinylpyrrolidone K-30

Synonym: Plasdone K-30, Agrimer, Albigen A, Hemodesis, K30, Luviskol K30, Plasdone, Povidone, PVPP, PVP-K 30; PVP; Polyvinylpyrrolidone; Povidone K-30;

Chemical name: Poly(1-vinyl-2-pyrrolidinone)

Molecular formula: $(C_6H_9NO)_n$

Molecular weight: ~ 40,000



Melting point: 150°C

Solubility: Soluble in water, chloroform, alcohol, chlorinated hydrocarbons, amines, nitro pariffins, lower weight fatty acids.

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Excess heat, incompatible materials

Incompatibility with various substances: Reactive with oxidizing agents.

Storage: Keep container tightly closed and cool.

Precautions: Keep away from heat. Keep away from sources of ignition. Keep away from incompatibles such as oxidizing agents.

Flammability of the Product: May be combustible at high temperature.

Toxicity to Animals: Acute oral toxicity (LD_{50}) was 40000 mg/kg for mouse.

Other Toxic Effects on Humans: Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation.

Regulatory status: HMIS (U.S.A.):

Health Hazard : 1 (Irritation or minor reversible injury possible)

Fire Hazard: 1 (Materials that must be preheated before ignition will occur. Includes liquids, solids and semi solids having a flash point above 200 °F)

Reactivity: 0 (Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose , condense, or self-react. Non-explosives)

Personal Protection: E (Protective Equipment: safety glasses, gloves and dust respirator)

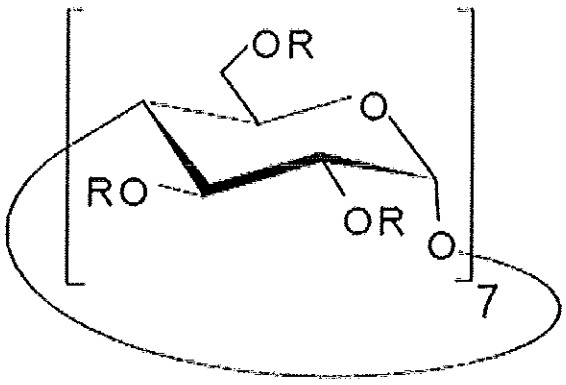
2. CAVASOL® W7 M Pharma

Synonym: Methylated-β-cyclodextrin, Methylated-β-CD

Chemical name: Methyl-β-cyclodextrin cyclomaltoheptaose, methyl ether

Degree of substitution (per anhydro glucose unit): 1.7-1.9

Molecular weight: ~ 1,310



R = CH_3 or H

Melting point: 160-190 °C

Description: Cyclodextrin is cyclic oligosaccharides containing D-(+)-glucopyranose units attached by α (1→ 4) glucoside bonds. Structure of β-cyclodextrin compose 7 glucose units. Methylated-β-cyclodextrin is prepared from β-cyclodextrin by the methylation. Methylated-β-cyclodextrin occur as a fine, amorphous powders, white and practically odorless. Methylated-β-cyclodextrin have been used to form inclusion complexes with a variety of drug molecules resulting in improvements to dissolution due to enhanced solubility and thereby improved its bioavailability.

Solubility: Good solubility in methanol, ethanol, acetone, pyridine, dimethyl sulfoxide, dimethyl formamide. Solubility for methylated-β-cyclodextrin in water is > 200 g in 100 ml at 25 °C.

Bulk density: ~ 0.2-0.3 g/ml

Stability and storage conditions: Methylated-β-cyclodextrin is stable in the solid state if protected from high humidity under normal temperatures and pressures. Storage at room temperature in tightly sealed containers under dry conditions in is recommended. Store in a cool place, well-ventilated area away from incompatible substance and protected from light. CAVASOL® W7 M Pharma has a shelf life of twelve months from date on the delivery note.

Safety: The oral administration of methylated-β-cyclodextrin in aqueous solution at doses up to 3 g/kg to mice resulted in no toxic symptoms.

Regulatory status: Included in oral and rectal pharmaceutical formulations licensed in Europe, Japan and the US.

VITAE

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Proceedings

Chompoonut Pechniramon and Nattha Kaewnopparat (2008). Enhanced solubility and dissolution of simvastatin by complexation with dimethyl beta-cyclodextrin. Proc. of the 14th International Cyclodextrin Symposium. May 8-11, 2008. Kyoto, Japan. (Full text proceedings).