DISSOLUTION AND BIOAVAILABILITY ENHANCEMENT OF MANIDIPINE HYDROCHLORIDE SOLID DISPERSION PREPARED BY MELT TECHNIQUE

By

Miss Benchawan Chamsai

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

Doctor of Philosophy Program in Pharmaceutical Technology

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การเพิ่มการละลายและชีวประสิทธิผลของระบบกระจายตัวของแข็งของมานิดิพีนไฮโดรคลอไรด์ที่เตรียมโดยใช้เทคนิคการหลอม

โดย

นางสาวเบญจวรรณ แจ่มใส

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต

สาขาวิชาเทคโนโลยีเภสัชกรรม

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

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The Graduate School, Silpakorn University has approved and accredited the thesis title of "Dissolution and bioavailability enhancement of manidipine hydrochloride solid dispersion prepared by melt technique" submitted by Miss Benchawan Chamsai as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Technology.

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BENCHAWAN CHAMSAI : DISSOLUTION AND BIOAVAILABILITY ENHANCEMENT OF MANIDIPINE HYDROCHLORIDE SOLID DISPERSION PREPARED BY MELT TECHNIQUE. THESIS ADVISORS : PROF. PORNSAK SRIAMORNSAK, Ph.D. AND ASSOC. PROF. SRISAGUL SUNGTHONGJEEN, Ph.D. 120 pp.

Manidipine hydrochloride (MDP) is generally used clinically as an antihypertensive agent; however, the bioavailability of orally administered MDP is limited due to its very low water solubility. The objective of this research was, therefore, to increase the solubility and bioavailability of MDP by the formation of ternary solid dispersion (tSD) containing MDP, polyethylene glycol (PEG) 4000 or D-α-tocopherol 1000 succinate (TPGS), and copovidone. Solid ternary phase diagram was constructed in order to find the miscibility of MDP in the solid carriers. The tSD powders were determined for their physicochemical properties using differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR) spectroscopy and hot stage microscopy. The solubility, dissolution, stability and pharmacokinetics of MDP from the tSD were also investigated. The results demonstrated that tSD obtained from solid ternary phase diagram divided into homogeneous and non-homogeneous regions. In the homogenous region, the transparent characteristics of tSD was observed and considered as a glass solution, which have a higher MDP solubility than that in non-homogenous region. The hot stage microscopy demonstrated that, when increasing the temperature, PEG4000/copovidone or TPGS/copovidone blends created miscible combination and MDP can be dissolved at lower processing temperature. PXRD patterns of tSD revealed halo pattern with the absence of MDP characteristic peaks at 2θ 11.1 and 17.7 while a crystalline form was observed only in MDP or its physical mixture. DSC results also supported the results of PXRD; the absence of MDP melting peak was observed in all formulations. The solubility study revealed that in PEG4000/copovidone blends, the solubility of MDP increased with the increased copovidone concentration. A decrease in solubility was observed when the copovidone concentration in TPGS/copovidone blends was increased. However, a good linear correlation between MDP solubility and copovidone concentration was observed. FTIR analysis demonstrated strong hydrogen bonding between amine groups of MDP and carbonyl groups of copovidone which supported a higher solubility and dissolution of tSD. After storage under accelerated condition (40°C/75%RH), the tSD still showed a good appearance and higher solubility than MDP powder; crystalline drug disappeared in both tSD formulations using PEG/copovidone and TPGS/copovidone blends. The pharmacokinetic study in Wistar rats showed that the tSD containing MDP, TPGS and copovidone had the greatest effect on oral bioavailability. The absorption of MDP, from tSD, in both fasted and fed state was higher than MDP powder (4.39 and 2.13 folds, respectively).The food effect of MDP in fed state compared to fasted state, was reduced by using solid dispersion formulations. In summary, the improvement of solubility, stability and oral bioavailability of MDP could be achieved by using solid dispersion technique.

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มานิดิพีนไฮโดรคลอไรด์เป็นยาลดความดันโลหิตที่มีค่าการละลายน้ำต่ำมากส่งผลให้ยามีค่าชีวประสิทธิผลต่ำ วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อเพิ่มค่าการละลายและชีวประสิทธิผลของมานิดิพีนไฮโดรคลอไรด์โดยเตรียมให้อยู่ในระบบกระจายตัวของแข็งไตรภาคของมานิดิพีนไฮโดรคลอไรด์และพอลิเอทิลีนไกลคอล 4000 หรืออนุพันธ์ซัคซิเนตของวิตามินอี (TPGS) และโคพอวิโดน แผนภาพวัฏภาคของแข็งไตรภาคสร้างขึ้นเพื่อหาสภาพผสมเข้ากันได้ของการกระจายตัวของแข็งไตรภาคของมานิดิพีนไฮโดรคลอไรด์ในตัวพาของแข็ง ศึกษาสมบัติทางเคมีฟิสิกส์ของผงกระจายตัวของแข็งไตรภาคโดยเครื่องวัดความจุความร้อนจำเพาะของสาร เครื่องวิเคราะห์การเลี้ยวเบนของรังสีเอ็กซ์ เครื่องมือวิเคราะห์ด้วยอินฟราเรดชนิดฟูเรียร์ทรานสฟอร์ม และกล้องจุลทรรศน์ชนิดแท่นความร้อน รวมถึงการประเมินค่าการละลาย การทดสอบความคงสภาพ และเภสัชจลนศาสตร์ของมานิดิพีนไฮโดรคลอไรด์จากระบบกระจายตัวของแข็งไตรภาค ผลการศึกษาแสดงให้เห็นว่าระบบกระจายตัวของของแข็งไตรภาคแบ่งออกเป็นส่วนที่เป็นเนื้อเดียวกันและไม่เป็นเนื้อเดียวกัน ระบบกระจายตัวของแข็งไตรภาคของมานิดิพีนไฮโดรคลอไรด์ในส่วนที่เป็นเนื้อเดียวกันมีลักษณะโปร่งใสและเรียกว่าสารละลายแก้ว ซึ่งมีค่าการละลายของมานิดิพีนไฮโดรคลอไรด์สูงกว่าในส่วนที่ไม่เป็นเนื้อเดียวกัน จากการวิเคราะห์ด้วยกล้องจุลทรรศน์ชนิดแท่นความร้อน แสดงให้เห็นว่าการเพิ่มอุณหภูมิมีผลให้สารผสมระหว่างพอลิเอทิลีนไกลคอล 4000 และโคพอวิโดน หรือ TPGS และโคพอวิโดนเข้ากันได้และมีผลทำให้มานิดิพีนไฮโดรคลอไรด์สามารถละลายได้หมดที่อุณหภูมิต่ำกว่าอุณหภูมิที่ใช้ในการเตรียมระบบกระจายตัวของแข็ง การวิเคราะห์การเลี้ยวเบนของรังสีเอ็กซ์ แสดงให้เห็นรูปแบบฮาโล ไม่พบพีกสำคัญของมานิดิพีนไฮโดรคลอไรด์ที่ตำแหน่ง 11.1 และ 17.7 องศา 2θ ในขณะที่พบรูปแบบผลึกในมานิดิพีนไฮโดรคลอไรด์หรือของผสมทางกายภาพเท่านั้น ผลการศึกษาโดยเครื่องวัดความจุความร้อนจำเพาะของสารก็สนับสนุนผลของการวิเคราะห์การเลี้ยวเบนของรังสีเอ็กซ์ คือไม่พบพีกการหลอมเหลวของมานิดิพีนไฮโดรคลอไรด์ในทุกตำรับ การศึกษาค่าการละลายพบว่าค่าการละลายของมานิดิพีนไฮโดรคลอไรด์เพิ่มขึ้นเมื่อเพิ่มความเข้มข้นของโคพอวิโดนในสารผสมระหว่างพอลิเอทิลีนไกลคอล 4000 และโคพอวิโดน ในขณะที่ค่าการละลายของมานิดิพีนไฮโดรคลอไรด์ลดลงเมื่อเพิ่มความเข้มข้นโคพอวิโดนในสารผสมระหว่าง TPGS และโคพอวิโดน อย่างไรก็ตามค่าการละลายของมานิดิพีนไฮโดรคลอไรด์และความเข้มข้นของโคพอวิโดนมีความสัมพันธ์เชิงเส้นตรงที่ดี ผลการวิเคราะห์ด้วยอินฟราเรดชนิดฟูเรียร์ทรานสฟอร์มแสดงให้เห็นถึงพันธะไฮโดรเจนที่แข็งแรงระหว่างหมู่เอมีนของมานิดิพีนไฮโดรคลอไรด์และหมู่คาร์บอนิลของโคพอวิโดนซึ่งสอดคล้องกับการเพิ่มค่าการละลายและการละลายของมานิดิพีนไฮโดรคลอไรด์จากระบบกระจายตัวของแข็งไตรภาค การศึกษาความคงสภาพที่สภาวะเร่ง (40 องศาเซลเซียสและความชื้นสัมพัทธ์ร้อยละ 75) ระบบกระจายตัวของแข็งไตรภาคยังคงมีลักษณะภายนอกที่ดีและมีค่าการละลายสูงกว่าผงยามานิดิพีนไฮโดรคลอไรด์ และไม่พบผลึกยาทั้งในสารผสมระหว่างพอลิเอทิลีนไกลคอล 4000 และโคพอวิโดนและสารผสมระหว่าง TPGS และโคพอวิโดน การศึกษาเภสัชจลนศาสตร์ของยาในหนูขาวใหญ่พบว่าสารผสมระหว่าง TPGS และโคพอวิโดนมีผลต่อค่าชีวประสิทธิผลของมานิดิพีนไฮโดรคลอไรด์มากที่สุด การดูดซึมของยามานิดิพีนในสภาวะอดอาหารและได้รับอาหารมีค่าสูงกว่าผงยามานิดิพีนไฮโดรคลอไรด์ 4.39 และ 2.13 เท่า ตามลำดับ ระบบกระจายตัวของแข็งสามารถลดผลของสภาวะได้รับอาหารเมื่อเทียบกับสภาวะอดอาหาร โดยสรุปการเพิ่มค่าการละลาย ความคงสภาพ และค่าชีวประสิทธิผลของมานิดิพีนไฮโดรคลอไรด์สามารถทำได้โดยใช้เทคนิคการกระจายตัวของแข็ง

สาขาวิชาเทคโนโลยีเภสัชกรรม บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
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ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ 1..... ..... 2.....

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% v/v	percent volume by volume
% w/v	percent weight by volume
% w/w	percent weight by weight
%	percentage
α	alpha
µg	microgram(s)
µL	microliter(s)
µm	micrometre(s)
<	less than
>	more than
±	plus-minus
°	degree
^{o}C	degree Celsius
®	registered trademark
2θ	2 theta
×	magnification
ANOVA	one-way analysis of variance
AUC	area under the plasma concentration time curve
BCS	biopharmaceutical classification system
C_{max}	maximum concentration
C	carbon
Cl	chloride
cm	centrimeter(s)
cm^2	square centrimeter
COO^-	carboxylate functional group
COOH	carboxylic acid functional group
DSC	differential scanning calorimetry
e.g.	for example
et al.	and others
FTIR	Fourier transform infrared spectroscopy
g	gram(s)
h	hour(s)
H	hygrogen
HCl	hydrochloride
HPLC	high performance liquid chromatography

i.e.	id est (Latin); that is
log	logarithm
KCl	potassium chloride
kg	kilogram(s)
KH_2PO_4	monobasic potassium phosphate
log *P*	partition coefficient
M	molar
MDP	manidipine hydrochloride
mg	milligram(s)
min	minute(s)
mL	milliter(s)
mm	millimetre(s)
mM	millimolar(s)
MW	molecular weight
N	nitrogen
NaCl	sodium chloride
NaCMC	sodium carboxymethylcellulose
NaOH	sodium hydroxide
NH_2	amidogen functional group
NH_3^+	ammonium functional group
nm	nanometer(s)
No.	number
PEG	polyethylene glycol
pKa	negative logarithm of an acid dissociation constant
PXRD	powder X-ray diffraction
RH	relative humidity
rpm	revolutions per minute
s	second(s)
S.D.	standard deviation
SGF	simulated gastric fluid USP without pepsin
S.E.	standard error
SEM	scanning electron microscope
T_g	glass transition temperature
USP	United States Pharmacopoeia
UV	ultraviolet

CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the research problem

In the field of drug delivery system development for oral application, the enhancement of solubility of drugs is becoming increasingly challenge as the number of innovative (Amidon et al., 1995). The dissolution rate of poorly water-soluble drugs often becomes a rate-limiting step in their absorption. There are many attempts to improve the dissolution and absorption of poorly water-soluble drugs, *e.g*., the use of surfactant (Eerdenbrugh et al., 2009), complex formation with β-cyclodextrin (Buchanan et al., 2007), micronization (Brynjelsen et al., 2002; Cha et al., 1994), salt formation (Shevchenko et al., 2012; Tao et al., 2009), nanoparticles (Burapapadh et al., 2012; Choi et al., 2012; Jafarinejad et al., 2012), self-emulsifying drug delivery system (Hong et al., 2006; Weerapol et al., 2015) and solid dispersions (Chowdary et al., 2000; Janssens et al., 2008a,b,c,d; Jung et al., 1999).

The solid dispersion can increase drug solubility and dissolution by creating a large surface area to contact dissolution medium. Therefore, it is desirable that the drug is embedded in a carrier, often a polymer, in such a way that the drug is molecularly dispersed. The formulation of solid dispersions can be prepared by the melting, solvent, or melting-solvent method. In the case of the solvent method, both drug and carrier must be dissolved completely in organic solvent and then evaporated to changes in drug solubility performance. However, the solvent method introduces residual solvent, which may bring up the environmental issues. Also, amount of residual solvents may be present in solid dispersions, which could be toxic. The melting method is another way to prepare solid dispersions; drug and carrier are melted together until miscible mixture was occured, after that the molten mixture is rapidly solidified. The benefits of melting technique are to reduce or avoid using organic solvent and environmental friendly, which can reduce toxicity. Furthermore, this method requires less processing steps, which is easy to scale up for commercial purpose. Melting method is, however, suitable for low melting point drug and thermo stable drug.

For melting method, the selection of the carrier has a significant impact in the success of enhancing dissolution rate of the drugs. The antiplasticizing effect of polymer may promote the change polymorphic forms of drug. The ease of alteration crystalline form to amorphous state depends on the free energy difference between the amorphous and the crystalline state. Thus, the selection of proper polymers benefits to stabilize the stability of amorphous drug as well as, with respect to dissolution, for the shelf-life stability (Van den Mooter, 2011). One of the polymers that has been used extensively as a carrier for solid dispersions is polyethylene glycol (PEG), which is a semi-crystalline polymeric carrier having low melting point. The enhancement mechanism of drug solubility is due to the dissolution of drug within the amorphous carrier, rather than in the crystalline or semi-crystalline fraction of the carrier. Therefore, a decrease in crystallinity of PEG could result in a higher solubility of drug. One of the ways to achieve the amorphous state can be done by blending PEG with a third component, in order to reduce its crystallinity, i.e., hydroxypropylmethylcellulose (HPMC) (Janssens et al., 2008c), polyvinylpyrrolidone (PVP) (Urbanetz, 2006), sucrose laurate (Szűts et al., 2011), copovidone (Janssens et al., 2008a and Wang et al., 2005). The increase in solubility of poorly water-soluble drugs and carriers occur when combine with surfactant, for example, Myrj® 52 (Wang et al., 2005), TPGS® 1000 (Janssens et al., 2008a) or used with another polymer, i.e., Inutec® SP1 (Janssens et al., 2008b), Eudragit® E100 (Six et al., 2005). Many researches revealed that the ternary solid dispersions system leads to the good solubility (Janssens et al., 2008a,b,c,d) and bioavailability (Al-Obaidi et al., 2011 and Li et al., 2012) of poorly water-soluble drugs. However, there is a few reports about stability of ternary solid dispersion system. Although solid dispersion system can vastly improve dissolution rate but not many solid dispersion products have reached the market. This is mainly due to physical instability of products such as phase separation (Urbanetz and Lippold, 2005) and recrystallization of amorphous phase or polymorphic transition (Yoshinari et al., 2002). In this research, we attempted to increase the dissolution rate, bioavailability and stability of a model poorly water-soluble drug, manidipine hydrochloride (MDP) using melting method in order to evaluate the potential of the ternary solid dispersion system. PEG, copovidone and TPGS were investigated as solid dispersion carriers in this study.

MDP is a lipophilic, third-generation, highly vasoselective, dihydropyridine calcium channel antagonist having long-lasting activity for the treatment of essential hypertension (Luque Otero and Martell Claros, 2005). The drug is practically insoluble in water and exists in the solid form as yellow crystals. Additionally, after oral administration of MDP in human at clinical dose, the plasma concentration of MDP is very low (Ghagare et al., 2012; Jing et al., 2007). The poorly water-solubility of MDP results in a reduced drug dissolution rate in gastrointestinal fluid following oral administration, and consequently a reduced bioavailability..

1.2 Objectives

The objectives of this research were:

(1) To develop ternary solid dispersions containing MDP prepared by melting method

(2) To investigate physicochemical characteristics, solubility and dissolution behavior of ternary solid dispersions

(3) To examine the stability of ternary solid dispersion at ambient temperature and/or under accelerated condition (40ºC/75%RH)

(4) To evaluate absorption and bioavailability of MDP-loaded solid dispersions in gastrointestinal tract of animal model

CHAPTER 2
LITERATURE REVIEW

2.1 Poorly water soluble drugs and solubility enhancement approaches

2.2 Solid dispersions

- 2.2.1 Definition
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2.3 Manidipine hydrochloride (MDP)

- 2.3.1 Physicochemical and pharmacological properites
- 2.3.2 Pharmacokinetic properties

2.1 Poorly water-soluble drugs and solubility enhancement approaches

The biopharmaceutics classification system (BCS) categorizes drugs into four biopharmaceutical classes according to their water solubility and membrane permeability characteristics (Fig. 2.1). Class I and III drugs are high water-soluble drugs but class II and IV are considered poorly soluble.

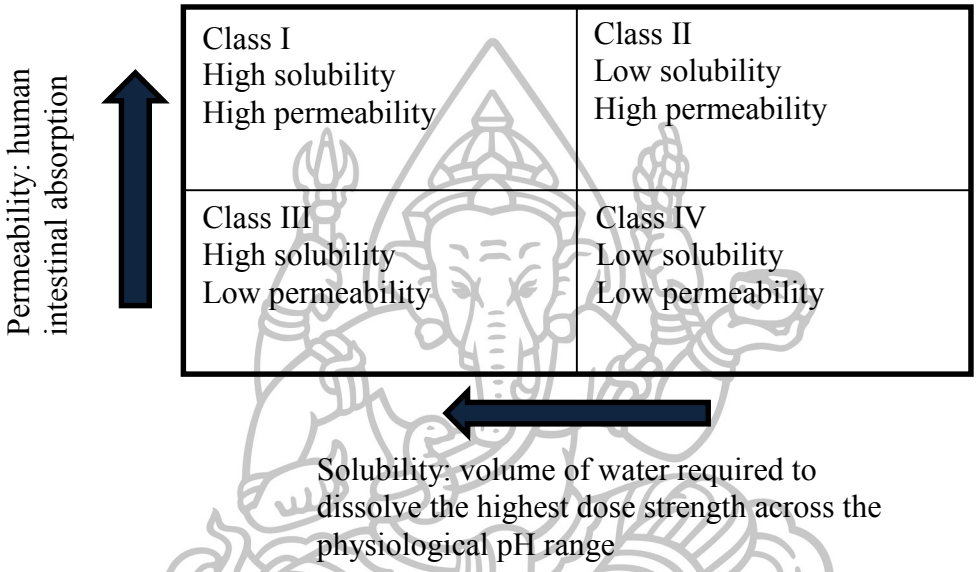


Figure 2.1 The biopharmaceutics classification system (Dahan et al., 2009)

Nowadays, 40% or more of new active substances are poorly soluble in water. A fundamental problem of insoluble drugs, including class II and IV drugs, is often insufficient bioavailability, as the drug absorption after oral administration is poor and very often below the therapeutic level (Chaumeil, 1998).

Several approaches have been attempted to improve the solubility and dissolution characteristics of poorly water-soluble drugs. The pH adjustment and pharmaceutical buffers are commonly used in solution formulations. As pH changes, the drug ionization increases; as a result, the drug solubility increases. The use of cosolvent can significantly increase drug solubility. Common cosolvents include ethanol, propylene glycol (PG), PEG300, PEG400, glycerin, N,N-dimethyl acetamide (DMA), etc. (Li and Zhao, 2007). These cosolvents are used with aqueous solutions

for oral and for parenteral dosage forms. A complexation with cyclodextrin can enhance drug solubility. Decreasing the particle size of drugs can also improve their rate of dissolution. Particle size and crystallinity are important physicochemical properties to affect solubility and absorption of drug. The dissolution behavior and bioavailability of water insoluble drugs could be improved by producing them in more soluble amorphous form (Biradar et al., 2006). Solid dispersions and solid solutions are one of solubility enhancement technique to enhance the solubility and dissolution rate of poorly soluble drugs.

2.2 Solid dispersions

2.2.1 Definition

Solid dispersions were introduced by Chiou and Riegelman who defined solid dispersions as "a dispersion of one or more active ingredients in an inert carrier at the solid state, prepared by either the melting, the solvent or the melting-solvent method" (Chiou and Riegelman, 1971). Nowadays, solid dispersions can be more narrowly defined as dispersion of drug in an amorphous polymer matrix where the drug is preferably in the molecularly dispersed state (Huang and Dai, 2014).

2.2.2 Classification

Solid dispersions have been classified by Chiou and Riegelman (1971) into four groups as follows.

2.2.2.1 Simple eutectic mixtures

A eutectic mixture is a mixture of sparingly water-soluble drug and highly water-soluble carrier. It may be regarded thermodynamically as an intimately blended physical mixture of its two crystalline components. These components are assumed to crystallize simultaneously in very small particulate sizes. The increase in specific surface area is mainly responsible for the increase rate of dissolution of poorly water-soluble drugs. The other factors such as increased wettability, reduction or absence of aggregation, and solubilization of the drug by the carrier at the site of diffusion layer also affect to the increase the dissolution of drug. Sekiguchi and Obi (1961) noted that the formulation of eutectic mixtures improves the rate of drug

release and the bioavailability of poorly water-soluble drugs. Chiou and Niazi (1976) prepared a fused composition of griseofulvin and succinic acid, and showed that the dissolution of griseofulvin is inversely proportional to the concentration of griseofulvin in the dispersion.

2.2.2.2 Solid solutions

Solid solutions consist of a solid solute dissolved in a solid solvent. If a carrier is crystalline, a mixed crystal is formed because the two components crystallize together in a homogeneous one-phase system. Perhaps, particle size is reduced in solid solution to molecular level. This system would be expected to have higher rate of dissolution than eutectic systems. Chiou and Riegelman (1971) reported a marked increase in dissolution rate of sparingly water-soluble drugs (e.g., digitoxin, 17-methyl testosterone, hydrocortisone acetate, and prednisolone acetate) when dispersed in PEG6000. This is due to the formation of colloidal or molecular dispersion of the drug in the carrier.

2.2.2.3 Glass solutions

The carrier is amorphous and the solute molecules are dispersed molecularly in the amorphous carrier. Glass solutions are a homogeneous one-phase system in which a glassy or a vitreous form of the carrier solubilizes drug molecule in its matrix, the dissolution and absorption rate of drug will be increased. In the past, sugar and urea were used as amorphous carriers. Recently, organic polymers such as PVP and cellulose derivatives are commonly used. Glass solutions of digitoxin with PVP (Stupak and Bates, 1973), griseofulvin with hydroxypropylmethyl cellulose acetate succinate (HPMCAS) (Al-Obaidi et al., 2013) have been reported.

2.2.2.4 Amorphous precipitates of a drug in crystalline carrier

Amorphous precipitation occurs when the drug precipitates as an amorphous form in the inert carrier. The high energy state of the drug in this system generally produces much greater dissolution rate than the corresponding crystalline drug. The conversion of a drug to an amorphous form of co-precipitation results in an increased dissolution. The examples of this system have been reported, for example, sulfisoxazole-PVP and chloramphenicol-PVP or hydroxypropylcellulose (HPC).

Based on the molecular distribution of solid dispersion, three different types of fully amorphous solid dispersions can be distinguished: (1) glass solutions, (2) glass suspensions, (3) combination of (1) and (2). The first type, glass solutions, consists of an amorphous carrier in which the drug is molecularly dispersed (Chiou and Riegelman, 1971). This type of solid dispersions is homogeneous on a molecular level. Therefore, only one phase is present and only one glass transition temperature (T_g) will be observed. The second type is glass suspensions. It consists of an amorphous carrier in which the drug is dispersed as amorphous clusters. This type of solid dispersions is not homogeneous on a molecular level and consists of two phases. Hence, glass transitions temperature of both carrier and drug are observed. The third type is a combination of both. Two phases are present: one consists of carrier in which a part of the drug is molecularly dispersed; the other consists of amorphous drug clusters. Which one of the three types is obtained depends on the miscibility of drug and carrier and on the preparation method (Breitenbach et al., 1999). Ideally, a molecular dispersion should be kinetically stable at the storage temperature. Such stability can be achieved by carefully selecting polymer excipients, the polymer/drug ratio and the processing temperature (Huang and Dai, 2014).

The classification of solid dispersion by the type of carriers divided into 3 generations, as shown in Fig. 2.2. First generation solid dispersions were prepared using crystalline carriers, including of urea and sugars, which were the first carrier to be employed in solid dispersions. They have the disadvantages of forming crystalline solid dispersions, which were more thermodynamically stable and did not provide the fastest drug release as amorphous ones. Second generation of solid dispersions contains amorphous carriers instead of crystalline ones. Polymeric carriers have been the most successful carriers for solid dispersions, because they are able to originate amorphous solid dispersions. They are divided into (1) fully synthetic polymers, e.g., PVP, PEG and polymethacrylates, and (2) natural product-based polymers, e.g., HPMC, ethylcellulose (EC), HPC. The drug was solubilized in the carriers, leading to the supersaturated state. The particles size of drug was reduced to nearly a molecular level. Third generation solid dispersions contain a surfactant carrier, or a mixture of amorphous polymers and surfactants as carriers. These third generation solid

dispersions are intended to achieve the highest degree of bioavailability for poorly soluble drugs and to stabilize the solid dispersion, avoiding drug recrystallization. The use of surfactants such as, inulin, Inutec® SP1, Compritol® 888 ATO, Gelucire® 44/14 and Poloxamer® 407, as carriers was shown to be effective in originating high polymorphic purity and enhanced *in vivo* bioavailability. The combination of amorphous polymers and surfactants has also been reported. The dissolution rate and bioavailability of biochanin A (poorly soluble bioflavonoid), was improved after being dispersed in a mixture of solutol® HS15 and HPMC. The bioavailability of this solid dispersion was 13-fold higher, compared to biochanin A (Han et al., 2011). Copovidone was also associated with Inutec® SP1 to prepare an amorphous itraconazole solid dispersion (Janssens et al., 2008d). The inclusion of surfactants in the formulation containing a polymeric carrier may help to prevent precipitation and/or protect a fine crystalline precipitate from agglomeration into much larger hydrophobic particles.

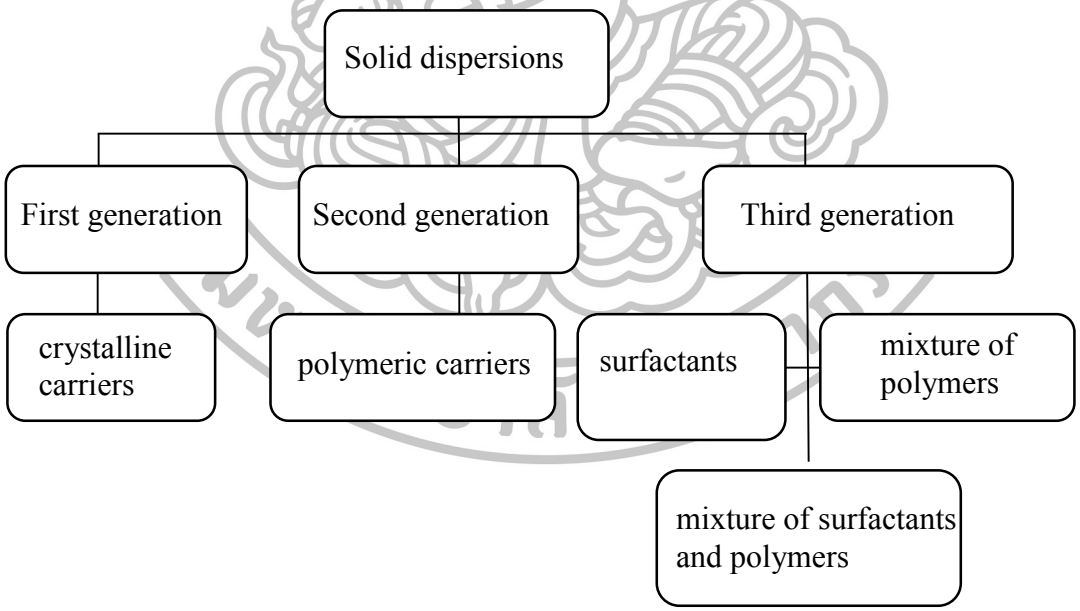


Figure 2.2 The classification of solid dispersions. (Vasconcelos et al., 2007)

2.2.3 Preparation of solid dispersions (Habib, 2001; Vasconcelos et al., 2007)

Generally, there are only two methods of preparing solid dispersions: melting and solvent process. However, a third method of melting-solvent method may be described.

2.2.3.1 Fusion or melting method

In the melting method, the carrier is heated to a temperature just above its melting point and the drug is incorporated into the melting. The mixture is cooled with constant stirring to homogeneously disperse the drug throughout the matrix. Several mechanisms could operate during the process of dispersion. If the drug has a high degree of solubility in the carrier, the drug could remain "dissolved' in the solid state that known as a solid solution. Particle size reduction under these conditions proceeds to the ultimate level, leading to molecular dispersion of the drug in the carrier matrix. These systems show very high drug dissolution rate, compared to control samples. On the other hand, the solubility of the drug in solid state is not so high; crystallites of the drug become dispersed in the matrix. The system shows only moderate increase in dissolution rate. A third mechanism is the conversion of a drug to an amorphous form in the presence of the matrix, again exhibiting different dissolution rate and solubility. Other factors that may play a role include solubilizing effect conferred by the carrier itself, improved wetting or decreased surface hydrophobicity, complexation, and crystallization of the drug in a metastable polymorphic form of altered thermodynamic properties. An important limitation of the fusion method of preparation is the exposure of drug to elevated temperature, particularly if the carrier is a high-melting solid and the drug is heat-sensitive.

Technically, this method is less difficult method of preparing solid dispersions, and provided that the drug and carrier are miscible in the molten state. Certain drug carriers, i.e., tolbutamide-mannitol (El et al., 1975) display a miscibility gap within their phase diagram, and consequent irregular crystallization may lead to only moderate increase in dissolution rate and difficulties in formulation. Goldberg et al. (1965) highlighted other potential problems, such as, thermal degradation, sublimation, and polymorphic transformation since metastable modification of the

drug may be formed, which convert to more stable form during storage. Small crystallites may be obtained by quenching cooling. But the solidification temperature will affect crystallization rate and may alter both the crystallite size and the hardness of the dispersion. The solidified melt may be tacky and unhandable, and consequently novel formulation techniques are required to permit formulation into elegant dosage forms. Decomposition should be avoided during melting, but is often composition-dependent and affected by melting time and the rate of cooling. Therefore, to maintain decomposition at an acceptable level, melting may be effected at a temperature just in excess of that which completely melts both drug and carrier, although it is feasible to prepare dispersion at just above the eutectic temperature when the carrier level is to the excess of the eutectic composition.

2.2.3.2 Solvent method

In the solvent method of preparation, the carrier and the active ingredient are dissolved in a suitable organic solvent and the solvent is evaporated at an elevated temperature or under vacuum. As the solvent is being removed, supersaturation occurs followed by simultaneous precipitation of the constituents, resulting in a solid residue. The co-precipitate is then dried under vacuum to drive out any solvent freely adhering to the particle surface. However, there is a possibility of the formation of a solvent with any of the constituents, which could hold some solvents within the crystal lattice. This presents a problem in terms of pharmaceutical acceptance since most of the solvents used are non-aqueous (organic) and toxic. Hence, removal of even trace amounts of the solvent is implied. Highly sensitive technique, i.e., differential scanning calorimetry (DSC), differential thermal analysis (DTA), thermogravimetric analysis (TGA), and less sensitive procedures, like gravimetry and spectroscopy, can be used to demonstrate complete solvent removal.

The choice of solvent and its removal rate are critical to the quality of dispersion. Since the chosen carriers are generally hydrophilic and the drugs are hydrophobic, the selection of a common solvent is difficult and its complete removal, necessitated by its toxic nature, is imperative. Certain solvents may plasticize polymeric carriers, i.e., PVP, resulting in the complete removal even more difficult.

Another disadvantage of the solvent method is that the volume of solvents used may be excessive and the cost of their recovery prohibitive.

2.2.3.3 Melting-solvent method

In this method, carriers are melted and the drug is incorporated in a form of solution. If the carrier is capable of holding a certain proportion of liquid yet maintaining its solid properties, and if the liquid is innocuous, the need for solvent removal is eliminated. This method is particularly useful for drugs that have high melting point or that are non-thermolabile. The feasibility of the method has been demonstrated for spironolactone and griseofulvin dispersions in PEG6000 (Klein, 2010), nifedipine solid dispersions with ethylcellulose and/or Eudragit® RL (Huang et al., 2011).

The formulation of solid dispersions prepared by the melting, solvent, or melting-solvent method is generally accepted as a method to enhance the dissolution characteristics of poorly water-soluble drugs. The increase in solubility of poorly water-soluble drug using solid dispersion technology have been reported, i.e., the increase in solubility of itraconazole using 5% PEG6000/HPMC 2910 E5 in methanol/ dichloromethane 50/50 (v/v) solution (Janssens et al., 2008d), PEG6000/PVPVA 64 (copovidone) in dichloromethane solution (Janssens et al., 2008b) or copovidone/Inutec® SP1 in dichloromethane solution (Janssen et al., 2008a) with spray drying technology. Binary mixture of PEG2000 and nimodipine or ternary mixture of PEG2000, nimodipine and povidone K17 were also prepared by melting method (Urbanetz and Lippold, 2005). Solid dispersions of itraconazole/D-α-tocopherol polyethylene glycol 1000 succinate (TPGS)/Aerosil® 200 prepared by spray drying were also reported (Eerdenbrugh et al., 2009). The use of non-ionic surfactant as a carrier may help to avoid drug recrystallization and to stabilize the systems, i.e., sucrose laurates (Szűts et al., 2011), Inutec® SP1, Poloxamer® 407.

2.2.4 Characterization of solid dispersions

The dissolution enhancement of poorly water-soluble drugs in solid dispersions can be proven by the standard dissolution method and solubility studies (Vo et al., 2013). Other properties of solid dispersions such as the physical states of

drugs, the drug–carrier interaction and the physical and chemical stability of drugs should also be evaluated. The crystalline state of drugs is commonly characterized by the following techniques: thermoanalytical techniques such as DSC, powder X-ray diffraction (PXRD) and other instrumental techniques such as Fourier transform Infrared (FTIR) spectroscopy and solid state nuclear magnetic resonance. Microscopy techniques such as optical microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM) are also used to qualitatively characterize the crystalline states of drug.

DSC is the most commonly used thermal technique for solid dispersion characterization. This technique provides accurate information about melting point, glass transition temperature as well as the energy changes associated with the phase transitions, including crystallization and fusion process. The lack of a drug melting peak in the DSC thermogram of a solid dispersion indicates that the drug exists in an amorphous form. PXRD is the standard technique for studying the crystalline or amorphous nature of drugs in solid dispersions and solid solutions. This method can detect material with long-range order as well as expose sharp diffraction peaks that indicate crystalline compound. However, the molecular mobility increased, thus promoting recrystallization and the formation of small crystals that might not be detected via PXRD or DSC (Kalivoda et al., 2012). For lower loadings, identification of this solid-state transition can become challenging using bulk scale techniques such as PXRD and DSC, due to their sensitivity limits. A new technology of nanothermal analysis (nano-TA), which in conjunction with other techniques, provides a powerful analytical strategy for characterizing nano- and micro-scale heterogeneity in the solid-state properties of drug–polymer formulations. Zhang et al., (2009) investigated the heterogeneity of low (5%) and high (50%) levels of carbamazepine in HPMC solid dispersion formulations using a novel nanothermal analysis technique. The results showed that 5% sample, a solid solution is formed, however, in the 50% sample, some of the carbamazepine precipitates into nano-crystals, visible through phase imaging as 50-nm domains and through direct observation of thermal transitions with localized thermal analysis. This technique has demonstrated a promising capability for imaging

and quantitatively characterizing the nanoscale properties of solid dispersion formulations.

FTIR spectroscopy is a common technique used to investigate the intermolecular interaction, physical and chemical reaction between drug and carrier. FTIR spectroscopy can explain hydrogen bonding between drug and carrier, which indicate the physical state and the stability of drugs in solid dispersions. AFM is capable of resolving individual molecules so that differences in size (length, diameter, etc.) and conformation (stiffness, aggregation, and association, mode of adsorption to a substrate, etc.) between neighboring polymers can be visualized directly, thus making possible the characterization of the heterogeneity of a molecular population at the level of single polymers. AFM has become a standard tool for the characterization of heterogeneity at the nanoscale, finding applications in every aspect of surface and interface science (Sriamornsak et al., 2008). AFM gave useful structural information on the molecular topography of the outer surface of the isolated samples (Benítez et al., 2004) and can produce subnanometre scale images of individual biopolymers, and has proved to be a useful tool for characterizing complex, irregular and heterogeneous samples at single molecule level. It has been widely employed in the study of individual polymers of glycans such as cellulose, starch, mucins and a variety of bacterial polysaccharides. There are several researches that used AFM to characterize the nanostructure of polymer, such as the study on the microstructure changes, including aggregates and branches of sodium carbonate-soluble pectin of peach during storage at atmosphere by Yang et al. (2006). Nanostructures of liposomes, pectin, and pectin-liposome nanocomplexes were observed by AFM. (Sriamornsak et al., 2008).

2.2.5 Marketed products of solid dispersions (Démuth et al., 2015)

Despite the large interest in solid dispersions, only 8 tablet-based products have been brought to the market containing the drug as an immediate release (Table 2.1). This can be related to several factors, including scale-up limitations of the manufacturing process such as degradation during melt processes or residual organic solvent during solvent-based processes as well as the stability of drug during oral formulation and storage.

Table 2.1 Marketed products containing an immediate release solid dispersions (Démuth et al., 2015).

Product	**Drug**	**Preparation method**	**Carrier polymer**	**indication**
Kaletra®	Lopinavir Ritonavir	Melt-extrusion	PVPVA64	HIV
Norvir®	Ritonavir	Melt-extrusion	PVPVA64	HIV
Gris-PEG®	Griseofulvin	Melt technology	PEG	Dermatophytosis
Certican®	Everolimus	Spray drying	HPMC	Rejection of organs
Intelence®	Etravirine	Spray drying	HPMC	HIV
Zelboraf®	Vemurafenib	Solvent-controlled coprecipitation	HPMCAS	Melanoma
Incivek®	Telaprevir	Spray drying	HPMCAS-M	Hepatitis
Fenoglide®	Fenofibrate	Melt-spraying	PEG	High cholesterol level

2.2.6 Carriers for solid dispersions

Carriers are one of the most important components of the solid dispersion formulation (Li et al., 2013). Their choice affects micro level properties of solid dispersion such as drug–carrier miscibility, intermolecular interactions and various relaxations associated with the amorphous state (Singh and Mooter, 2016). Crystalline first generation solid dispersions used urea and sugars such as sorbitol and mannitol. Second generation solid dispersions utilized amorphous polymers such as PVP, PEG, copovidone, and polymethacrylates. Third generation solid dispersions used surfactants such as poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Poloxamer®), glyceryl dibehenate (Compritol® 888 ATO), lauroyl polyoxyl-32 glycerides (Gelucire®), inulin lauryl carbamate (Inutec® SP1) and polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®).

The most importance for the selection of carriers is the supersaturation maintenance ability and maintaining higher drug concentrations with higher absorption and hence supersaturation maintenance potential of the carrier. Many carriers can inhibit precipitation by adsorbing onto the surface of nuclei and hinder crystal growth by providing steric stabilization and blocking access to the active surface (Alonzo et al., 2012).

It is well known that two factors that have the influence on absorption of oral drugs are the dissolution of the drug at the site of absorption and the transport of the drug through the gastrointestinal mucosa to the systemic blood circulation. For poor solubility drugs that have a good permeability according to the BCS, the rate-determining step to absorption is often the dissolution of the drug in the small intestine (Wagner et al., 2012). Only dissolved drug will be absorbed to the site of action. Compendial dissolution media often fail to yield *in vitro-in vivo* correlation (IVIVC) for BCS class 2 drugs because relevant physiological parameters are not taken into account. A suitable *in vitro* model should include a medium that mimics as much as possible the gastrointestinal (GI) contents after food intake. Six et al. (2005) reported a lack of IVIVC of three solid dispersion formulations of itraconazole in human volunteers in comparison with Sporanox®. Solid dispersions made up of itraconazole (40%, w/w) and HPMC 2910, Eudragit® E100 or a mixture of Eudragit® E100-copovidone were manufactured by hot-stage extrusion and filled in gelatin capsules. The *in vitro* release behavior of the solid dispersions showed the opposite of what was observed *in vivo* since the formulations based on Eudragit® E100 or a mixture of Eudragit® E100-copovidone showed the lowest mean AUC and C_{max}. The poor predictability of the absorption behavior based on *in vitro* dissolution data was speculated that the difference is either due to (i) reduction of itraconazole solubility as the drug left the stomach, (ii) incomplete disintegration of the administered tablets *in vivo*, or (iii) a change in the physical state of the drug (amorphous to crystalline transformation) when the solid dispersion was granulated and tableted. This study also points to the fact that the use of *in vitro* dissolution data in view of prediction of *in vivo* behavior is of limited value and needs careful interpretation. Then the prediction of the *in vivo* behavior with biorelevant media is more favored.

2.3 Biorelevant media simulating the environment in the upper small intestine

The food and drinks, gastric juices, bile, pancreatic juices, bacterial fermentation as well as water re-uptake all combine to influence the composition of the GI fluids at various points in the gut. The dissolution of drug and excipient released depend on the composition in GI tract. In general, the poorly water-soluble drug could be the best absorbed in this segment. Biorelevant dissolution testing designed with appropriate simulated media and hydrodynamics are useful from the early stages of drug development for identifying the biopharmaceutical performance of compound (i.e., solubility problems, food effects, precipitation in the small intestine) through the later stages of development to assist in formulation strategies and the establishment of IVIVC that will lead to reduction of the number of animal experimentation, bioavailability and bioequivalence studies.

The FaSSIF and FeSSIF have recently been fine-tuned to bring the compositions and characteristics closer to those of the human small intestinal environment. Later, the composition of the updated FaSSIF, so-called FaSSIF-V2, was changed closely to the composition of *in vivo* data, while the buffer has been changed from phosphate to maleate for practical reasons (Jantratid and Dressman, 2009). Table 2.2 demonstrates the composition of FaSSIF and FaSSIF-V2.

Table 2.2 Composition of the biorelevant medium used to simulate fasted state conditions in the small intestine, FaSSIF and FaSSIF-V2 (Klein, 2010).

Composition	FaSSIF	FaSSIF-V2
Sodium taurochlorate (mM)	3	3
Lecithin (mM)	0.75	0.2
NaH_2PO_4 (g)	3.43	-
Maleic acid (g)	-	2.22
NaCl (g)	6.18	4.01
NaOH (g)	0.38	1.39
DI water	qs ad 1L	qs ad 1L
pH	6.5	6.5
Osmolarity (mOsmol/kg)	~270	~180
Buffer capacity (mEq/pH/L)	~12	~10

In the fed state, the environment in the small intestine also changed after a meal, compared with the fasting conditions. The updated FeSSIF medium is called FeSSIF-V2, combines the postprandial changes in pH, buffer capacity, osmolality, and bile component concentration, while the concentrations of lipolysis products that reflect aqueous phase values in the aspirates (Table 2.3). There are lower amounts of bile components in FeSSIF-V2 than in FeSSIF, but this is compensated for by adding meal digestion products, including monoglycerides and free fatty acids, both of which can enhance solubility and dissolution of poorly soluble compounds. The study of Janssens et al. (2008e) demonstrated that the oral absorption of glibenclamide tablets in the fasted and fed state is not significantly different in FaSSIF-V2 and FeSSIF-V2 whereas with the previous version of the media, results indicated that there would be a food effect.

Table 2.3 Composition of the biorelevant medium used to simulate fed state conditions in the small intestine FeSSIF and FeSSIF-V2 (Klein, 2010).

Composition	FeSSIF	FeSSIF-V2
Sodium taurochlorate (mM)	15	10
Lecithin (mM)	3.75	2
CH_3COOH (g)	8.65	-
Glyceryl monooleate (mM)	-	5
Sodium oleate (mM)	-	0.8
Maleic acid (g)	-	6.39
NaCl (g)	11.87	7.33
NaOH (g)	4.04	3.27
DI water	qs ad 1L	qs ad 1L
pH	5.0	5.8
Osmolarity (mOsmol/kg)	~670	~390
Buffer capacity (mEq/pH/L)	~72	~25

Rosillon et al. (1998) reported that food has a significantly improved the absorption of MDP by the solubilization of food and bile secretions. The bioavailability of nifedipine spontaneous emulsifying powder that can enhance C_{max} and AUC in both fasted and fed rats was studied by Weerapol et al. (2015). Then, the presence of ingested food in the GI tract may result in delayed, reduced or increased systemic drug availability.

2.4 Excipients of interest

2.4.1 Copovidone

PVP is amongst the most frequently investigated hydrophilic polymeric carriers for solid dispersion. PVP is made up of N-vinylpyrrolidone monomer units and is available in different grades. One of the severe drawbacks of PVP is its high hygroscopicity. It can take up more water during storage in humid environment (Weuts et al., 2005). Therefore, combining PVP with water insoluble vinyl-acetate results in a copolymer which is still appreciably soluble in a variety of solvents but

slightly more hydrophobic. Copovidone contains vinylpyrrolidone and vinyl acetate in the ratio of 6:4 is one of interest carriers for solid dispersion (Fig.2.3). It is a widely used as excipient in the pharmaceutical industry, serving as a soluble binder and film-forming agent, particularly for solid dosage forms. It is only minimally absorbed following oral administration (approximately 2% of the administered dose) and largely excreted in the feces. The copolymer has demonstrated no acute toxicity and is not irritating to the skin or mucous membranes Moreover, the carcinogenicity and chronic toxicity of copovidone in rats and dogs showed the absence of any significant toxicological and no-observed-adverse-effect levels of 2800 mg/kg body weight/day in rats and 2500 mg/kg body weight/day in dogs, the highest doses tested (Mellert et al., 2004).

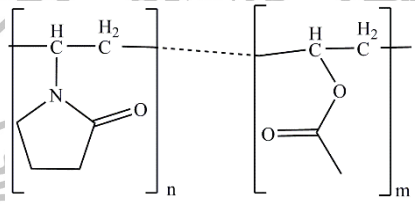


Figure 2.3 Structure of copovidone.

2.4.2 Polyethylene glycol (PEG)

PEG is also known as macrogols. The molecular weight of this synthetic, semi-crystalline polymer usually lies between the range of 200 and 300,000. Their low melting point (approximate 65°C) and good solubility in both aqueous and organic solvents make them good candidates for both solvent and fusion based methods. The PEG chain length, molecular weight and drug loading influence the drug solubility and dissolution rate of the drug.

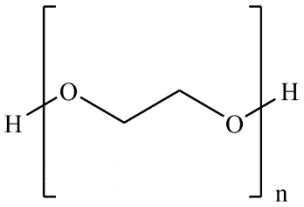


Figure 2.4 Structure of PEG

2.4.3 D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)

TPGS (Fig. 2.5) is a water-soluble derivative of natural vitamin E, which is formed by esterification of vitamin E succinate with polyethylene glycol (PEG) 1000. It has an average molecular weight of 1513, an amphiphilic structure of lipophilic alkyl tail and hydrophilic polar head with a hydrophilic/lipophilic balance value of 13.2 and a relatively low critical micelle concentration (CMC) of 0.02% w/w. It is a waxy solid (melting point 37–41 °C) and completely dissolves in water (Guo et al., 2013). TPGS has been approved by FDA as a drug solubilizer in oral, parenteral, topical, nasal, and rectal/vaginal therapies. TPGS has shown promise as a solublizer due to surfactant property. TPGS displayed significant surface activity that can solubilize a variety of both water-soluble and water-insoluble compounds.

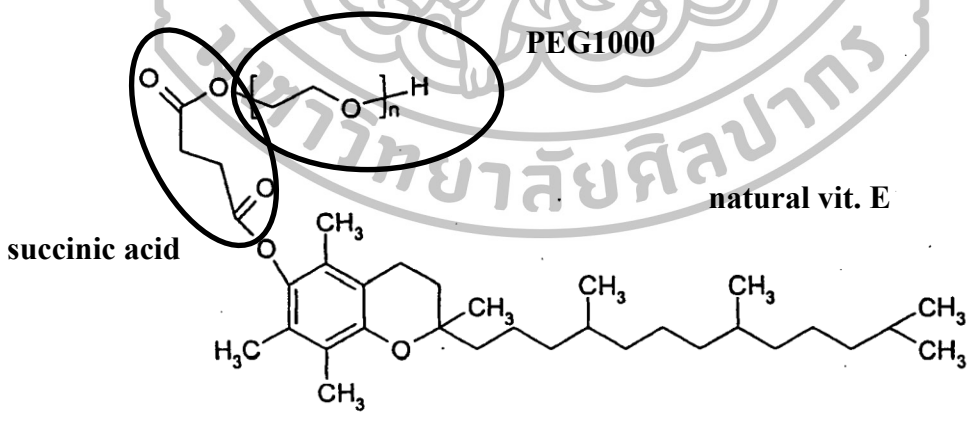


Figure 2.5 Structure of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (Robin, 2015).

TPGS has been used in solid dispersions to increase the drug solubility, dissolution rate and also enhance the drug oral bioavailability. It may be attributed to the fact that the micellar formation can increase the solubility of drug, enhance the separation of drug particle and interaction between polymer and drug, and improve wettability and partial crystalline drug transferred to the amorphous form (Rajebahad et al., 2006). Shin and Kim (2003) prepared solid dispersions of furosemide and TPGS using solvent method. They found that the aqueous solubility and the dissolution rate of furosemide were rapid and markedly enhanced, compared to pure drug and physical mixture. Palitaxel was loading into the TPGS, the solubility and bioavailability of paclitaxel is significantly increased (Varma and Panchagnula, 2005). Berberine bioavailability enhancement by TPGS has been studied on intestinal absorption in rats; TPGS was a good absorption enhancer capable of enhancing intestinal absorption (Chen et al., 2011).

2.5 Manidipine hydrochloride (MDP)

2.5.1 Physicochemical and pharmacological properties of MDP

Manidipine hydrochloride (MDP), (±)-2-[-(diphenylmethyl)-1-piperazinyl] ethylmethyl-1,4dihydro-2,6-dimethyl-4(*m*-nitrophenyl)-3,5-pyridinedicarboxylate methyl ester (Fig. 2.6) is a lipophilic, third-generation, highly vasoselective, dihydropyridine calcium channel antagonist having long-lasting activity for the treatment of essential hypertension. It classify in BCS class II drug that extremely lipophilicity resulting in a number of undesirable physicochemical and biopharmaceutical properties such as a very poor aqueous solubility and a strong tendency to get adsorbed on glass and plastic surfaces. Its dihydrochloride salt (MDP.2HCl) is actually marketed as once a day 20-mg tablets for the treatment of different kinds of hypertension. Like other dihydropyridine derivatives, MDP exhibits high clearance and first pass metabolism and, hence, a low systemic bioavailability (Mielcarek and Szamburska, 2005).

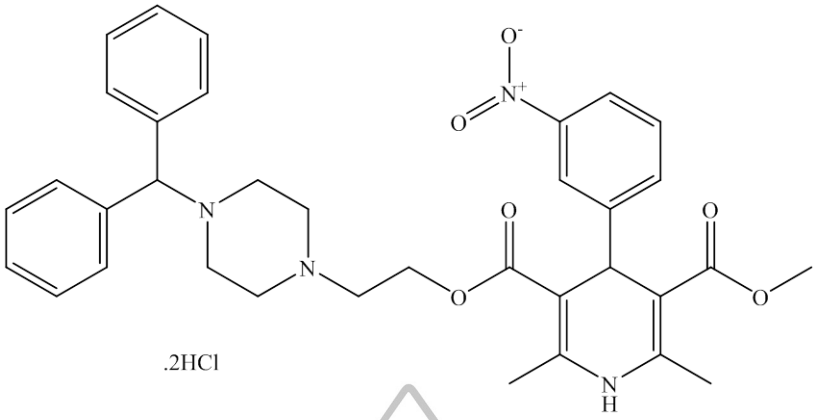


Figure 2.6 Chemical structure of MDP.

2.5.2 Pharmacokinetic properties

After oral administration, maximum plasma concentration is achieved in 2-3.5 h. MDP undergoes first-pass metabolism. Binding to plasma proteins is 99%. The medicinal product is widely distributed to the tissues and is extensively metabolized, mainly by the liver. It is mainly eliminated through feces (63%) and, to a lesser extent, through urine (31%). No accumulation is noted after repeated administration. The drug pharmacokinetics is not modified in patients with renal failure. Absorption of MDP increases in the presence of food in the gastrointestinal tract.

CHAPTER 3
TERNARY SOLID DISPERSIONS OF MANIDIPINE IN PEG4000/COPOVIDONE BLENDS

3.1 Introduction

Nowadays, 35-40% of all new chemical entities suffer from poor aqueous solubility; hence enhancement of the solubility of poorly water-soluble drugs is challenging innovation in drug development. The use of solid dispersions is one of strategies that can improve the solubility and dissolution rate of poorly soluble drugs by dispersing drugs in an inert carrier or matrix at solid state, as either molecularly or fine particles (Habib, 2001). If the drug solubility in the carrier is high enough, a so-called solid solution can be obtained. Numerous methods of preparing solid dispersion systems have been proposed, including precipitation, solvent evaporation, melting (fusion), hot-melt extrusion, spray drying, supercritical fluid technology, etc. (Vasconcelos et al., 2007). In the case of the solvent method, both drug and carrier must be dissolved completely in a suitable organic solvent; this solvent is evaporated at an elevated temperature or under vacuum. As the solvent is being removed, supersaturation occurs followed by simultaneous precipitation of the constituents, resulting in a solid residue (Habib, 2001). However, the solvent method introduces residual solvents, which may bring up the toxic and environmental issues. The melting method is an alternative way to prepare solid dispersions; carrier is heated to a temperature just above their melting point and the drug is incorporated into the matrix. The mixture is rapidly solidified to homogeneously disperse the drug throughout the matrix. The benefits of melting technique are to reduce or avoid using organic solvent, and environmental friendly. Furthermore, this process requires fewer processing steps and less time; it is easily to scale up for commercial purpose.

Although the use of solid dispersions has been reported frequently in the literature, very few marketed products count on the solid dispersion strategy (Vasconcelos et al., 2007). The main reason is its physical instability, such as phase separation, crystal growth or conversion from the amorphous (metastable) to the crystalline state during storage, resulting in decreased solubility and dissolution rate. Hydrophilic polymers are critical component of solid dispersion formulation since they act as a carrier for the drug and inhibit crystallization (Al-Obaidi et al., 2011; Martins et al., 2012; Rashid et al., 2015). Also, they can prevent recrystallization (either nucleation and/or growth) in the solid formulation during storage and during

the dissolution process. Thus, the selection of proper polymers benefits to stabilize the stability of amorphous drug as well as, with respect to dissolution, for shelf life stability (Van den Mooter, 2011). One of the polymers that has been used extensively as a carrier for solid dispersion is polyethylene glycol (PEG). PEG, a semi-crystalline polymeric carrier, has been used to increase drug solubility because of its high hydrophilicity, ability to solubilize some compounds, ability to improve wettability, and ability to induce water uptake. Its melting point lies below 65°C, which is advantageous for the manufacture of solid dispersions by melting method. However, the stability of PEG-based solid dispersions is limited (Urbanetz, 2000). Therefore, it may be more appropriate to use combination of polymers to ensure adequate stability during storage and optimum performance during dissolution (Xie and Taylor, 2016). One of the ways to achieve this is by blending PEG with a third component, in order to reduce its crystallinity, that is, PVP (Barmpalexis et al., 2013), sucrose ester (Szűts et al., 2011), vinylpyrrolidone-vinyl acetate copolymers or copovidone (Janssens et al., 2008a), HPMC (Janssens et al., 2008b), etc.

Copovidone having hydrophilic property and high thermal stability is a suitable carrier to prepare solid dispersions. Janssens et al. (2008b) prepared binary solid dispersion (bSD) containing itraconazole and copovidone, and ternary solid dispersion (tSD) containing itraconazole and PEG6000/copovidone blends by spray drying. They found that the ratio of PEG6000/copovidone and percentage of drug loading have an influence on the physical appearance of solid dispersion and dissolution of itraconazole. Moreover, the dissolution of tSD was improved, compared to the bSD. However, to the best of our knowledge, there was no tSD prepared by melting technique.

Recently, Gumaste and colleagues (2016) used ternary phase diagram to systematically screen polymer-drug-surfactant miscibility by using the film casting method. They found that the use of ternary phase diagram is relatively simple and practical for screening miscibility of different components. In this study, we applied the solid ternary phase diagram, which commonly existed for many metallic alloys and ceramics, for preparing tSD. In ternary phase diagram, the homogeneous and non-homogeneous region of solid dispersions could be differentiated. The molten solid

dispersion was subsequently solidified at low temperature by quenching substances (Sugimoto et al., 2012).

Therefore, the objective of this study was to prepare the enhanced dissolution and improved stability of MDP, a poorly water-soluble drug, by the formation of tSD with PEG4000/copovidone blend, using melting technique. Solid ternary phase diagram was constructed to find homogeneous solid dispersion region after melting and solidifying at low temperature with different quenching substances (i.e., ice, liquid nitrogen, and dry ice). The physical stability of tSD was also determined under accelerated condition at 40°C/75% relative humidity (RH) for 6 months..

3.2 Materials and methods

3.2.1 Materials

MDP, dihydrochloride salt, was supported by Sriprasit Pharma Co., Ltd. (Bangkok, Thailand). Macrogol (25)-cetostearyl ether polyethylene glycol 1100 mono (hexadecyl/octadecyl) ether (Cremophore® A25), poloxamer 407 or poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Lutrol® F127), polyoxyethylene-polyoxypropylene block copolymer (Lutrol® micro68), copovidone (Kollidon® VA64) were a gift from BASF (Thai) Co., Ltd. (Bangkok, Thailand). PEG4000 was purchased from Merck-Schuchardt (Germany). Sorbitan monopalmitate (Span® 40) and sorbitane monostearate (Span® 60) were purchased from Srichand United Dispensary Co., Ltd. (Thailand). Distilled water was purchased from General Hospital Products Public Co., Ltd. (Thailand). All other chemicals used in this study were of pharmaceutical grade or analytical grade and used as received without further purification.

3.2.2 Preparation of bSD

MDP and carriers were accurately weighed and physically mixed using mortar and pestle. The mixed powders (including 5-30% w/w MDP) were then melted at temperature range of 95-105, 145-155 or 170-180°C and continuously stirred until all materials were completely melted. The clear molten mixture was immediately solidified in an ice bath. The solid dispersion obtained was ground and kept at -20°C

until further analysis. Various carriers (i.e., PEG4000, Cremophore® A25, Span® 40, Span® 60, Lutrol® micro68, Lutrol® F127) were investigated.

3.2.3 Preparation of tSD

The tSD was prepared by incorporating copovidone into selected bSD formulations (from section 3.2.2). Ternary mixture can easily be prepared using triangular diagram or solid ternary phase diagram, as described by Gumaste and coworkers (2016). In this study, PEG4000 and copovidone were mixed in different weight ratios, ranging from 1:8 to 8:1, 1:7 to 7:1 and 1:6 to 6:1 for 10, 20 and 30% w/w of MDP, respectively, and MDP was then added and mixed using mortar and pestle. The mixed powders were then melted at selected temperature range (i.e., 170-180°C) and continuously stirred until all materials were melted completely. The clear molten mixture was solidified and kept in the same manner as described in section 3.2.2. The homogeneous and non-homogeneous regions of solid dispersions were investigated by visual observation and hot-stage microscopy. The effect of quenching substance, that is, ice (melting temperature, 0°C), dry ice (sublimation temperature, -79°C) and liquid nitrogen (boiling temperature, -196°C), on the solubility and physicochemical properties (e.g., PXRD and DSC) was also investigated.

3.2.4 Solubility test

The saturation solubility of MDP powder, bSD and tSD in distilled water was determined by adding an excess amount of MDP, bSD and tSD (approximately 200 mg) to 0.5-mL distilled water in microcentrifuge tubes. The tubes were equilibrated at ambient temperature (28°C) in a thermostatically controlled bath for 48 h. After equilibrium, the tubes were centrifuged at 3,500 rpm for 15 min; clear supernatants were analyzed by high performance liquid chromatography (HPLC).

3.2.5 HPLC analysis

HPLC analysis was performed with a JASCO PU-2089 plus quaternary gradient inert pump, and a JASCO UV-2070 plus multiwavelength UV-vis detector (Jasco, Japan). Luna 5u C18 column (5 mm, 4.6 nm × 150 mm) (Phenomenex, USA) column was used. The mobile phase consisted of acetonitrile:potassium dihydrogen phosphate solution (49:51, v/v) was filtered through a nylon membrane filter (0.45

μm), and degassed in a sonicator bath before use. The flow rate was 1.0 mL/min, and the UV detection wavelength was 228 nm. The analyses were carried out in triplicate.

3.2.6 Physicochemical characterization

3.2.6.1 DSC analysis

The thermal properties of the solid dispersion were observed by differential scanning calorimeter (model Sapphire, Perkin Elmer, Germany). An accurately weighed amount of sample (2 mg) was placed inside standard crimped aluminum pan and heated from 25 to 250°C at a heating rate of 10°C/min under nitrogen flow (20 mL/min).

3.2.6.2 PXRD

The crystallinity of solid dispersion was investigated using powder X-ray diffractometer (model MiniFlex II, Rigaku, Japan) at 30 kV, 40 mA over the range of 5-45 degree 2θ by the scanning speed of 2 degree/min using Cu Kα radiation wavelength of 1.5406 Å.

3.2.6.3 FTIR spectroscopy

The possible interaction between drug and excipient was obtained by FTIR spectrophotometer (model Nicolet 4700, Thermo Electron Corporation, USA). The sample was blended with dry potassium bromide (KBr), ground well using mortar and pestle. KBr disk was compacted by hydraulic press machine at a pressure of 5 tons. The disk was placed in the FTIR sample holder and the spectral values of the samples were obtained by scanning from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. FTIR spectral parameters of the samples were obtained using a software package (OMNIC FTIR software, version 7.2a, Thermo Electron Corporation, USA).

3.2.6.4 Hot stage microscopy

Additional information on the thermal behavior of the solid dispersions was obtained by visual identification with hot stage microscopy (model FP82HT, Mettler Toledo, Switzerland). A change in the morphology of crystalline drug was noted as a function of temperature. The polarization properties of drug were also

observed during the experiment using a polarized filter (Model CX-AL, Olympus, Japan) to investigate the crystal properties.

3.2.7 *In vitro* dissolution study

The tSD containing MDP (equivalent to 3 mg of MDP, in order to keep sink condition) was accurately weighed. The dissolved drug from solid dispersions was determined using dissolution apparatus II (Model DT-720, Erweka, Germany) with 900 mL of 0.1 N HCl as dissolution medium, at 37±0.5°C. The paddle speed was adjusted to 50 rpm. The samples were withdrawn from dissolution vessels at 5, 10, 20, 30, 45, 60, 90 and 120 min and passed through 0.45-µm nylon membrane before analysis with HPLC to determine the drug dissolved. The dissolution experiments were carried out in triplicate.

3.2.8 Stability study

The stability study was conducted for selected tSD formulations that provided good solubility, physicochemical and dissolution properties. In general, the stability study is a requirement for the products that may be prone to phase separation, absorbing moisture, crystal growth or a change from metastable form to stable crystalline form, leading to the reduction of drug solubility and dissolution rate. Accelerated stability studies were performed according to International Conference on Harmonization (ICH) guidelines at 40°C±2°C and 75%±5%RH for 6 months. The tSD was placed into opened glass vials and kept at accelerated conditions (40ºC/75%RH). After storage for 6 months, the samples were characterized for their solubility, thermal property and crystallinity.

3.3 Results and discussion

Solid dispersion formulation was relatively simple and the cooled masses of solid dispersions prepared by melting technique were fragile enough to be ground easily. This could be an advantage from industrial aspects because the pulverization of solid dispersions was one of the major problems encountered in melting method. Moreover, this method was relatively more feasible to prepare solid dispersions because of the ease in controlling the processing variables.

3.3.1 Selection of melting temperature range and carriers

To select the melting temperature range, the melting temperature and thermal degradation of the drug and carriers were taken into consideration. From the DSC thermograms, it is observed that the melting temperature of MDP, PEG4000 and copovidone was 222, 59 and 60.7°C, respectively (Fig. 3.1a). From the preliminary study, MDP showed three events of mass loss, according to the thermogravimetric analysis; the first event between 146 and 202°C, the second one between 202 and 394°C and the third one between 400 and 541°C (Fig. 3.1a), suggesting that MDP degraded immediately after melting. These findings agree well with other published results (Hosaka et al., 2005). Therefore, the melting process should be prepared at temperature lower than its melting point to avoid degradation of drug.

In this study, the temperature range used was divided into three ranges, that is, 95-105°C, 145-155°C and 170-180°C, based on the melting point of MDP and carriers. Table 3.1 shows the appearance of bSD prepared at different process temperatures. After melting process, almost all formulations were opaque. This is probably because the drug did not dissolve completely in the carrier. Only the bSD formulation with 5% w/w MDP, prepared at process temperature of 170-180°C, demonstrated a transparent, yellowish, and homogenous melt, indicating complete drug dissolution or molecular dispersion in the carrier. Fig. 3.1b shows the PXRD patterns of MDP, PEG4000, physical mixture of MDP and PEG4000, and bSD formulations prepared at various process temperatures. The characteristic peaks of MDP at 10.9, 22.0, 22.7 and 22.8° 2θ were clearly observed, indicating the crystalline nature of the drug. Typical peaks for PEG4000 were detected at ca. 19 and 23° 2θ, according to its semi-crystalline nature (Janssens et al, 2008b). The bSD formulations displayed less intense and highly diffused peaks as compared to the MDP or physical mixture of MDP and PEG4000. The PXRD patterns of bSD prepared at temperature range of 170-180°C revealed a reduction in crystallinity of MDP in solid dispersion, which may influence drug dissolution from bSD. The disappearance of drug crystallinity may be due to the molecular dispersion in the carrier or the complete change from crystalline structure to amorphous form. Hence, the temperature range of 170-180°C was further used in subsequent experiments.

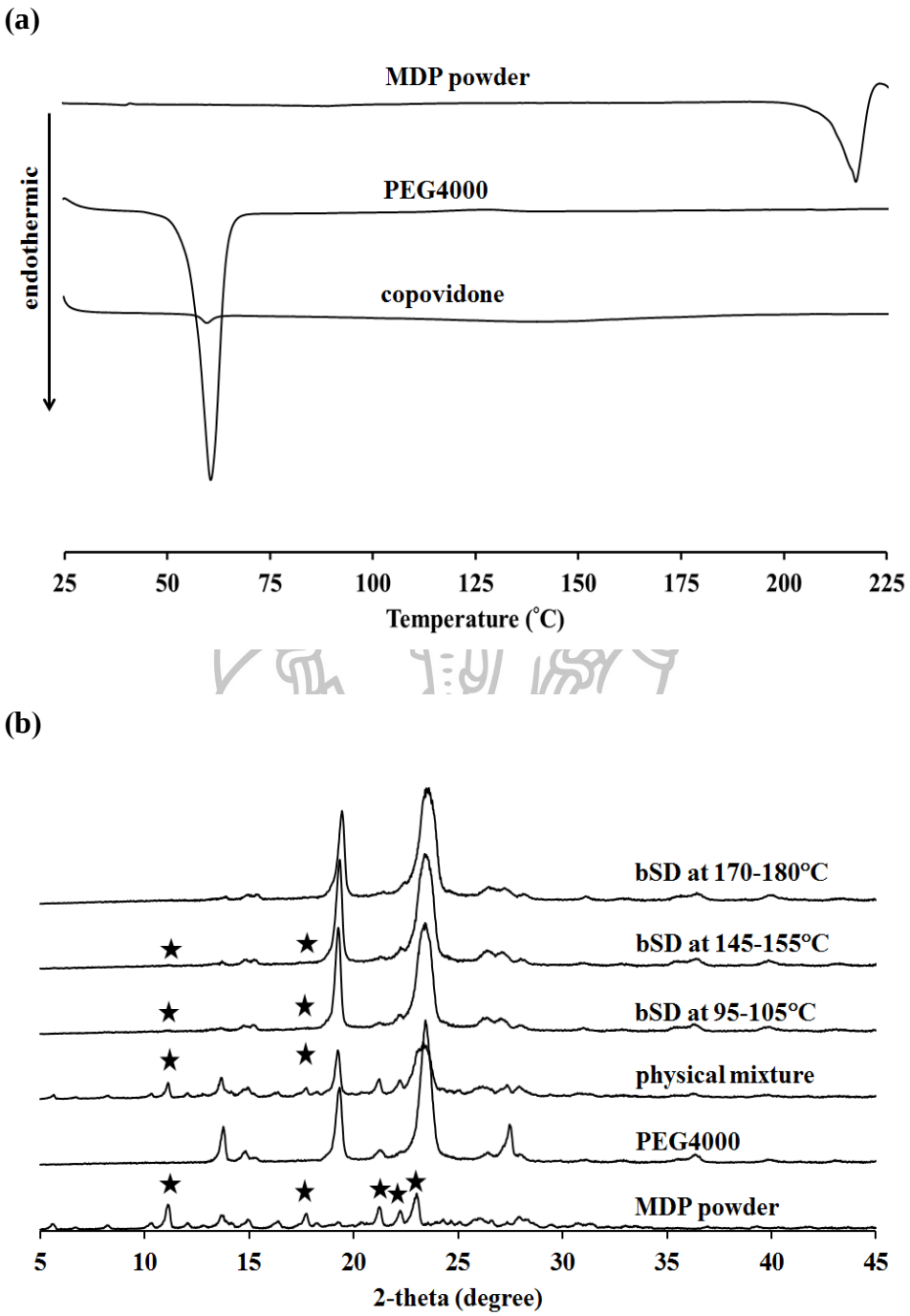


Figure 3.1 (a) DSC thermograms of MDP, PEG4000 and copovidone; (b) PXRD patterns of MDP, PEG4000, physical mixture (PM) of MDP and PEG4000, and bSD formulations prepared at various temperatures.

Table 3.1 Appearance of bSD prepared at different process temperatures.

Temperature (°C)	Drug loading (% w/w)			
	5	10	20	30
95-105	opaque	opaque	opaque	opaque
145-155	opaque	opaque	opaque	opaque
170-180	transparent, yellowish	opaque	opaque	opaque

Various carriers (i.e., PEG4000, Cremophore® A25, Span® 40, Span® 60, Lutrol® micro68, and Lutrol® F127) were selected for preparing bSD because they are solid at room temperature and their melting points are low. MDP was added at different concentrations of 1, 2, 3, 4, 5, 10, 20 and 50% w/w. The appearance of the bSD containing different carriers is shown in Table 3.2. Most of the carriers investigated did not produce clear and transparent solid dispersions. On the other hand, a clear, transparent and yellowish solid dispersion was obtained when PEG4000 was used as a carrier, at MDP concentration of 1-5% w/w. This is probably due to the hydrophilicity of PEG4000, which can increase wettability of MDP and subsequently enhance MDP dissolution. Urbanetz and Lippold (2005) reported similar results; light yellow and transparent solid dispersions of nimodipine and PEG2000 were prepared. From the above results, therefore, PEG4000 was selected as the carrier for preparing the tSD.

Table 3.2 Appearance of bSD prepared by melting method, using process temperature of 170-180°C, with various carriers.

Drug loading (% w/w)	Carrier					
	PEG4000	Cremophore® A25	Span® 40	Span® 60	Lutrol® F 127	Lutrol® micro 68
1	transparent, yellowish	opaque	opaque	opaque	-	-
2	transparent, yellowish	opaque	opaque	opaque	-	-
3	transparent, yellowish	opaque	opaque	opaque	-	-
4	transparent, yellowish	opaque	opaque	opaque	-	-
5	transparent, yellowish*	opaque	opaque	opaque	opaque	opaque
10	opaque*	opaque and sticky	opaque	opaque	opaque	opaque
20	opaque*	opaque and sticky	opaque	opaque	opaque	opaque
30	opaque*	opaque and sticky	-	-	-	-

* Duplicated data, taken from Table 3.1, for comparison purpose.

3.3.2 Preparation and physicochemical characterization of tSD

It has been found that the stability of bSD containing MDP and PEG4000 is not stable, which is due to the semi-crystalline structure of PEG4000, resulting in the recrystallization of MDP when kept for a long period. Therefore, tSD was used to improve the stability as well as the dissolution of MDP. Copovidone was chosen to be used as the third component to form tSD together with PEG4000, due to its hydrophilicity and good processability. It is commonly used for manufacturing of solid dispersions, in order to increase the dissolution rate of drug and stabilize the drug in solid glassy hydrophilic polymer (Vaka et al., 2014).

Solid ternary phase diagram was used as a screening approach for the proper selection of MDP, PEG4000 and copovidone ratio that can form homogeneous tSD. From the solid ternary phase diagram, homogeneous and non-homogeneous solid dispersions can be identified (Fig. 3.2). All ratios of bSD of MDP and PEG4000 gave opaque solid dispersion while the bSD of MDP and copovidone demonstrated transparent but very sticky solid dispersion at all ratios. Thus, the appearance of both bSD of MDP and PEG4000, and MDP and copovidone was not suitable for the further development. The heterogeneous region appeared when less than 60% w/w of copovidone was used in the formulation. The transparent and yellowish solid dispersion was observed when more than 60% w/w of copovidone was used in the formulation. In this case, it is likely that MDP was molecularly dispersed within the matrix of PEG4000 and copovidone, and a thermodynamically stable, homogeneous solution was formed. This is the most desirable structure of solid dispersions.

Hot stage microscopy was used to follow the phase transformation of sample as a function of temperature. The observation of the behavior of samples during the heating process with hot stage microscope could provide additional information of the prepared bSD and tSD. For the bSD of MDP and PEG4000, it is observed that MDP crystals in PEG4000 began to melt at a temperature of 59.4°C; small crystals of MDP were still observed inside melted droplets (Fig. 3.3a). At 210°C, all MDP crystals still remained (not dissolved). Fig. 3.3b shows hot stage microscopic images of tSD containing MDP, PEG4000 and copovidone at a ratio of 1:7:2. MDP crystals did not completely dissolve at 210°C, which is similar to that of bSD. However, the tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6 showed different behavior, compared with that at a ratio of 1:7:2, as shown in Fig. 3.3c. The hot stage microscopic images showed a homogeneous sample; MDP crystals began to melt at 85.5°C and completely dissolved at around 160°C. From the above results, it is suggested that MDP in tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6 was molecularly dispersed (Vasa et al., 2014).

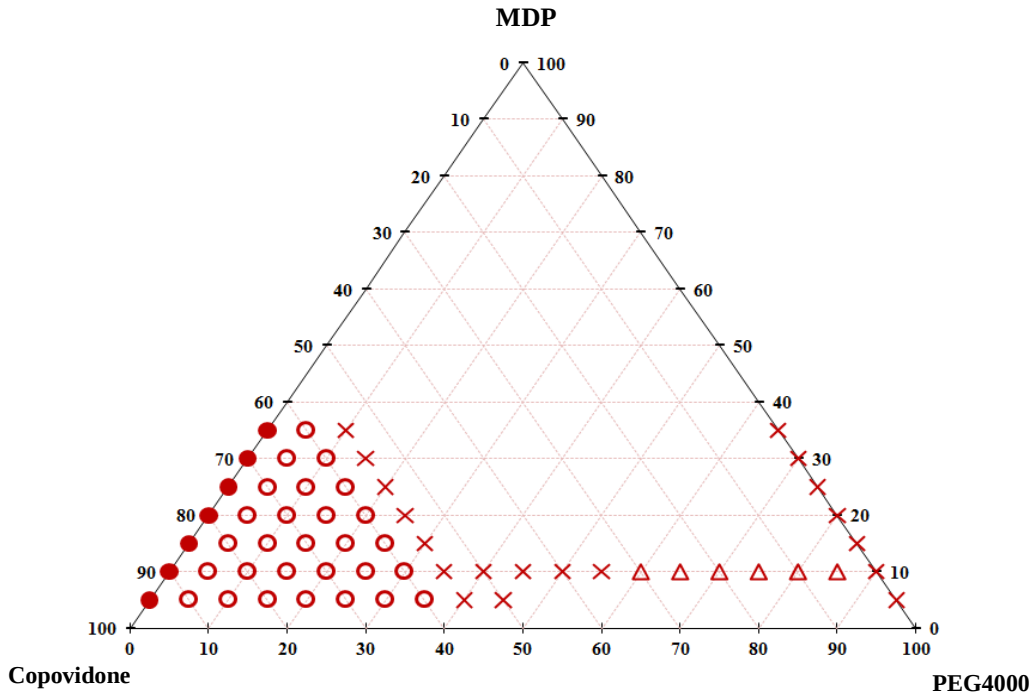


Figure 3.2 Ternary phase diagram of MDP, PEG4000, and copovidone. Note: ● (closed circle), transparent and sticky; ○ (open circle), transparent; × (cross), opaque, △ (triangle), phase separation.

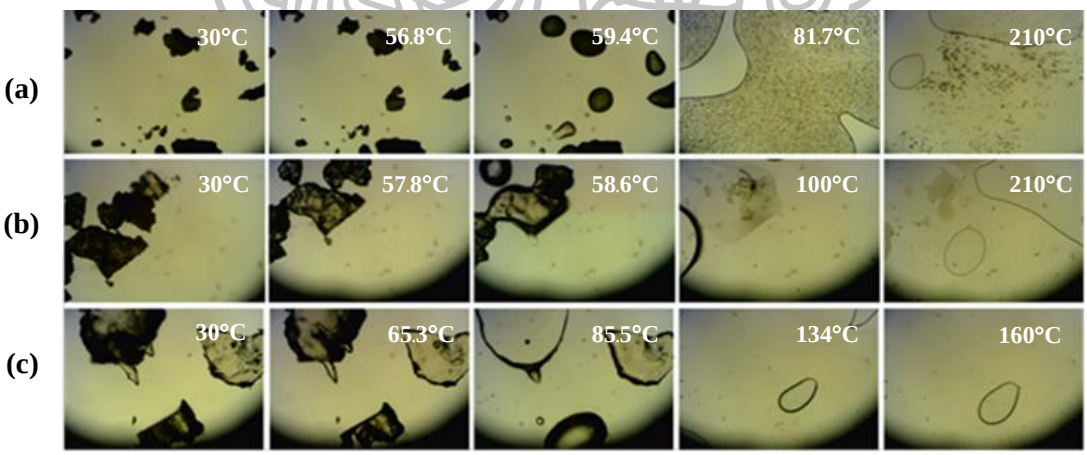


Figure 3.3 Hot stage microscopic images of solid dispersions; (a) bSD containing MDP and PEG4000 at a ratio of 1:9, (b) tSD containing MDP, PEG4000 and copovidone at a ratio of 1:7:2, and (c) tSD containing MDP, PEG4000, and copovidone at a ratio of 1:3:6.

Fig. 3.4a demonstrated the PXRD patterns of tSD containing MDP, PEG4000 and copovidone, showing the effect of MDP to PEG4000 ratio, at a fixed amount of copovidone (60% w/w). The PXRD pattern of physical mixture of MDP, PEG4000 and copovidone revealed the characteristic peaks of MDP at 10.9, 22.0, 22.7 and 22.8° 2θ, and those of PEG4000 at ca. 19 and 23° 2θ, indicating the that both were present in a crystalline state. When the amount of MDP in MDP, PEG4000, copovidone was increased from 0.5:3.5:6 to 1:3:6, the characteristic peaks for crystalline MDP were not present, only the two broad peaks for PEG4000 can be observed. This could be explained by the amorphization of MDP in tSD (Barboza et al., 2014). In case that the amount of MDP in MDP, PEG4000, copovidone was increased to 2:2:6 or 3:1:6, the characteristic peaks of MDP were observed (Fig. 3.4a). This phenomenon suggested that MDP may be in the crystalline state. The results agreed with the previous report (Li et al., 2016) that, with the increased drug loading, drug is likely to be recrystallized from the solid dispersions. The effect of PEG4000 to copovidone ratio, at a fixed amount of MDP (10% w/w), was shown in Fig. 3.4b. The tSD formulations with different of PEG4000 to copovidone ratios exhibited characteristic diffraction peaks of MDP and PEG4000 but of reduced intensity, indicating decreased drug crystallinity. When the ratio of PEG4000 to copovidone was decreased from 8:1 to 1:8, the intensity of the characteristic peaks at ca. 19 and 23° 2θ decreased; the characteristic peaks of MDP disappeared when the amount of copovidone was over 40% w/w (i.e., PEG4000 to copovidone ratio ranged from 5:4 to 1:8). This is likely due to strong miscibility between MDP and carriers, leading to an increase in the molecular mobility of both MDP and carriers (Jog et al., 2016).

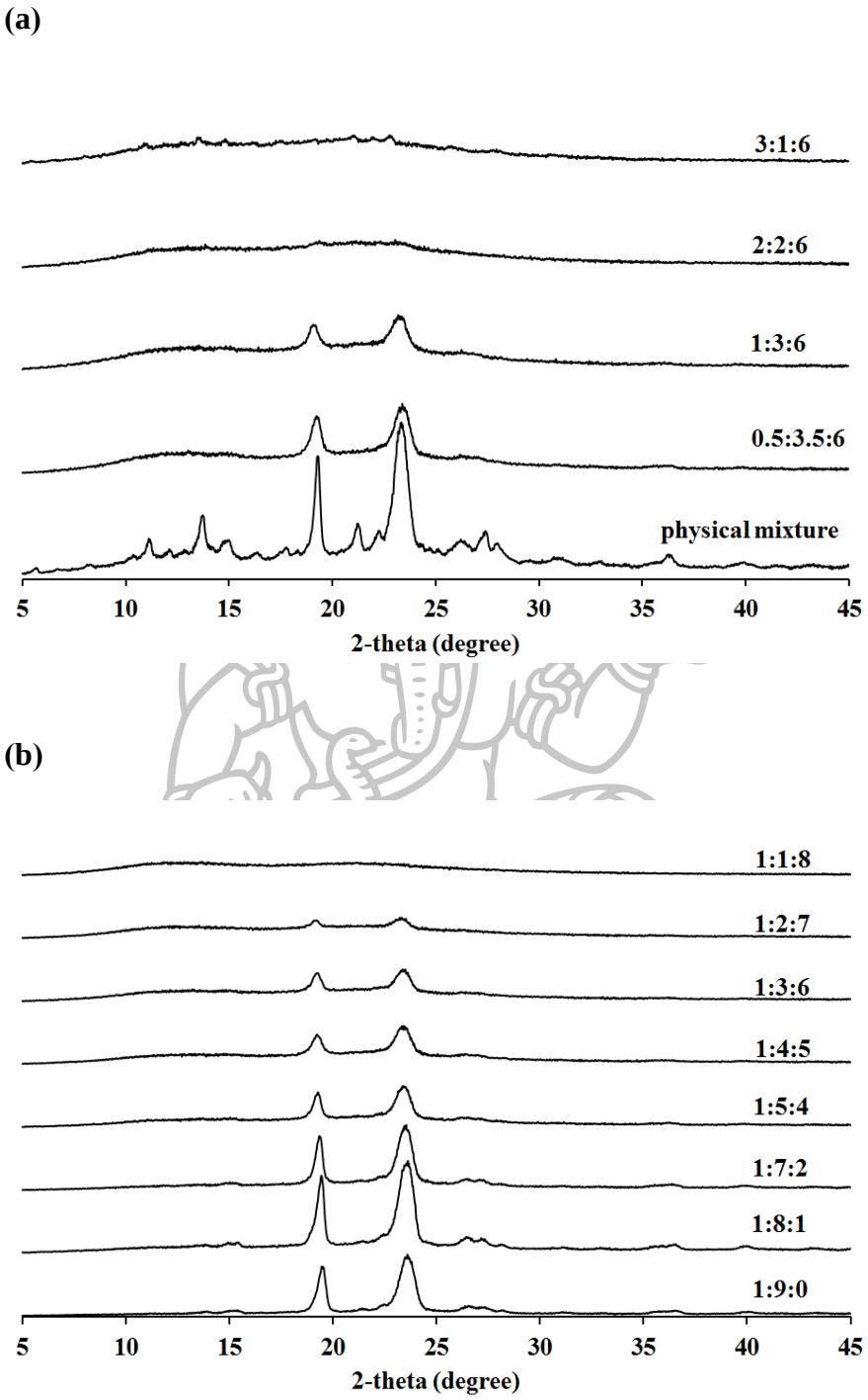


Figure 3.4 PXRD patterns of the tSD containing MDP, PEG4000 and copovidone, showing the effect of **(**a**)** MDP to PEG4000 ratio, at a fixed amount of copovidone (60**%** w/w), and **(**b**)** PEG4000 to copovidone ratio, at a fixed amount of MDP (10**%** w/w).

The DSC thermograms of physical mixture and tSD at different ratios of MDP, PEG4000 and copovidone are shown in Fig. 3.5a. Physical mixture showed relatively weak endothermic peaks at about 50°C and about 216°C, indicating the melting of PEG4000 and crystalline MDP, respectively. No melting peak of MDP was observed in all tSD formulations presented in the homogeneous (transparent) region of solid ternary phase diagram. In this region, the thermal behavior of MDP was the same even though the amount of MDP in tSD was increased, from 0.5:3.5:6 to 3:1:6 (MDP:PEG4000:copovipone). This is attributable to the complete miscibility of MDP in the molten carrier. However, the DSC results found here were not consistent with the PXRD observations in which the crystallinity of MDP was observed at the increased amount of MDP in tSD (Fig. 3.4a). It is likely that, during DSC scanning, MDP dissolved into the molten PEG4000 (starting from the temperature of 60°C). Similar results were reported by Fini et al. (2005) that diclofenac dissolves into the molten PEG6000 during DSC determination and no more undissolved diclofenac was present in the system after test.

FTIR spectroscopy was used to investigate the solid state properties of tSD and detect possible interaction of MDP and the carrier. Fig. 3.5b shows the FTIR spectra of MDP, PEG4000, copovidone, physical mixture of MDP, PEG4000 and copovidone, and tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6. The FTIR spectrum of MDP presented a typical N–H stretching band at 3341 cm^{-1}, C=O stretching band at 1719 cm^{-1}, NO_2 asymmetric and symmetric stretching bands at 1533 and 1348 cm^{-1}, aromatic C=C stretching band at 1480 cm^{-1}, C–N stretching band at 1219 cm^{-1}, and out-of-plane bending of aromatic C–H bonds at 756 and 707 cm^{-1}. The N–H group may act as a hydrogen donor, which is capable of forming hydrogen bond with an appropriate acceptor group, such as a carbonyl (C=O) group (Song et al., 2013). PEG4000 showed a broad band at 3445 cm^{-1}, which was attributed to the presence of O–H stretching. The C–H_2 stretching bands at 1467 cm^{-1} and C–O stretching bands at 1059 cm^{-1} were also observed. Copovidone showed a broad band at 3479 cm^{-1}, the C=O stretching at 1737 cm^{-1} of the vinyl acetate moiety, C=O stretching of the amide function at 1667 cm^{-1}, C–C binding at 1450–1480 cm^{-1}, and doublet peaks at fingerprint region of 600-750 cm^{-1}. The presence of more numbers of

O–H stretching groups was attributed to the presence of water as both of these polymers are hygroscopic in nature and can absorb moisture from the environment. All the polymers have a hydrophilic surface with lots of hydrophilic groups, resulting in a diffusion of dissolution medium and accelerating release of MDP.

Physical mixture of MDP, PEG4000 and copovidone presented band assignment at the same FTIR wavenumber range of raw materials; they did not show any new band. The absence of any significant change in the FTIR spectra indicated the absence of interaction between the MDP and carriers. In the case of tSD containing MDP, PEG4000 and copovidone, N-H stretching band of MDP disappeared. This may be due to the formation of intermolecular hydrogen bonds between N-H stretching band at 3341 cm^{-1} of MDP and the stretching vibration of carbonyl (C=O) group of copovidone, resulting in a shift of carbonyl group towards higher wavenumber from 1667 to 1669 cm^{-1}. The results were also supported by the shift of the ester band towards higher wavenumber (going from 1719 to 1735 cm^{-1}). The results was also supported by the research of Eerdenbrugh and Taylor (2011), which suggested that copovidone has a strong acceptor strength as indicated by pK_{BHX} value. The very strong (1-methyl-2-pyrrolidone) and medium (ethyl acetate) acceptor value of 2.38 and 1.07, respectively. The acceptor functional group of copovidone could form hydrogen bonding with amine group of pyridinedicarboxylate methyl ester of MDP that act as electron donor group. The intermolecular interaction between MDP and carriers led to better dispersion of MDP in the polymer matrix and reduction in size of MDP particle, resulting in an enhanced dissolution of tSD.

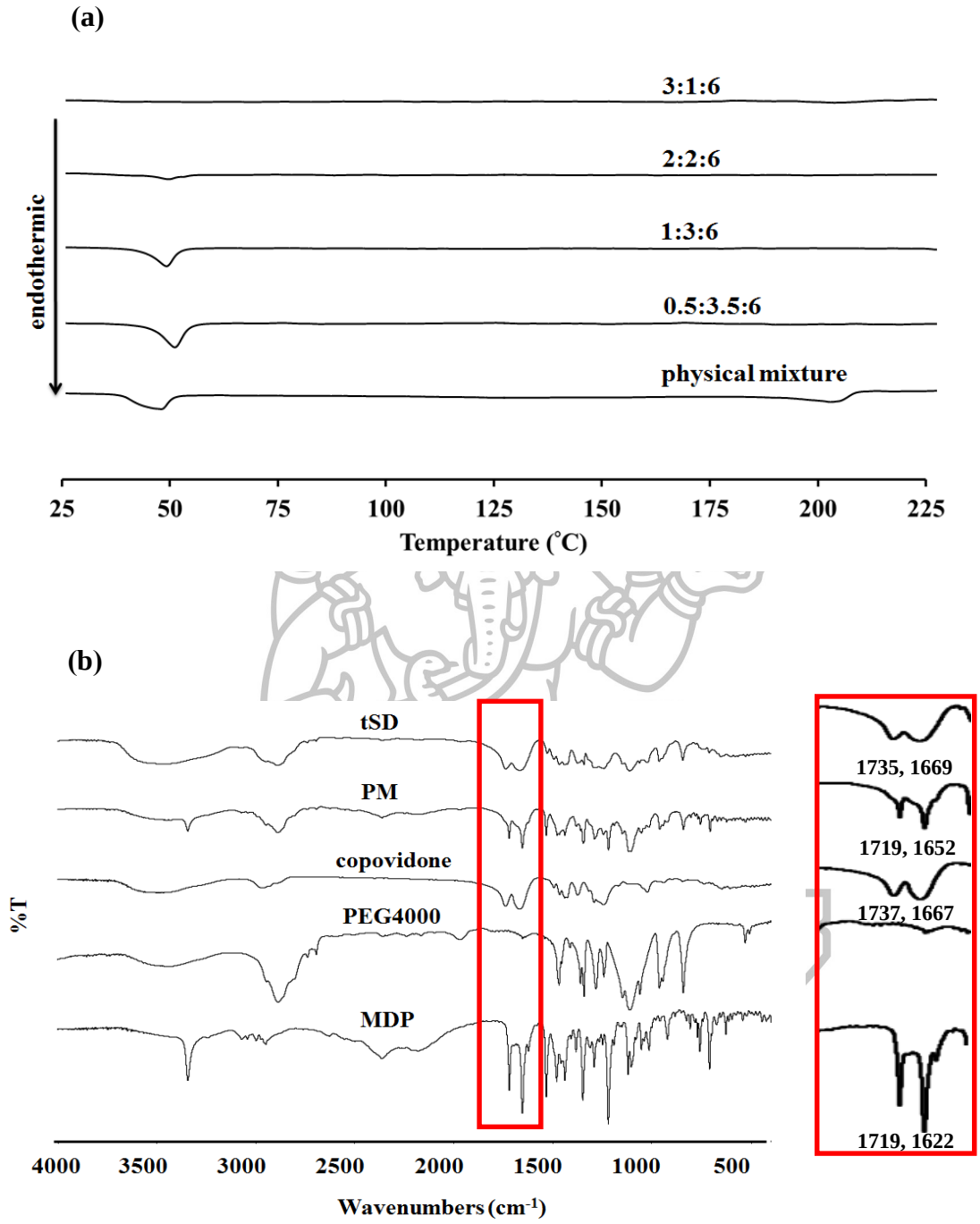


Figure 3.5 (a) DSC thermograms of physical mixture and tSD at different ratios of MDP, PEG4000 and copovidone; (b) FTIR spectra of MDP, PEG4000, copovidone, physical mixture of MDP, PEG4000 and copovidone, and tSD containing MDP, PEG4000 and copovidone a ratio of 1:3:6.

3.3.3 Solubility study

The solubility in water of MDP and its tSD was evaluated at ambient temperature. MDP powder showed a low solubility (0.04 mg/mL), which is considered as practically insoluble. The higher values of solubility (i.e., 49-315 folds) were obtained in the case of tSD formulations containing 5-35% w/w MDP (Table 3.3). The enhanced solubility of MDP was likely due to a combination of solubilization, surface activity and wetting effect of hydrophilic carriers (i.e., PEG4000 and copovidone). When the tSD came in contact with water, the polymer particles hydrated rapidly into polymer solution, solubilizing the MDP particles and subsequently releasing the MDP into the medium (Newa et al., 2007). All tSD displayed higher solubility than MDP powder. The solubility of MDP in tSD increased after dispersing them in the polymer matrix, compared to the physical mixture. Increasing the proportion of copovidone in the tSD demonstrated an improvement in drug solubility; the linear increase in MDP solubility was found (Fig. 3.6a). Similar results were reported by Ghareeb et al. (2009). The coefficient of determination (r^2) of solubility of tSD containing 10% w/w MDP was 0.9161.

Fig. 3.6b shows solubility of different tSD formulations containing different concentrations of MDP (10-35% w/w of MDP). At higher concentration of MDP (30-35% w/w), the solubility tended to decrease when the proportion of copovidone in tSD was increased. In contrast, at lower concentration of MDP (10-20% w/w MDP), the MDP solubility increased when the proportion of copovidone in tSD was increased. This is probably due to the complete dissolution of MDP in the molten carrier, when the high amount of copovidone was used, agreeing well with the DSC results discussed above. Table 3.4 shows regression coefficient and coefficient of determination (r^2) of MDP solubility of tSD containing different concentrations of MDP. The results showed strong linear relationship between MDP solubility and proportion of copovidone, in each concentration of MDP. The regression coefficient of the linear regression of the tSD containing different concentrations of MDP was different; it seemed that the increased concentration of MDP resulted in lower regression coefficient. The tSD with 10% w/w MDP demonstrated the highest

regression coefficient, indicating the highest rate of change of MDP solubility relative to change in MDP concentration.

Table 3.3 MDP solubility of tSD containing different concentrations of MDP, PEG4000 and copovidone.

MDP (% w/w)	PEG4000 (% w/w)	Copovidone (% w/w)	Solubility (mg/mL)
5	95	0	1.57±0.34
	35	60	2.72±0.18
	30	65	3.30±0.06
	25	70	3.38±0.12
	20	75	3.78±0.71
	15	80	3.61±0.20
	10	85	3.86±0.22
	5	90	4.05±1.13
10	90	0	1.71±0.08
	30	60	4.70±0.01
	25	65	4.50±0.07
	20	70	5.03±0.02
	15	75	6.28±0.43
	10	80	5.96±0.75
	5	85	11.57±0.91
15	85	0	1.35±0.17
	25	60	7.09±0.53
	20	65	9.36±0.46
	15	70	10.88±1.99
	10	75	10.93±0.02
	5	80	12.63±2.15
20	80	0	1.00±0.10
	20	60	5.49±0.84
	15	65	6.12±0.76
	10	70	7.46±0.38
	5	75	9.86±1.15
25	75	0	0.76±0.05
	15	60	5.91±0.91
	10	65	5.95±0.92
	5	70	7.49±1.21
30	70	0	0.49±0.02
	10	60	5.81±0.34
	5	65	5.43±0.53
35	65	0	0.29±0.00
	5	60	3.51±0.47
	0	65	2.31±0.66

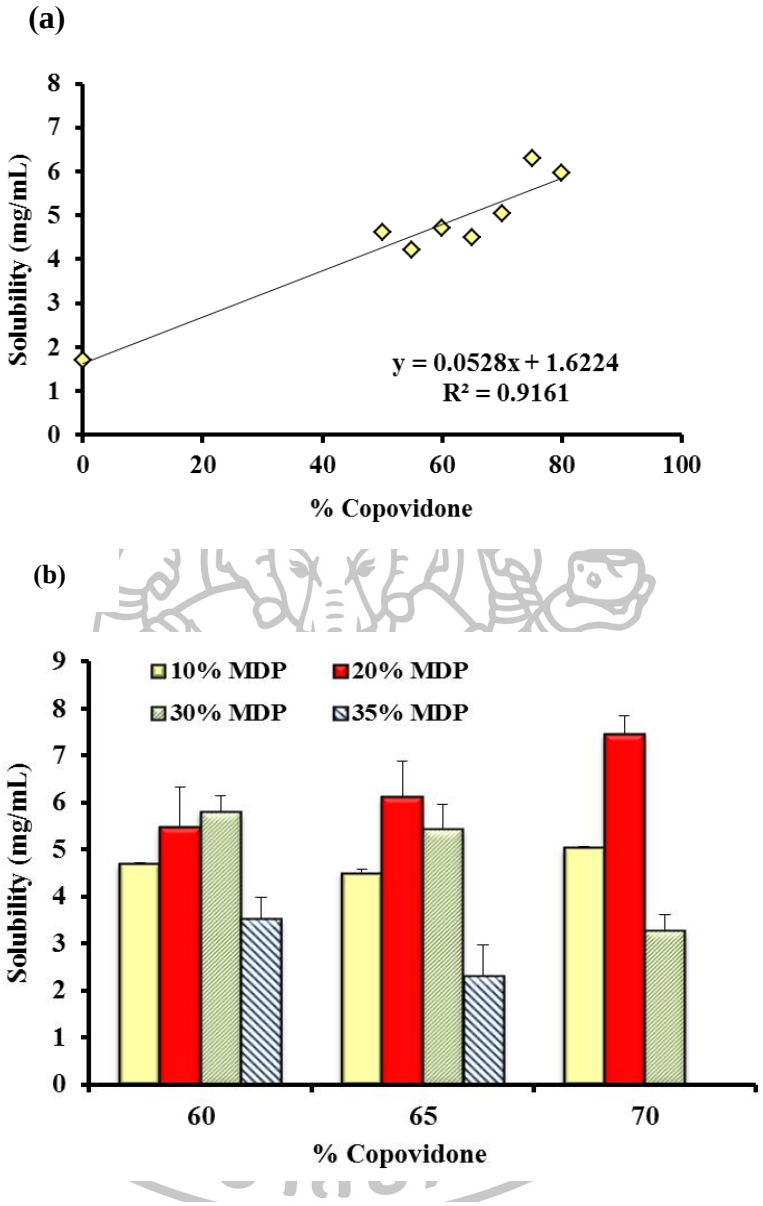


Figure 3.6 **(**a**)** Scatter plot showing linear regression analysis between MDP solubility and proportion of copovidone in tSD containing MDP (10% w/w), PEG4000 and copovidone; **(**b**)** solubility of different tSD containing different concentrations of MDP (10, 20, 30 and 35**%** w/w)**.** Note: N/A, not applicable.

Table 3.4 Regression coefficient and coefficient of determination (r^2) of MDP solubility of tSD containing different concentrations of MDP.

Concentration of MDP (% w/w)	Regression coefficient (slope)	Coefficient of determination, r2
5	1.3328	0.7270
10	1.6224	0.9161
15	0.5505	0.9013
20	0.6505	0.8708
25	0.7025	0.9758
30	0.5277	0.9823
35	0.3514	0.8108

3.3.4 *In vitro* dissolution study

In vitro dissolution study was carried out to investigate the pharmaceutical performance of tSD. The dissolution profiles of MDP powder and tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6 are shown in Fig. 3.7. It is clearly seen that the MDP dissolution from the tSD was faster than that of MDP powder; the amount of MDP dissolution at 10 min was 18% and 50% for MDP powder and tSD, respectively. The addition of the PEG4000 and copovidone in the tSD improved the MDP dissolution. The dissolution enhancing effect of tSD is most likely due to the molecular dispersion of MDP in solid carriers (Rashid et al., 2015) and increasing of wettability of MDP by copovidone at the diffusion layer. Moreover, PEG4000 and copovidone could delay or prevent supersaturation in the dissolution medium and avoid formation of poorly soluble MDP during dissolution study (Ullah et al., 2015). The PXRD and DSC results also supported these results.

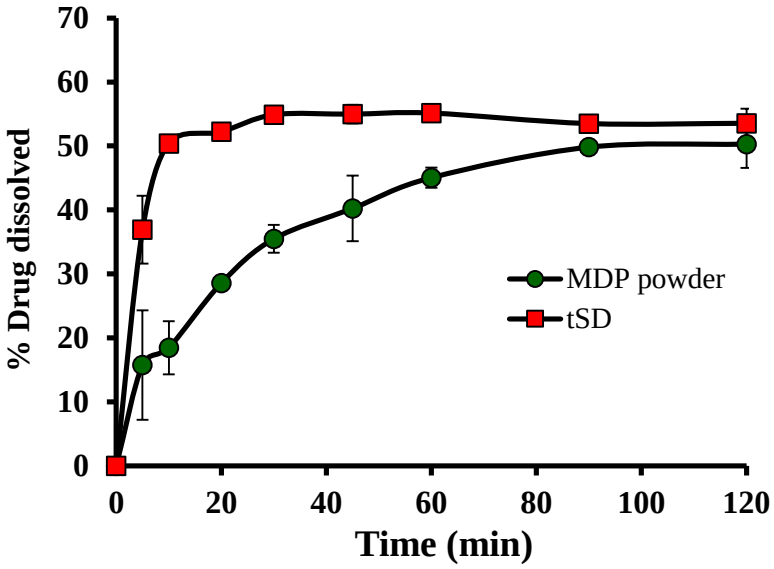
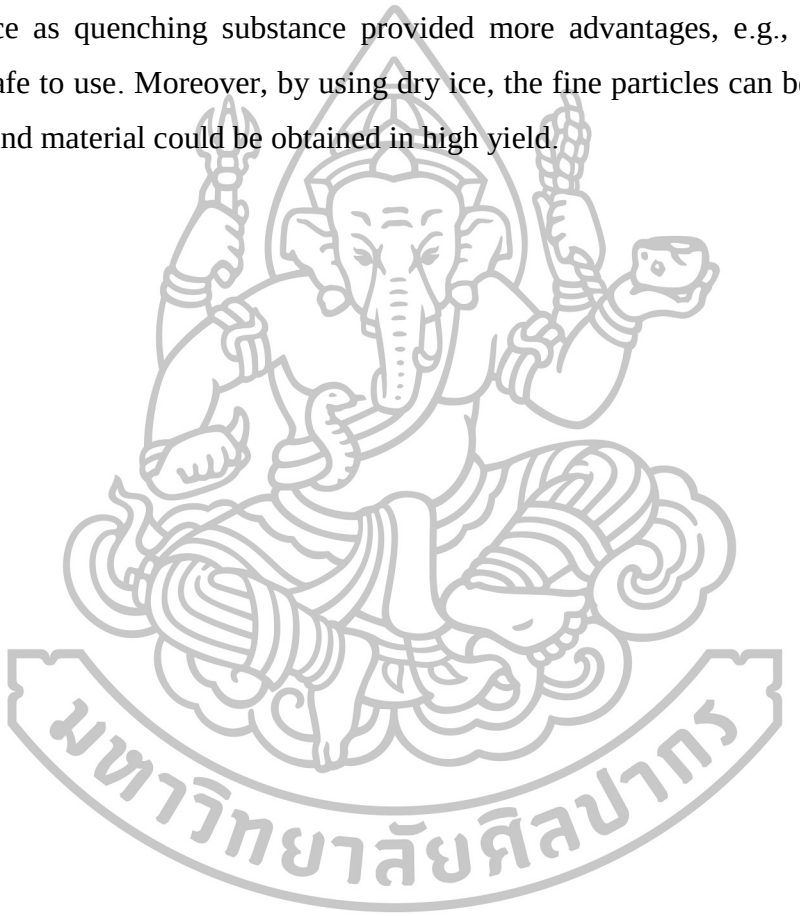


Figure 3.7 Dissolution profiles of MDP powder and the tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6.

3.3.5 Effect of quenching substance

In order to investigate the effect of quenching substance on the physicochemical properties and MDP solubility of tSD, the different quenching substances (i.e., ice, liquid nitrogen, dry ice) were used. Fig. 3.8 displays PXRD patterns and DSC thermograms of physical mixture and tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6, quenched by different substances. The PXRD patterns of the tSD quenched by different substances showed the same halo pattern; no crystalline drug was observed. DSC thermograms of the tSD quenched by ice showed a small and shifted endothermic peak of MDP to lower temperature at around 200°C. This result was attributed to the gradual dissolution of the MDP into the carrier during DSC heating ramp. Similar results have been reported recently (Gupta et al., 2015; Li et al., 2015). The scatter plot showing linear regression analysis between MDP solubility and proportion of copovidone in tSD containing MDP (10% w/w), PEG4000 and copovidone, quenched by ice, liquid nitrogen and dry ice, is presented in Fig. 3.9. An increase in MDP solubility was observed with increased proportion of copovidone. The good linear relationship between MDP

solubility and proportion of copovidone in tSD was observed. The regression coefficient of the linear regression of the tSD quenched by dry ice was the highest, suggesting the highest rate of change of MDP solubility relative to change in the proportion of copovidone in tSD. For example, at 70% w/w of copovidone, the MDP solubility increased to 5.04±0.01 mg/mL (126 folds), 6.55±0.73 mg/mL (163 folds), and 8.01±0.67 mg/mL (200 folds) for the tSD quenched by ice, liquid nitrogen and dry ice, respectively, compared to MDP powder. From the above results, it seems that using dry ice as quenching substance provided more advantages, e.g., the highest solubility, safe to use. Moreover, by using dry ice, the fine particles can be produced, and the ground material could be obtained in high yield.



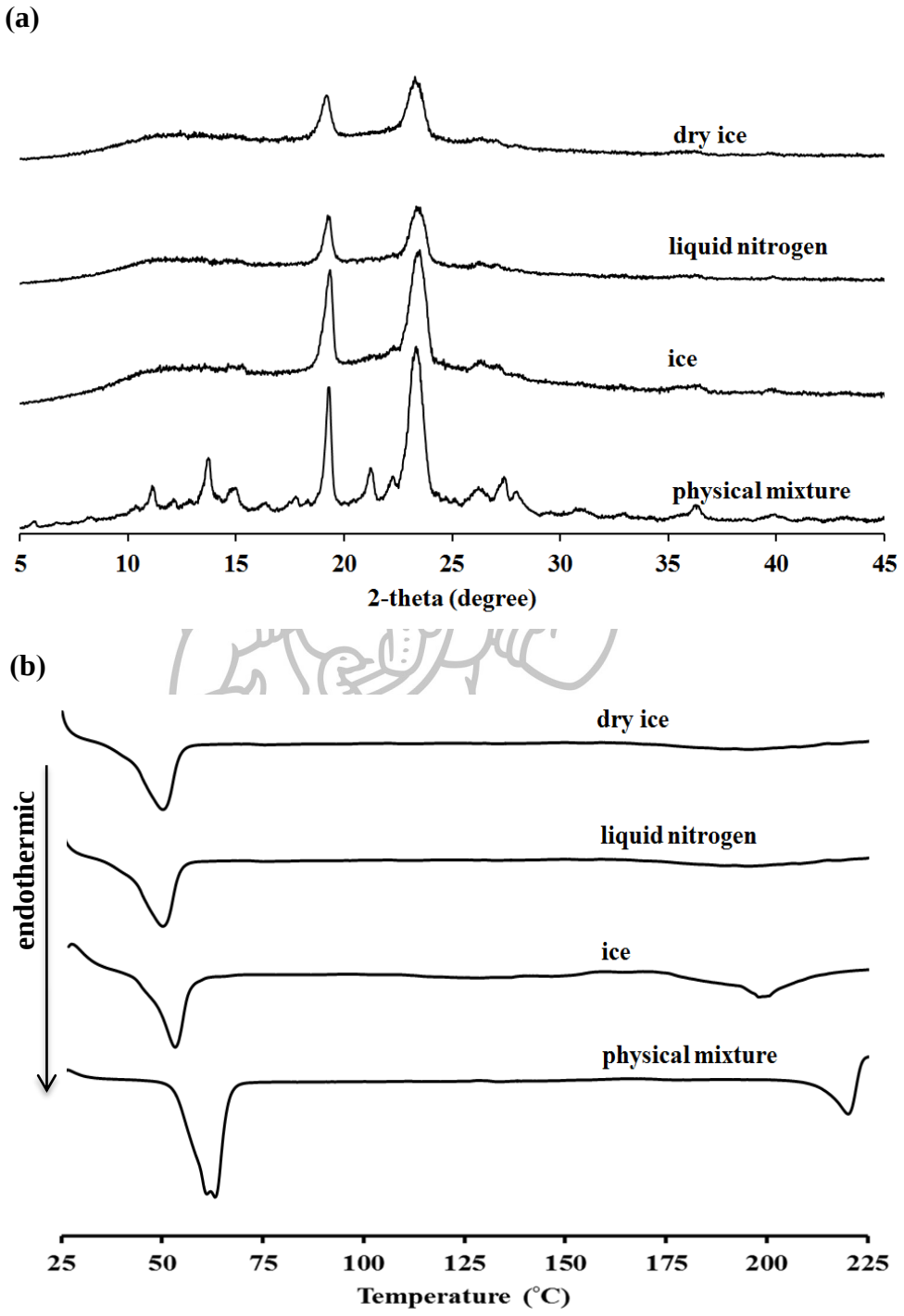


Figure 3.8 **(**a**)** PXRD patterns and **(**b**)** DSC thermograms of physical mixture and tSD containing MDP, PEG4000 and copovidone at a ratio of 1**:**3**:**6.

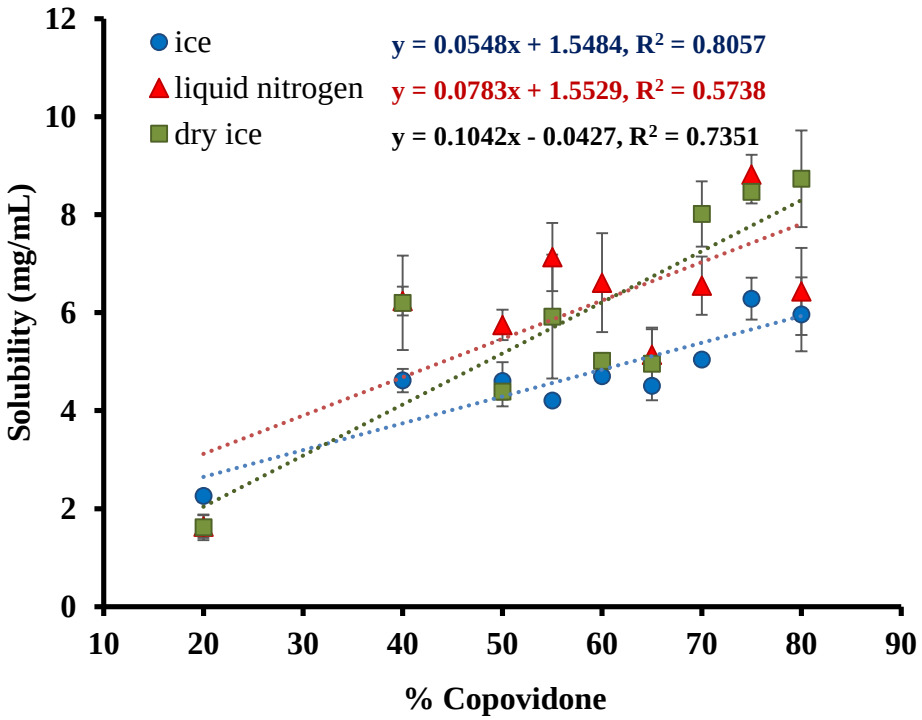


Figure 3.9 Scatter plot showing linear regression analysis between MDP solubility and proportion of copovidone in tSD containing MDP (10% w/w), PEG4000 and copovidone, quenched by ice, liquid nitrogen and dry ice.

3.3.6 Stability test

Moisture and temperature have more of deteriorating effect on solid dispersions than on physical mixtures. Thus, the stability study under an accelerated condition (40°C/75%RH) would provide evidence on how the quality of a drug substance. Table 3.5 shows the solubility of tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6, quenched by different quenching substances, at initial and after storage at 40°C/75%RH for 6 months. There was no statistical significantly difference in MDP solubility of tSD, between at initial and after storage for 6 months, for all quenching substances. The aged sample had similar PXRD diffraction features, compared to the freshly prepared sample (Figures 3.10a), indicating that the MDP molecules in the solid dispersion did not recrystallize over such accelerated stability test conditions. DSC thermogram of the tSD after storage at 40°C/75%RH for 6

months also showed the same pattern as the freshly prepared sample. No endothermic peak of MDP at 222°C was observed. From the PXRD and DSC results, it confirmed that the molecular dispersion of MDP was not changed after storage at accelerated condition for 6 months.

Table 3.5 Solubility of tSD containing MDP, PEG4000 and copovidone at a ratio of 1:3:6, quenching with ice, liquid nitrogen or dry ice, at initial and after storage at 40°C/75%RH for 6 months.

	Solubility (mg/mL)	
	Day 0	40°C/75%RH, 6 months
Ice	4.70 ± 0.01	3.14 ± 0.11
Liquid nitrogen	3.52 ± 0.10	3.85 ± 0.63
Dry ice	5.02 ± 0.16	4.82 ± 1.00

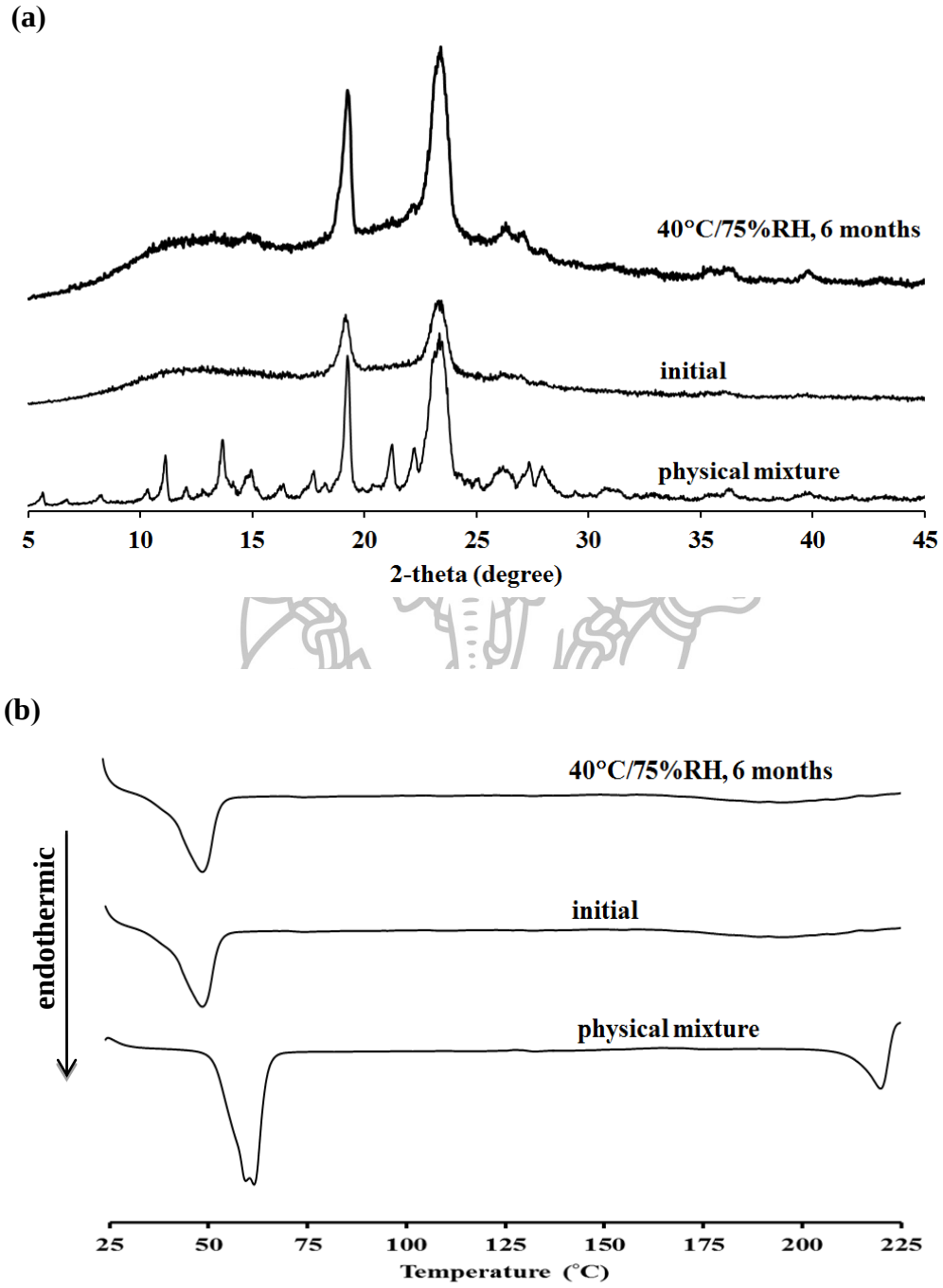
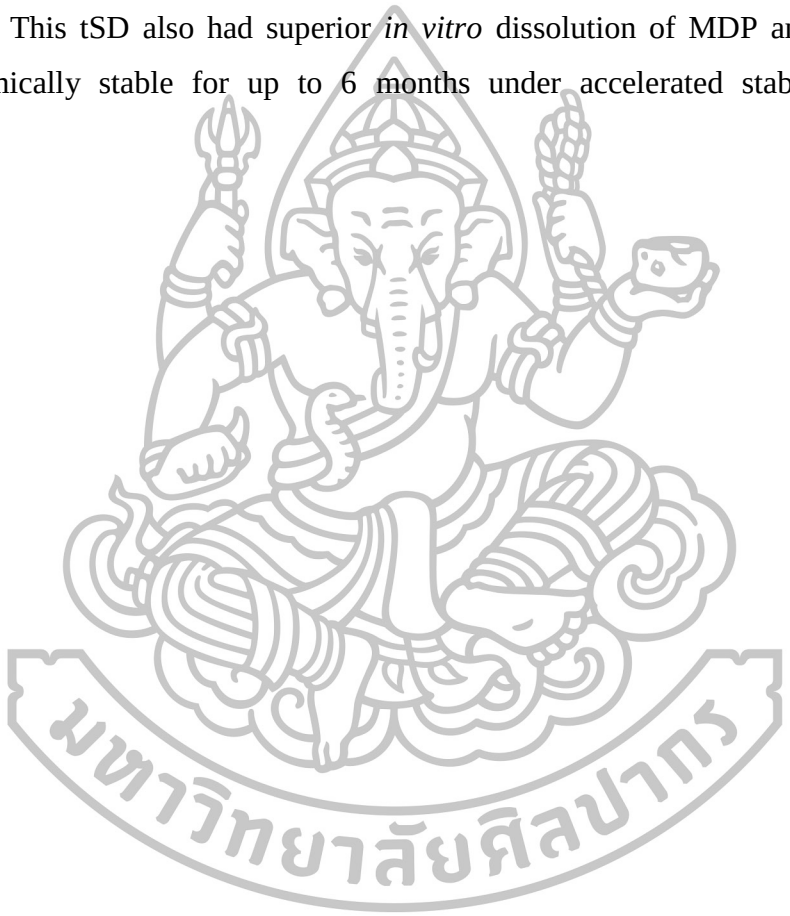


Figure 3.10 **(**a**)** PXRD patterns and **(**b**)** DSC thermograms of physical mixture and tSD containing MDP, PEG4000 and copovidone at a ratio of 1**:**3**:**6, at initial and after storage at 40°C/75**%**RH for 6 months

3.4 Conclusion

The selection of carrier and processing temperature are important concerns in the preparation of tSD by melting method. In the current study, it was clearly demonstrated that the tSD formulations containing MDP, PEG4000 and copovidone were successfully prepared using solid ternary phase diagram. In the homogeneous region of ternary phase diagram, MDP was molecularly dispersed in PEG4000/copovidone blend. The solubility of MDP was significantly increased from this system. This tSD also had superior *in vitro* dissolution of MDP and remained physicochemically stable for up to 6 months under accelerated stability testing conditions.



CHAPTER 4
TERNARY SOLID DISPERSIONS OF MANIDIPINE IN TPGS/COPOVIDONE BLENDS

4.1 Introduction

Numerous drug candidates was suffered from low aqueous solubility and bioavailability. Various formulation strategies have been researched to improve the solubility and the rate of dissolution and thus the oral bioavailability. These strategies include the solubilization with surfactants, particle size reduction, complexation with cyclodextrins, formation of solid dispersions, etc. Solid dispersion is defined as the dispersion of one or more active ingredients in an inert carrier. Particularly, a solid dispersion can be narrowly termed as dispersion of drug in an amorphous polymer, where the drug is rather in the molecularly dispersed state (Huang and Dai, 2014). The dispersed state has included many forms such as eutectic mixtures, solid solutions, glass solutions, and amorphous/glass suspensions (Chiou and Riegelman, 1971; Sekiguchi and Obi, 1961). Many water-soluble polymer and water-miscible polymer have been used to prepare solid dispersions. Among them PEG was the most commonly used according to their good solubility in water and many solvents, and low melting points. Others polymers include PVP, polyvinylalcohol (PVA), HPMC, hydroxypropyl cellulose (HPC), copovipone, etc. (Craig, 2002; Huang and Dai, 2014). These polymers can act as a barrier to prevent drug crystallization during storage (Al-Obaidi et al., 2011; Rashid et al., 2005). Among these water-soluble polymers, copovidone was widely used as a carrier for solid dispersions because of its high aqueous solubility, high physiological tolerance, and low toxicity (Wang et al., 2005). Also, it has been able to maintain a supersaturated drug concentration for an extended period of time to allow optimal absorption (Huang and Dai, 2014).

A dispersion of drug into a polymer can be called as bSD. The bSD provides a decrease in crystallinity of drug and enhance drug dissolution. For example, the solubility of meloxicam increases when bSD is prepared with poloxamer 188 (Ghareeb et al., 2009). However, bSD represents a thermodynamically unstable system; it is likely to convert into a more stable crystalline state (Serajuddin, 1999). Therefore, some researches try to use an additional additive (e.g., bile salts, lecithin, TPGS and Gelucire® 44/14) for preparing tSD in order to improve solubility, dissolution behavior and physical stability of drug from the system (Liu and Wang, 2007; Zaki et al., 2013). Previously, Janssens et al. (2008) used a combination of

copovidone and TPGS as carrier in order to make tSD of itraconazole by spray drying (solvent process). TPGS, a nonionic surfactant, has been used owing to its stabilizing, emulsifying, and solubilizing effects (Shin and Kim, 2003).

MDP is a lipophilic and highly vasoselective, dihydropyridine calcium channel antagonist, having long-lasting activity for the treatment of essential hypertension (Luque Otero, Martell Claros, 2005). The drug is practically insoluble in water and exists in the solid form as yellow crystals. After oral administration of MDP in human at clinical dose, the plasma concentration of MDP is very low (Jing et al., 2007). The poor water-solubility of MDP results in a reduced drug dissolution rate in gastrointestinal fluid following oral administration, and consequently a reduced bioavailability.

In this study, we have constructed the solid ternary phase diagram to check the homogeneity of the tSD. The homogeneous solid dispersions (glass solutions) can be differentiated by the phase diagram. Different ratios of MDP, TPGS and copovidone were investigated. Hot stage microscopy, DSC, PXRD and FTIR spectroscopy, solubility measurement and dissolution study were conducted to characterize the tSD. The *in vivo* study in Wistar rat model was also performed to compare the pharmacokinetics of tSD with MDP powder and marketed product.

4.2 Materials and methods

4.2.1 Materials

MDP was obtained from Sriprasit Pharma Co., Ltd. (Bangkok, Thailand). D-α-tocopherol polyethylene glycol 1000 succinate (referred to as TPGS) was purchased from Sigma-Aldrich® (USA). Copovidone or vinylpyrrolidone-vinyl acetate copolymers (Kollidon® VA64) was a gift from BASF (Thai) Co., Ltd. (Bangkok, Thailand). Distilled water was purchased from General Hospital Products Public Co., Ltd. (Thailand). All other chemicals used in this study were of pharmaceutical grade or analytical grade, and used as received without further purification.

4.2.2 Preparation of tSD using ternary phase diagram

The tSD was prepared by physical mixing the MDP with TPGS and copovidone, at different weight ratios, using mortar and pestle. Based on the ternary phase diagram, the mixing ratios of TPGS and copovidone ranged from 1:8 to 8:1, 1:7 to 7:1, 1:6 to 6:1, 1:5 to 5:1, and 1:4 to 4:1 for 10, 20 30, 40 and 50% w/w of MDP, respectively. Then, the mixed powders were melted at the temperature of 170-180°C and continuously stirred until all materials were completely melted. The clear molten solution was immediately solidified using dry ice. The solid dispersion was ground and kept at -20°C until further analysis.

4.2.3 Solubility measurement

The equilibrium or thermodynamic solubility represents the saturation solubility of a compound in equilibrium with an undissolved substance in the solvent at the end of the dissolution process. It is an essential requirement for the successful development of solid dispersion. In this study, the equilibrium solubility of tSD was determined using shake-flask method. Excess amount of the tSD sample (~200 mg) was added to 500 μL of distilled water in a 1.5-mL microcentrifuge tube and vortexed for about 1 min. The tubes were later agitated in a thermostatically controlled shaker at 150 rpm for 48 h, at ambient temperature (28°C). After 48 h, the tubes were vortexed, and then centrifuged (model Universal 320R, Hettich, Germany) at 3,500 rpm for 15 minutes.The clear supernatants were analyzed with HPLC.

4.2.4 Hot stage microscopy

The samples were placed on the glass slide and heated by a hot-stage unit (model FP82HT, Mettler Toledo, Switzerland) at the rate 5°C/min and observed under an optical microscope (model CX41, Olympus, Japan). The changes in the morphology (melting or crystallization) were noted as a function of temperature. The polarization properties of MDP were also observed during the experiment using a polarized filter (model CX-AL, Olympus, Japan) to investigate the crystal properties.

4.2.5 Scanning electron microscopy

The morphology of samples was observed using a scanning electron microscope (SEM, model Maxim-2000, CamScan Analytical, England), operating at

the accelerating voltage of 15 kV. The samples were attached to aluminum stub with double-sided adhesive carbon tape and then gold-coated with a sputter coater before investigation. Micrographs with different magnifications were recorded to study the morphology of the different samples.

4.2.6 Physicochemical characterization

4.2.6.1 DSC analysis

DSC analyses of samples were conducted using differential scanning calorimeter (model Sapphire, Perkin Elmer, Germany). An accurately weighed amount of sample was placed inside a standard crimped aluminum pan and heated from 25 to 250°C at a heating rate of 10°C/min under nitrogen flow (20 mL/min).

4.2.6.2 PXRD analysis

The crystallinity of samples was examined using a powder X-ray diffractometer (model MiniFlex II, Rigaku, Japan). The analysis was carried out using a Cu Kα monochromatic radiation wavelength of 1.5406 Å with 40 mA current and 30 kV voltage. The PXRD patterns were recorded in the range of 5°-45° 2θ, using a step size of 0.04°/s and a scan speed of 10°/min.

4.2.6.3 FTIR spectroscopy

The FTIR spectroscopic analysis was performed to determine the potential interaction between MDP and excipients. The FTIR spectra of samples were recorded using Nicolet FTIR spectrophotometer (model 4700, Thermo Nicolet, Japan). All samples were prepared using potassium bromide (KBr) disc method. Each sample was thoroughly blended with dry KBr powder and ground well in mortar and pestle. Then, the powders were compressed to a disc with pressure of 5 tons. The spectral value of a sample was obtained by scanning from 4000 to 400 cm^{-1}. FTIR spectra were obtained using a software package (Omnic FTIR Software, version 7.2a, Thermo Electron Corporation, USA).

4.2.7 Dissolution study

Dissolution test was performed in 900 mL of 0.1 N hydrochloric acid (HCl), using an USP apparatus II (model DT-720, Erweka, Germany), at 37±0.5°C.

The paddle speed was adjusted to 50 rpm. The total amount of MDP in each formulation was equal (approximately 3 mg), in order to keep sink condition. The samples were withdrawn from dissolution vessels at 5, 10, 20, 30, 45, 60, 90 and 120 min and passed through 0.45-μm nylon membrane before analysis with HPLC to determine the drug dissolved. The withdrawn volume was replaced by fresh medium to keep the total volume constant. The dissolution experiments were carried out in triplicate.

4.2.8 HPLC analysis

The chromatographic system consisted of JASCO PU-2089 plus quaternary gradient inert pump equipped with JASCO UV-2070 plus multiwavelength UV-vis detector (Jasco, Japan). The separation was performed on Luna 5u C18 column (5 μm, 4.6 mm×150 mm, Phenomenex, USA) was used. The mobile phase consisting of acetonitrile:potassium dihydrogen phosphate solution (49:51, v/v) was filtered through a 0.45-μm nylon membrane filter, and degassed in a sonicator bath before use. The flow rate was 1.0 mL/min, and the UV detection wavelength was 228 nm. The sample was prepared in triplicate.

4.2.9 Stability study

The tSD formulations were filled into opened glass vials and kept at either ambient condition (25°C) or accelerated condition (40ºC/75%RH, according to ICH guideline) for 3 months. After storage for 1 and 3 months, the samples were characterized for their solubility, thermal property and crystallinity.

4.2.10 Statistical analysis

The difference between sample groups was analyzed by ANOVA and Levene's test for homogeneity of variance. *Post hoc* testing ($p<0.05$) of the multiple comparison was performed by either the Scheffé or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively.

4.3 Results and discussion

4.3.1 Preparation of the tSD

Although the drug solubility and dissolution can be improved by bSD containing drug and carrier, the drug may recrystallize (either nucleation and/or growth) during storage or precipitate during dissolution process. In attempt to extend the stability of drug in bSD, an appropriate third component has been added, in order to form tSD. In this study, the solid dispersion of MDP was prepared by melting technique using TPGS and copovidone as nonionic surfactant and hydrophilic carrier, respectively. Both TPGS and copovidone could increase the solubility of MDP and stability of the solid dispersion system. Similar results were reported by Janssens et al. (2008); an increase in the stability of supersaturated itraconazole solutions was achieved when both TPGS and copovidone were combined as carrier in order to make tSD of itraconazole by spray drying.

The homogeneous one-phase systems (or glass solutions) can be formed when the drug molecules are molecularly dispersed in the amorphous carrier (e.g., polymer) (Xie and Taylor, 2016). However, the distribution of drug molecules in the carrier may be irregular due to a high viscosity of glass solutions, and a homogeneous distribution within the glass solution needs to be ensured. It is often observed that the miscibility of drug molecules in amorphous carrier is limited, and as the drug content increases, phase separation can occur. In this form, the drug is still present in the amorphous form but has a high chance for recrystallization of the amorphous drug (Laitinen et al., 2014). In the current study, the solid ternary phase diagram was applied to investigate the homogeneous and non-homogeneous regions, indicating the formation of glass solutions and phase separation in the solid dispersions, respectively. Fig. 4.1 demonstrates a ternary phase diagram of MDP, TPGS and copovidone, in which any point represented a fixed composition of three components. The homogeneous tSD was observed when high concentration of copovidone was used (≥ 50% w/w), demonstrating a light yellow clear solution. This may be due to the fact that MDP can dissolve completely in the copovidone and TPGS in solid state, leading to a stable system without phase separation (Six et al., 2004). In non-homogeneous region, both opaque (e.g., formulations containing 40-50% w/w of

copovidone) and phase separation (e.g., formulation containing ≤ 40% w/w of copovidone) were observed, indicating that MDP did not completely dissolve in the carriers.

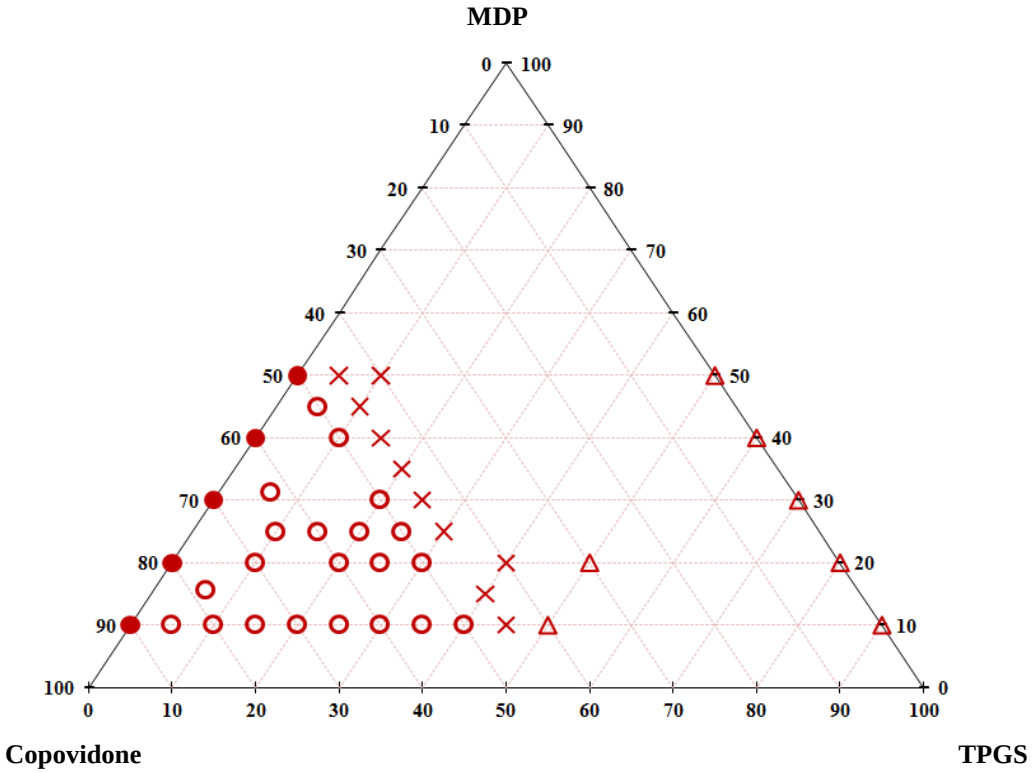


Figure 4.1 Ternary phase diagram of MDP, TPGS and copovidone. Note**:** ● **(**closed circle**)**, transparent and sticky; ○ **(**open circle**)**, transparent; × **(**cross**)**, opaque; △ **(**triangle**)**, phase separation

4.3.2 Hot stage microscopy

Hot stage microscopy is a powerful tool that is widely used for the visual characterization of all kinds of thermal transitions (changes in crystalline form, crystal structure and growth rates, size and number of crystals, etc.). Additionally, the observation of sample behavior during heating process may provide additional information of the phase transformation of sample as a function of temperature. In this study, the samples were put on the heating unit and heated to 210°C using heating rate of 5°C/min to ensure its complete melting. The microscopic images of the starting materials, that is, MDP powder, TPGS, and copovidone, were taken as a function of

time (Fig. 4.2a-c). MDP powder melted at around 250°C and decomposed immediately after melting, showing good agreement with the previous study (Todeschini et al., 2014). TPGS began to melt at 34.9°C and completely melted at 39°C. Copovidone started to melt at 135°C and completely melted at 161°C.

The hot stage microscopy was also used to investigate the difference between two regions (non-homogeneous and homogeneous) of tSD. For non-homogeneous zone, the phase-separated sample (tSD containing MDP, TPGS and copovidone at a ratio of 2:5:3) and opaque sample (tSD containing MDP, TPGS and copovidone at a ratio of 2:4:4), were presented in Fig. 4.2d and 4.2e, respectively. The tSD sample was opaque when the heating temperature was 180°C or below. The sample was gradually changed to transparent at the temperature of 222 and 208.2°C for tSD containing MDP, TPGS and copovidone at a ratio of 2:5:3 and 2:4:4, respectively. This is probably because TPGS and copovidone could not provide the miscible blends at low temperature, resulting from a limited dispersion of MDP in the carriers (Vasa et al., 2014). The tSD containing higher amount of copovidone presented in the homogeneous zone in the solid ternary phase diagram, suggesting that MDP was completely miscible with the carriers. Fig. 4.2f shows the hot stage microscopic images of the tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5. At temperature around 123.9°C, the MDP crystals became smaller and eventually disappeared at 170°C. This fact is a clear proof that, with the increased temperature, the addition of TPGS in the copovidone matrix created miscible blends and MDP can then be dissolved during melting. The results corresponded with the study by Papadimitriou et al. (2012).

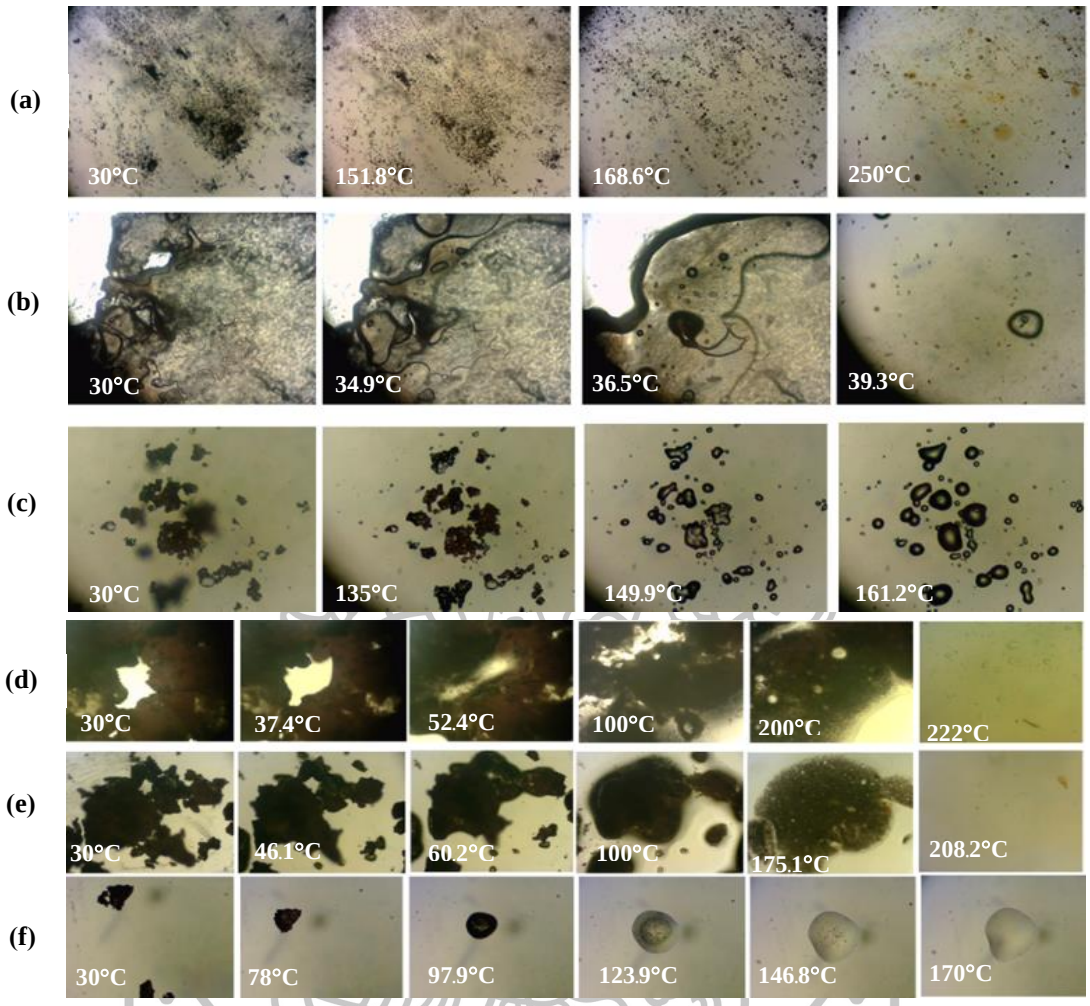


Figure 4.2 Hot stage microscopic images of (a) MDP, (b) TPGS, (c) copovidone, and tSD containing MDP, TPGS and copovidone at a ratio of (d) 2:5:3, (e) 2:4:4 and (f) 2:3:5.

4.3.3 Morphology study

As morphology of drug particles would have an impact on dissolution behavior, the morphology of samples was then investigated using SEM. Fig. 4.3a shows the surface morphology of MDP powder. Crystalline nature of MDP with irregular shape was observed. The morphology of TPGS and copovidone is displayed in Fig. 4.3b and 4.3c, respectively. The irregular shaped agglomerates of MDP in

copovidone (bSD) were clearly seen in Fig. 4.3d. It is evident that surface of tSD was smooth, uniform, and homogeneous; no drug crystal was observed (Fig. 4.3e). The homogeneous phase observed from tSD indicated the molecular dispersion of MDP in the blends of copovidone and TPGS (Bikiaris et al., 2005).

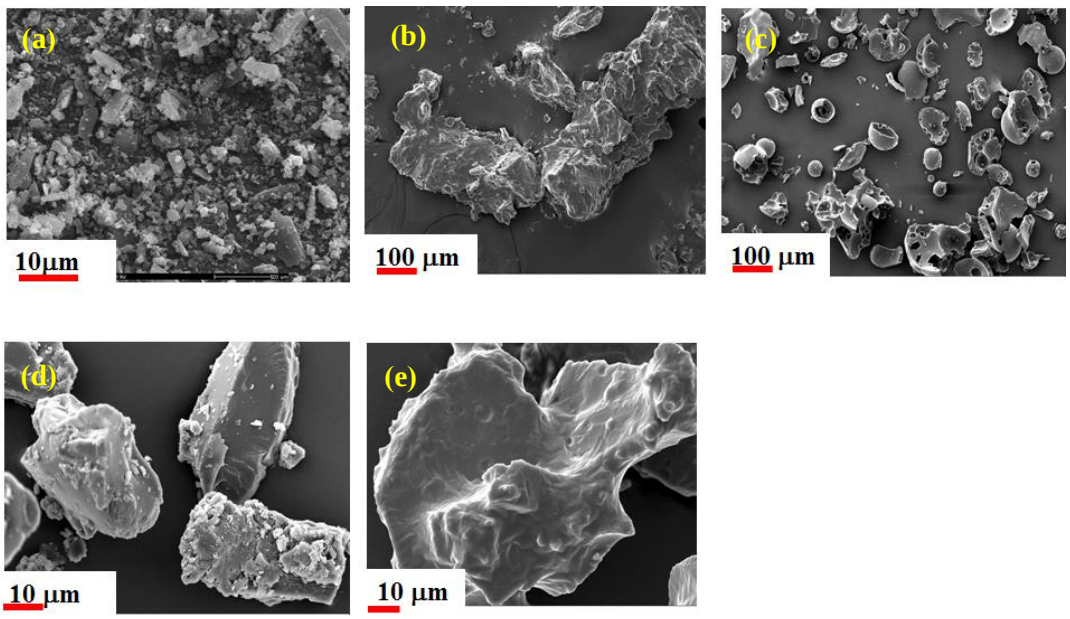


Figure 4.3 Scanning electron microscopic images of (a) MDP, 100×; (b) TPGS, 100×; (c) copovidone, 100×; (d) bSD, 1000×; (e) tSD 1000×

4.3.4 Physicochemical characterization

Fig. 4.4a shows the PXRD patterns of MDP, TPGS, copovidone, physical mixture of three components, and tSD containing 10 %w/w MDP. Major characteristic peaks of MDP were observed at 2θ diffraction angle of 10.9, 22.0, 22.7 and 22.8 (Rao et al., 2012), indicating the presence of crystalline MDP. PXRD pattern of TPGS demonstrated two prominent peaks with a high intensity at 2θ diffraction angle of 19 and 23, according to semi-crystalline nature of PEG. The major characteristic peaks of crystalline MDP completely disappeared in the PXRD pattern of tSD containing 50% w/w of copovidone (presented in homogeneous region in ternary phase diagram), only the peaks of TPGS at 19 and 23 2θ were observed. It is

likely that the crystalline MDP was transformed into an amorphous state or molecularly dispersed in the carriers by melting (Bikiaris et al., 2005; Shin and Kim, 2003). On the other hand, the peaks of the crystalline MDP were clearly observed in the PXRD patterns where the concentration of copovidone was 30 and 40%, in the non-homogeneous region in ternary phase diagram, suggesting that MDP was still in crystalline state.

DSC is useful for investigating properties of small molecular drug and polymer blend, providing information not only about the drug physical state in the blend, but also polymer behavior. In this study, DSC was performed to examine drug-polymer interaction and the physical state of MDP in tSD. Fig. 4.4b shows the DSC thermograms of MDP, TPGS, copovidone, physical mixture of three components, and tSD containing 10%w/w MDP. MDP presented a single sharp endothermic melting peak at 222°C. The DSC thermogram of physical mixture of MDP and carriers also showed the characteristic endothermic peak, corresponding to MDP melting. MDP melting temperature decreased was observed in the case of tSD containing 30% and 40% w/w copovidone. The presence of TPGS (30-50% w/w) in the formulation led to broad and shallow peaks at the lower melting temperature of MDP (around 200°C). It appeared that the crystallized drug in the solid dispersions, if any, redissolved in the carriers inside the DSC pans during heating (Gumaste et al., 2016). In case of tSD containing 50% w/w of copovidone, the melting peak of MDP was disappeared. This is probably due to the increased solubility of MDP in carriers at elevated temperatures. The results demonstrated that MDP was molecularly dispersed in TPGS and copovidone. The carrier system was able to depress the melting point of MDP and, therefore, interaction between MDP and carriers were present.

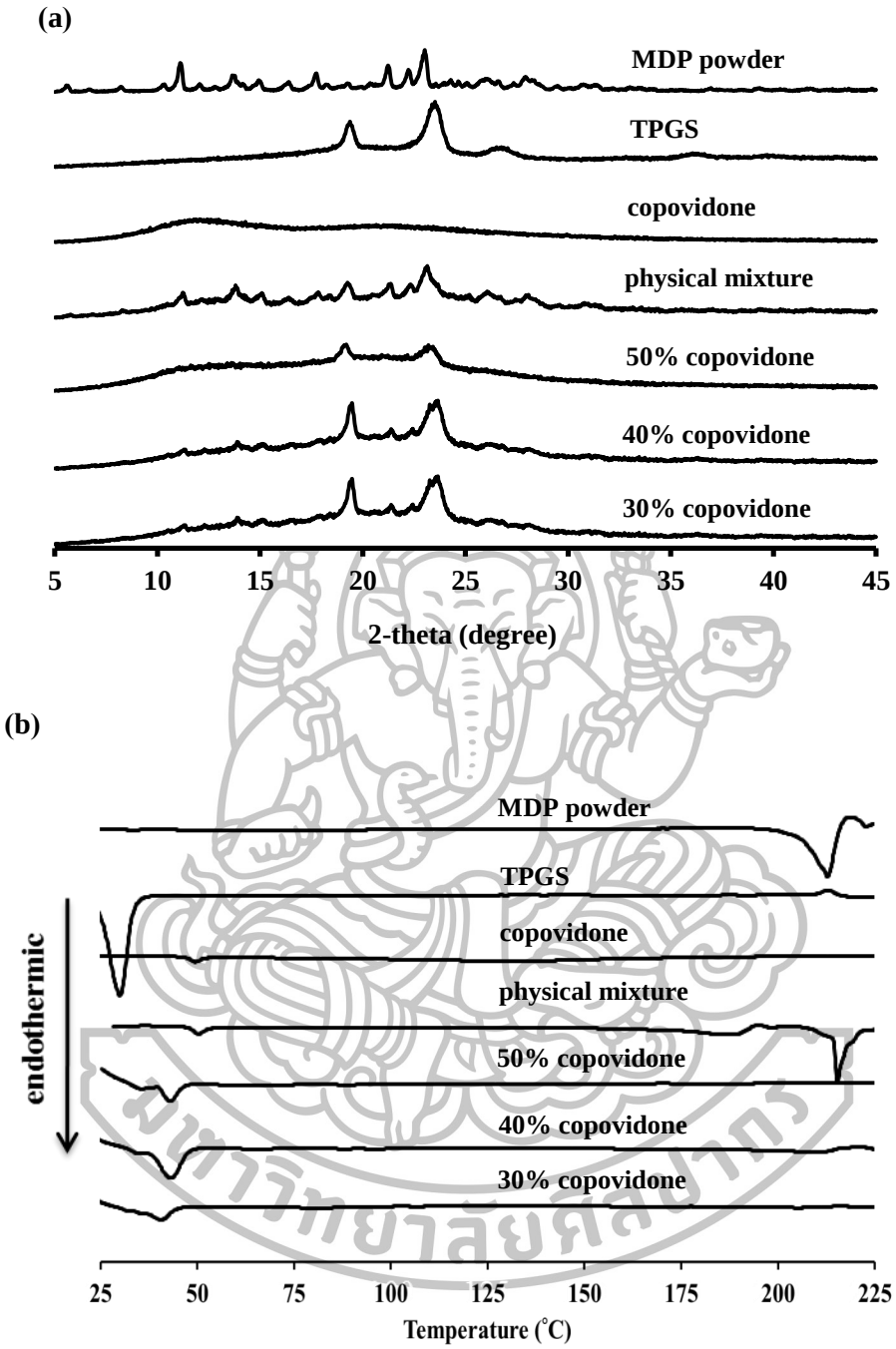


Figure 4.4 **(**a**)** PXRD diffractograms and **(**b**)** DSC thermograms of MDP, TPGS, copovidone, physical mixture of MDP, TPGS and copovidone, and tSD containing 10**%** w/w MDP**.**

In this research, FTIR spectroscopy was also used to characterize the possible interaction between MDP and TPGS or copovidone in the solid state. Fig. 4.5 demonstrates the FTIR spectra of MDP, TPGS, copovidone, physical mixture of MDP, TPGS and copovidone, and tSD of MDP, TPGS and copovidone at a ratio of 2:3:5. The FTIR spectrum of MDP presented a typical N–H stretching peak for crystalline MDP at 3341 cm^{-1}, C=O stretching of ester band at 1719 cm^{-1}, NO_2 asymmetric and symmetric stretching bands at 1533 and 1348 cm^{-1}, aromatic C=C stretching band at 1480 cm^{-1}, C–N stretching band at 1219 cm^{-1}, and out-of-plane bending of aromatic C–H bands at 756 and 707 cm^{-1}. N–H group may act as a hydrogen donor which is capable of forming hydrogen bonds with an appropriate acceptor group such as a carbonyl (C=O) group. TPGS showed an absorption band at 3480 cm^{-1} due to the hydroxyl group. Copovidone showed a broad band at 3479 cm^{-1}, the C=O stretching at 1737 cm^{-1} of the vinyl acetate moiety, C=O stretching of the amide function at 1667 cm^{-1}, C–C band at 1450-1480 cm^{-1}, and doublet peaks at fingerprint region of 600-750 cm^{-1}. The presence of more numbers of –OH stretching groups was attributed to the presence of water as both of these polymers are hygroscopic in nature and can absorb moisture from the environment (Chen et al., 2012). All the polymers have a hydrophilic surface with lots of hydrophilic groups, resulting in a diffusion of dissolution medium into the systems and accelerating drug release.

In case of a physical mixture, no shift was observed for the peak at 1719 cm^{-1} (C=O of MDP), indicating that MDP was not involved in the interaction with carriers. For the tSD, the changes in the carbonyl region at 1600-1800 cm^{-1} were clearly observed. As copovidone has proton acceptor group (C=O) at 1737 cm^{-1} and MDP has one proton donor group (N–H) at 3341 cm^{-1}, the hydrogen bonding was expected to occur between these two moieties. The shift of N–H band in the tSD and the peak shift of the C=O groups from 1719 to 1734 (non hydrogen bonding, C=O group) and 1652 to 1682 cm^{-1} (hydrogen bonding, C=O group) represented the formation of intermolecular hydrogen bonding between the N–H and C=O group of the tSD (Kothari, K., 2015).

The higher in MDP loading was observed in TPGS/copovidone blend that was due to the hydrogen bonding between MDP and copovidone, as described in Chapter 3. The addition hydrogen bonding might occur between amine group of pyridinedicarboxylate methyl ester of MDP and carboxyl group of TPGS molecule, which is not presented in PEG4000.

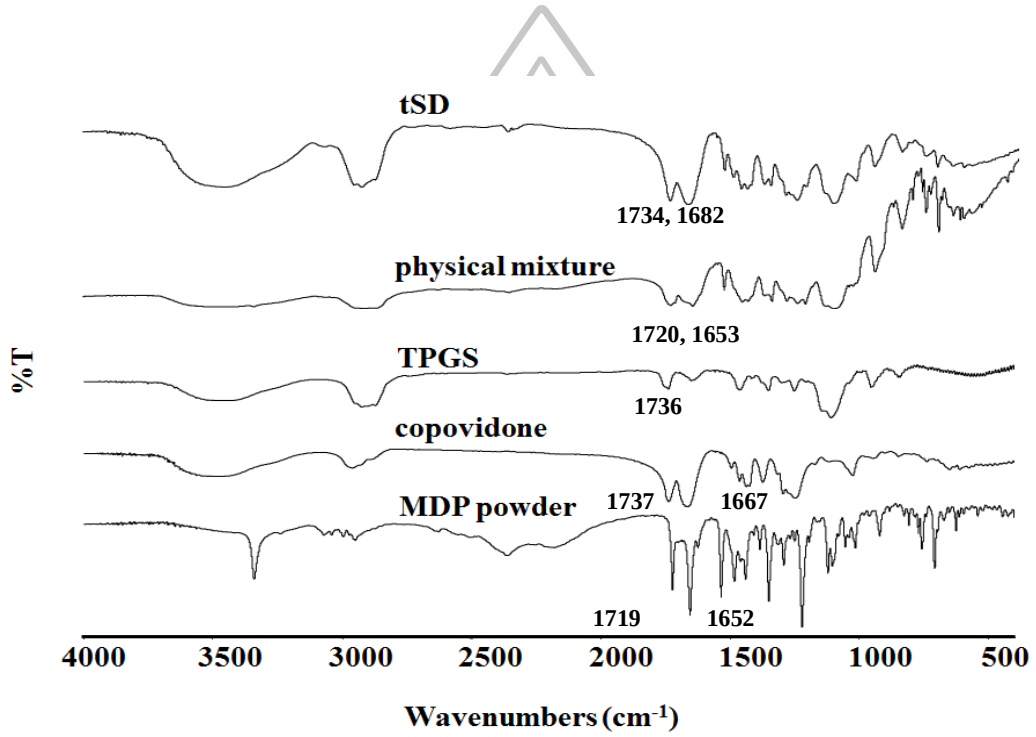


Figure 4.5 FTIR spectra of MDP, TPGS, copovidone, physical mixture of MDP, TPGS and copovidone, and tSD of MDP, TPGS and copovidone at a ratio of 2:3:5.

4.3.5 Solubility study

The presence of TPGS in the tSD could not only lower the interfacial surface tension of the drug but also increase the drug solubility by dispersing MDP inside the polymer matrix. In this study, the MDP solubility of tSD containing MDP, TPGS and copovidone, in distilled water, was determined after equilibrium condition.

The solubility of crystalline MDP at equilibrium was determined to be 0.034 ± 0.004 mg/mL (at 25°C). Taking the crystalline MDP solubility value as reference, the solubility of the tSD formulations was significantly increased ($p<0.05$). The improved MDP solubility was attributed to a combination of solubilizing property and surface activity of TPGS and copovidone. The increased concentration of copovidone in tSD resulted in a lower MDP solubility (Fig. 4.6). This is probably due to the increased amount of TPGS in tSD. Moreover, a better linear relationship between the MDP solubility and concentration of copovidone was observed for the tSD with 20% w/w MDP loading ($r^2=0.8997$), compared to that with 10% w/w MDP loading ($r^2=0.5855$). According to a higher drug loading, the tSD with 20% w/w MDP loading was chosen for further experiments.

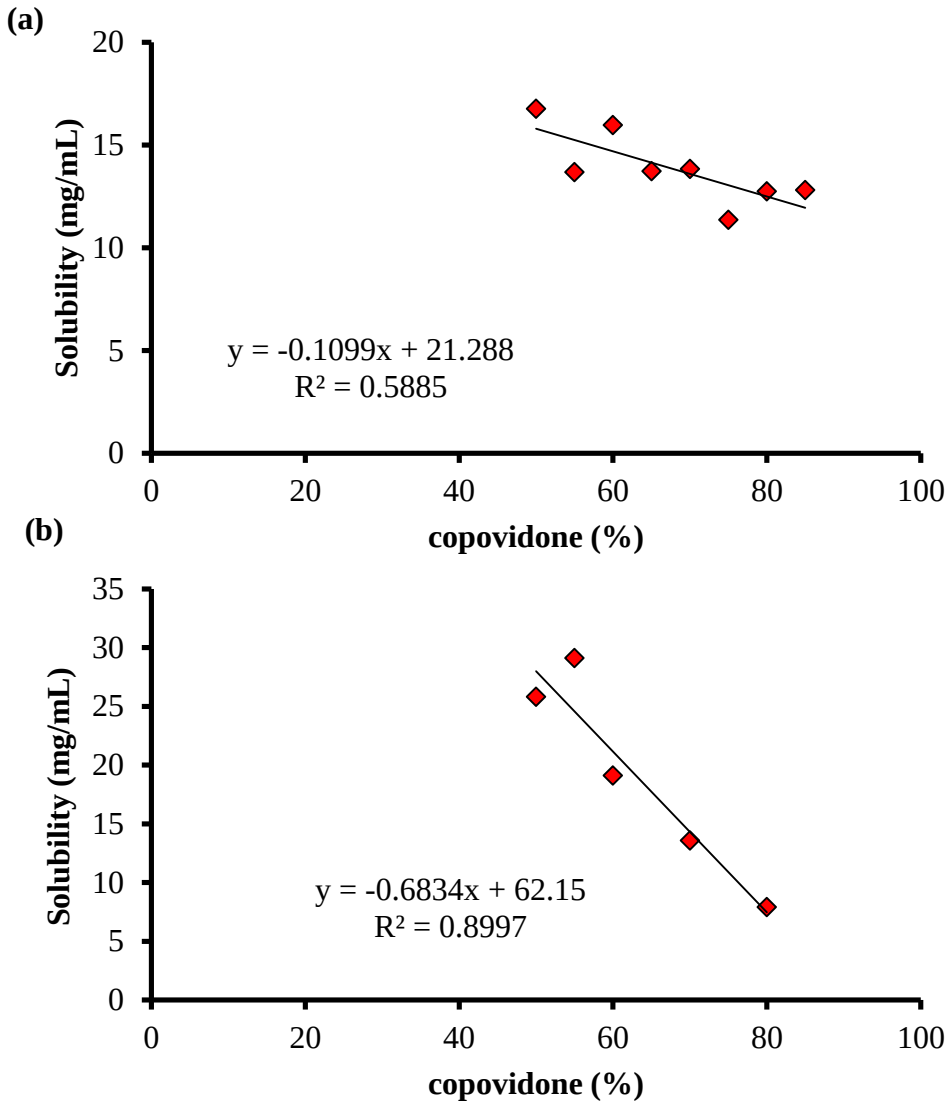


Figure 4.6 Scatter plots showing linear regression analysis between MDP solubility and proportion of copovidone in tSD containing MDP, PEG4000 and copovidone; (a) 10% w/w MDP and (b) 20% w/w MDP.

4.3.6 Dissolution study

Fig. 4.7 shows dissolution profiles, in 0.1 N HCl, of MDP powder, commercial product, bSD containing MDP and copovidone at a ratio of 2:8, and tSD

containing MDP, TPGS and copovidone at a ratio of 2:3:5. The MDP powder demonstrated a low dissolution percentage because of its low aqueous solubility. The commercial product exhibited the highest drug dissolution. During the first 45 min, the dissolution of MDP from bSD was significantly lower than MDP powder ($p<0.05$), resulting from the dominant effect of copovidone in the formulation. The bSD composes of a high amount of copovidone (80%), leading to the sticky characteristics after contact with the dissolution medium. The prepared tSD exhibited a significant higher drug dissolution than MDP powder ($p<0.05$). Moreover, the tSD showed a markedly enhanced dissolution, compared to the bSD. This may be due to the effect of TPGS with copovidone. The addition of TPGS with surface active property could reduce the surface tension of MDP and probably form micelles to increase MDP dissolution (Guo, et al., 2013). Lang and coworkers (2016) reported that itraconazole dissolution is greatly increased when TPGS level is increased from 10 to 40%. Besides, the addition of TPGS may exhibit more porous structure, leading to a higher dissolution rate (Lang et al., 2016). However, all formulations showed incomplete drug dissolution. This may be due to the viscous nature of the molten polymer and the partial precipitation of drug in the dissolution medium (Truong et al., 2016).

4.3.7 Stability studies

Stability studies can provide evidence on how the quality of solid dispersions varies with time under ambient or accelerated conditions. Fig. 4.8a shows PXRD patterns of tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, after storage at ambient condition (28°C) and under accelerated condition (40°C/75%RH) for 1 and 3 months. It is clearly seen that, after storage under both conditions up to 3 months, the PXRD patterns of the tSD presented the halo pattern, suggesting the molecular dispersion of MDP in the carriers even after storage for 3 months. Moreover, the peaks at 2θ diffraction angle of 19 and 23 were not observed. The disappearance of these peaks indicated a decrease in crystallinity of TPGS. This corresponds with the study reported by Gupta et al. (2012) who found a decrease in crystallinity of naproxen solid dispersion after storage at 40ºC/75%RH for 4 weeks, corresponding to the increase in hydrogen bonding between naproxen and carriers.

Also, from the DSC thermograms, the MDP melting peak was not seen after storage for 3 months in both conditions (Fig. 4.8b), indicating that the MDP were still molecularly dispersed in the carriers, same as after preparation. The melting peak of TPGS also disappeared after storage, corresponding to the PXRD results.

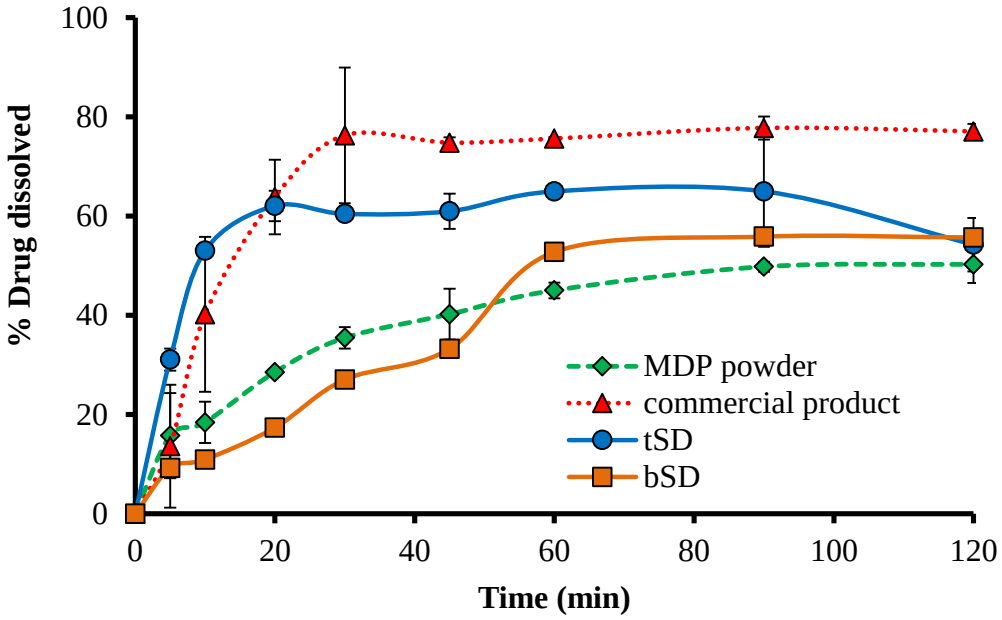


Figure 4.7 Dissolution profiles, in 0.1 N HCl, of MDP powder, commercial product, bSD containing MDP and copovidone at a ratio of 2:8, and tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5.

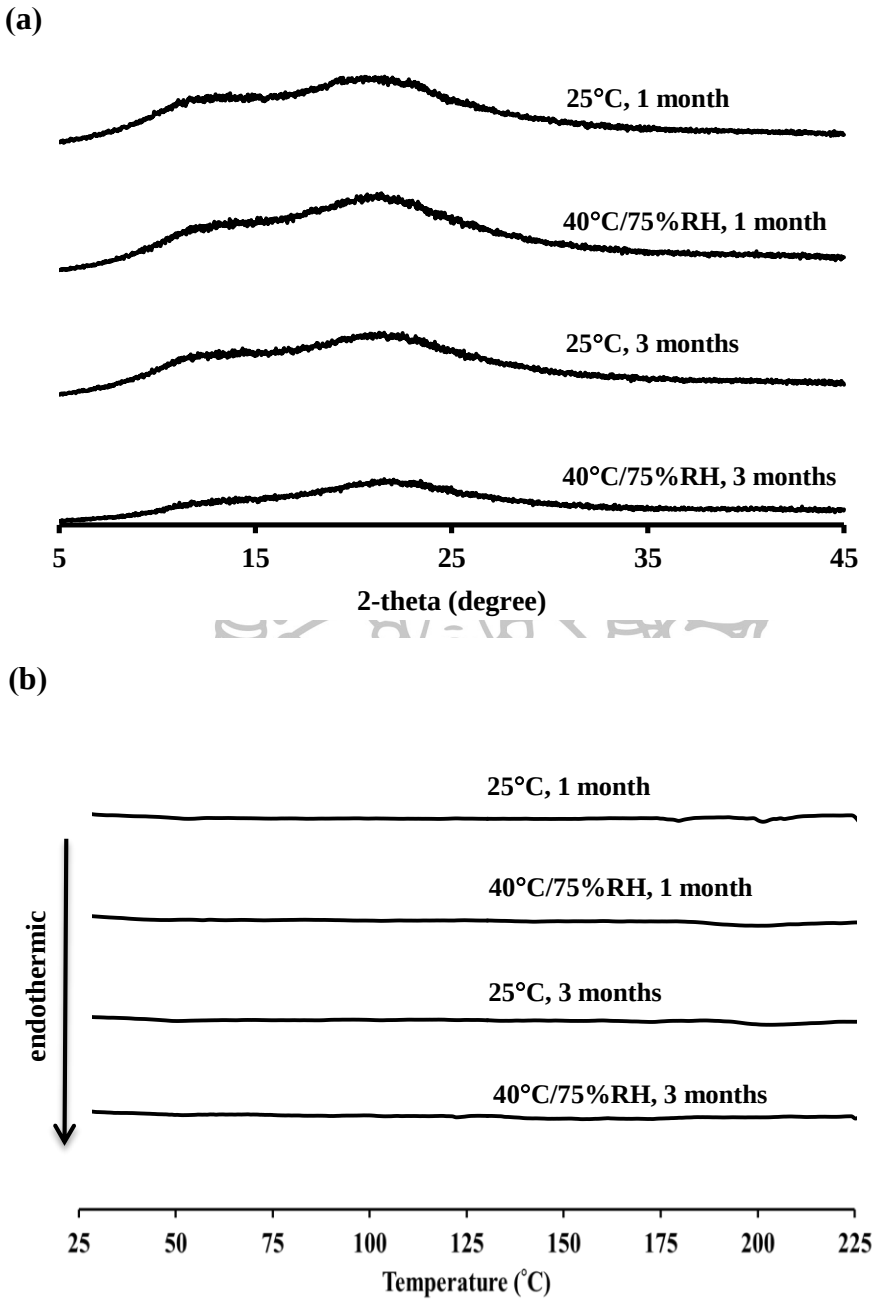


Figure 4.8 (a) PXRD patterns and (b) DSC thermograms of tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, after storage at ambient condition (28°C) and accelerated condition (40°C/75%RH) for 1 and 3 months.

As shown in Table 4.1, the solubility of tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, after storage at ambient temperature for 1 and 3 months, was significantly increased ($p<0.05$), compared to that of freshly prepared tSD. The higher in solubility after storage may be due to the increase of hydrogen bonding during storage (Gupta et al., 2002). After storage under accelerated condition (40ºC/75%RH), the solubility of tSD also increased significantly ($p<0.05$), and slightly higher (insignificance, $p>0.05$) than that kept at ambient temperature. Moreover, the solubility of tSD after storage for 1 and 3 months was not significantly different ($p>0.05$).

Table 4.1 Solubility of the tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, in water, after storage at ambient temperature (28°C) and under accelerated condition (40ºC/75%RH) for 1 and 3 months (n=3).

	Solubility (mg/mL) ± S.D.	
	After storage for 1 month	After storage for 3 months
Ambient temperature (28°C)	37.91 ± 1.99*	38.28 ± 2.02*
Accelerated condition (40ºC/75%RH)	39.93 ± 2.60*	41.17 ± 5.96*

Note: Solubility of freshly prepared tSD (0 month) was 25.81±1.15 mg/mL.
*$p<0.05$ (compared to freshly prepared tSD)

4.4 Conclusion

In the present study, solid ternary phase diagram was an implement for screening the suitable composition for tSD. The dissolution enhancement of tSD of MDP, with TPGS/copovidone was achieved by melting technique at low temperature it has relatively easy, simple, quick, inexpensive, and reproducible manner using melting method. The tSD could be a promising approach to improve solubility and

dissolution of MDP**.** The prepared tSD was stable at least 3 months that the drug still molecularly dispersed in the carriers system**.**

CHAPTER 5
EFFECT OF DIETARY STATE ON ORAL BIOAVAILABILITY OF SOLID DISPERSIONS CONTAINING MDP

5.1 Introduction

The BCS classifies drugs into four classes depending on the solubility and membrane permeability of the drug. Most oral formulations, first dissolve in the gastrointestinal tract, absorbs through the membrane of the small intestine thus enter the circulation. It was recently estimated that 40% of new chemical entities identified in drug discovery programs are insufficiently soluble in aqueous media. Thus, the design of oral formulations to allow the rapid dissolution of poorly water-soluble drugs is important. The term solid dispersion is defined as dispersion of drug in an amorphous polymer matrix, the dispersion of one or more active ingredients in an inert matrix in the solid state prepared by melting, solvent or melting-solvent method with the goal of enhancing oral bioavailability.

The pharmacokinetics of some drugs can be significantly impacted by the presence of food. This food effect has been known to occur through a variety of mechanisms such as modulation in gastric emptying time or splanchnic blood flow, binding of drug to food, and decreased absorption due to increased chyme viscosity. These collective mechanisms can result in an increase or decrease in drug bioavailability. One approach to improve the oral bioavailability of poorly-water soluble drug, is to administer with a meal (Rosillon et al., 1998; Giersbergen and Dingemanse, 2007). However, the food effect on drug absorption is rather complex, as it can involve specific interactions between the drug and food as well as the physiological changes in the gastrointestinal tract between the prandial states. Various strategies for predicting food effects on intestinal absorption have been reported in the literature (Jones et al., 2006; Klein, 2010).

In vitro dissolution tests using biorelevant media has proven to be a useful tool to predict *in vivo* performance of drug products (Dressman et al., 1998) and food effects on the dissolution and availability of orally administered drugs. It has been observed that biorelevant media can provide a more accurate simulation of pharmacokinetic profiles than simulated gastric fluid or simulated intestinal fluid. The use of biorelevant media can have a great impact on the pharmacokinetic studies performed to optimize dosing conditions and product formulation. Fasted-state simulated intestinal fluid (FaSSIF) is described more specific intestinal conditions. It

has a pH of 6.5 and contains lecithin and sodium taurocholate as the bile salt and has a more specific application in terms of simulation of the fasting intestine. On the other hand, fed-state simulated intestinal fluid (FeSSIF) simulates the juices of the intestine after food intake. It has a lower pH than FaSSIF, but multiple times higher amounts of simulated bile components, therefore, contains high amounts of sodium taurocholate and lecithin and has a pH of 5.8.

The application of FaSSIF and FeSSIF with respect to the *in vivo* predictiveness. The media predicted correctly the oral absorption of glibenclamide tablets in the fasted and fed states (Löbenberg et al., 2000).

The objective of the present study was to compare the dissolution of MDP from biorelevant media, i.e., FaSSIF and FeSSIF. The influence of food intake on drug absorption in rats was also investigated.

5.2 Materials and methods

5.2.1 Materials

MDP was supported by Sriprasit Pharma Co., Ltd. (Bangkok, Thailand). TPGS (batch no. BCBL2588V), Sigma-Aldrich (USA) was purchased from SM Chemical supply, Thailand. Copovidone (Kollidon® VA64, lot no. 51524456PO) was a gift from BASF (Thai) Co., Ltd. (Bangkok, Thailand). Madiplot® 10 mg (lot no. A162, Takeda, Japan). Other chemicals were of reagent or analytical grade and used without further purification. SIF® powder FaSSIF-V2 and FeSSIF-V2 (Biorelevant.com, United Kingdom) were used as test medium.

5.2.2 Preparation of bSD and tSD

Selected bSD (MDP and copovidone at a ratio of 2:8) and tSD (MDP, TPGS, and copovidone at a ratio of 2:3:5) formulations were prepared as described in section 4.2.2.

5.2.3 *In vitro* dissolution study

Dissolution test was performed using an USP apparatus II (model DT-720, Erweka, Germany). The dissolution media were FaSSIF and FeSSIF (500 mL). The temperature set as 37±0.5°C. The paddle speed was adjusted to 50 rpm. The amount

of MDP in each formulation was equal (approximately 3 mg), in order to keep sink condition. The samples were withdrawn from dissolution vessels at 5, 10, 20, 30, 45, 60, 90 and 120 min and passed through 0.45-µm nylon membrane before analysis with HPLC, as described in section 4.2.8, to determine the drug dissolved. The withdrawn volume was replaced by fresh medium to keep the total volume constant. The dissolution experiments were carried out in triplicate.

5.2.4 Pharmacokinetic study

5.2.4.1 Care of animals

All animal experiments were approved by the ethics committee for the use of laboratory animals, Faculty of Pharmacy, Silpakorn University, under the permission number 001/2015. Male Wistar rats weighing between 200 and 250 g were placed under a controlled environment at 22±2°C, with 60±10%RH, and had free access to food and water.

5.2.4.2 Animal experiment

The rats were divided into 2 groups, i.e., fasting group and feeding group. In case of fasting group, the rats were fasted for 16 h before experiment in order to avoid food influence on drug absorption (Nielsen et al., 2013). For the feeding group, the rats were fed with standard feeds. Immediately before dosing, the formulations were suspended in 0.3% w/w sodium carboxymethylcellulose using a magnetic stirrer. The animals were given four different formulations (i.e., MDP powder, bSD containing MDP and copovidone at a ratio of 2:8, tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, and commercial product at a dose equivalent to 10 mg/kg of MDP) by oral gavage to each group of rats (n=6). Before collecting the blood sample, the catheters were flushed with heparin solution not more than 1 day. Blood samples of approximately 600 µL were collected from jugular vein at 0, 0.5, 1, 2, 4, 6, 12 and 24 h after dosing. Plasma was separated from blood samples by centrifugation (Model Universal 320R, Hettich, Germany) at 10,000 rpm, 25°C for 10 min and stored at -20°C until analysis. The rats were euthanized after collection of the last sample.

5.2.4.3 Plasma analysis

Prior to extraction, frozen plasma samples were thawed at ambient temperature (28°C). A 200-µL plasma was pipetted into 1.5-mL conical centrifuge tube, then 800-µL of acetonitrile was added and vortexed in order to fully precipitate protein. After that, the mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was transferred to a clean centrifuge tube and dried under a vacuum oven at 40°C for 72 h. The residue was dissolved in 150-µL methanol and injected into the HPLC system for analysis (as described in section 4.2.8). The plasma concentration versus time data were plotted and various pharmacokinetic parameters, that is, maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the curve from time zero to 12 h ($AUC_{0–12h}$), elimination half-life ($t_{1/2}$), and elimination rate constant (K_{el}) were calculated from plasma concentration-time profile curve. The area under plasma concentration-time curve was calculated according to log trapezoidal method. The $t_{1/2}$ associated with K_{el} was computed by the following formula.

$$t_{1/2} = 0.693/K_{el} \quad (1)$$

5.2.5 Evaluation of blood pressure by tail-cuff method (Indirect blood pressure measurement) (Kubota et al., 2006)

The rats were given four different formulations (i.e., MDP powder, bSD containing MDP and copovidone at a ratio of 2:8, tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, and commercial product at a dose equivalent to 10 mg/kg of MDP) by oral gavage to each group of rats (n=3) as described in section 5.2.4.2. Normal control rats received distilled water *ad libitum* only. The blood pressure (BP) and heart rate (HR) of rats was monitored at 0, 0.5, 1, 4 and 24 h by tail cuff plethylsmometer (Model Digital Plethysmometer LE7500; Panlab Harvard Apparatus, Barcelona, Spain). The rats were placed in plastic restrainers, and a cuff with a pneumatic pulse sensor was attatched to the tail. Rats were allowed to habituate to this procedure for 7 days before experiments were performed. BP and HR values were recorded using ad instrument NIBD controller (Ad instrument NIBD controller, Australia) without heating and were averaged from at least three readings obtained from each rat.

5.2.6 Statistical analysis

The difference between sample groups was analyzed by ANOVA and Levene's test for homogeneity of variance. *Post hoc* testing ($p<0.05$) of the multiple comparison was performed by either the Scheffé or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively.

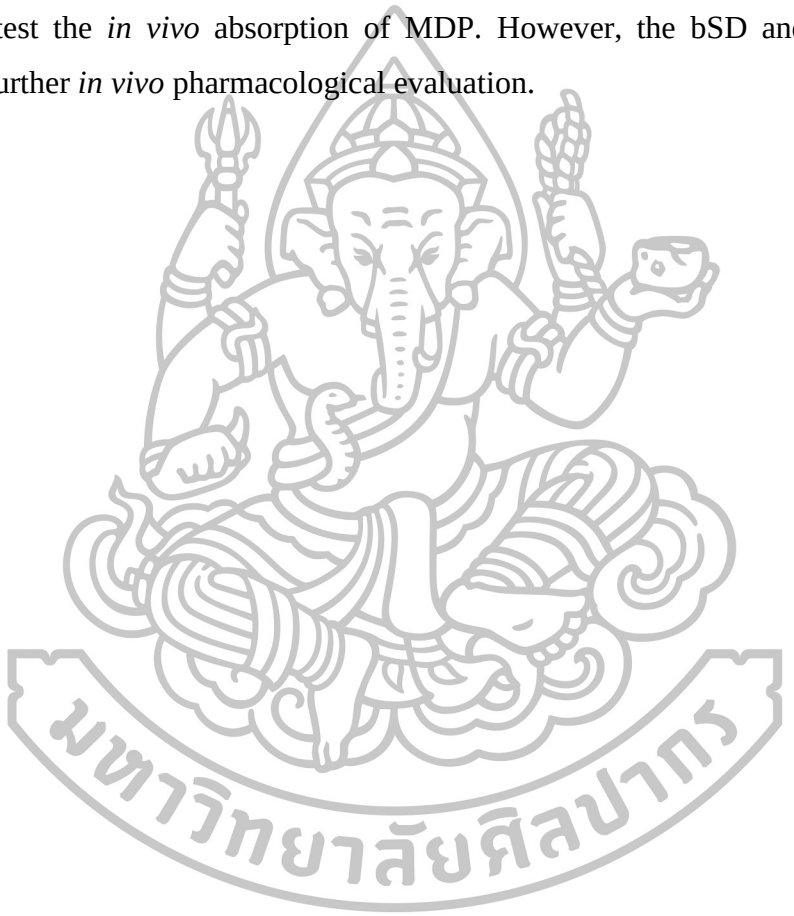
5.3 Results and discussion

5.3.1 *In vitro* dissolution

Food taken has an impact on drug bioavailability through a variety of mechanisms, including drug solubilization and prolonged gastric residence time (Raman and Polly, 2016). Then, in this research, the *in vitro* dissolution studies were performed in both FaSSIF and FeSSIF, biorelevant media, which could simulate the conditions in the human intestine environment in the fasted and fed states, and may provide valuable information on *in vivo* drug permeation. The dissolution profiles of MDP powder, bSD of MDP and copovidone at a ratio of 2:8, tSD of MDP, TPGS and copovidone at a ratio of 2:3:5 and commercial product are shown in Fig. 5.1. In FaSSIF, only 20% of MDP dissolved from MDP powder. The MDP dissolution at 120 min from tSD and bSD was 35% and 48%, respectively, as seen in Fig. 5.1a. On the contrary, the drug dissolution at 120 min from MDP powder and tSD was 41 and 42%, respectively, in FeSSIF. Surprisingly, the MDP dissolved from bSD was around 56% in FeSSIF (Fig.5.1b), which is significantly higher than that from MDP powder ($p<0.05$). The commercial product showed the highest drug dissolution (about 71%) for both biorelevant media. MDP powder was highly dissolved in FeSSIF, compared to FaSSIF. This is plobably due to a fact that MDP is a weak base with a pK_a value of 9.4 and fully protonated in acidic pH medium. A similar results was found by Nicolaides et al., (1999). The bSD showed a slight higher drug dissolution than tSD in both biorelevant media. These results might be the bSD contained higher concentration of copovidone than tSD, leading to the great increasing of wettability (Yasser et al, 2012). Lee et al., (2015) found that the addition of TPGS in the formulation did not induce a significant difference in the drug release at all pH. Thus, in this study, copovidone had a dominant effect on the drug dissolved more than

TPGS. The dissolution results in biorelevant media disagreed with the results in 0.1 N HCl (Chapter 4, section 4.3.6), which bSD presented the lowest dissolution profile. This distingue might be due to the effect of bile salt and lipolytic product of the biorelevant media (Janssens et al., 2008e).

The calculated area under the curve ($AUC_{0\text{-}2h}$) of MDP dissolved in both biorelevant media were presented in Table 5.1. The data revealed that tSD showed the highest value of $AUC_{0\text{-}2h}$ in both fasted and fed state. The highest value of tSD might be the greatest the *in vivo* absorption of MDP. However, the bSD and tSD were chosen for further *in vivo* pharmacological evaluation.



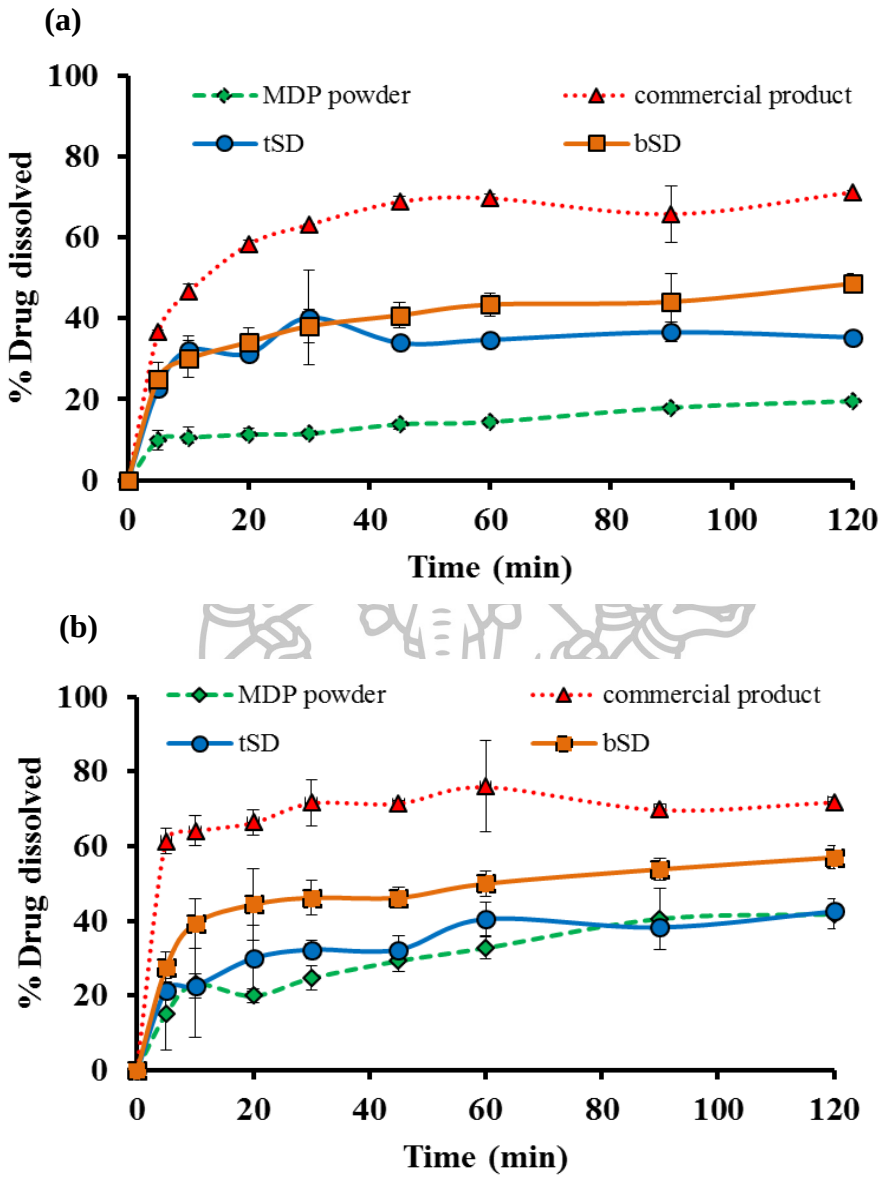


Figure 5.1 Dissolution profiles of MDP powder, the bSD containing MDP and copovidone at a ratio of 2:8, the tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, and commercial product in (a) FaSSIF and (b) FeSSIF.

Table 5.1 The area under the curve (AUC_{0-2h}) of dissolution of MDP powder, the bSD containing MDP and copovidone at a ratio of 2:8, the tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, and commercial product in (a) FaSSIF and (b) FeSSIF.

	AUC_{0-2h} (mg min)	
Formulations	FaSSIF	FeSSIF
MDP powder	0.07±0.01	0.34±0.13
bSD	0.41±0.05	0.69±0.86
tSD	1.42±0.10	3.28±5.06
Commercial product	0.99±0.33	1.71±0.16

5.3.2 Stability study

Fig. 5.2 shows the solubility of tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, in FaSSIF and FeSSIF, after storage at 28°C and 40°C/75%RH for 1 and 3 months. The results exhibited that the solubility of tSD at initial and after storage for 1 and 3 months was around 25 mg/mL. Althought, the solubility of tSD in FeSSIF at 1 month was lower than that initial state ($p<0.05$), at the end of test (3 months), the solubility of MDP was also the same as the initial. The solubility in FaSSIF was also lower than the initial, similar to the FaSSIF but it was not significantly different ($p<0.05$). These results might be due to the error of the test. This means that copovidone not only inhibit the crystallization upon dissolution of the tSD but also improve the physical stability of the formulation during storage (Knopp et al., 2016). The possible causes were attributed to hydrogen bonding between drug and polymer that can provide thermodynamic stability of the dispersed drug molecules, leading to the stable system for at least 3 month.

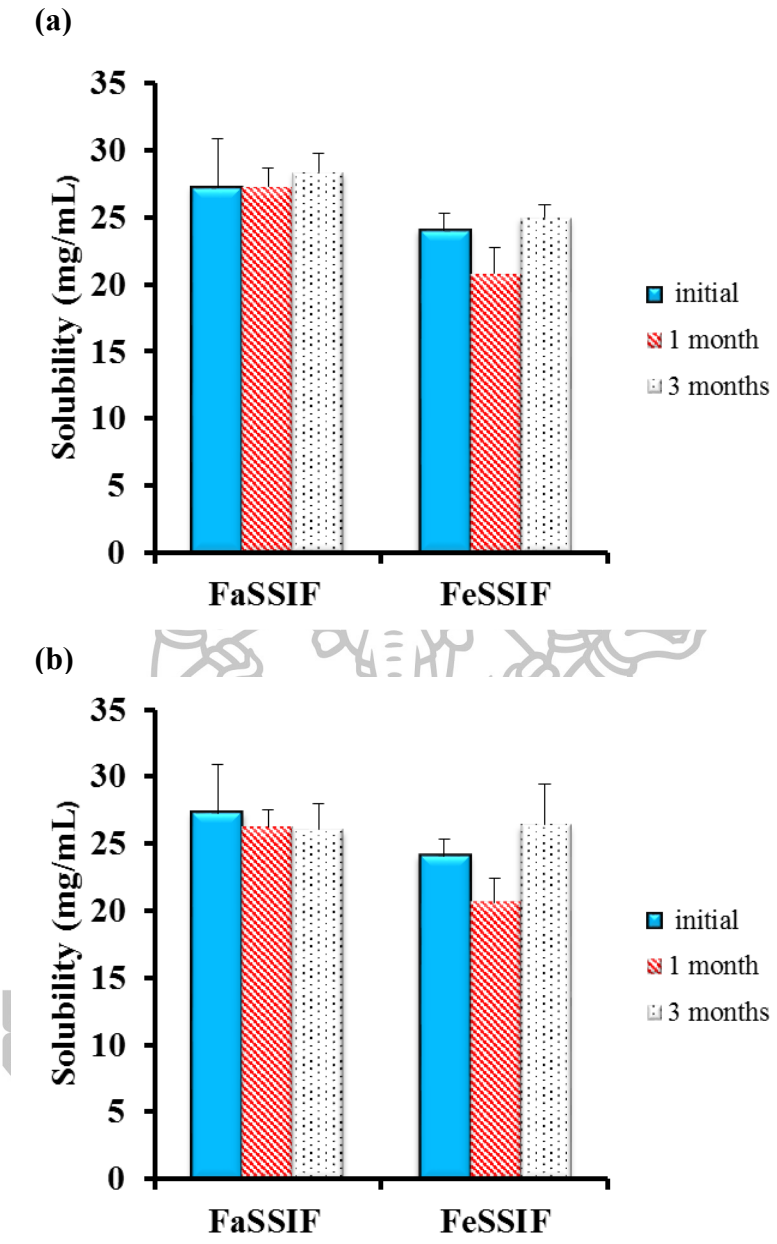


Figure 5.2 Solubility of tSD containing MDP, TPGS and copovidone at a ratio of 2:3:5, in FaSSIF and FeSSIF, after storage at (a) 25°C and (b) 40°C/75% RH, for 1 and 3 months.

5.3.3 *In vivo* absorption study in rats

The *in vivo* study was performed in order to compare the drug plasma concentration-time profiles of bSD and tSD with those of MDP powder and commercial product. Selected formulations were tested in both fasted and fed rats. The *in vivo* plasma concentration-time profiles, after oral administration in fasted and fed rats, of different formulations are demonstrated in Fig. 5.3 and the relevant pharmacokinetic parameters, i.e., C_{max}, T_{max}, $AUC_{0\text{-}12h}$, $t_{1/2}$, and K_{el} are listed in Table 5.2.

In the fasted state, MDP powder presented a low plasma concentration. The median T_{max} for MDP powder was 2 h, ranging from 1 to 12 h. The C_{max} and $AUC_{0\text{-}12h}$ of MDP powder were 0.31 μg/mL and 2.85 h μg/mL, respectively. The $AUC_{0\text{-}12h}$ of commercial product was slightly higher than that of MDP powder ($p>0.05$). For both bSD and tSD, the $AUC_{0\text{-}12h}$ of MDP was significantly improved ($p<0.05$) when compared to MDP powder, that is, 2.82 and 4.39 folds, respectively. The bSD and tSD also showed significantly higher C_{max} than MDP powder ($p<0.05$) but no difference in the median T_{max}. The high value of C_{max} and the shortened T_{max}, could be attributed to the hydrophilic property of carriers (TPGS and/or copovidone), leading to the improvement in bioavailability of MDP. The data also indicated that the *in vivo* oral absorption of MDP was markedly higher in the tSD than the bSD ($p<0.05$), due to the difference in composition of the bSD and tSD, resulting in the difference in bioavailability.

In vivo plasma concentration-time profiles following administration to fed rats are shown in Figure 5.3b. The absorption of MDP powder under fed condition was slightly higher than in fasted condition. No significant difference was observed between the median T_{max} in the fed rats. The $AUC_{0\text{-}12h}$ and C_{max} after administration of bSD, tSD and commercial product was found to be significantly higher than that MDP powder ($p<0.05$). This finding was in good agreement with the study of Rosillon et al., (1998), which showed the effect of food on the oral bioavailability of MDP in 12 male healthy subjects. The $AUC_{0\text{-}12h}$ value of MDP were in order of tSD > commercial product > bSD > MDP powder. It was different from the *in vitro* dissolution results in 5.3.1 that agreeed with Six et al., (2005), who found the poor

predictability of the absorption behavior due to (i) reduction of solubility of drug that left in the stomach, (ii) incomplete disintegration of the administered drug *in vivo*, or (iii) a change in the physical state of the drug (amorphous to crystalline transformation) when the solid dispersion was granulated and tableted. This study also pointed to the fact that the use of *in vitro* dissolution data in view of prediction of *in vivo* behavior is of limited value and needs careful interpretation.

However, the tSD showed the highest $AUC_{0\text{-}12h}$ while MDP powder showed the lowest $AUC_{0\text{-}12h}$ in both fasted and fed states. These results indicated that both TPGS and copovidone provided the improved oral absorption of MDP. Although TPGS did not produce different drug release patterns (Fig. 5.2), the increase in oral drug absorption may be due to the plasticizing properties of TPGS, which can reduce process temperature and melt viscosity during the melting process. The passive diffusion mechanism was obtained when TPGS was incorporated in the formulation (Duan et al., 2016; Guo et al., 2013; Lee et al., 2015). It generally known that TPGS is one of P-gp inhibitors, that may enhance the drug permeation across transmucosal of many drug such as cyclosporine A, vancomycin hydrochloride and doxorubicin (Chang, 1996; Prasad et al., 2003; Zhang et al., 2015). Lang et al., (2016) found that T_{max} in the fasted state seems to be longer than in the fed state; it took around 1.5-4 h in fasted state, and only 0.5-1 h in the fed state when used TPGS in the formulation. The higher of K_{el} value in bSD and tSD (both fasted and fed conditions) lead to the rapid elimination of MDP. The shorter $t_{1/2}$ of bSD and tSD was observed, compared to MDP powder and commercial product.

The plasma MDP concentrations of MDP powder and commercial product under fed condition was higher than those fasted condition, i.e., AUC ratio (fed/fasted) of 1.36 and 2.20, respectively. The results suggested that the food effect on bioavailability of MDP appeared to be significant. However, the AUC ratio was 0.83 and 0.67 for bSD and tSD, respectively. It is suggested that the reduction of food effect was found when using solid dispersion formulations. Moreover, in fasted state, the absorption of MDP was higher than in fed state. These might be due to the high concentration of TPGS in the fed state. According to the study by Malik et al., (1975), at low concentration, TPGS can increase the absorption of poorly water-soluble drug

by altering the membrane permeability, resulting in highly absorption of drug. On the contrary, the high concentration of TPGS can decrease the absorption of drug because the drug molecule entrapped into the micelle of TPGS caused the unavailable drug to absorb.

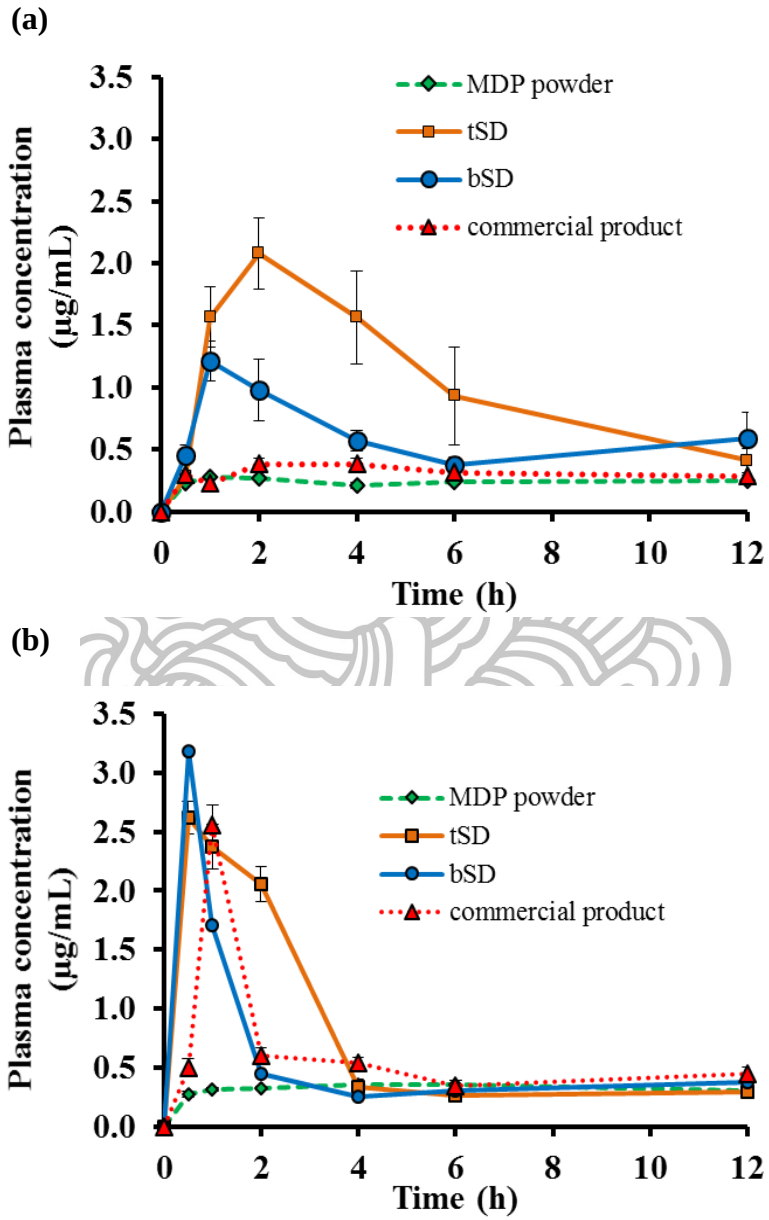


Figure 5.3 *In vivo* plasma concentration–time profiles of MDP powder, commercial product, bSD and tSD formulations in (a) fasted condition and (b) fed condition (bars indicate S.E.; n= 6).

Table 5.2 Pharmacokinetic parameters of different formulations in fasted and fed conditions *in vivo* (n=6)

Parameter	Fasted conditions					Fed conditions					AUC ratio (fed/fasted)
	AUC_{0-12h} (h µg/mL)	C_{max} (µg/mL)	T_{max} (h) $^{\delta}$	K_{el} (h^{-1})	$t_{1/2}$ (h)	AUC_{0-12h} (h µg/mL)	C_{max} (µg/mL)	T_{max} (h) $^{\delta}$	K_{el} (h^{-1})	$t_{1/2}$ (h)	
MDP powder	2.85±0.15	0.31±0.01	2[1-12]	0.10	6.39	3.89±0.10	0.35±0.04	4 [1-6]	0.01	39.15	1.36
bSD	8.04±0.44*,**	1.29±0.33*,**	1.5 [1-4]	0.24	2.89	6.66±0.71*	3.18±0.39*	0,5 [0.5-1]	0.41	1.69	0.83
tSD	12.50±2.31*,**	2.22±0.77*,**	2 [1-6]	0.14	4.89	8.32±0.42*	2.62±0.13*	0.5 [0.5-1]	0.20	3.47	0.67
commercial product	3.13±0.20	0.43±0.10	3 [2-6]	0.05	13.86	6.89±0.30*	2.55±0.77*	1 [1]	0.10	6.39	2.20

Notes: Each value represents the mean±S.E. (n=6).

$^{\delta}$ Median range in brackets

*p<0.05 when compared to MDP powder.

**p<0.05 when compared to commercial product.

5.3.4 Blood pressure measurement

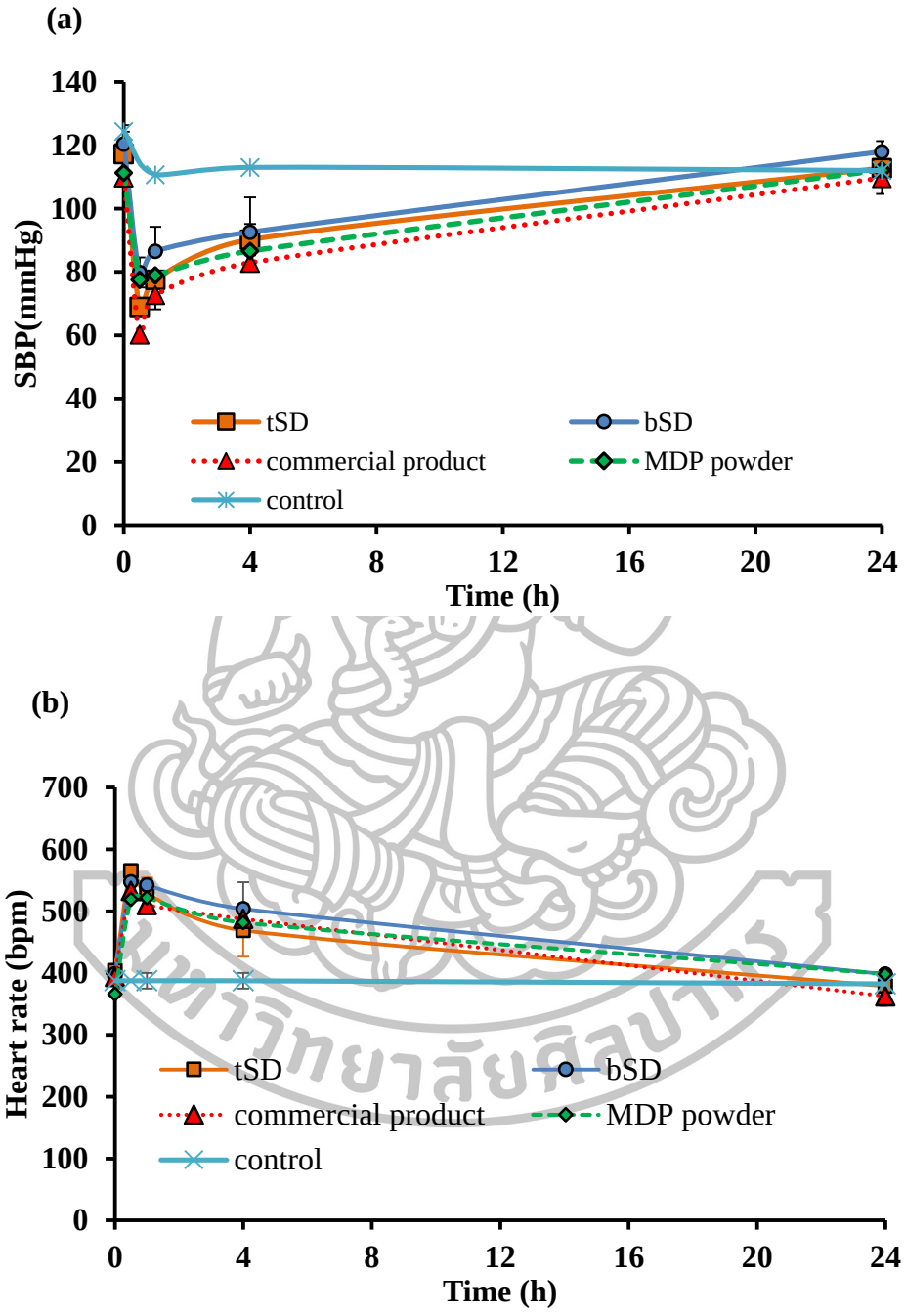


Figure 5.4 Effect of oral administration of MDP on (a) SBP (b) HR in rats. Each point represents the mean±S.E. (n=3).

Tail-cuff method is non-invasive measurement of blood pressure in conscious rats. It also simple and inexpensive technique. The normal SBP and HR of rats are in the range of 110-120 mmHg and 380 beats per minute (bpm), respectively (Fritz and Rinaldi, 2008; Geleta et al., 2016; Van den Buuse, 1999). From Fig. 5.4a the SBP at initial and 24 h are in the range of normal SBP. The significantly decreased in SBP from all formulations (MDP powder, bSD, tSD and commercial product) were observed in 30 min after oral administration of MDP when compared to the control group ($p<0.05$). But there are not significant difference between the formulations ($p>0.05$). For the HR, the same results as SPB was observed as seen in Fig. 5.4b. At initial and 24 h, the HR of rats are in the range of normal (380 bpm). After oral administration of MDP, the HR were significantly increased when compared to the control group ($p<0.05$), but there are not significant difference between the formulations ($p>0.05$). MDP is dihydropyridine calcium channel blocker that tend to be more potent vasodilators (Frishman, 2007). The decrease in blood pressure may be due to the vasodilators effect of MDP that results in the inhibitory effect on smooth muscles through blocking of calcium channel and then reflex on tachycardia. In this study, when the rats received the antihypertensive drug, MDP, not only the gradually decreased in SBP, but the HR also increased in 30 min. From *in vivo* study (section 5.3.3) revealed that MDP has the highest K_{el} value, then the rapidly excreted of it has occurred. Finally, the blood pressure and HR came to normal range in 24 h

5.4 Conclusion

In conclusion, the tSD containing MDP, TPGS and copovidone showed the greatest effect on the oral bioavailability, as indicated by the pharmacokinetic parameters, i.e., $AUC_{0\text{-}12h}$, C_{max}, T_{max}, K_{el} and $t_{1/2}$. The dominant composition, that is TPGS, had an enormous effect on the absorption of MDP from tSD in both fasted and fed states. The food effect of MDP in fed state, compared to fasted state, was reduced by using solid dispersion formulations. The best absorption of MDP powder and commercial product was found especially giving with food. Moreover, it was stable

for three months in accelerated stability test. Then the tSD is a promising way to enhance MDP bioavailability.

CHAPTER 6

SUMMARY AND GENERAL CONCLUSION

The solid dispersion method is one of the effective approaches to enhance the solubility of poorly water-soluble drugs. In developing a new solid dispersion system, it is important to understand the physicochemical properties of the drug and carrier. The preparation method and the amount of the carrier also play a vital role in the enhancement of drug dissolution rate. In this research, two excipients were combined in order to provide the molecular dispersion of MDP in homogeneous solid dispersions by melting method.

The selection of carrier and processing temperature is important concerns in the preparation of tSD (Chapter 3). Although the drug solubility and dissolution has been improved by bSD containing drug and carrier, the drug may change the polymorphic from during storage or precipitate during dissolution process. In attempt to extend the stability of drug in bSD, the third component has been added, in order to form tSD. In this part, it is clearly demonstrated that the tSD formulations containing MDP, PEG4000 and copovidone are successfully prepared. The solid ternary solid phase diagram has been constructed, in order to find the miscibility of MDP in the carrier system. The ternary phase diagram can be divided into homogeneous and non-homogeneous regions. In the homogeneous region of ternary phase diagram, MDP is molecularly dispersed in PEG4000/copovidone blend. The solubility of MDP is significantly increased by this system. This tSD also has superior *in vitro* dissolution of MDP and remains physicochemically stable for up to 6 months under accelerated stability testing conditions.

As mentioned above, the solid solubility of MDP in PEG4000/copovidone is often limited, only 10% w/w of MDP are molecularly dispersed. Therefore, this part has aimed to reduce the crystallinity of MDP and also increase the solubility and stability. The use of nonionic surfactant TPGS, instead of PEG4000 could solve these problems, due to the amphiphilic structure of lipophilic alkyl tail and hydrophilic polar head with a hydrophilic/lipophilic balance value of 13.2. It has a low melting point (37-41°C) and a relatively low critical micelle concentration of 0.02% w/w. In

Chapter 4, solid ternary phase diagram of MDP, TPGS copovidone has been constructed. More drug loading can be achieved in homogeneous region of the ternary phase diagram. The molecular dispersion of drug in these carrier blends could be a promising approach to improve solubility and dissolution of MDP. The prepared tSD is stable for at least 3 months under accelerated stability testing conditions, MDP still molecularly disperses in the carrier system.

The effect of dietary state on oral bioavailability of solid dispersion was investigated *in vitro* and *in vivo* in Chapter 5. The *in vitro* dissolution of tSD in biorelevant media was lower than that in 0.1 N HCl (Chapter 4) may be due to the effect of bile salt and lipolytic product in the biorelevant media. In this chapter, the tSD containing MDP, TPGS and copovidone shows the greatest effect on the oral bioavailability, as indicated by the pharmacokinetic parameters, i.e., $AUC_{0\text{-}12h}$, C_{max}, T_{max}, K_{el} and $t_{1/2}$. The dominant composition, i.e., TPGS, has an enormous effect on the absorption of MDP, both in fasted and fed state. The effect of dietary state, fasted and fed state, on oral bioavailability of solid dispersions is reduced by using solid dispersion formulations. The absorption of MDP, from tSD formulations, in both fasted and fed state is higher than MDP powder (4.39 and 2.13 folds, respectively) and commercial product (3.99 and 1.20 folds, respectively). Summing up the results, it is clearly seen that the tSD is a promising approach to enhance MDP bioavailability.

Future direction of research

This study has developed the tSD of drug and polymer blend, and drug-surfactant-polymer system, which can increase dissolution and absorption of MDP. The other surfactants or polymer-surfactant systems could be applied to tSD for dissolution improvement and the ternary phase diagram could be used to find the appropriate proportion of each component. Moreover, the ternary phase diagram of other BCS class II drugs should be investigated to confirm the capability of the developed tSD system.

Even though the current study recommended that tSD would be a good option for increasing solubility as well as bioavailability but the clinical study has been required before its clinical application in the upcoming days. Furthermore, the

correlation between *in vitro* dissolution study and *in vivo* absorption study, IVIVC, should be performed in order to predict *in vivo* results based on *in vitro* data.

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APPENDIX

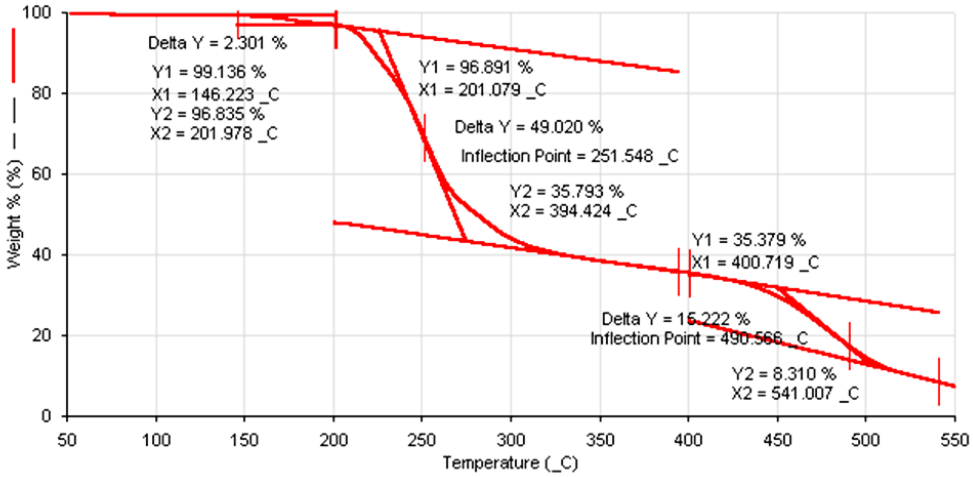

Figure A.1 Thermogravimetric Analysis (TGA) of MDP

Standard curve of MDP in methanol

Method : HPLC analysis

Analytical column : Phenomenex Luna C18 column, 5 μm, 4.6 mm × 150 mm

Mobile phase : acetonitrile: potassium dihydrogen phosphate solution (49:51% v/v)

Flow rate : 1.0 mL/min

UV Detector : wavelength 228 nm

Table A.1 The concentration of MDP in methanol.

Concentration (mg/mL)	area
0.0005	13006.50
0.0010	36898.00
0.0020	51494.50
0.0040	95194.50
0.0080	194990.50
0.0159	392491.00
0.1270	3139518.50
0.2540	6371918.50
0.5080	12734536.00

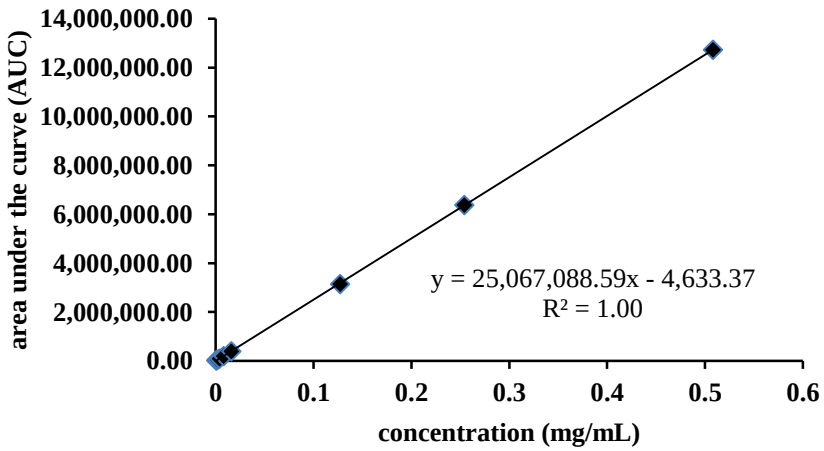

Figure A.2 Standard curve of MDP in methanol.

Standard curve in plasma

Method : HPLC analysis

Analytical column : Phenomenex Luna C18 column, 5 μm, 4.6 mm × 250 mm

Mobile phase : acetonitrile: potassium dihydrogen phosphate solution (49:51% v/v)

Flow rate : 1.0 mL/min

UV Detector : wavelength 228 nm

Table A.2 The concentration of MDP in plasma.

Concentration (mcg/mL)	area
0.505	10805
1.010	22265.67
2.020	37817.17
10.10	168641.7
50.50	851275.3

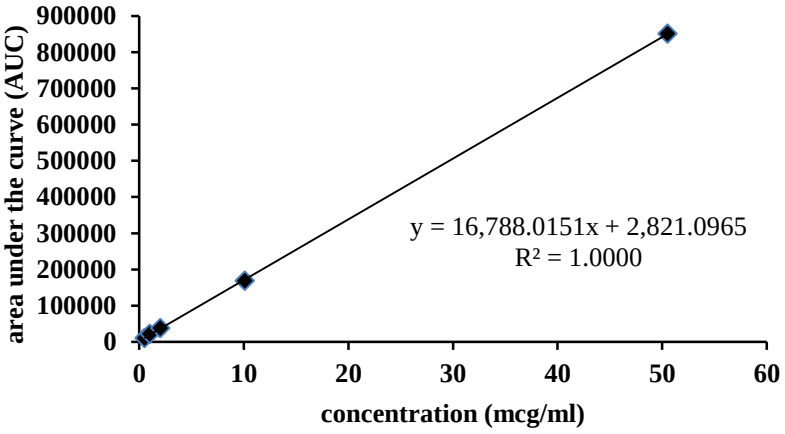

Figure A.3 Standard curve of MDP in plasma.

Certification of approval for the care and use laboratory animals at Silpakorn University

Certification of Approval for the Care and Use of Laboratory Animals at Silpakorn University

by Ethics Committee for the Use of Laboratory Animals
Faculty of Pharmacy, Silpakorn University

Title of Project	Dissolution and bioavailability enhancement of manidipine hydrochloride solid dispersion prepared by melt technique
Principal Investigator	Professor Pornsak Siramornsak, PhD
Protocol Number	001/2015
Name of Faculty	Faculty of Pharmacy, Silpakorn University.

The aforementioned protocol have been reviewed and approved by Ethics Committee for the Use of Laboratory Animals, Faculty of Pharmacy, Silpakorn University.

Date of Approval 3 December 2015

(Pornsak Sriamornsak, Ph.D.)
Chairman
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Faculty of Pharmacy, Silpakorn University

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Presentations (poster)

1. **Benchawan Chamsai** and Pornsak Sriamornsak. Exploding microcrystalline cellulose pellets as a mean of increasing the dissolution rate of poorly water- soluble drugs. The 27th Annual Research Conference in Pharmaceutical Sciences, 3 December 2010, Bangkok, Thailand.

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