FLUID PROPERTIES, SOLVENT EXCHANGE AND TRANSFORMATION OF *IN SITU* FORMING GEL AND MICROPARTICLES USING BETA-CYCLODEXTRIN AS A MATRIX FORMER

By
Mr. Sai Myo Thu REIN

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy PHARMACEUTICAL ENGINEERING
(INTERNATIONAL PROGRAM)
Graduate School, Silpakorn University
Academic Year 2018

คุณสมบัติของไหล การแลกเปลี่ยนตัวทาละลาย การเปลี่ยนแปลงเป็นเจล และไมโครพาติเคิลชนิดก่อตัวเองโดยใช้ เบต้า-ไซโคลเด็กซ์ตรินเป็นสารก่อเมทริกซ์

โดย
Mr.Sai Myo Thu Rein

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปรัชญาดุษฎีบัณฑิต
สาขาวิชาวิศวเภสัชกรรม ปริญญาปรัชญาดุษฎีบัณฑิต แบบ 1.1 (หลักสูตรนานาชาติ)
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2561

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Field of Study	PHARMACEUTICAL ENGINEERING (INTERNATIONAL PROGRAM)
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58365803 : Major PHARMACEUTICAL ENGINEERING (INTERNATIONAL PROGRAM)
Keyword : Fluid Properties, Solvent Exchange, Matrix Forming, Betacyclodextrin, In situ forming gel, In situ forming microparticles

MR. SAI MYO THU REIN : FLUID PROPERTIES, SOLVENT EXCHANGE AND TRANSFORMATION OF *IN SITU* FORMING GEL AND MICROPARTICLES USING BETA-CYCLODEXTRIN AS A MATRIX FORMER THESIS ADVISOR : ASSOCIATE PROFESSOR THAWATCHAI PHAECHAMUD, Ph.D.

β-cyclodextrin (β-CD) is interesting for using as a matrix former of the solvent exchange-induced *in situ* forming drug delivery system because of its apparent high solubility in some injectable solvents including 2-pyrrolidone (PYR), N-methyl pyrrolidone (NMP) and dimethyl sulfoxide (DMSO) while it exhibits limited aqueous solubility. The objective of this research was to investigate the fluid properties of β-CD solutions, physicochemical properties of β-CD precipitates prepared from three injectable solvents, physical properties of various solvents and oils used for in situ forming gel (ISG) and *in situ* forming microparticles (ISM) preparations. In addition, this research aimed to develop and evaluate the meloxicam (Mex)-loaded β-CD solvent exchange-induced ISG and ISM using β-CD as a matrix former for periodontitis treatment. Fluid properties of β-CD solution in NMP, DMSO and PYR were evaluated for pH, density, surface and interfacial tensions, contact angle, apparent viscosity and rheological behavior, water resistantance, matrix formation rate and injectability. The higher the concentration of β-CD resulted in the increased viscosity with the higher force and energy of injectability. β-CD in DMSO exhibited the highest ease of injectability and highest rate of matrix formation. The study of physicochemical properties of β-CD dried precipitates from above three solvents with different techniques revealed that there was a partial complexation of β-CD with respective solvents. According to the fluid and physicochemical properties, DMSO was more suitable for formulation development because of high concentrated β-CD loading capacity. The understanding of properties of solvents and oils used in ISM was also important. Therefore, the characterization of fluid and physicochemical properties of NMP, PYR, DMSO, glycofurol, triacetin, olive oil, camellia oil and isopropyl myristate (IPM) were also evaluated. The intrinsic viscosity of β-CD solution in DMSO was the lowest value of 0.07 dL/g while that in NMP was 0.084 dL/g. The high viscosity dissipation of PYR and triacetin slowed the diffusion of the amaranth solution. The miscibility test revealed the phase separation between DMSO and oils including camellia oil, olive oil and IPM. DMSO showed antibacterial activities against *E.coli* and C. albicans whereas all tested solvents exhibited the larger inhibition zones against *C. albicans* than bacteria. PYR inhibited methicillin-resistant *Staphylococcus aureus* (MRSA) such as *S. aureus* ATCC 43300 strain more effectively than NMP and glycofurol, respectively. DMSO showed its lowest cytotoxic activity against colorectal HCT 116 cell line which was followed by PYR, NMP, triacetin and glycofurol, respectively. β-CD solution in DMSO was used as the *in situ* forming system for Mex and used as the internal phase for Mex-loaded ISM whereas the camellia oil comprising 5% glycerol monostearate (GMS) was used as the external phase. Mex/β-CD solution showed the phase inversion into the matrix like after exposure to simulated crevicular fluid by solvent exchange. The attained

prolonged release of Mex with minimized initial burst was achieved for Mex-loaded ISG. In addition, the external phase provoked the slower drug release of ISM than ISG. The drug release obeyed the Fickian diffusion in both Mex-loaded ISG and ISM systems comprising 40% β-CD. Upon exposure to simulated crevicular fluid, the ISM system comprising 40% β-CD with 5% GMS of external phase could initiate the system transforming into the microparticles. Therefore, the prepared Mex-loaded ISM system comprising 40% β-CD exhibited the potential controlled release delivery system for periodontitis.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest appreciation to my thesis advisor, Assoc. Prof. Dr. Thawatchai Phaechamud for his supervision, patience, valuable guidance, kindness and encouragement throughout my study. He provided me various opportunities and supported and guided me not only in research but also in social life. His ultimate mentorship helped me to improve my knowledge and to accomplish my research on time effectively.

My appreciation and deepest gratitude go to Assist. Prof. Dr. Torrsak Intaraphairotic, Assoc. Prof. Dr. Wichai Santimaleeworagund, Assoc. Prof. Dr. Manee Luangtanaanan, Assoc. Prof. Dr. Sontaya Limmatvapirat, Assoc. Prof. Dr. Prasert Akkaramongkolporn and Assoc. Prof. Dr. Wiwat Pichayakorn for the valuable comments and advices for thesis development.

I would like to manifest the gratefulness to Faculty of Pharmacy, Silpakorn University for the scholarships, laboratory equipment and other facilities to perform research work effectively and smoothly. I would like to express my much appreciation and gratitude to every teachers and staffs in Faculty of Pharmacy, Silpakorn University for the knowledge and generous support. I also would like to pass my heartfelt thanks to all my friends: Ms. Tiranit Chuenbarn, Mr. Takron Chantadee, Mr. Nutdanai Lertsuphotvanit and colleague for their invaluable suggestions, encouragement and help throughout the research. I am gratefully thanks to Ministry of Health, Myanmar and University of Pharmacy, Mandalay, Myanmar for giving permission to study Doctor of Philosophy Program of Pharmaceutical Engineering at Faculty of Pharmacy, Silpakorn University. I also would like to thank Ajarn Anthony Phonpituck for kind guidance with my presentation and writing techniques for the conferences. My grateful thanks will be with Ms. Siripong Srisukha and Dr. Wai Wai Lwin for valuable comments and help.

Finally, I would like to express my deepest gratitude and sincere appreciation to my parents, Dr. Sai Tun Kyi and Dr. Phyu Aye Aye, my eldest brother and youngest sister for their love, support and encouragement throughout my life.

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LIST OF ABBREVIATIONS

%w/w	percent weight by weight
%	percent
°C/min	degree Celsius per minute
°C	degree Celsius
cm^{-1}	per centimeter
α	alpha
β	beta
r^2	coefficient of determination
®	trademark
µg	Microgram(s)
µL	Microliter(s)
µg/mL	microgram per milliliter
µm	Micrometer(s)
k	release rate
n	solvent release exponent value
±	plus per minus
2θ	two theta
Abs	absorbance
ANOVA	way analysis of variance
ATCC	American Type Culture Collection
β-CD	Beta-cyclodextrin
β-MexDMSO ISG	meloxicam-loaded ISF gel comprising β-CD dissolved in DMSO
β-CD ISM	*in situ* forming microparticles containing β-cyclodextrin
β-CDMex	meloxicam-loaded in suit forming gel containing β-cyclodextrin

40% β-CD ISM	*in situ* forming microparticles containing 40% β-cyclodextrin
cm	centimeter(s)
Cam oil	camellia oil
CD	cyclodextrin
CDs	cyclodextrins
Conc.	concentration
CO., LTD.	Company Limited
cPs	centipoise
BCS	biopharmaceutics classification system
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
COX-2 inhibitor	cyclooxygenase- 2 inhibitor
CuK	copper K
Dcyn/cm	Dyne per centimeter
DF	drug free
dL/g	deciliter per gram
DSC	Differential scanning calorimetry
DTG	differential thermogravimetric analysis
e.g. (Latin);	for example
Eq.	equation
et al.	and others
etc. et cetera	and other things/ and so forth
ELISA	enzyme-linked immunosorbent assay
FDA's GRAS	Food and Drug Admistration's Generally Regarded As Safe
Fig.	figure

$F_{\text{max deformation}}$	maximum deformation force
$F_{\text{remaining}}$	remaining force
FT-IR	Fourier-transform infrared
g	gram (s)
g kg^{-1}	gram per killogram
g/kg	gram per kilogram
g/Dl	gram per deciliter
G (needle)	gauge
GMS	Glyceryl monostearate
GCF	gingival crevicular fluid
h	hour(s)
HCl	hydrochloride
HSM	hot stage microscope
HPMC	hydroxypropyl methyl cellulose
ISF	*in situ* forming systems
ISFI	*in situ* forming implant
ISG	*in situ* forming gel
ISM	*in situ* forming microparticles
IPM	Isopropyl myristate
KBr	Potassium bromide
KH_2PO_4	potassium dihydrogen phosphate
Kv	killo volt
L	liter(s)
LD_{50}	median lethal dose
LSD	least significant difference

mg	Milligram(s)
mm	millimeter
mA	milli Angstrom
ml/Kg	milliliter per kilogram
min	minute(s)
mL	milliliter(s)
MW	molecular weight
Mex	meloxicam
MexβCD 40% ISG	meloxicam-loaded ISF gel containing 40% β-cyclodextrin
MexβCD 40% ISM	meloxicam-loaded ISM containing 40% β-cyclodextrin
MMP8	matrix metalloproteinase 8
MRSA	methicillin-resistant *Staphylococcus aureus*
msc	model selection criterion
M_0	microparticles containing 40% w/w β-CD
M_{net}	the weight of dry sample without residual solvent and Meloxicam
MHA	MullerHintonagar
N	newton
mN/m	meter newton/meter
Nm	newton meter
nm	namometer(s)
NMP	N-methyl pyrrolidone
No.	number
ND	not determine
NSAIDs	non-steroidal anti-inflammatory drugs
o/o	oil in oil

o/w	oil in water
OH	hydroxyl
PBS	phosphate buffer solution
PD	probing depth
PGE_2	prostaglandin E_2
pH	potentia hydrogenii (Latin); power of hydrogen
PI	plaque index
PLA R202H	Poly(lactide)
PLC	poly (dl-lactide co-caprolactone)
PLGA/PLG	Poly(D,L-lactide-co-glycolide)
PLGA RG 503H	Poly(lactide-co-glycolide)
ppts	Precipitates
PVP	polyvinyl pyrollidone
PYR	2-pyrrolidone
Rpm	revolutions per minute
s	sec(s)
S.D.	standard deviation
SEM	scanning electron microscopy
SPSS	statistical package for social sicence
TG	thermogravimetric
TGA	Thermogravimetric analysis
UV-vis	Ultraviolet-visible
UK	United Kingdom
USA	Uinted States of America

CHAPTER 1
INTRODUCTION

1. Statement and significance of the research problem

The destructive inflammatory occurred in the periodontal disease could lead to the dramatic effects on supporting tissues of the teeth such as the loss of attachment level and bone resorption, the formation of periodontal pockets and/or gingival retraction thereafter a loss of teeth in the dental arches. Additionally, the appearance of periodontal pockets leads to the creation of the favorable environment for pathogenic microbes especially anaerobic bacteria. Thus, this inflammatory process connects to the multiple pathogens and host immune response (Vyas et al., 2000). The potential pathogenic bacteria are primarily Gram negative, facultative anaerobic species colonized in periodontal pocket, especially *Porphyromonas gingivalis* (Marcotte and Lavoie, 1998). The major concerns in the treatment of the periodontitis are drug partitioning which is not largely pronounced in periodontal pockets and rapid elimination of drug by gingival crevicular fluid (Pascale et al., 1986). For the treatment of periodontitis, the scaling and root planning followed by the administration of systemic antibiotics or application of local antibiotics are the current recommended practice. However, after the process of the conventional scaling and root planning, number of pathogens are left behind such as the inaccessibility and recolonization of pathogens. On the point of systemic administration, they can give easy and simple administration; nevertheless, the side effect of systemic administration such as hypersensitivity, gastrointestinal intolerance, lower concentration at the target sites (periodontal tissues) and the subsequent insufficient therapeutic efficacy limit its usefulness and effectiveness (Schwach-Abdellaoui et al., 2000). Additionally, the limited therapeutic drug concentration at the site of action and the rapid drug elimination are the major challenges for the effective treatment for periodontitis. The local drug delivery system is more promising for periodontitis because the locally administered drug can reach the base of the periodontal pocket as the target site and maintain an adequate time for its therapeutic effects whereas the controlled drug release can minimize the undesired side effect which can occur in the systemic drug administration (Schwach-Abdellaoui et al., 2000).

Typically, many approaches are employed for local drug delivery system for periodontitis treatment such as fibers, films, injectable systems, gels, strips and compacts, vesicular systems, microparticle systems, nanoparticle systems and *in situ* forming systems (Jain et al., 2008). Among them, the injectable *in situ* forming biodegradable drug delivery systems have gained the great attention because of their simple preparation, less stressful manufacturing conditions and easy administration (Packhaeuser et al., 2004). These are liquid formulations, which after administration with an injection into the periodontal pockets, transform into a solid-like depot.

Among various mechanisms of in situ forming (ISF) systems such as phase separation systems, crosslinked systems and solidifying organogels (e.g. solubility change) (Wischke and Schwendeman, 2008), the phase separating system based on a solvent exchange appears to be the most attractive one which requires not only the use of solvent/solvent systems without the need for critical temperature (for thermoresponsive ISF implants) but also not necessary for presence of ions (for charge sensitive ISF implants) and change in pH (for pH sensitive ISF implants(to trigger the in situ formation (Fredenberg et al., 2011). Thus, the present research concentrated on the solvent exchange-induced ISF.

Recently, the *in situ* forming gel (ISG) and *in situ* forming microparticles (ISM) have become attractive and interesting ISF since they could sustain the drug liberation effectively, localize the drug delivery at target site, reduce the dose and frequency of administration and improve a patient compliance (Hatefi and Amsden, 2002). For solvent exchange-induced ISG system, the aqueous-insoluble polymer is dissolved in a pharmaceutically acceptable organic solvent and the drug is incorporated into this polymeric solution. The major challenges encountered in the ISG system is the injectability and burst drug release. As an alternative to reduce the viscous polymer solutions of typical polymeric ISG and to increase the injectability, a novel ISM have been developed (Rungseevijitprapa and Bodmeier, 2009). The internal phase of ISM comprising the drug dissolved in a polymeric solution is emulsified with the external phase. The viscosity of the internal phase is controlled by the external oil phase; thus, the viscosity of the system is reduced owing to the lubricating effect of oil. Additionally, the oil included in the external phase retards the

burst drug release effectively which encountered this undesirable manner in the ISG system. Moreover, the ISM system has significantly reduced the myotoxicity. Upon contact with the environmental fluid at the injection site, this ISG and ISM transformed into gel (Xiong et al., 2011) or microparticles (Rungseevijitprapa and Bodmeier, 2009) easily and rapidly without pain using the proper needle. Additionally, these ISG can maintain the effective high level of drug in the gingival crevicular fluid of the periodontal pocket for a prolonged period of time (Medlicott et al., 1994).

Various polymers have been used as the main matrices for solvent exchange-induced ISG and ISM. The ethyl cellulose, bleached shellac and eudragit RS have been reported to be employed as the polymeric matrix of antimicrobial-loaded ISF(Srichan and Phaechamud, 2017). Additionally, poly(D,L-lactide-co-glycolide) (PLGA) (Kranz and Bodmeier, 2008), poly(lactide-co-glycolide) (PLGA RG 503H) and poly(lactide) (PLA R 202H) (Luan and Bodmeier, 2006b) have also been used to control the drug delivery of the ISM systems. The mechanism of phase transformation of these solvent exchange-induced ISF and ISM is the precipitation of the polymeric solution into solid-like matrix after solvent exchange and consequently the entrapped drug in the matrix releases in a controlled release manner**.** Thus, the controlled release mechanism of these systems is regulated mainly by the type and concentration of polymers**.** Beta-cyclodextrin (β-CD) exhibits the ability to form complex aggregates with the guest molecules which the drug molecules are encaged in the cavity of β-CD (Giordano et al., 2001). In some case, cyclodextrin (CD) moleucles are packed linearly on top of each other with infinite channels including guest molecules (Stella and He, 2008). Besides, owing to the limited solubility of β-CD (Magnúsdóttir et al., 2002), it has been interested as matrix former of solvent induced ISG and ISM**.** Thus, β-CD is investigated as the potential matrix-like of ISG and ISM in this study to control the anti-inflammatory drug release for periodontitis treatment. Since the inflammatory process connects with the development of the multiple pathogens (Vyas et al., 2000), non-steroidal anti-inflammatory drugs (NSAIDs) can give the promising effect for the treatment of the inflammatory destruction in (Jeffcoat et al., 1988; Reddy et al., 1993). Among NSAIDs, meloxicam (Mex) was used as the model drug in the present study.

The main aim of this present research is to investigate the matrix forming behaviour of β-CD in the ISG and ISM systems**.** Mex was used as a model drug**.** Thus, this study explained and introduced the use of β-CD as a matrix former by solvent exchange mechanism to acquire the better understanding fluid properties of β-CD solution, physicochemical properties, single phase and interfacial behaviour of the solvent exchange ISF containing β-CD**.** The development and evaluation were conducted for the Mex-loaded β-CD solvent exchange-induced ISG and ISM for periodontitis treatment.

1.1 OBJECTIVES

- To investigate the fluid properties of β-CD solutions in the injectable solvents used in ISG**.**
- To examine the physicochemical properties of β-CD precipitates prepared from injectable solvents used in ISG**.**
- To investigate the physical properties of various solvents and oils used for ISG and ISM preparations**.**
- To develop and evaluate the Mex-loaded β-CD solvent exchange-induced ISG and ISM for periodontitis treatment

1.2 HYPOTHESIS:

- Types of injectable solvents and amount of β-CD could influence the fluid properties of β-CD solutions including viscosity and injectability.
- Fluid properties of injectable solvents and the amount of β-CD could affect the properties of ISG and ISM formulations including matrix formation.
- Physicochemical properties of various solvents and oils could affect the properties of ISM formulation prepared from β-CD.
- Controlled released Mex-loaded ISG and ISM comprising β-CD as matrix former could be developed for the periodontitis treatment.

CHAPTER 2
LITERATURE REVIEW

2.1 Periodontitis

2.1.1 Introduction to periodontitis

2.1.2 Treatment

2.2 Local controlled delivery systems for the treatment of periodontitis

2.3 Solvent exchange *in situ* drug delivery system

2.4 Polymers used in *in situ* forming system (ISF)

2.4.1 β-Cyclodextrin (β-CD)

2.5 Fluid properties of solvents and oils used in ISG and ISM

2.5.1 Dimethyl sulfoxide (DMSO)

2.5.2 N-methyl-2-pyrrolidone (NMP)

2.5.3 2-pyrrolidone (PYR)

2.5.4 Triacetin

2.5.5. Glycofurol

2.5.6 Olive oil

2.5.7 Isopropyl myristate (IPM)

2.5.8 Camellia oil

2.5.9 Glyceryl monostearate (GMS)

2.6 Drug

2.6.1 Meloxicam (Mex)

2.1 Periodontitis

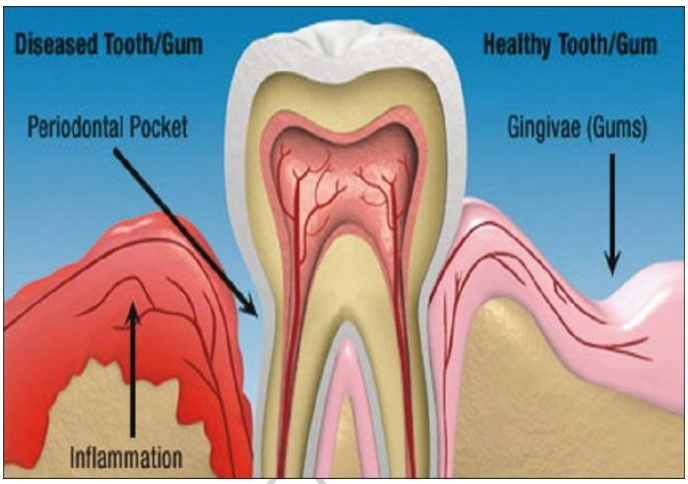
2.1.1. Introduction

Typically, a local inflammation is occurred in the periodontal pocket which is in the area between the teeth and the gum and it is the favorable environment for pathogenic anaerobic bacteria. The loss of attachment level and bone resorption, the formation of periodontal pockets and/or gingival retraction are the events occurred in the periodontal disease. If untreated, the periodontitis can lead to the loosening and subsequent loss of the teeth and bone resorption. The consequence is the primary cause of tooth loss in adults. Severe periodontitis, which may result in the tooth loss, is found in 5-20% of most adult population worldwide (Albandar, 2005). The expending of the inflammation in tooth-supporting structures can lead to the tissue destruction and tooth loss (Kinane, 2000). Therefore, it is necessary to inhibit the progression of periodontal disease by reducing the inflammation in supportive structure of the tooth by blocking the important inflammatory pathways involved in periodontal tissue destruction.

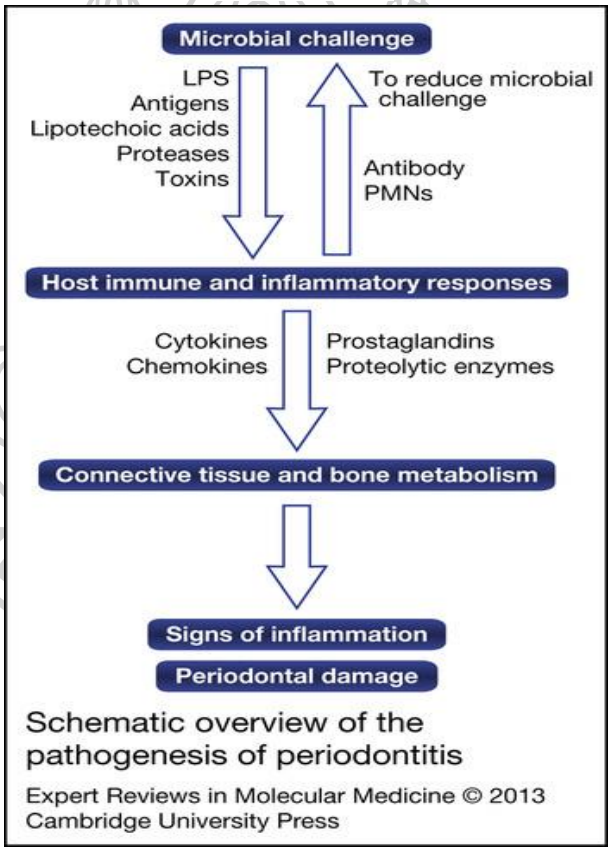
2.1.2 Treatment

Typically, the main aim for the treatment of the periodontal diseases is to interrupt the disease progression, prevent its recurrence and preserve the teeth in a healthy state (Pihlstrom et al., 2005). There are two primary treatments for periodontitis such as surgical and non-surgical treatment.

The scaling and root planning is the first and essential therapy for the treatment of periodontal diseases (Berezow and Darveau, 2011) in which the normal oral flora will be restabilized and will stop the gingival inflammation. Although this treatment can be achieved by decreasing the tissue inflammation and the clinical periodontal attachment (Pihlstrom et al., 2005). Nevertheless, it is not enough to attain the desirable clinical outcomes (Berezow and Darveau, 2011).



(A)



(B)

Figure 1 Structure showing healthy/infected tooth (Nair and Anoop, 2012) (A), Schematic overview of the pathogenesis of periodontitis (Yucel-Lindberg and Bage, 2013) (B)

Thus, the antimicrobial therapy as an adjunct process to scaling and root planning is often used (Berezow and Darveau, 2011). The choice of the antibacterial agents is based on the bacterial etiology of the infection (Schwach-Abdellaoui et al., 2000). There are many studies of several antiseptics and antibiotics for their clinical and microbiological efficacy in periodontal diseases such as chlorhexidine, sanguinarine, metronidazole, clindamycin, ofloxacin, tetracycline, minocycline, doxycycline hyclate etc.(Do et al., 2014).

In case of the failure of non-surgical treatment, the surgery may be done for restoration of the impaired periodontal anatomy by reducing periodontal pocket depth, gaining access for debridement of residual dental plaque and stimulating the regeneration of lost periodontal support (Pihlstrom et al., 2005).

Although the systemic therapy has been applied for the treatment for severe periodontitis, there are some disadvantages such as poor patient compliance, low drug concentration at periodontal pockets, microbial resistance, requiring of multiple doses and risk of adverse drug reaction (Ryan, 2005) whereas the local delivery system provides a long-term effective treatment at the site of infection at much smaller doses and it provides the drug in a time-controlled manner in the periodontal pockets during prolonged periods of time. Therefore, the localized drug delivery system is interesting for periodontitis treatment.

2.2 Local controlled delivery systems for the treatment of periodontitis

Practically, the periodontal pockets filled with gingival crevicular fluid (GCF) act as a natural reservoir for the administration of antimicrobial agents to the periodontal tissues and it is the ideal route for local antimicrobial therapy for periodontitis (Goodson, 2003; Nair and Anoop, 2012). Although the flushing action of GCF leads to a rapid removing of substances from gingival sulcus, the administration of controlled release drug delivery system into periodontal environment can compensate this effect (Goodson, 2003; Nair and Anoop, 2012). The local drug delivery system for periodontitis should be easy to place into the periodontal pocket and remain within the pocket during the whole treatment time to maintain the local drug concentration, convenient to be administered, be ensure good retention of the device after placement, deliver drug into the periodontal pocket with sufficient concentration, sustain the drug concentration for a sufficient length of time.

Additionally, it should be biodegradable so that it can erode after a certain period without any surgical procedure to remove the device remnants (Finkelman and Williams, 1998).

Figure 2 Administration of different drug delivery systems within the periodontal pocket (Jain et al., 2012)

There are numerous studies designing and developing as the local drug delivery systems for the treatment of periodontitis and the formulations for local drug delivery systems including fibers (Goodson et al., 1983; Tonetti et al., 1990), films (Perugini et al., 2003), strips (Addy and Langeroudi, 1984; Paolantonio et al., 2008), injectable gel (Polson et al., 1997). ISG (Phaechamud et al., 2016; Rungseevijitprapa and Bodmeier, 2009)etc.

The Actisite®, which consists of fibers of ethylene vinyl acetate containing 25% tetracycline HCl prolonged the release of tetracycline for 9 days *in vitro* (Radvar et al., 1996). However, this dosage form required the anesthesia (Do et al., 2014). Although the lipid-like gel Elyzol® containing 25% metronidazole can be placed easily into periodontal pocket by a provided syringe, there is the poor retention of Elyzol® gel within periodontal pocket (Schwach-Abdellaoui et al., 2000). Besides, Dentomycin®, the 2% minocycline gel still lack biodegradability (Do et al., 2014). The Atridox® is one of the commercially available biodegradable injectable *in situ* forming system which a biodegradable polymer such as PLA is dissolved in a biocompatible solvent NMP with 10% doxycycline hyclate drug loading. Upon

solvent exchange with the liquid in the surrounding environment, it transforms into solid state after injection into periodontal pocket. This implant can sustain a drug release over 7 days (Drisko, 1998).

2.3 Solvent exchange *in situ* drug delivery system

Among local drug delivery systems, *in situ* drug delivery system is revolution in therapeutics for periodontitis which is an injectable liquid that transformed at active site into a semisolid or solid depots (Packhaeuser et al., 2004). The advantages of *in situ* delivery systems are ease of administration, reduced frequency of administration, improved patient compliance and comfort. According to their mechanism of formation, these systems can be classified into ;

1) *in situ* cross-linked polymer systems,

2) *in situ* solidifying organogels, and

3) *in situ* phase separation (Kempe and Mader, 2012).

The interest on the present study is the *in situ* phase spearation. In this *in situ* phase speartion mechanism, the polymers included in the formulations undergo an abrupt decrease in their solubility due to changes in their environmental factors such as temperature, pH or solvent exchange (Kempe and Mader, 2012). This research was mainly focused on the solvent exchange mechanism which is the dynamic diffusion process between solvent in polymer solution and water in surrounding environment at target site (Graham et al., 1999). In this system, the insoluble polymer and drug are dissolved in a water-miscible organic solvent with low toxicity (Srichan and Phaechamud, 2017). In the present study, the ISG has been used for periodontitis treatment. ISG is in the sol form before administration, but once administered, it turns into gelation *in situ* to form gel after the solvent exchange with water occurs leading to the precipitation of polymer at the target sites, and thereafter, the drug releases with a controlled manner. The relatively lower production cost and simple manufacturing procedure support the solvent exchange ISG to be a great commercial potential (Hatefi and Amsden, 2002). The polymer-solvent affinity, solvent miscibility, viscosity and formulation are the influencing factors in solvent exchange process (Kranz and Bodmeier, 2008). ISG has the prospect of maintaining effective high levels of drug in the gingival crevicular fluid for a prolonged period of time to gain the desirable clinical benefits (Medlicott et al., 1994).

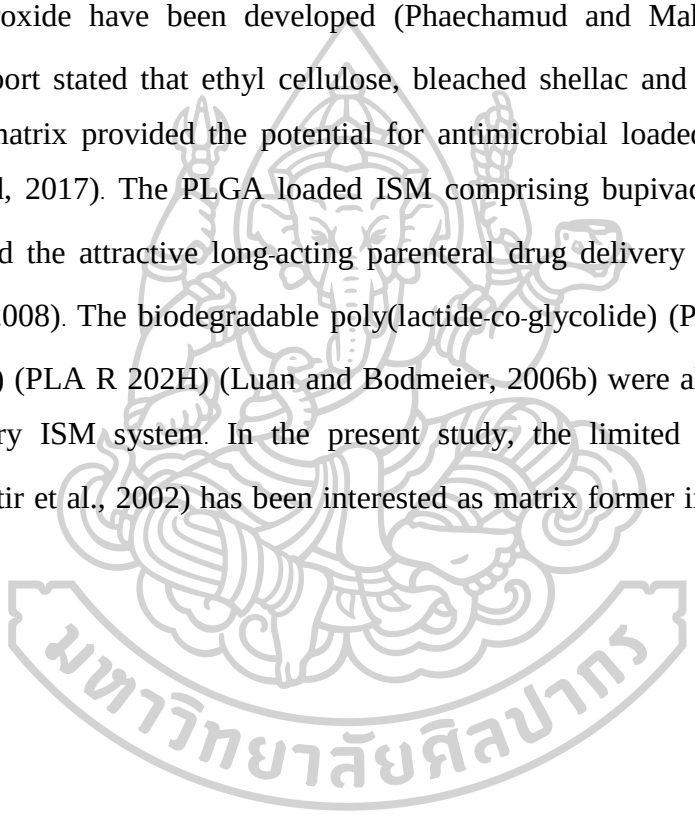
Recently, to overcome the complicated and high cost of the conventional microsphere preparation (Kranz and Bodmeier, 2008), the ISM systems are manipulated by two-syringe technique which loaded the internal phase and the external phase in each syringe whereas the syringes are coupled with a connector and pushed for 50 mixing cycles (Kranz and Bodmeier, 2007; Luan and Bodmeier, 2006a). ISM has advantages over the ISG such as lower cytotoxicity, greater reproducibility and minimized burst release (Luan and Bodmeier, 2006a). In addition, owing to the external phase (oil) performed as a lubricant and its action is dominant over the internal phase, ISM exhibited the better injectability than ISG system. ISM system is an injectable emulsion in which the drug dissolved in polymer solution for the internal phase and the oil with stabilizer was used for the external phase. The two phases were mixed together using two syringe connectors before administration (Voigt et al., 2012). After injection, the polymer phase became hardened and gradually formed into ISM. ISM system comprising internal phase of poly (lactide-co-glycolide) in NMP and the external phase of peanut oil with 2% w/w span 80 exhibited the more easily injectable system with less painful and better patient compliance (Rungseevijitprapa and Bodmeier, 2009)

2.4 Polymers used in *in situ* forming system (ISF)

In the treatment of periodontitis by the ISF, it is necessary to maintain the effective high level of drug in the gingival crevicular fluid for a prolonged period of time to produce the desirable clinical benefits (Medlicott et al., 1994;1994). After administration of ISF, the solvent in the formula was exchanged with the fluid at the target site, thereby the precipitation of the polymer occurred which the entrapped drug released in a controlled release manner. There are different types of polymers have been applied in the preparation of ISF. The hydroxyethylcellulose and polyvinylpyrrolidone have been used in the preparation of tetracycline-loaded bioadhesive semisolid system (Jones et al., 1996). The metronidazole-loaded systems were prepared using the chitosan (Akncbay et al., 2007) and carbopol 974P, hydroxyethylcellulose and polycarbophil (Jones et al., 1996). Additionally, the secnidazole-serratiopeptidase pH-sensitive ISG has been formulated using alginate combined with HPMC (Priyanka and Meenakshi, 2011). The gellan gum and sodium alginate hydrocolloids have been applied for an ion activated system of moxifloxacin

(Kunche et al.). The Pluronic F-127, HPMC K-100, Carbopol 934P, PVP-K-30 were also been used in the formulation of ornidazole ISG implant (Rawat et al., 2010). The hydrophilic nature of the above polymers resulted in the rarely sustainable drug release (Phaechamud and Mahadlek, 2015).

Thus, the solvent exchange-induced ISF using water insoluble polymer has been developed in order to obtain the system with a controlled release mechanism. Recently, the ethyl cellulose ISG comprising metronidazole, doxycycline hyclate, benzoyl peroxide have been developed (Phaechamud and Mahadlek, 2015). The previous report stated that ethyl cellulose, bleached shellac and eudragit RS as the polymeric matrix provided the potential for antimicrobial loaded ISF (Srichan and Phaechamud, 2017). The PLGA loaded ISM comprising bupivacaine hydrochloride demonstrated the attractive long-acting parenteral drug delivery system (Kranz and Bodmeier, 2008). The biodegradable poly(lactide-co-glycolide) (PLGA RG 503H) or poly(lactide) (PLA R 202H) (Luan and Bodmeier, 2006b) were also used for control drug delivery ISM system. In the present study, the limited solubility of β-CD (Magnúsdóttir et al., 2002) has been interested as matrix former in ISF drug delivery system.



2.4.1 β-Cyclodextrin

Cyclodextrins **(**CDs) is composed with a variable number of glucose units, commonly 6 to 8 units which are linked by α-1,4 bonds. It is cyclic oligosaccharides and the inner cavity of its possesses lipophilic nature whereas the outer surface showed hydrophilic behavior due to hydroxyl group on the outer surface. CDs resemble molecular containers whereas non-polar, nonionic guest molecules can be holed in its inner cavity. Thus, it can form the inclusion complex with many different types of appropriately size, especially nonpolar molecules (Del Valle, 2003)**.** Regarding to the hydrophilic outer surface, the complexation contributed to increased solubilization capacity of guest molecules (Das et al., 2013)**.** Thus, this behavior can be useful for a compound with low solubility whereas the inclusion complex increased the apparent solubility and drug dissolution rate thereafter the oral bioavailability can increased (Challa et al., 2005; Hirayama and Uekama, 1999;)**.** The another advantage of CDs as soluble complex is to improve oral absorption of BCS class II (Loftsson et al., 2016). Moreover, the labile drugs can be prevented against dehydration, hydrolysis, oxidation, and photo-decomposition by complexation with CDs thereby increase in the shelf life of drugs (Loftsson and Brewester, 1996). Not only CDs provide for the enhancement of the aqueous solubility of hydrophobic compounds but also reduce or eliminate unpleasant taste and smell without changing aqueous solubility of CDs themselves (Wei et al., 2017). Typically, for CDs having molecular weight of 122-1500 g/mol, the drug doses less than 200 mg should be used for good complexation properties (Loftsson and Brewester, 1996). In most cases, the complexation is formed by 1:1 guest/host complex (Brewster and Loftsson, 2007)**.** Inside the cavity of CDs, the guest molecules are entrapped by cage (Giordano et al., 2001) or by the channels (He et al., 2008). During complexation, electrostatic, hydrophobic, and hydrogen-bonding interactions are the major contribution factors for stability of biological macromolecules.

CDs dissolve well in DMSO and DMF, but it is practically insoluble in common organic solvents such as methanol, ethanol, isopropanol, acetonitrile and tetrahydrofuran. Among natural CDs, beta-cyclodextrin (β-CD) showed high number of intramolecular hydrogen bonds which interactions make the structure more rigid and prevent a hydration by water molecules (Loftsson et al., 2005a). Thus, when

compared, β-CD has the lowest solubility (Chatjigakis et al., 1992). The solubility of CDs is shown in Table 1.

Table 1 Solubility of CDs (Loftsson et al., 2005a)

Cyclodextrin	**Substitution[a]**	**MW[b]**	**Solubility in water (mg/ml)[c]**	**Application**
α-Cyclodextrin	-	972	145	Oral, parenteral, topical
β-Cyclodextrin	-	1135	18.5	Oral, topical
2-Hydroxylpropyl β-Cyclodextrin	0.65	1400	>600	Oral, parenteral, topical
Randomly methylated β-Cyclodextrin	1.8	1312	>500	Oral, topical[d]
β-CDSulfobutyl ether sodium salt	0.9	2163	>500	Oral, parenteral, topical
γ-Cyclodextrin	-	1297	232	Oral, parenteral, topical[d]
2-Hydroxylpropyl -γ-Cyclodextrin	0.6	1576	>500	Oral, parenteral, topical

[a]Average number of substituents per glucopyranose repeat unit.
[b]MW in Daltons
[c]Solubility in pure water at approx..25°C.
[d]very limited amounts

β-CD is seven membered sugar ring molecules and can be readily available with least toxic among natural CDs (Chowdary and Kamalakara, 2002). Moreover, it shows pharmaceutically useful complexation characteristics with the range of drugs. The guest molecules with molecular weight between 200-800 g/mol can fit inside the cavity of β-CD (Szente and Szejtli, 2004)**.** The Van der Waals forces, non-covalent forces, hydrophobic interactions and other forces are the major responsible for formation of the stable β-CD complex (Loftsson and Brewester, 1996)**.** It has been widely used to prepare the inclusion complexes in pharmaceutical, food, biotechnological, chemical, and cosmetic industry**.** β-CD can solubilize the poorly soluble drugs and allow these drugs to partition into the lipophilic membrane. Thus, it was used as the penetration enhancers (Kurkov and Loftsson, 2013; Loftsson and Brewster, 2011; Loftsson and Stefansson, 2017). β-CD was also used in the formulation of topical and oral dosage forms (Loftsson et al., 2005b)**.** However, β-CD cannot be given parenterally because of its low aqueous solubility and nephrotoxicity

(Brewster and Loftsson, 2007). When given orally, the non-toxic effect level of β-CD was found to be 0.7–0.8 g/kg/day in rats and about 2 g/kg/day in dogs (Bellringer et al., 1995). The acceptable daily oral intake of β-CD for human is 0.35 g for β-CD (Antlsperger and Schmid, 1996). β-CD cannot be hydrolysed by human salivary and amylases (Koutsou et al., 1999) but it can be subjected to fermentation by the intestinal microflora (Loftsson et al., 2005a).

The market β-CD based formulations are sublingual tablets (PGE_2/β-CD & nitroglycerin/β-CD), capsules (benexate/β-CD), gargling solution (Iodine/β-CD), ointment (dexamethasone/β-CD), tablets (new oral cephalosporin-ME1207/β-CD, nimesulide/β-CD & chlordiazepoxide/β-CD), chewable tablet (diphenhydramine HCl+ chlortheophylline/β-CD) (Giordano et al., 2001). Moreover, owing to the taste masking ability of β-CD complex, it was formulated in fast disintegrating tablet of cetirizine dihydrochloride (Katewongsa et al., 2017).

Its limited solubility of β-CD (Magnúsdóttir et al., 2002) has been interested as a matrix former in *in situ* forming drug delivery system of this research**.** When contact with aqueous at the target site, the solution of drug-loaded *in situ* forming drug delivery system has transformed into a solid-like matrix by solvent exchange mechanism which entrapped the drug from which the liberating the drug molecule as the controlled release manner (Phaechamud and Mahadlek, 2015). This solvent-exchange process is crucial for *in situ* forming drug delivery systems (Sanghvi et al., 2008) which is probably driven initially by water influx and onward by a solvent efflux.

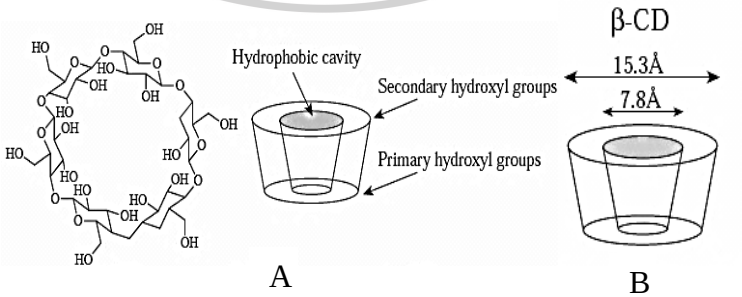


Figure 3 Chemical structure of β-CD (A) and β-CD cavity size (B) (Ahuja et al., 2011)

2.5 Fluid properties of solvents and oils used in ISG and ISM

The physicochemical characteristics of solvents such as density, viscosity, pH, injectability and wettability influence directly on the properties of pharmaceutical dosage forms. The solvent affinity to the polymer could affect the injectability of the ISF systems. For a good solvent, the polymer-solvent interaction should dominate over the polymer-polymer interaction which leads to lowering the viscosity (Parent et al., 2013). Therefore, the study of the viscosity of the solvents used for *in situ* drug delivery system is necessary. Typically, the preparation with the applied injection force through needle lower than 50 N was acceptable as the injectable dosage forms (Philippot et al., 2005). The ease of injection is critical factors together with an optimal rheological behavior of the injectable dosage forms that should be the Newtonian or pseudoplastic flows. Theoretically, the density is the one of the crucial factors in the stability of emulsion. The internal and external phase of the density in the emulsion system should not be much difference to inhibit the phase separation process (Mieth, 1984). In addition, the contact angle, surface tension and interfacial tension are important parameters for emulsion system such as oil in oil emulsion of ISM. Furthermore, the wettability data is the indicative compatibility among formula vehicle, and it is described by the value of contact angle of a liquid on a solid surface (Phaechamud and Savedkairop, 2012).

The injectable solvent exchange-induced ISF system, when exposes to the surrounding environment, the water miscible organic solvent exchanges with water leading to the formation of a solid/semisolid depot at the site of injection followed by a sustained release of the drug. Between the time of injection and complete depot formation, the initial drug burst release occur which can be controlled by modulating the hydrophobicity of solvent (Prabhu et al., 2005). The diffusion pattern of the solvents might be assessed using the water soluble dye solution and oil soluble color**.**

The biodegradable ISM systems are injectable emulsion in which the internal phase composes of polymer solution whereas the oil mixed with proper stabilizer is used as the external phase. The ideal solvent for ISF systems needs to gain the appropriate properties in term of the proper water affinity, viscosity, ability to dissolve the polymer and its safety. In the present study, the various solvents such as *N*-methyl pyrrolidone (NMP), 2-pyrrolidone (PYR), dimethyl sulfoxide (DMSO),

triacetin and glycofurol were interested as the injectable solvents as the internal phase and the oils such as olive oil, camellia oil and isopropyl myristate (IPM) were selected as the oil phase of ISM.

DMSO (Figure 4) is amphipathic molecule and composes of a polar domain characterized by the sulfinyl and two nonpolar methyl groups**.** Thus, DMSO can solubilize the polar and nonpolar substances and transpose hydrophobic (De Abreu Costa et al., 2017). In addition, it is miscible with water and organic solvents and it has highly acceptable safety and toxicology profiles (Gad, 2016). It was used as the solvent in the formulation of simvastatin ISF implant and this system showed the higher rate of a drug release than those using NMP (Gad, 2016). It is the most common solvent for *in vivo* administration of several water-insoluble substances (Santos et al., 2003). Regarding to its biocompatibility and thermostability, DMSO has been reported as a vehicle for an ISG (Parent et al., 2013).

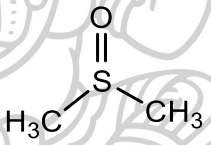


Figure 4 Chemical structure of DMSO

NMP (Figure 5) is the most frequently used organic solvent because of its excellent solubilizing power and low viscosity (Strickley, 2004)**.** It is thermally stable, biocompatible and it can be used in parenteral and oral medications as an acceptable solvent (Jouyban et al., 2010). NMP is used for the enhancement of hydrophobic molecules for transdermal delivery (Lee et al., 2005)**.** It is also used in the topical formulation (Fujii et al., 2000) and the ophthalmic drug delivery systems as a permeability enhancer. Moreover, it is used in a controlled released delivery system comprising parenteral formulations (Jouyban et al., 2010)**.** The solubility of NMP is similar to those of ethanol and DMSO, thus, it has the potential to solubilize the lipid in cell membrane (Seyedlar et al., 2014).

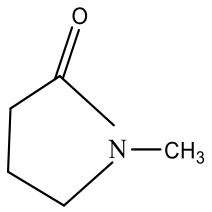


Figure 5 Chemical structure of NMP

PYR **(**Figure 6**)** has a high-polar property and it is miscible with a wide variety of solvents such as water, ethanol and chloroform**.** In pharmaceutical research, PYR is used as a plasticizer and a solvent for dissolving the incorporated drug in ISG system (Parent et al., 2013) and ISM (Kranz et al., 2001)**.**

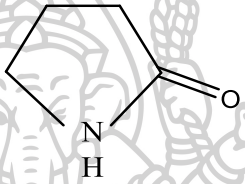


Figure 6 Chemical structure of PYR

The other biocompatible solvents such as triacetin and glycofurol were also investigated in this study**.** Triacetin, the triester of glycerol (Figure 7) and acetylating agents, such as acetic acid and acetic anhydride (Kong et al., 2016) have been used as a plasticizer in the ISF implants system (Ahmed et al., 2014)**.** Using triacetin as a solvent can reduce a burst effect and extend the drug release of ISF biodegradable PLGA microspheres (Jain et al., 2000). The initial burst release was reduced when triacetin was used as the cosolvent in a hydrophilic polymer depot containing either NMP or DMSO as the solvents (Liu and Venkatraman, 2012). Triacetin was recognized as safe (FDA's GRAS list) (Ahmed et al., 2014) which not toxic in short-term inhalation or parenteral studies (Fiume, 2003). Moreover, it was used as a potential parenteral nutrient (Bailey et al., 1991; Karlstad et al., 1992). The LD_{50} of triacetin is higher than 2 mL/kg which is acceptable for used in the injectable implant systems (Kranz and Bodmeier, 2007).

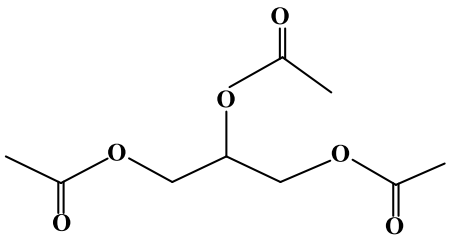


Figure 7 Chemical structure of triacetin

Glycofurol (Figure 8) was known as a parenteral solvent (Mottu et al., 2000; Mottu et al., 2001) which has the ability to dissolve a wide range of water insoluble drugs (Bonacucina et al., 2006). It was used as a co-solvent for two polymers, Eudragits L and RS, to obtain the high viscosity systems of the controlled release properties (Bonacucina et al., 2006), as an injectable solvent in ISF implant system (Algın and Baykara, 2010) and also used as a vehicle for naproxen-loaded topical gel (Barakat, 2010) and for an intranasal preparation of insulin (Bechgaard et al., 1996) and bumetamide (Nielsen et al., 2000) to enhance a drug absorption. Glycofurol is a nontoxic and nonirritant material when it is diluted with water. The LD_{50} of Glycofurol by intravenous injection in the mouse is 3.78 g/kg (Spiegel and Noseworthy, 1963).

Figure 8 Chemical structure of glycofurol

In the present study, the olive oil and IPM were used as the external phase of oil in oil emulsion of ISM. Olive oil has been used as an oily vehicle in an injectable solution (Nema and Ludwig, 2010). The main compositions of olive oil are the mixed triglyceride esters of oleic acid and palmitic acid (Beltran et al., 2004). IPM (Figure 9) consists of the esters of isopropyl alcohol and the saturated high MW fatty acids, principally myristic acid (Engelbrecht et al., 2012). IPM has been used as a vehicle for injectable solution as an alternative of vegetable oil (Engelbrecht et al., 2012; Nema and Ludwig, 2010). In addition, IPM showed the very low irritability and no sensitizing properties in animals after topical and parenteral administrations (Platcow and Voss, 1954).

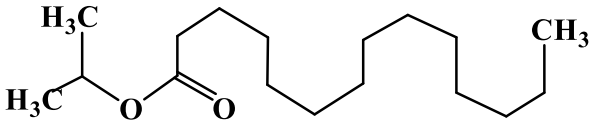


Figure 9 Chemical structure of IPM

Camellia oil, obtained from *Camellia oleifera*, has several therapeutic effects and it has the comparable health benefits to olive oil and better than sunflower oil (Sahari et al., 2004). This oil is able to reduce the blood lipids. Moreover, it possesses a remarkable antioxidant activity (Lee and Yen, 2006). Because it has the fatty acid as the constituents such as palmitic, linoleic, oleic and stearic acids, this oil is important in cosmetics and used for skin and hair. Therefore, the objective of the present study is to investigate the physicochemical properties of various solvents and oils used in ISM preparation**.**

Glycerol monostearate (Figure 10), commonly known as GMS, is a monoglyceride commonly used as an emulsifier in foods and pharmaceutical products. It is a white, odorless, and sweet-tasting flaky powder that is hygroscopic. GMS, the partial glycerides is self-assembling amphiphilic molecules. Regarding to its ability for formation of varieties of crystalline structures, it has special interest in drug delivery (Mengesha et al., 2013). The versatile structural appearance of GMS offers a wide range of different possibilities for pharmaceutical formulation purposes. In addition, GMS is bio-degradable and physiologically non-toxic (Windbergs et al., 2009). Chemically it is the glycerol ester of stearic acid having a specific hydrophilic head and a hydrophobic tail, GMS is an important non-ionic surfactant with low hydrophile–lipophile balance value (HLB) which particularly use in “water-in-oil” emulsions (Sharma et al., 2017). The lipophilic property of GMS could render the matrix system more lipophilic. Increase in lipophilicity retarded the rate of water penetration which caused in a slower rate of drug release. Thus, retardation of drug release from the GMS matrix system was due to lipophilicity of GMS (Peh et al., 2000). Upon addition of short-chain alcohol, the viscosity of GMS microemulsions was lowered because alcohol decreased the polarity of water and provided an environment with a balance of lipophilic–hydrophilic properties (Antona et al., 2000). Glycerol monooleate having HLB value 2.8 is more effective as destabilizer than

GMS having HLB of 3.8 (Goff and Jordan, 1989). GMS is a food additive and it is used as an emulsifying agent for oils, waxes, and solvents. Moreover, it is used as a protective coating for hygroscopic powders and a solidifier and control release agent in pharmaceuticals (Sharma et al., 2017). The emulsion containing GMS showed no phase separation over more than 1h because the fine matrix of amorphous-like and rod-like GMS crystals were embedded in the oily continuous phase (Voigt et al., 2012). In addition, the anhydrous β crystalline dispersion of GMS structured olive oil to form an organogel (or oleogel as an edible organogel). GMS was also employed as an organogelator which its thickening ability controlled the oil migration upon mixing with the internal phase (Daniel and Marangoni, 2012).

Figure 10 Chemical structure of GMS

2.6 Drug

2.6.1. Meloxicam

The non-steroidal anti-inflammatory drugs have promising effects on the treatment of inflammatory destruction in periodontitis (Jeffcoat et al., 1988; Reddy et al., 1993). Among them, the meloxicam (Mex) (Figure 11), selective cyclooxygenase-2 inhibitor (COX-2 inhibitor), is a highly potent NSAID (Engelhardt, 1996). The melting point of meloxicam is 254ºC. It is practically insoluble in water, slightly soluble in acetone, soluble in dimethyl formamide, very slightly soluble in ethanol (96%) and in methanol (Sweetman, 2002). It could prevent the alveolar bone loss in an experimental periodontitis in rats when administered subcutaneously (Bezerra et al., 2000). Moreover, Mex is a potent inhibitor for acute exudation in periodontal tissues and bone and cartilage destruction (Engelhardt, 1996). When the adjunct to scaling and root planning, the oral administration of Mex can reduce the matrix metalloproteinase 8 (MMP8) level of GCF which is a main factor in the connective tissue destruction (Buduneli et al., 2002).

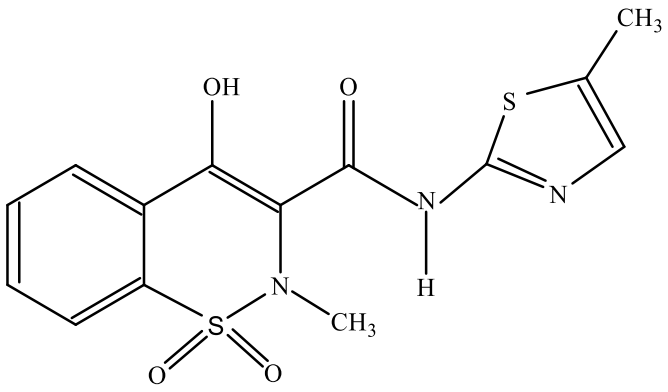


Figure 11 Chemical structure of Mex

Although Mex is the potent NSAID for the treatment of inflammation of the periodontitis, its usefulness is limited by its solubility**.** Being BCS class II, mex has limited aqueous solubility of 9.4 μg/mL (Santos et al., 1998) and the rate of absorption is relatively slow after oral administration (Busch et al., 1998; Hanft et al., 2001; Turck et al., 1996). Inclusion complexes of Mex with α-, β- and γ-CDs have been investigated for increase the drug solubility (Lu et al., 2009). To improve dissolution and permeation, Mex was formulated as freeze-dried solid dispersion in polyvinyl pyrrolidone K-30 (El-Badry and Fathy, 2006). In addition, Mex gel was prepared using hydroxypropyl cellulose (Jantharaprapap and Stagni, 2007). The Mex/CD binary system both in solution and solid state have also been prepared by complexation with β, γ-CD (Naidu et al., 2004). The inclusion complexation with β-CD-based nanosponges has been studied as an alternative method to increase the solubility and bioavailability of Mex (Shende et al., 2015). Mex/β-CD complexes increased the solubility of Mex when compared with other carriers such as PVP and urea. However, the Mex -loaded β-CD-based ISM has not been reported previously.

In the present study, β-CD was dissolved in various solvents such as NMP, DMSO, PYR, triacetin and glycofurol. The fluid properties of β-CD in injectable solvents without drug and the complexation behavior of β-CD precipitates prepared from injectable solvents were investigated. Then, Mex was added as a model drug to the β-CD solution. The physical properties of various solvents and oils used for ISM were also studied. This study was focused on preparing the Mex-loaded ISG and ISM using β-CD as the matrix former.

CHAPTER 3
Physicochemical properties of β-CD solutions and precipitates prepared from injectable vehicles

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3.5 Conclusion

3.1 Introduction

The β-cyclodextrin (β-CD) has a limited aqueous solubility (Loftsson and Brewester, 1996; Magnúsdóttir et al., 2002) meaning that complexes resulting from interaction of lipophiles with this CD exhibit the limited solubility resulting in precipitation of solid β-CD complexes from water and other aqueous systems. Its low aqueous solubility character is interesting for using as a matrix former in solvent exchange-induced ISG. The main controlling step for the solvent exchange process is probably driven initially by water influx and onward by solvent efflux. This behavior is crucial for solvent-exchanged induced ISG (Sanghvi et al., 2008). In the periodontal pocket, the precipitation of polymer occurred after exposure the aqueous environment at injection site inducing the formation of a depot for consequent entrapping the drug with the controlled release manner (Phaechamud and Mahadlek, 2015). Water is typically employed as the solvent for inclusion complexation, but this can be accomplished in a co-solvent system and in the presence of any non-aqueous solvents (Del Valle, 2003; Frömming and Szejtli, 1994). In the present study, the high concentrated β-CD solutions were prepared in the injectable solvents including dimethyl sulfoxide (DMSO), N- methyl pyrrolidone (NMP) and 2-pyrrolidone (PYR). DMSO is miscible with water and organic solvents and it can be used as a polymer vehicle in ISG and ISM (Ahmed et al., 2014). NMP is used as the organic solvent because of its solvating ability. Interestingly, a polar disubstituted cyclic amide group in NMP molecules has responsible for its interaction with water molecules for their complete miscibility. It acts as co-solvent owing to presence of the non-polar carbons of NMP which weakens the hydrogen-bonded structure of water (Sanghvi et al., 2008). It has been used in parenteral and oral dosage forms with thermally stable, biocompatible and biodegradable properties (Jouyban et al., 2010; Strickley, 2004). It is also used as an acceptable solvent which the solubility of NMP is similar to that of ethanol and DMSO (Hansen and Just, 2001; Jouyban et al., 2010). NMP is used as solvent of Atridox ® gel for delivering antimicrobial drug for periodontitis treatment. PYR is miscible with a wide variety of other solvents; therefore, it is used as a plasticizer and polymer solvent of ISG system (Parent et al., 2013) and ISM (Kranz et al., 2001). The objective of the present study was to investigate physicochemical properties of β-CD solutions in DMSO, NMP and PYR which have not been reported

before. Moreover, the β-CD precipitates obtained from these solvents were characterized. These obtained data will be useful for applying the concentrated β-CD solution in these injectable solvents as the ISG as delivery system to deliver the active compounds for periodontitis treatment.

3.2 Materials and methods

3.2.1 Materials

β-CD (lot no. 70P360) was kindly supported by Pharma nueva Co., Ltd., Bangkok, Thailand. NMP (lot no. A0251390, Fluka, New Jersey, USA), DMSO (lot no. 453035, Fluka, Switzerland) and PYR (lot no. BCBF5715V, Fluka, Germany) were used as the solvents for β-CD. Potassium bromide (KBr) (spectrograde) was purchased from Fisher Scientific UK limited, UK and phosphoric acid (Merck KGaA, Darmstadt, Germany), potassium dihydrogen phosphate (KH_2PO_4) (ARgrade, lot no. P5104-1-1000, QREC Chemical CO, LTD, Chonburi, Thailand) were used as received.

3.2.2 Preparation of β-CD solutions

The 30%–60% (w/w) β-CD solutions were prepared in NMP, DMSO and PYR by stirring until the clear solutions were obtained.

3.2.3 Preparation of β-CD precipitates

The 20 g of 30% (w/w) β-CD solution were titrated with distilled water until the initial phase separation was evident with precipitate formation. The amount of distilled water was further added untill no apparent precipitation. Then, the obtained precipitate was filtered and washed with cool distilled water several times to eliminate the solvent from precipitate and was dried in hot air oven at 60°C for 2 days and kept in desiccator containing dried desiccant before test.

3.3 Evaluations

3.3.1 Characterization of β-CD solutions prepared from three solvents

3.3.1.1 Gel appearance, density and pH measurement

The appearance of the prepared formulations was examined visually, and the pH values of β-CD solutions were measured using a pH meter (Ultra 24 Basic UB-10, Denver Instrument, Bohemia, New York) (n = 3). All samples were compared with pure solvents and distilled water. In addition, both pure solvents and β-CD solutions

were investigated for the density using pycnometer (Densito 30PX, Mettler Toledo Thailand Ltd, Portable- Lab TM) (n = 3).

3.3.1.2 Apparent viscosity

The apparent viscosity and rheology of the prepared β-CD solutions were investigated at 25°C using Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories. Inc., USA) (n = 3). The apparent viscosity of pure solvents (DMSO, NMR and PYR) was also determined.

3.3.1.3 Determination of intrinsic viscosity

Intrinsic viscosity can be measured from the flow time of a solution through a simple glass capillary such as Ubbelohde viscometer. The time it takes a volume of polymer solution to flow through a thin capillary is compared to the time for a solvent flow. Intrinsic viscosity can be calculated from the relative viscosity (η_r) and specific viscosity (η_{sp}) using Huggin's and Kraemer's equations.

$$\text{Reduced viscosity} = \eta_r = \eta_{sp}/C \text{ (Huggin's equation)} \quad \text{.....(1)}$$

$$\text{Inherent viscosity} = \eta_{inh} = \ln \eta_r / C \text{ (Kraemer's equation)} \quad \text{.....(2)}$$

Double extrapolation plots of reduced viscosity (equation 1) against concentration and inherent viscosity (equation 2) against concentration was plotted by calculating the corresponding reduced viscosity and inherent viscosity. Then the obtained viscometry data were extrapolated to the zero polymer concentration. The intrinsic viscosity is given by the common ordinate intercept of the graphs. The experiment was carried out in triplicate (n =3).

3.3.1.4 Measurement of water resistance

In order to determine the resistance of β-CD solutions for inducing phase separation with water, the titration method with distilled water was used. The procedure was the same as the preparation of β-CD precipitates described in 3.2.3. The amount of used water was recorded and expressed as the percentage to indicate the water resistance (n = 3).

3.3.1.5 Matrix formation

The observation of β-CD matrix formation due to water diffusion into formulations was studied by filling each 150 µl β-CD solutions into the well with diameter of 6 mm of the 0.6% (w/v) agarose gel containing PBS pH 6.8 which mimic artificial periodontal pocket (Do et al., 2014). When water diffused into the sample, the apparent system was changed from transparent to opaque insoluble β-CD matrix.

The distance of matrix formation (opaque front) with time was recorded under a stereo microscope (Motic SMZ-171 Series). The rate of matrix formation into β-CD solutions was correlated as opaque distance and time as Eq. (3):

$$\text{Rate of matrix formation} = \frac{\text{Distance of opaque front (mm)}}{\text{Time (min)}} \quad (3)$$

3.3.1.6 Solvent diffusion

To determine the characteristic of solvent diffusion, the 0.6% (w/v) agarose gel containing PBS pH 6.8 with the cylindrical well (diameter of 6 mm) was filled with 150 µl β-CD solutions dyed with amaranth (0.1g/10 ml). During solvent exchange, the change was captured with stereo microscope (Motic SMZ-171 Series) every 5 min for 30 min. The distance of amaranth diffusion front was measured in triplicate. The rate of solvent diffusion together with amaranth into agarose gels was calculated by Eq. (4).

$$\text{Rate of solvent diffusion into gels} = \frac{\text{Distance of amaranth color front distance (mm)}}{\text{Time (min)}} \quad (4)$$

3.3.1.7 *In vitro* gel formation

The study of *in vitro* gel formation was carried out to investigate the gel formation of the prepared solutions when exposed to phosphate buffer saline (PBS pH 6.8). Firstly, PBS pH 6.8 was prepared and 1 mL of sample was injected into test tube containing 5 mL PBS solution with 18-guage needle. Then the turbid gel formation was observed visually at various times intervals such as 0, 5, 15, 30 and 60 min respectively.

3.3.1.8 Injectability test

For injectable formulation, injectability of the systems is an important factor to be considered for the ease of administration to periodontal pocket. Injectability test was determined by using texture analyzer (TA.XT plus, Stable Micro Systems, UK) in compression mode. The test sample was filled into 1 ml syringe with 27-guage needle which is widely used in the dental field (Sato et al., 2012)and then this needle was clamped with stand. The upper probe of the texture analyzer moved downward until it moved into contact with the syringe barrel base. A constant speed of 1.0 mm/s and constant force of 0.1 N were applied. The distance required to expel the contents for a barrel length of 20 mm was measured and recorded. The work of injection was measured in triplicate at room temperature.

3.3.1.9 Contact angle, surface and interfacial tension study

The contact angle on the glass slide and the surface tension of the diluted solvents were tested using the pendent drop method by a goniometer (FTA 1000, First Ten Angstroms, USA) in triplicate under room temperature. The interfacial tension was conducted for various formula components using a goniometer. Total measurements were recorded in triplicate under room temperature.

3.3.2 Characterization of dried β-CD precipitates

3.3.2.1 Determination of morphology by inverted microscope

The precipitates after dispersed in distilled water were dropped onto the slide and visually recorded their morphology every 10s for 3 min under an inverted microscope (Nikon DXM 1200, Japan).

3.3.2.2 Determination of topography by scanning electron microscopy (SEM)

The topography of precipitates was observed by SEM. The precipitates were coated with gold prior to examination using SEM (Maxim 200 Camscan, Cambridge, England) at an accelerating voltage of 15 kV at magnifications of ×200, ×500, and ×2000.

3.3.2.3 Morphology change under hot stage microscope (HSM)

Change of precipitate morphology was also studied using a hot stage microscope (HSM Mettler Toledo, model FP82HT, SNR 5130346892, Switzerland) at room temperature to 300°C. The sample of 0.1 mg was placed on glass slides and heated at 5°C/min.

3.3.2.4 Melting point

A small quantity of powder was placed into a capillary tube and the tube was placed in the capillary melting apparatus (Melting point M-560, BUCHI, Switzerland) and the temperature was gradually increased automatically. The temperature at which powder started to melt and the temperature when all the powder completely melted were recorded (n=3).

3.3.2.5 Powder X-ray diffractometry (XRD)

The X-ray diffraction (XRD) was performed upon the intact β-CD and three β-CD precipitates. The precipitates were filled into a glass sample holder with 0.2 mm depth and then loaded to an powder X-ray diffractometer (Miniflex II, Rigaku Corp. Tokyo, Japan). The CuK α source (λ=1.54˚A) operated at 30 kV and 15 mA was used to record powder XRD patterns in the 2θ range of 4°– 60° at a step size of 2°/min.

3.3.2.6 Differential scanning calorimetry (DSC)

The thermal properties of samples were determined using DSC (Pyris Sapphire DSC, Standard 115 V, Perkin Elmer instruments, Japan). The intact β-CD and three β-CD ppts were weighed accurately 5 mg into aluminum pan (PerkinElmer, Inc., MA, USA), sealed and operated under dry nitrogen atmosphere which the measurement temperature was set at the ranges of 0°C to 300°C with heating rate of 10°C/min.

3.3.2.7 Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTGA)

Thermal properties of in-tact β-CD and three β-CD ppts were determined using the thermal gravimetric analysis (TGA) (Pyris TGA, PerkinElmer, USA). The 15-mg intact β-CD and three β-CD precipitates were placed on an aluminum pan, and heated from 30°C to 600°C at 10 °C/min. The weight differences were recorded, and the percent weight loss was calculated. Then the differential thermogravimetric analysis (DTGA) was also calculated.

3.3.2.8 Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of the intact β-CD and β-CD ppts were recorded using KBr method using FT-IR spectrophotometer (FT/IR-4100, JASCO International Co., Ltd., Tokyo, Japan). Approximately 2 mg each sample was mixed and ground with 100 mg

KBr before compressed into pellet using KBr die kit. The test was performed with the wave number ranges of 400–4000 cm^{-1} with 32 scans at 4 cm^{-1} resolution.

3.3.2.9 Statistical analysis

For statistical analysis of the measurements, the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test was performed using SPSS for Windows. The significant level was set at $p< 0.05$.

3.4 Results and discussions

3.4.1 Characterization of β-CD solutions prepared from three solvents

3.4.1.1 The gel appearance, density and pH measurement

The 30% (w/w) β-CD was readily dissolved in all three injectable solvents. The 35%-40% (w/w) β-CD was also dissolved in three different solvents but it was taken time about 2 h to be completely dissolved. The rather clear solutions were obtained for 45% (w/w) β-CD in NMP and PYR. The 45%-60% (w/w) β-CD solutions required heating at temperature of 150°C and stirring process to obtain the clear solution in DMSO which they were still clear solution after cooling down at room temperature. PYR and its β-CD solutions showed the highest density and that of DMSO and its systems were slightly lower. NMP and its related solutions gave the lowest density among three solvents. The more the concentration of intact β-CD, the more increased density of the solution were attained (Figure 13). The high density of PYR related with its high viscosity and could affect the diffusion property of this solvent. In addition, the higher density of the developed solutions than water indicated that they could transform into matrix-like after exposure an aqueous environment and could deposit at bottom of target site such as periodontal pocket properly. The pH of NMP was highest and followed by PYR and DMSO, respectively (Figure 12). The increased amount of β-CD in the solution containing DMSO gradually decreased the pH of solution. However, the prominent reduction in pH was observed in the β-CD solution containing NMP and PYR. This might be due to the hydrogen formation of β-CD with lone pair electrons in NMP and PYR which led to the increase in acidity. But the steric hindrance effect of NMP caused less acidity than PYR (Questel et al., 1992)

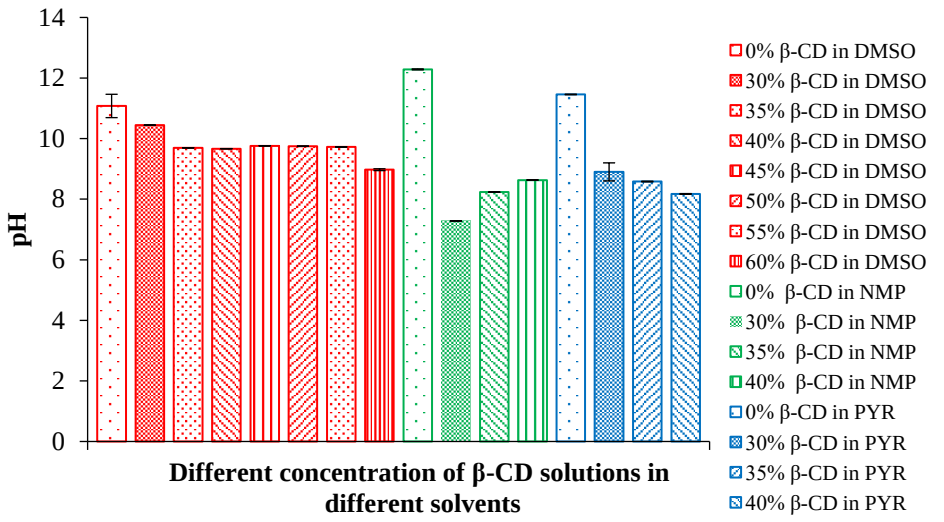


Figure 12 pH for β-CD solutions and its respective solvents (n=3)

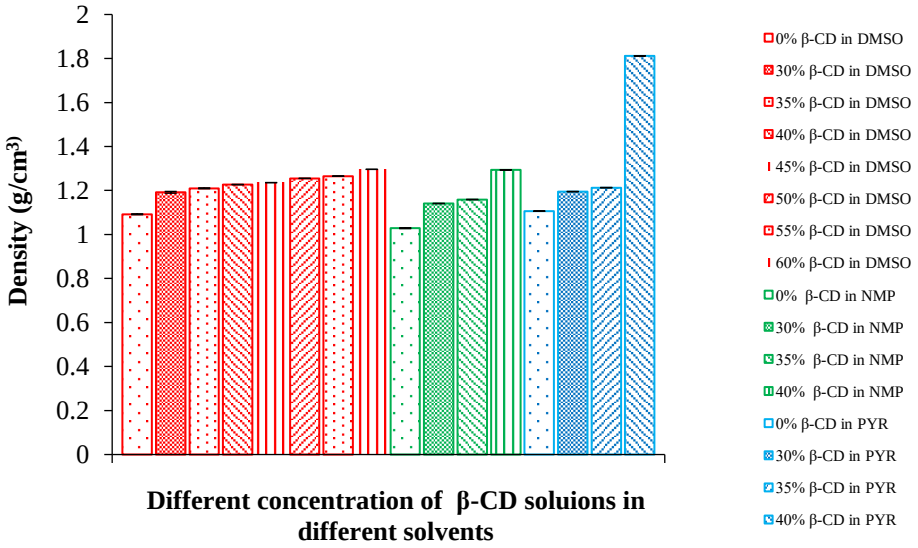


Figure 13 Density for β-CD solutions and its respective solvents (n=3)

3.4.1.2 Apparent viscosity

Viscosity, as an internal property of a fluid, offers the resistance to flow affecting abundant properties of ISF systems, especially the character of solvent exchange and diffusion rate (Phaechamud and Setthajindalert, 2017). PYR exhibited higher apparent viscosity than NMP and DMSO, respectively, and they were accounted for 10.61 ± 0.22, 8.01 ± 0.06 and 0.82 ± 0.04 cPs, respectively (Figure 14). This result corresponded with the density value as mentioned previously. The apparent viscosity of solvents was noticeably increased when β-CD was added. This proved the strong interaction of respective solvent with β-CD molecule. The 30% (w/w) β-CD solution in PYR showed the highest viscosity (99.67 ± 0.12 cPs) due to apparent higher viscosity of solvent itself while that of NMP and DMSO exhibited lower viscosity of 45.7 ± 8.6 cPs and 29.4 ± 8.40 cPs, respectively (Figure 14). The trend of apparent viscosity of β-CD solutions using different solvents were PYR >> NMP > DMSO. In this study, the curves moved to a higher shear stress value when concentration of β-CD was increased in all solutions signifying the compact structure of the gels. All the β-CD solutions showed the Newtonian flow (Figure 15). When compared, the β-CD solution in PYR showed the highest shear stress which was followed by β-CD solution dissolved in NMP and DMSO, respectively (Figure 16). Typically, for ease of injection, the optimal rheological behavior of the ISG should be a Newtonian or pseudoplastic flow (Priyanka and Meenakshi, 2011).

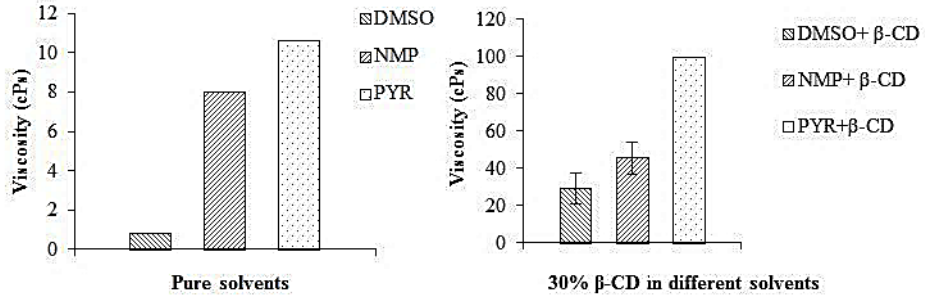


Figure 14 Apparent viscosity of the pure solvents and its solutions (n= 3)

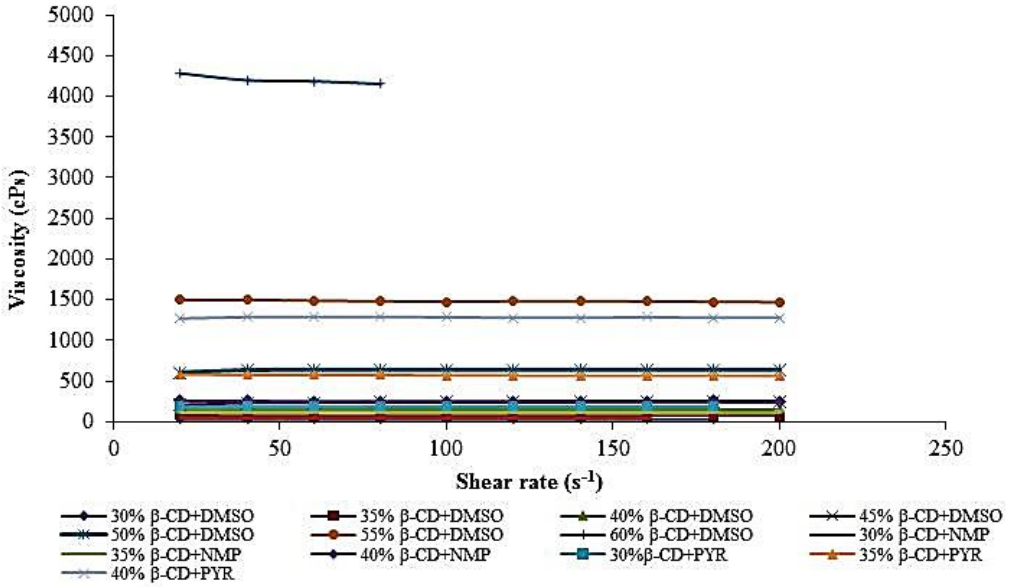


Figure 15 Viscosity studies of β-CD in three different solvents (n=3)

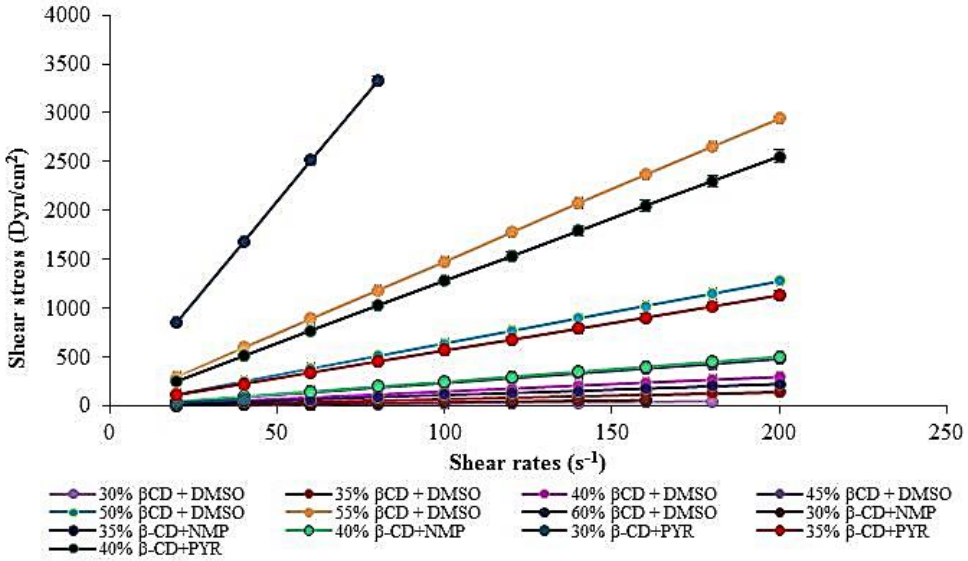


Figure 16 Rheological properties of β-CD solutions (n=3)

3.4.1.3 Intrinsic viscosity

The intrinsic viscosity of β-CD solution in DMSO exhibited the lowest value of 0.07 dL/g while that in NMP was 0.084 dL/g (Figure 17 and 18). The β-CD solution in PYR showed the highest intrinsic viscosity of 0.09 dL/g (Figure 19). A copolymer having an intrinsic viscosity below 0.05 was not suitable to significantly impart in a controlled release profile while the copolymer having the intrinsic viscosity above 0.8 was too viscous to be easily administered. Therefore, DMSO was more suitable solvent for β-CD for controlled drug release system with in turn of good injectability.

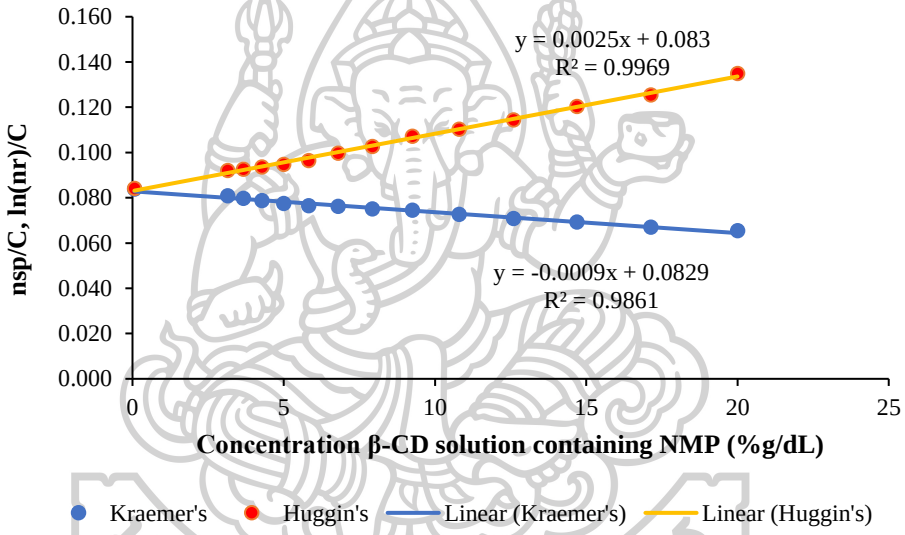


Figure 17 Intrinsic viscosity of β-CD solution in NMP (n=3)

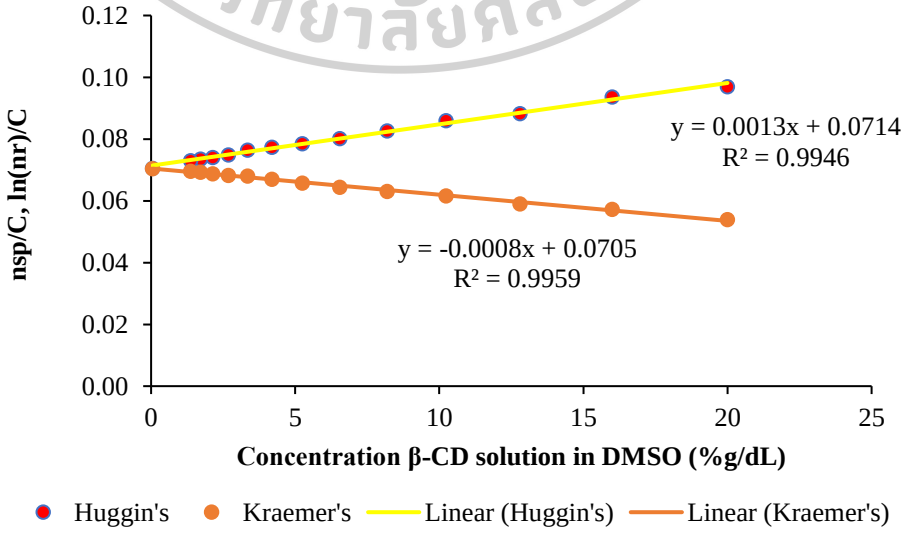


Figure 18 Intrinsic viscosity of β-CD solution in DMSO (n=3)

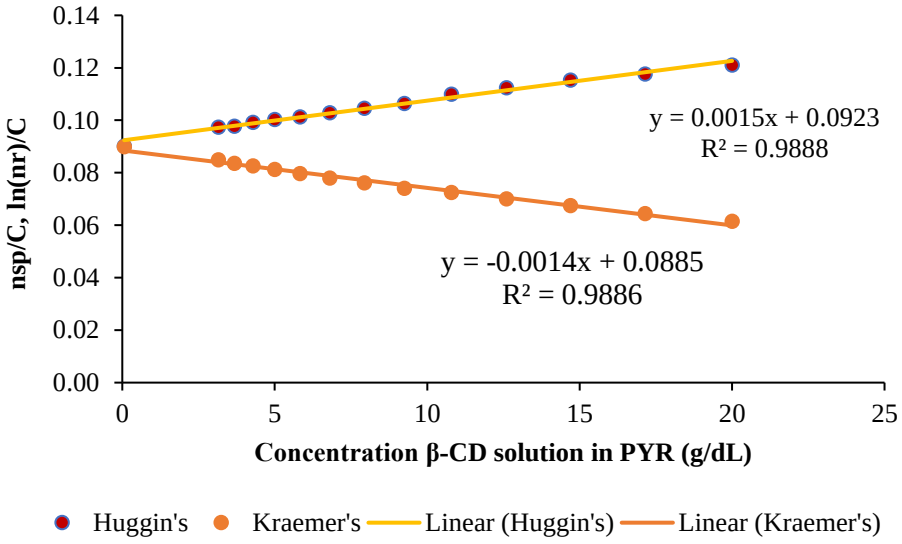


Figure 19 Intrinsic viscosity of β-CD solution in PYR (n=3)

3.4.1.4 Water resistance of β-CD solutions

The titration with distilled water was used to determine the resistance for phase separation of β-CD solutions. The different pairs of β-CD solvent exhibited different phase separation behavior or the precipitation pattern. The β-CD in PYR showed the highest tolerance for phase separation while β-CD in NMP gave the faster de-mixing rate compared to that from PYR. PYR has less affinity with water (Parent et al., 2013) and the system containing PYR was more viscous (Phaechamud et al., 2016) which retarded the diffusion rate of water into the β-CD solution. Thus, the amount of water used for phase separation of β-CD containing PYR was higher and the amount of water used for phase separation of 30% β-CD solution in PYR was 258.34% ± 2.89% (w/w). The polar nature of the solvents has high affinity with water as like dissolve like theory. The polarity of DMSO was higher than PYR (George and Sastry, 2004; Hollingsworth, 1952). Owing to the high affinity with water and lower viscosity than that of PYR, the de-mixing rate of β-CD from DMSO was more rapid than PYR. The amount of water used for phase separation of β-CD solution containing DMSO was 180.00% ± 7.55% (w/w) (Figure 20). The polar disubstituted cyclic amide group of NMP molecule can interact with water molecules causing the complete miscibility (Sanghvi et al., 2008). In addition, the less viscous behavior of

NMP allowed the water molecule to penetrate into β-CD solution easily and rapidly. Hence, the β-CD solution containing NMP showed the fastest de-mixing rate with the small volume of water (74.17% ±1.04%, w/w).

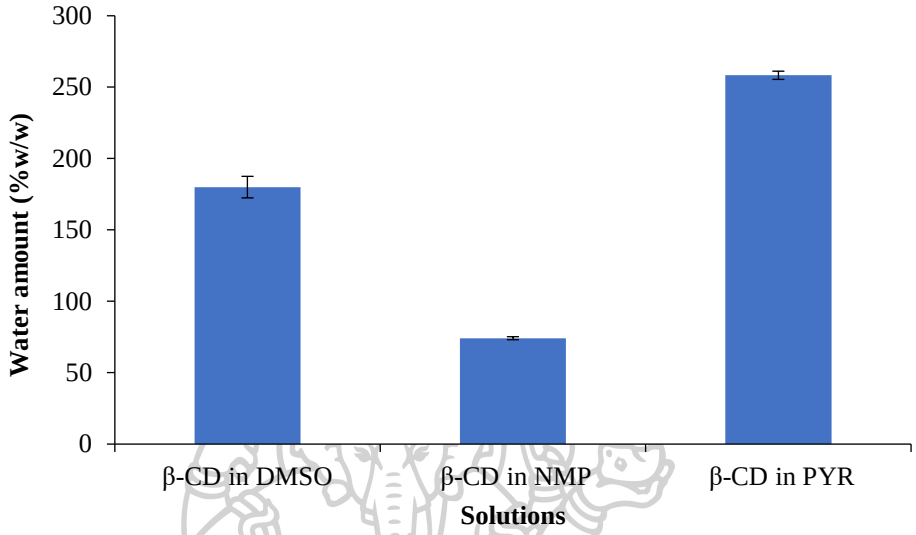


Figure 20 Water resistance of 30% β-CD solutions (n=3)

3.4.1.5 Matrix formation and solvent diffusion

The solvent exchange process was probably driven initially by water influx and onward by solvent efflux. This behavior is crucial for solvent-exchanged induced ISG or ISM to transform from solution or emulsion, respectively, into gel or microparticle (Sanghvi et al., 2008). The rate limiting step of structure change into gel formation or solid matrix was the diffusion rate of aqueous into the β-CD solution (Phaechamud and Setthajindalert, 2017). Therefore, the effect of solvents on diffusion rate and the β-CD matrix formation were studied. When the PBS diffused into the β-CD solution, the solvent exchange process happened, and the solvent diffused into the surrounding aqueous environment. Consequently, the water diffused into the β-CD solution and opaque ring was occurred as phase separation zone which represented the process of matrix formation from outer area to inner area which the enlargement of this band was increased by time. Owing to the strong water miscibility of NMP, the diffusion rate of water into β-CD solution and transformation rate into matrix was rapid (Figure 21 A and 21 B). The opaque band of β-CD solution using PYR as solvent was small because of the high viscosity of solvent itself. The DMSO has a high affinity with

water (Shikata et al., 2006). Thus, the β- CD solution containing DMSO as solvent promoted a rapid phase inversion more than that of the PYR owing to its less viscous nature (Packhaeuser et al., 2004). However, the transformed solid-like matrix behaved like a thicker barrier with time for the aqueous penetration into inner system. Thus, the diffusion path was lengthened, and the penetration of water was gradually slower with time. In ISG system, the solvent typically diffuses into the surrounding aqueous environment and water diffuses into the polymer matrix and then the polymer precipitates and there after the formation of a solid-like matrix is occurred (Phaechamud and Setthajindalert, 2017). Thus, the amaranth front movement was used to indicate the solvent diffusion. The diffusion of solvent together with amaranth as red front into the surrounding environment was investigated under stereomicroscope. The movement of solvent front was occurred after 5 min (Figure 22). Owing to the high affinity of water, rapid solvent exchange behaviour of NMP with water was observed. This led to the lowest distance of solvents front movement. DMSO exhibited lower diffusion of water and matrix formation rate which allowed movement of solvent front to be longer. Thus, the distance of solvent front of DMSO was higher than that of the NMP. The highest solvent front was occurred in the β-CD solution containing PYR.

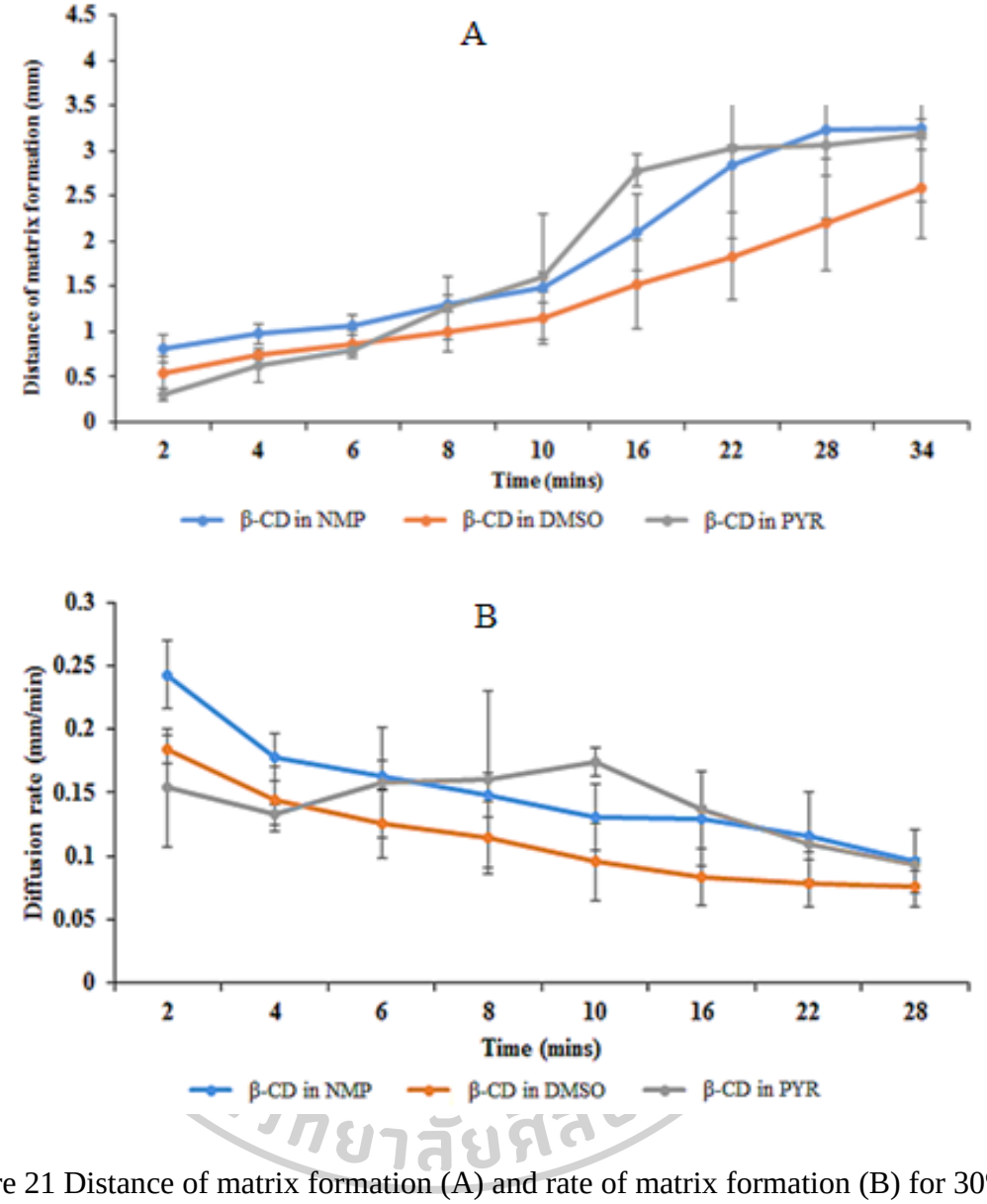


Figure 21 Distance of matrix formation (A) and rate of matrix formation (B) for 30% β-CD solution (n=3)

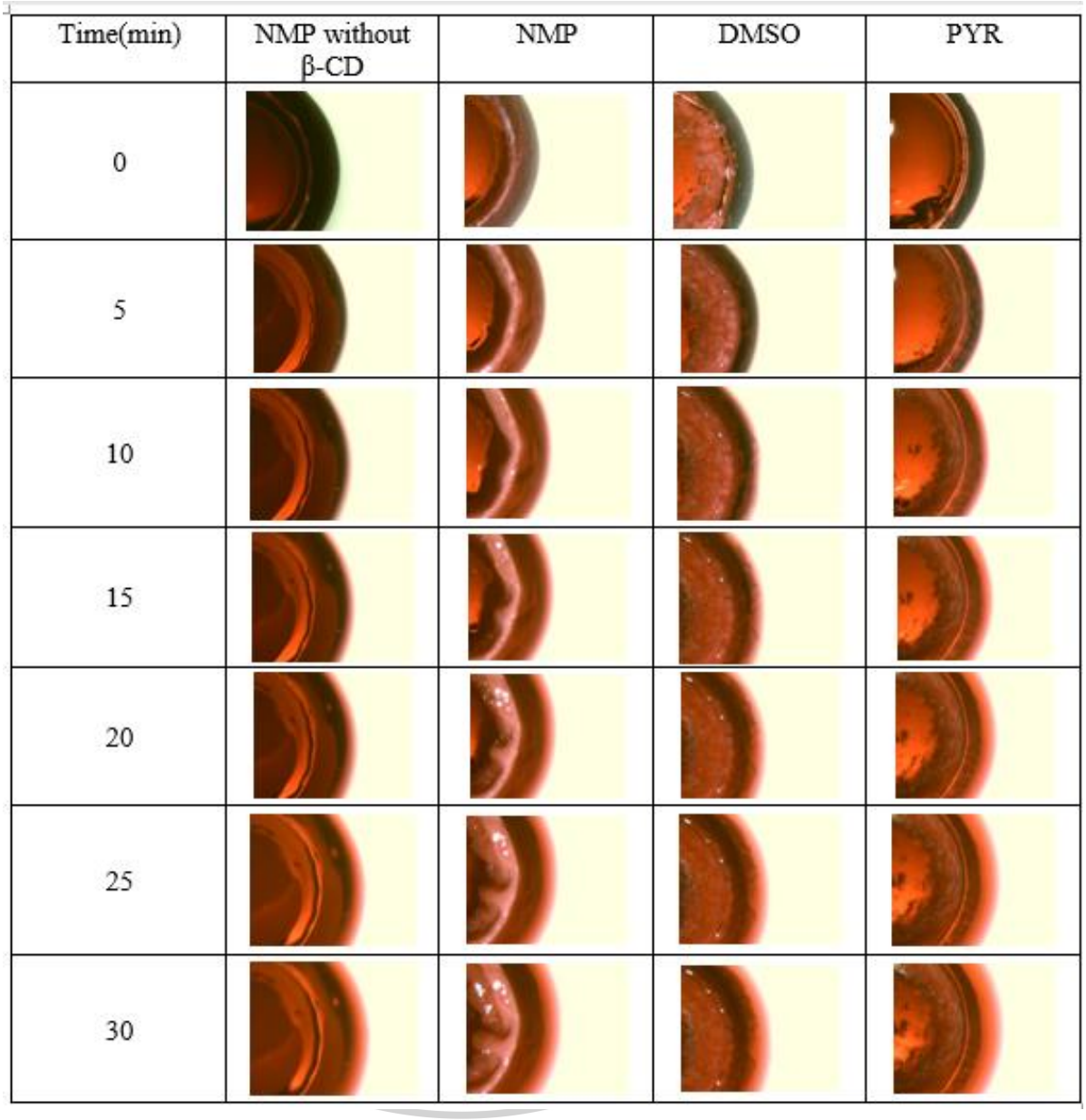


Figure 22 Visual images of solvent diffusion of systems comprising 30%β-CD prepared with different solvents containing amaranth as a colorant

3.4.1.6 *In vitro* gel formation

The β-CD solutions were prepared using the injectable solvents such as NMP, DMSO and PYR. When these drug free β-CD ISG solutions exposed to the aqueous liquid environment, the solvent exchange occurred and the solid deposition of the β-CD as the matrix was occurred from which the drug was released gradually. The present study investigated the *in vitro* gel formation of β-CD solutions. After

exposure with the PBS pH 6.8, the β-CD solution could transform into matrix-like. The lower amount of polymer (< 30%w/w) did not change into solid form due to the discontinuation of the polymer network (Abashzadeh et al., 2011). The complete gelation depended on the concentration of the β-CD (Srichan and Phaechamud, 2017). Moreover, after injection into the surrounding environment, the exchange of solvent with aqueous liquid occurred whereas the affinity of solvent to the surrounding environment may affect the exchange mechanism. When compared, PYR has less affinity with water (Parent et al., 2013). Thus, the slow transformation into gel was observed in β-CD solution in PYR. Increased in concentration of β-CD contributed to the thicker solid-like and opaque matrix (Figure 23). At 35%, both β-CD solution in NMP and DMSO formed solid-like while β-CD solution in PYR formed matrix at 40% w/w (Figure 24). The gelation was increased with time. When compared, β-CD solution in NMP and DMSO formed solid-like matrix rapidly while β-CD solution in PYR demonstrated the slowest formation into solid owing to the high viscosity of the PYR which rendered the solvent exchange and subsequent phase separation. Typically, the higher the polymer concentration the faster the precipitation rate (Phaechamud and Mahadlek, 2015). Thus, the increased β-CD contributed to the rapid formation of solid when contact with PBS solution. Therefore, the prepared β-CD solution could be easily injected into the periodontal pocket and the transformation of gel or matrix-like occurred suddenly.

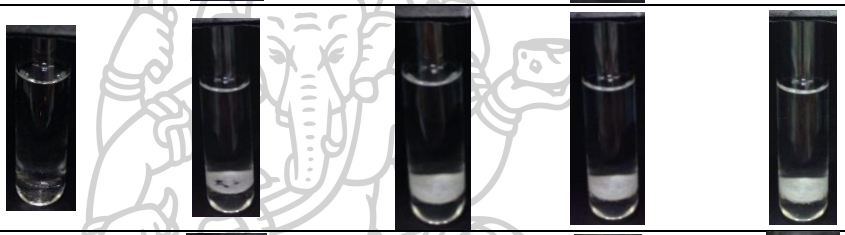
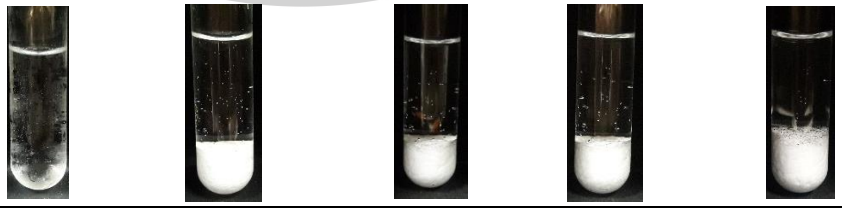
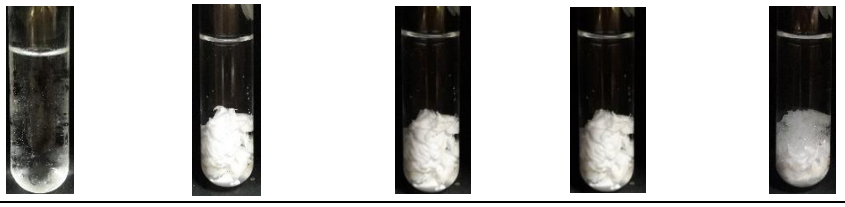
β-CD solutions	Time (min)				
	0	5	15	30	1440
30% β-CD					
35% β-CD					
40% β-CD					
45% β-CD					
50% β-CD					
55% β-CD					
60% β-CD					

Figure 23 Gel formation of β-CD in DMSO after exposure to PBS 6.8 at different time intervals

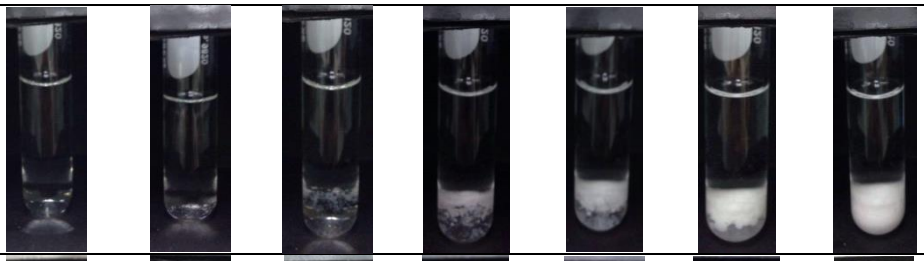
β-CD solution	Time (min)						
	0	1	5	10	15	30	60
NMP							
PYR							

Figure 24 Gel formation of 35 % w/w β-CD in NMP and PYR after exposure to PBS 6.8 at different time intervals

3.4.1.7 Injectability test

The present study indicates that the force of injectability between β-CD solutions was statistically different. The increasing β-CD content increased the work required for expulsion indicating lower injectability. The work force for injectability of the β-CD solutions and pure solvents are shown in Figure 25. Higher concentration of β-CD in NMP at 45% (w/w) and that of PYR > 40% (w/w) could not be expelled through the 27-gauge needle. By comparison, PYR exhibited particularly lower injectability than others owing to the highest viscosity of PYR. The solvent affinity to the β-CD could affect the injectability of the ISG. A good solvent is dominant the polymer-solvent interaction over the polymer-polymer which leads to lowering the viscosity (Parent et al., 2013). Typically, the preparations with the applied force lower than 50 N were acceptable as injectable dosage forms (Philippot et al., 2005). Among three solvents, DMSO solubilized the high amount β-CD and gave the higher injectability indicating the ease of administration of *in situ* forming system by injection.

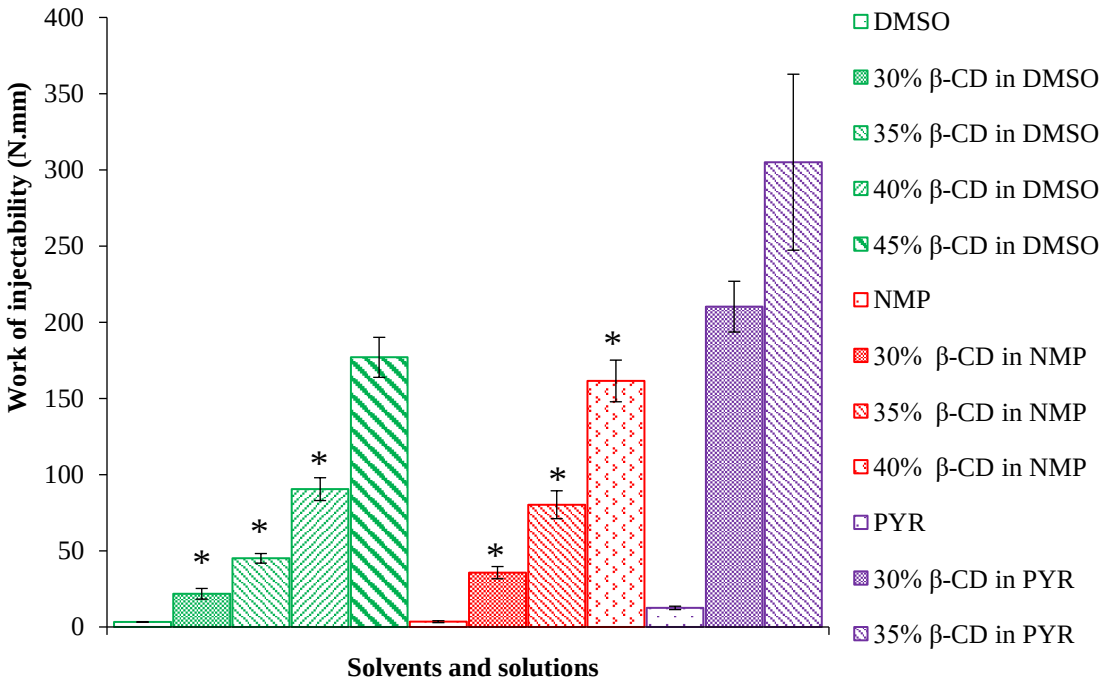


Figure 25 Injectability of pure solvents and β-CD solution (n=3)
The esterics (*)represent the significant differences ($p < 0.05$)

3.4.1.8 Contact angle, surface tension and interfacial tension of β-CD solutions

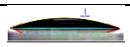
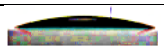
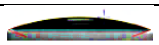
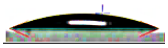
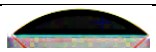
The increased β-CD amount in solvents led to the increased contact angle as shown in Table 2 due to the higher viscous dissipation lowering the wettability and in turn reducing the spreading rate (Phaechamud and Savedkairop, 2012). At 30% w/w, the β-CD solution in PYR exhibited the highest contact angle while β-CD solution in DMSO showed the lowest value which was in accordance with the apparent viscosity data as mentioned above. Typically, the lower contact angle and surface tension led to the better wettability (Phaechamud and Savedkairop, 2012; Yuan and Lee, 2013). The surface tension of 30% w/w β-CD solution in DMSO showed the highest value while β-CD solution in NMP exhibited the least surface tension (Table 3).

The determination of the interfacial tension is a useful parameter for stability of emulsions (Dalgleish, 2003; Herrera, 2012.; Phaechamud and Setthajindalert, 2017). Decreasing the interfacial tension between two phases caused the reducing of the free energy which led to the inherent thermodynamic stability of the system (Harkins and Humphery, 1916). For stability, any emulsion needs to minimize the interfacial energy by making the interfacial area between oil and water as small as

possible. The reduction in the interfacial tension facilitates the emulsion formation and prevents the immediate droplet recoalescence during preparation (Herrera, 2012.). The function of the adsorbed surfactant molecules is to lower the interfacial tension between the two phases of emulsion which consequently lowers the driving force for coalescence (Dalgleish, 2003).

Interfacial tension between β-CD solution in NMP and the oil phase could not be detected even at the high β-CD concentration. It may be due to the very low density gradient between these two phases which minimized the free energy after dropping solvent into oil. In case of β-CD solution in DMSO, the interfacial tension between the two phases could not be detected until the β-CD concentration was increased to 40%w/w (Table 4) because the density gradient between β-CD solution in DMSO and the oil phase was slightly lower. However, owing to the difference in density, the β-CD solution in PYR and the oil phase could determine the interfacial tension value. Differences in the density profile led to the increase in the interfacial tension between the two phases. Although the density gradient between DMSO and camellia oil, olive oil and IPM was less than that of PYR, the interfacial tension between the two phases was highest owing to the highest surface energy at air/liquid surface tension of DMSO. In addition, owing to the highest viscosity nature of IPM, the interfacial tension between the respective β-CD solutions and IPM seemed to be higher than that of the camellia oil and olive oil (Table 4).

Table 2 Contact angle of β-CD solutions (n=3)

β-CD Conc.	Contact angle (Mean ± SD) (°)					
	NMP		DMSO		PYR	
0	13.81 ± 0.33		18.49 ± 1.09		19.07 ± 0.08	
30	22.24 ± 0.44		20.56 ± 0.36		32.20 ± 0.46	
35	ND	ND	20.02 ± 1.70		41.02 ± 0.26	
40	ND	ND	30.48 ± 0.89		ND	ND
45	ND	ND	52.08 ± 1.58		ND	ND
50	ND	ND	58.25 ± 0.16		ND	ND
55	ND	ND	59.41 ± 0.67		ND	ND
60	ND	ND	60.62 ± 2.29		ND	ND

*ND = not determine

Table 3 Surface tension for β-CD solutions (n=3)

β-CD Conc. (%w/w)	Surface tension (Mean ± SD) (mN/m)					
	NMP		DMSO		PYR	
0	38.440 ± 0.380		40.263 ± 0.178		42.317 ± 0.099	
30	40.243 ± 0.372		45.190 ± 0.092		43.537 ± 0.085	
35	ND	ND	49.673 ± 0.129		48.663 ± 0.178	
40	ND	ND	50.437 ± 0.084		ND	ND
45	ND	ND	50.560 ± 0.040		ND	ND
50	ND	ND	51.270 ± 0.053		ND	ND
55	ND	ND	54.040 ± 0.036		ND	ND
60	ND	ND	54.503 ± 0.055		ND	ND

*ND = not determined

Table 4 Interfacial tension for β-CD solutions and different external oil phases (n=3)

Solution	Interfacial tension (Mean ± SD) (mN/m)			
	β-CD Conc.	Camellia oil	Olive oil	IPM
β-CD in NMP	0%	ND	ND	ND
	30%	ND	ND	3.176 ± 0.418
β-CD in DMSO	0	ND	ND	4.677 ± 0.044
	30	ND	ND	8.034 ± 0.020
	35	ND	ND	12.320 ± 0.036
	40	ND	ND	12.860 ± 0.053
	45	8.593 ± 0.058	10.230 ± 0.085	12.597 ± 0.042
	50	8.996 ± 0.058	10.207 ± 0.040	13.113 ± 0.097
	55	11.103 ± 0.025	11.503 ± 0.130	14.580 ± 0.187
	60	10.657 ± 0.275	12.123 ± 0.006	15.283 ± 0.137
β-CD in PYR	0	0	0	2.644 ± 0.062
	30%	5.298 ± 0.048	5.469 ± 0.017	5.947 ± 0.024
	35%	6.2830 ± 0.071	6.413 ± 0.035	7.119 ± 0.255

*ND = not determined

3.4.2 Characterization of β-CD dried precipitates

3.4.2.1 Morphology under inverted microscope

Intact β-CD exhibited the definite rhombic dodecahedral large crystals as previously reported (Millan et al., 1998). The precipitates obtained from DMSO exhibited the small irregular structure which was deformed with time (Figure 26). The smaller particle size of β-CD ppt obtained from DMSO was due to the solubility of β-CD in DMSO which was proved by the higher amount of water used to precipitate the β-CD in water resistant study. However, some well-defined structure was still occurred in DMSO precipitates which indicated that β-CD might partially complexed with DMSO. The substantially large and nearly planar non-polar region of NMP may

lead to hydrophobic interaction between NMP and β-CD molecule to form a complex (Sanghvi et al., 2008). On the other hand, the ppt obtained from PYR resembled in bundle of rhombic dodecahedral crystals which looked likes dendrites and it might be due to the aggregation formation between β-CD and PYR (Figure 26). Therefore, the obtained β-CD precipitates exhibited the quite different morphology from that of the intact β-CD.

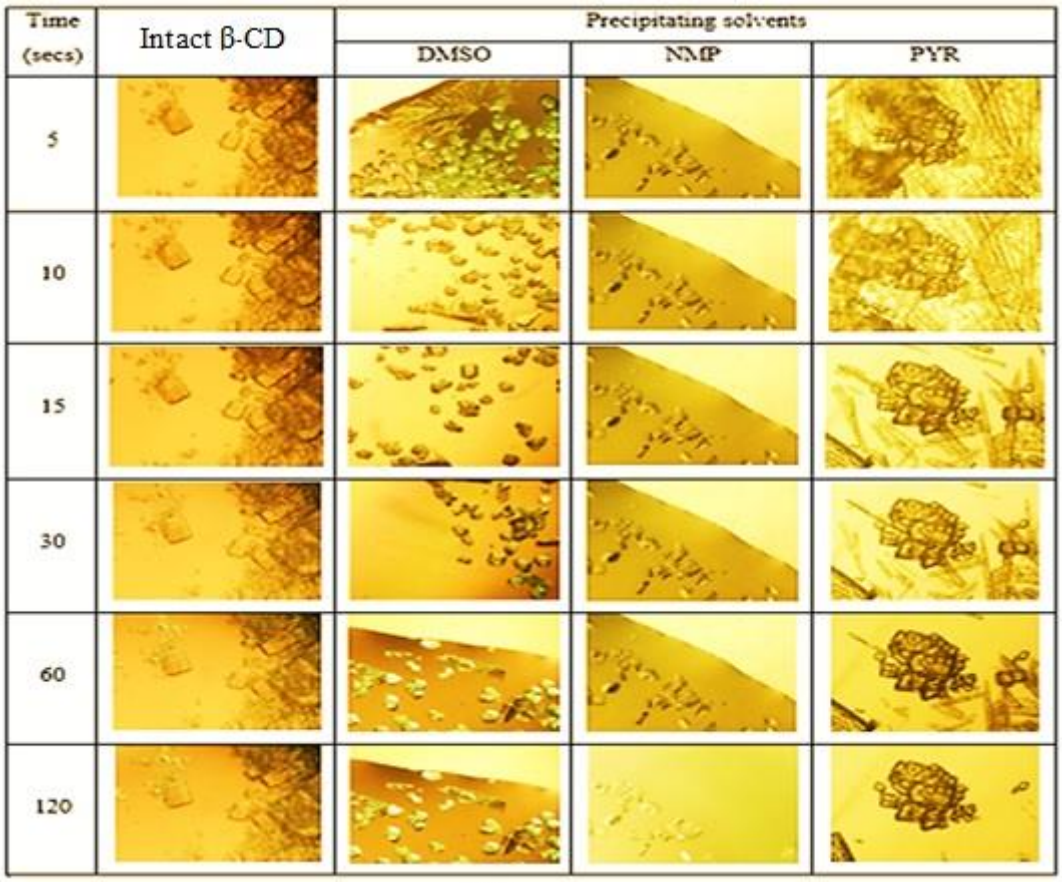


Figure 26 Morphology of dried β-CD precipitates after dispersed in distilled water under inverted microscope (10x)

3.4.2.2 Morphology by SEM

The large well-defined flake-type crystalline particles were found in the intact β-CD (Figure 27). The morphology of ppts from DMSO showed the well-defined surface similar to the intact β-CD and this proved partial interaction between DMSO and β-CD. When there is strong interaction between compound and CDs, it is difficult to differentiate the crystals of both components (Mura, 2015). In addition, the

guest/host inclusion complexes can increase the tendency of β-CD molecules to form aggregates (Messner et al., 2011). The agglomerate aggregated on flake type crystalline particles was observed in NMP precipitates while the rod-like aggregates were observed in PYR precipitates whereas the change in the morphology of intact β-CD was observed. This change in morphology of precipitates might be the indicative partial complexation or aggregation of β-CD with NMP and PYR.

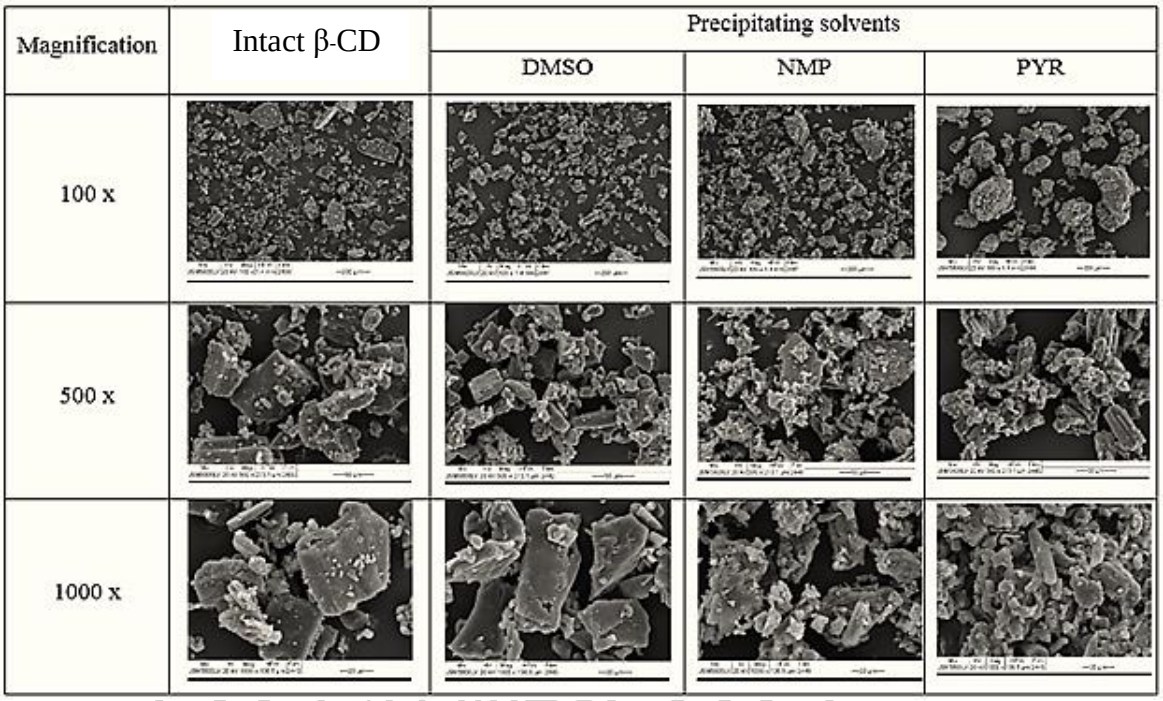


Figure 27 SEM photomicrographs of intact β-CD and β-CD precipitates from different solvents

3.4.2.3 Morphology from hot stage microscopy (HSM)

The HSM photomicrograph revealed that the crystalline structure of intact β-CD did not alter till higher temperature (Figure 28). For β-CD precipitates from NMP and DMSO, their crystalline structures were observed at low temperature. The behavior of precipitate obtained from DMSO showed similar characteristic to intact β-CD signifying the β-CD partially complexed with DMSO. But heating to higher temperature (290°C), the partial melting of crystals was occurred. In the case of the β-CD precipitate from DMSO, the small and large vesicles were also observed. The melting of the β-CD precipitate crystal from PYR was observed even at low

temperature. Thus, HSM proved the evidence of the changes for the character of the intact β-CD when it dissolved in solvents with the partial complexation with solvents.

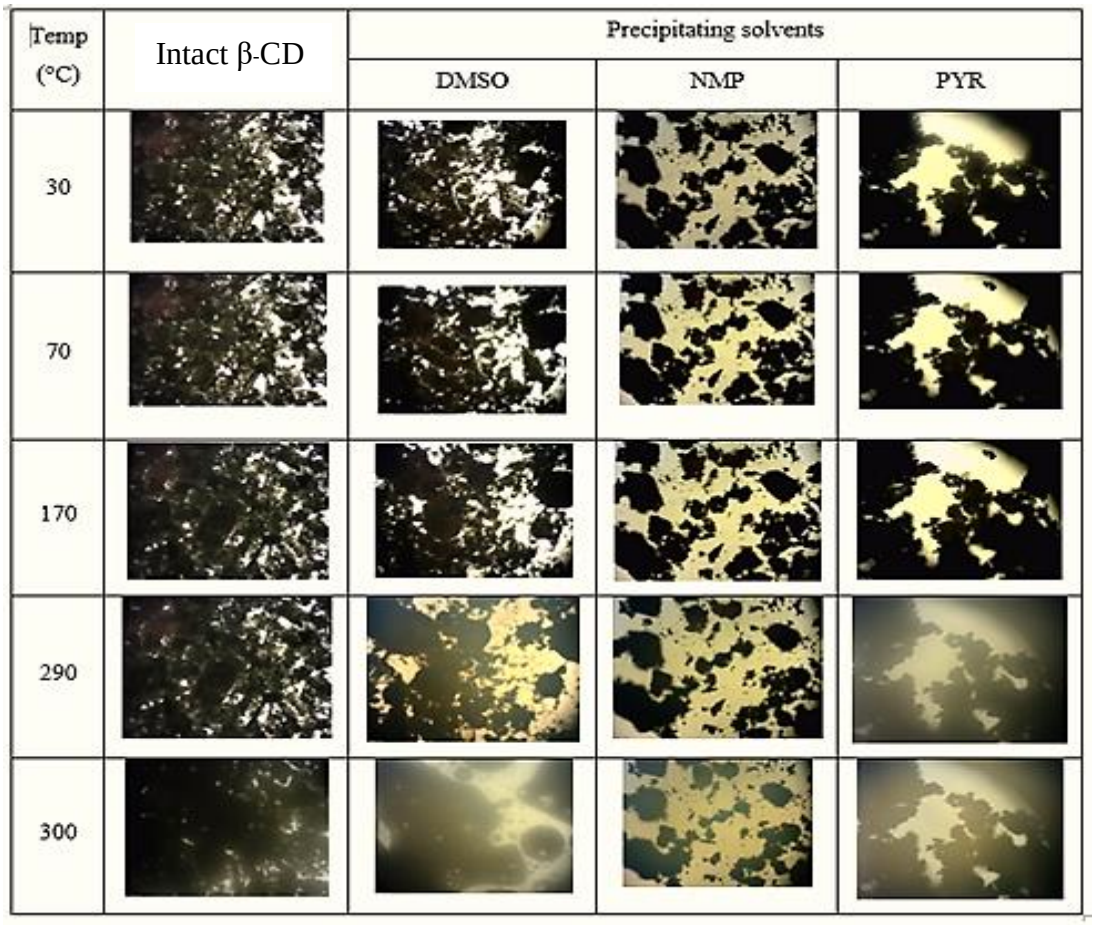


Figure 28 Morphology of intact β-CD and β-CD precipitates from different solvents under HSM (40x)

3.4.2.4 Melting point of β-CD precipitates

Although there is no definite melting point, CDs have begun to decompose from about 200 °C and above (Szejtli, 1988). In the present study, the melting point of intact β-CD was occurred within the range of 267.5–269.3°C. Comparison among β-CD precipitates, the β-CD in NMP gave the highest melting point of 296.8°C which was followed by β-CD in PYR and β-CD in DMSO. The value of their melting points was accounted for 293.0°C for PYR and 283.6°C for DMSO, respectively. The new arrangement of crystalline structure of β-CD precipitated from solvents as seen from above SEM results together with complexation between β-CD and respective solvent could occur because the melting point of β-CD precipitates was higher than that of

intact β-CD. The inclusion complexation could increase the thermal stability (Mura, 2015).

3.4.2.5 Powder X-ray diffractometry (XRD)

Diffractograms of intact β-CD exhibited a series of thin and intense lines corresponding to its crystallinity shown in Figure 29 A. When the respective solvent was introduced into the cavities of β-CD during the formation of inclusion complex, the similar diffraction with intact β-CD might be obtained (Katewongsa et al., 2017). The similar diffraction patterns with low intensities were observed in β-CD precipitates. Moreover, the intensity of sharp maximum peak presented in intact β-CD was reduced in DMSO and PYR precipitates and the new peak was also occurred in PYR precipitate which indicate the partial complexation behavior. The partial complexation between β- CD and PYR might be due to the hydrophobic part of solvent molecules incorporated into the β-CD cavity (Sanghvi et al., 2008) while the partial complexation between β-CD and DMSO might be the result of the of hydrogen bond formation to an oxygen atom of DMSO and hydroxyl group of β-CD (Shikata et al., 2006). The sharp maximum peak of intact β-CD was not occurred in precipitate obtained from NMP. Therefore, the different and lower intensities may be indicative of partial complexation.

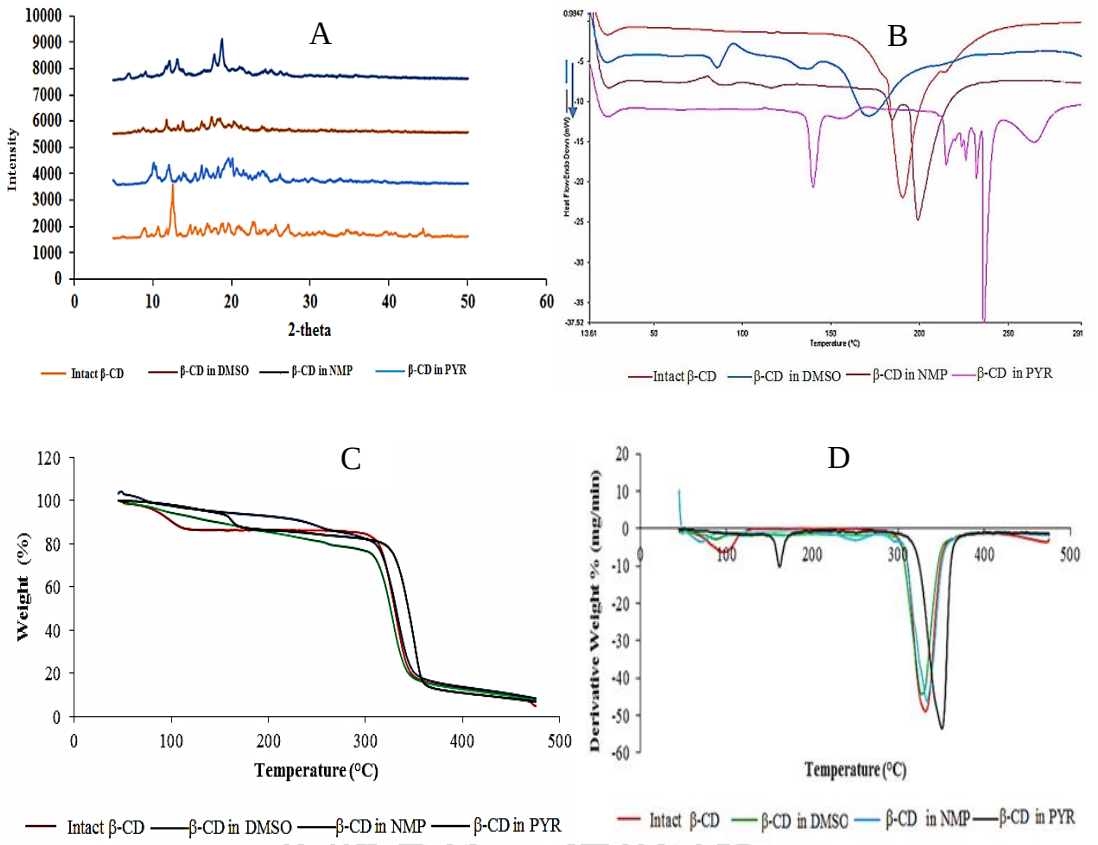


Figure 29 (A) XRD diffractograms; (B) DSC Thermograms; (C) TGA curves and (D) DTGA curve of intact β-CD and β-CD precipitates from different solvents (DMSO, NMP and PYR).

3.4.2.6 Differential scanning calorimetry (DSC)

The intact β-CD exhibited only a broad endothermic peak at 180°C and no peaks were observed below 150°C. The peak of DMSO precipitate was shifted to lower temperature and the residual presence of the solvent melting peak is the indication of the existence of solvent molecules which suggested that there was incomplete inclusion complexation (Al-Marzouqi et al., 2007) which was in accordance with the SEM study. The β-CD precipitate derived from NMP exhibited a broad endothermic peak which was similar to intact β-CD, but the peak was shifted to higher temperature. The PYR precipitate gave the residual peak of the pure solvent at 140°C and the sharp endothermic peak of β-CD was shifted to 240°C. The results are shown in Figure 29 B. The solid-states interactions caused the energy activation for the decomposition of β-CD which could lead to the change in the temperature peak of the β-CD dehydration band (Al-Marzouqi et al., 2007). Typically, the increased decomposition temperature might be assumed as the increased thermal stability of the

inclusion complex (Hees et al., 2002). Therefore, the DSC thermogram of β-CD precipitates indicated a sign of partial inclusion complex of β-CD with respective solvents.

3.4.2.7 Thermogravimetric analysis (TGA) and derivative of thermogravimetric analysis (DTGA)

TGA was carried out to identify the changes in weight percent with respect to temperature change. TGA reveals that intact β-CD exhibited two stages of weight loss (Figure 29 C and D). Loss of water molecules from intact β-CD cavity occurred at 76°C in the first stage. The degradation temperature of CD has been reported at about 300°C (Lee et al., 2017), which the weight loss related to the decomposition of intact β-CD was occurred at 305°C. The flat curve of TGA was observed between 120°C and 280°C and no mass loss was detected (Giordano et al., 2001). For β-CD precipitate derived from DMSO, the first stage of loss of water molecule occurred in intact β-CD was disappeared and it exhibited only one mass loss at 299°C. This different weight loss pattern occurred in precipitate derived from DMSO was the indicative of the partial complexation behavior. First weight loss for β-CD precipitate derived from NMP started at 73°C which was similar to the water loss of intact β-CD. The second mass loss for β-CD precipitate derived from NMP was decreased to 225°C which indicated the decrease in thermal stability of β-CD precipitate (Chen et al., 2006). In case of the mass loss for precipitate derived from PYR, it gave two stages of weight loss as occurred in intact β-CD except the water loss temperature was increased to 157°C and the second decomposition temperature was increased above 310°C. The residual weight of β-CD precipitate above 300°C after thermal decomposition exhibited similar weight to that of the intact β-CD which indicates the partial incorporation of β-CD in respective solvents. As the boiling point of NMP was 202°C (Sanghvi et al., 2008) and that of PYR was 245°C (Budavari, 1996), there was no residual weight of pure solvents were observed above 250°C. As the boiling point of NMP was 202°C (Sanghvi et al., 2008) and that of PYR was 245°C (Budavari, 1996), no residual weight of pure solvents was observed above 250°C. This observation was corresponded to the residual weight observed in β-CD precipitate above 300°C which was the component of β-CD; therefore, this thermal behavior indicated the complexation of β-CD with solvents. The two stages of weight loss were

occurred in intact β-CD and they were accounted for 10% and 50% weight loss at 100°C and 325°C, respectively. The two stages of %weight loss for precipitate obtained from DMSO was less than that of intact β-CD. The three stages of weight loss for precipitate obtained from NMP were occurred and the temperature for first weight loss was less than that of the intact β-CD. On the other hand, the prominent different weight loss pattern was obtained in precipitate form PYR which the weight loss was more than 10% and 50% at 160°C and 350°C, respectively. This phenomenon indicates that the β-CD precipitates had changed the thermal degradation properties of intact β-CD which proves that the morphology of the intact β-CD was changed.

3.4.2.8 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectrum of intact β-CD and precipitates is shown in Figure. 30. The prominent peak of intact β-CD in the region of 3000–3500 cm^{-1} was due to O-H stretching vibration (Bratu et al., 2004). The vibration of –CH and $–CH_2$ groups was occurred in the 2800-3000 cm^{-1} regions (Schalley, 2006). The C=O group gave the strong absorption near 1820-1660 cm^{-1}, C-O asymmetric stretching and OH bending were occurred in the range of 1200-1000 cm^{-1}, respectively (Bratu et al., 2004). Almost similar peak at wave number of the intact β-CD was observed in the obtained precipitates. However, overlapping of O-H and C-H group promoted in peak broadening of β-CD precipitates. Other corresponding peaks of O–H, C–H (bending), C=O, C–H (stretching) occurred in intact β-CD were evident at the lower frequencies in precipitates. Although the functional group of the intact β-CD was not changed, strong intensities in the area between 1800 cm^{-1} in PYR and NMP precipitates proved the insertion of molecules into the electron rich cavity of β-CD and it caused to increase the density of electron cloud, which led to the increase in frequency.

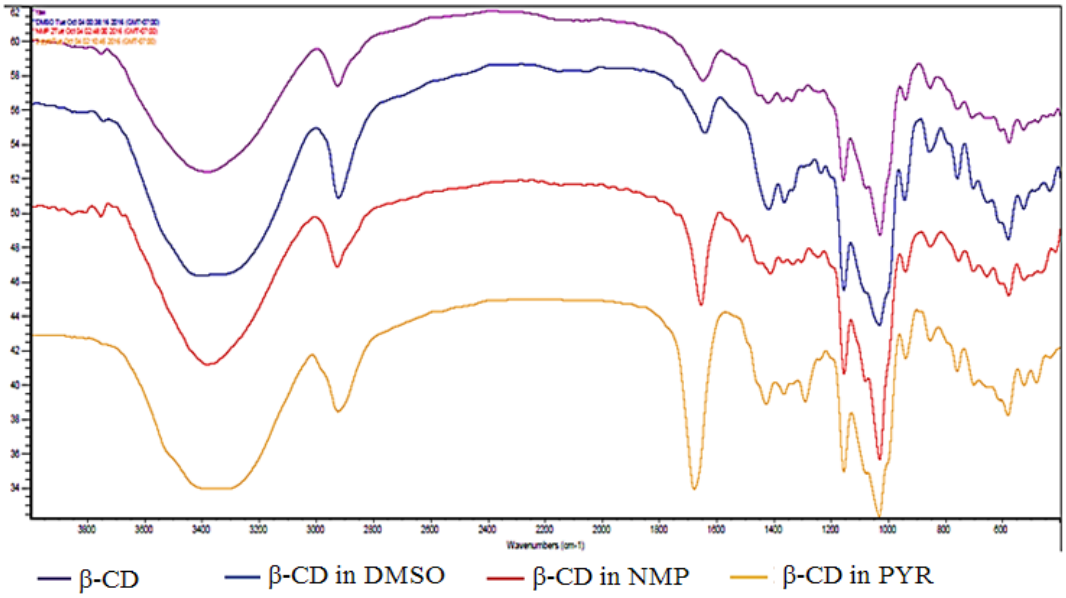


Figure 30 FT-IR spectra of intact β-CD and β-CD precipitates from different solvents

3.5 Conclusion

Owing to the highest viscosity of the solvent itself, the β-CD solutions containing PYR as solvent exhibited the highest viscosity which was followed by the β-CD solution prepared from NMP and DMSO, respectively. The β-CD dissolved in NMP and DMSO exhibited the higher rate of solvent diffusion and solid matrix formation. The matrix formation depended on the concentration of β-CD which β-CD solution in PYR showed the slower gelation rate when compared with β-CD solution in NMP and DMSO. In addition, DMSO presented the advantage of achievement for the highest β-CD loading which further reduced the injection volume and provoked the dense matrix for controlling the drug release whereas it also showed the proper viscosity with Newtonian flow and lower work of injectability. β-CD in PYR exhibited the highest water resistance and its matrix formation was lower than that in DMSO and NMP. From DSC, TGA, XRD and FT-IR data, β-CD precipitates prepared from these solvents showed the complexation between β-CD and solvent molecules. Therefore, these characteristic properties will be useful for further using β-CD solutions as matrix former in the ISG for periodontitis treatment. In addition, the β-CD solubility, intrinsic, apparent and relative viscosities, density, surface and interfacial tension, contact angle measurements provided the useful information of β-CD solution as potential internal phase of emulsion for further preparation of oil in oil emulsion of ISM.

Chapter 4

Fluid Properties of Solvents and Oils used in *In Situ* Forming Microparticles (ISM)

4.1 Introduction

4.2 Materials and methods

4.2.1 Materials

4.2.2 Measurement of density and pH

4.2.3 Viscosity measurement

4.2.4 Measurement of flow time

4.2.5 Injectability test

4.2.6 Measurement of surface tension and contact angle

4.2.7 Phase separation study

4.2.8 Measurement of diffusion pattern of color and miscibility test

4.2.9 Antimicrobial study

4.2.10 Determination of cytotoxic activity

4.3 Results and discussion

4.3.1 Density

4.3.2 pH

4.3.3 Viscosity

4.3.4 Flow time

4.3.5 Injectability

4.3.6 Contact angle and surface tension

4.3.7 Phase separation study

4.3.8 Measurement of color diffusion pattern and solvent/oil miscibility

4.3.9 Antimicrobial study

4.3.10 Determination of cytotoxic activity

4.4 Conclusion

4.1 Introduction

Pharmaceutical manufacturers use solvents as a vehicle, wetting agent and granulating fluid. The physicochemical characteristics of solvents such as density, viscosity, pH, injectability and solubility directly influence the properties of pharmaceutical dosage forms. The solvent affinity to the polymer could affect the injectability of the ISF systems. For a good solvent, the polymer-solvent interaction should be dominant over the polymer-polymer interaction which leads to lowering the viscosity (Parent et al., 2013). Therefore, the study of the viscosity of the solvents used for *in situ* drug delivery system is necessary. Typically, the preparations with the applied force lower than 50 N were acceptable as injectable dosage forms (Philippot et al., 2005). The ease of injection is a critical factor together with an optimal rheological behavior of the injectable dosage forms that should be a Newtonian or pseudoplastic flow. Theoretically, the density is one of the crucial factors in stability of emulsion. The density of the internal phase and the external phase of the emulsion system should not be that different to inhibit the phase separation process (Mieth, 1984). In addition, the contact angle, surface tension and interfacial tension are important parameters for an emulsion system such as an aqueous in oil emulsion of *in situ* forming microparticles (ISM). Furthermore, the wettability data is indicative in compatibility among a formula vehicle and it is described by the value of contact angle of a liquid on a solid surface (Phaechamud and Savedkairop, 2012).

The injectable solvent exchange-induced ISF system, when exposed to the surrounding aqueous environment, the water miscible organic solvent exchanged with water leading to the formation of a solid/semisolid depot at the site of injection followed by sustained release of the drug. Between the time of injection and complete depot formation, the initial drug burst release occurred which can be controlled by modulating the hydrophobicity of the solvent (Prabhu et al., 2005). The diffusion pattern of solute into the solvents could be assessed using the water soluble dye solution and oil soluble color.

The biodegradable ISM systems are injectable emulsions in which the internal phase is composed of polymer solution, whereas the oil mixed with proper stabilizer is used as the external phase. Practically, the solvent-exchange induced-ISM is in liquid form before administration but when in contact with body fluid, the influx of

water from environment and diffusion out of solvent leads to the phase separation of the polymer emulsion droplet into microparticles. By comparison with ISG, the advantage of ISM includes: the decreased myotoxicity (Kranz et al., 2001), the minimized burst-free drug release patterns (Kranz and Bodmeier, 2007) with better injectability (Rungseevijitprapa and Bodmeier, 2009) from a lower viscosity of the emulsion due to the presence of external oil phase.

The ideal solvent for ISF systems needs to gain appropriate properties in terms of proper water affinity, viscosity, ability to dissolve the polymer and its safety. In the present study, various solvents such as *N*-methyl pyrrolidone (NMP), 2-pyrrolidone (PYR), dimethyl sulfoxide (DMSO), triacetin and glycofurol showed interesting qualities as the injectable solvents as the internal phase and the oils such as olive oil, camellia oil and isopropyl myristate (IPM) were selected as the oil phase of ISM. Being the amphipathic molecule, DMSO is miscible with water and organic solvents which is a very efficient solvent for water-insoluble compounds via hydrogen-bound disruption (Santos et al., 1998). DMSO has been used in the formulation of an injection and subcutaneously implant (Lee and Wang, 1999; Strickley, 2004). The LD_{50} for intravenous and subcutaneous injections of DMSO in rat is 5.3 and 12g kg^{-1}, respectively (Rowe et al., 2009). In addition, it can be used as a polymer vehicle in ISFimplant and ISM (Ahmed et al., 2014). A polar disubstituted cyclic amide group present in NMP molecules is responsible for interactions with water molecules for their complete miscibility. In addition, it has the ability to act as a cosolvent owing to presence of the non-polar carbons which can weaken the hydrogen-bonded structure of water (Sanghvi et al., 2008). It is thermally stable, biocompatible and has a high solubilizing power which can be used in the parenteral and oral medications as an acceptable solvent (Jouyban et al., 2010; Strickley, 2004). Its solubility is similar to that of ethanol and DMSO (Hansen and Just, 2001). NMP shows low toxicity both orally and parenterally (Bartsch et al., 1976). The LD_{50} of NMP is 3.9 g kg^{-1} in rat when given by oral route (Rowe et al., 2009). The PYR is miscible with a wide variety of other solvents with greater solubilizing power than a conventional solvent such as ethanol, glycerin, or propylene glycol due to a complexation and/or cosolvency mechanism (Jain and Yalkowsky, 2007). PYR is often used as a solvent in the parenteral formulation including ISG system (Jain and Yalkowsky, 2007; Parent et

al., 2013) and ISM (Jain and Yalkowsky, 2007; Kranz et al., 2001) and was used as a plasticizer to improve mechanical properties and reduce the drug burst release of the spider silk films (Agostini et al., 2015). LD_{50} value, when tested in rat by oral route, of PYR is 6.5 g kg^{-1} (Raymond et al., 2009). The other biocompatible solvents such as triacetin and glycofurol were also investigated in this study. Triacetin, the triester of glycerol and acetylating agents, such as acetic acid and acetic anhydride (Kong et al., 2016), have been used as a plasticizer in ISF implant systems (Ahmed et al., 2014). Using triacetin as a solvent can reduce a burst effect and extend the drug release of ISF biodegradable PLGA microspheres (Jain et al., 2000). The initial burst release was reduced when triacetin was used as the co-solvent in hydrophilic polymer depot containing either NMP or DMSO as the solvents (Liu and Venkatraman, 2012). Triacetin was recognized as safe (FDA's GRAS list) (Ahmed et al., 2014) which is not toxic in short-term inhalation or parenteral routes (Fiume, 2003). Moreover, it was used as a potential parenteral nutrient (Bailey et al., 1991; Karlstad et al., 1992). The LD_{50} of triacetin is higher than 2 mL/kg which is acceptable for using in the injectable implant systems (Kranz and Bodmeier, 2007). Glycofurol was known as a parenteral solvent (Mottu et al., 2000; Mottu et al., 2001) which has the ability to dissolve a wide range of water insoluble drugs. It was used as a co-solvent for Eudragit L and Eudragit RS to obtain the high viscous systems of controlled release properties (Bonacucina et al., 2006), as the injectable solvent of the ISFimplant system (Algın and Baykara, 2010) and also used as the vehicle for the naproxen loaded topical gel (Barakat, 2010) and for the intranasal preparation of insulin (Bechgaard et al., 1996) and bumetamide (Nielsen et al., 2000) to enhance drug absorption. Glycofurol is a nontoxic and nonirritant material when it is diluted with water. The LD_{50} of glycofurol by intravenous injection in mice is 3.78 g/kg (Spiegel and Noseworthy, 1963). Olive oil has been used as an oily vehicle in the injectable solution (Nema and Ludwig, 2010). The main compositions of olive oil are the mixed triglyceride esters of oleic acid and palmitic acid (Beltran et al., 2004). Isopropyl myristate (IPM) has been used as a vehicle for injectable solution as an alternative of a vegetable oil (Engelbrecht et al., 2012; Nema and Ludwig, 2010). In addition, IPM showed very low irritability and no sensitizing properties in animals after topical and parenteral administration (Platcow and Voss, 1954). Camellia oil, obtained from *Camellia*

oleifera, has several therapeutic effects and it has the comparable health benefits to olive oil and better than sunflower oil (Sahari et al., 2004). Moreover, it possesses a remarkable antioxidant activity (Lee and Yen, 2006). Because of the fatty acid constituents such as palmitic, linoleic, oleic and stearic acids, this oil is important in cosmetics used for skin and hair. Chemical structure of NMP, PYR, DMSO, triacetin, glycofurol and IPM are shown in Figure 31. Therefore, the objective of the present study is to investigate physicochemical properties of various solvents and oils used in ISM preparation.

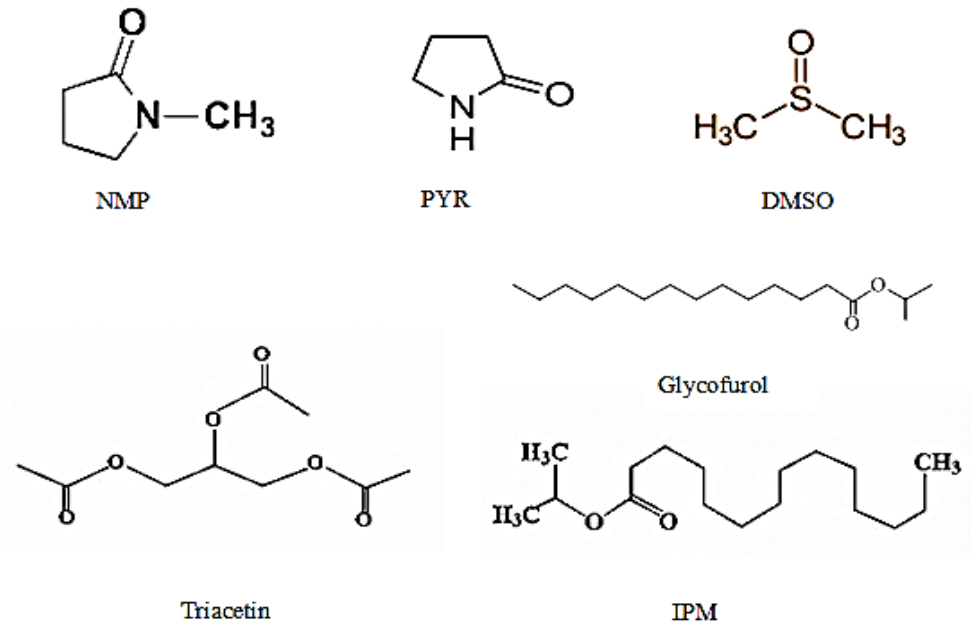


Figure 31 Chemical structures of solvents and IPM

4.2 Materials and methods

4.2.1 Materials

NMP (lot no. A0251390, Fluka, New Jersey, USA), DMSO (lot no. 453035, Fluka, Switzerland) and PYR (lot no. BCBF5715V, Fluka, Germany) were used as received. Glycofurol (T3396) was supplied by SIGMA-ALDRICH Co., St. Louis, USA. Olive oil (Lot no. L5506T H1700, ConAgra Foods Inc.), IPM (batch no. 1993 KNLE, PC drug centre Co. Ltd), camellia oil (Lot no. 11-1-20123-0185, Naturel, P.K. Trading, Thailand) and triacetin (Lot no. A0375797, SIGMA-ALDRICH) were used as received. Amaranth (lot No. A0293188, ACROS organic, New Jersey, USA) and

nile red (lot No. BCBV2046, SIGMA-ALDRICH, Germany) was used for measurement of diffusion pattern of solvents. Agarose (Lot H7014714, Vivantis, Malaysia) was used to study the solvent diffusion. Potassium dihydrogen orthophosphate (lot no. E23W60, Ajax Finechem, 10 Australia) and sodium hydroxide (lot no. AF 310204, Ajax Finechem, Australia) were used for preparation of phosphate buffer (PBS) pH 6.8.

For antimicrobial activity of solvents, the bacterial strains such as *Staphylococcus aureus* ATCC 25923, *S. aureus* DMST 6935, *S. aureus* ATCC 43300, *Klebsiella pneumoniae* ATCC 700603, *Enterococci faecium* UCLA192, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMST 37166 and *Stenotrophomonas maltophilia* DMST 19079 were purchased from the Department of Medical Science, Ministry of Public Health, Thailand (DMST). For determination of cytotoxic activity of solvents, HCT116 colorectal carcinoma cell line was purchased from American Type Culture Collection (ATCC) USA. The culture medium components including DMEM, L-glutamine, penicillin, streptomycin and fetal bovine serum were purchased from Invitrogen, Paisley, UK.

4.2.2 Measurement of density and pH

The density of solvents was determined by pycnometer (Densito 30PX, 21 Mettler Toledo (Thailand) Ltd, PortableLab TM) (n=3) at room temperature. The pH values of the such solvents were also measured with the pH meter (Seven compact, Mettler-Toledo, Thailand Ltd.) by setting the automatic reader to room temperature and the pH are recorded (n = 3).

4.2.3 Viscosity measurement

Viscosity measurement was carried out using a Brookfield viscometer (Brookfield DV-lll ULTRA programmable rheometer, BROOKFIELD ENGINEERING LABORATORIES, INC., Middleboro, United States). Shear rate was changed slowly and then the correlation between shear rate and shear stress was recorded.

4.2.4 Measurement of flow time

The determination of flow time for the various solvents was carried out using a Ubbelohde viscometer (Calibrated viscometer, model 9721-R53, Cannon) (n=3).

4.2.5 Injectability test

The injectability test was used to indicate the difficulty of injection using a texture analyzer in a compression mode (TA.XT plus, Stable Micro Systems, UK). Briefly, each solvent was inserted into a 1 mL syringe with a 27-gauge needle which was clamped with a stand. The upper probe of the texture analyzer moved downwards at a constant speed (1.0 mm s^{-1}) and a constant force of 0.1 N was applied to the base. Force displacement profiles were recorded, with the forces at a distance of 10-mm being selected for analysis (n=3).

4.2.6 Measurement of surface tension and contact angle

The surface tension and contact angle of solvents were measured by using a goniometer (FTA 1000, First Ten Angstroms, USA) at room temperature (n=3).

4.2.7 Phase separation study

In determination of phase separation between the internal and external phases, the internal phase was firstly prepared. The DMSO was colored with 0.1%w/w amaranth solution. Then different concentrations of β-CD were added into DMSO and mixed by magnetic stirrer. For the higher concentrations, increasing temperature range from 50°C to 250°C was required. For external phase, glyceryl monostearate (GMS)/Polyaquol was dissolved in camellia oil, olive oil and IPM separately by using magnetic stirrer at 150°C. Then, the emulsification of 1:1 for external and internal phases was done by back and forth movement of the syringe connector for 50 mixing cycles syringe. Then, connected syringes were stand vertically with the emulsion syringe in upward position in room temperature. The separation rate from the incompatible parts were measured from 0 hours to 24 hours each. Then, the rate of separation from oil phase was calculated (n=3).

4.2.8 Measurement of diffusion pattern of color and miscibility test

In order to observe the behavior of diffusion pattern in various solvents, the 0.3% w/w of water soluble dye (amaranth solution) and oil soluble fluorescent dye (nile red) were added into the 10 ml solvent filled glass bottle. Then the changes of diffusion pattern in 0, 1, 2, 3, 5, 10, 15, 20, 25, 30 min were recorded. The oil soluble colors used to investigate their diffusion patterns into the various solvents and oils were green, blue and red.

For miscibility study, 3 ml of each solvent dyed with 0.01% amaranth (distilled water, NMP, PYR, DMSO and glycofurol) was individually mixed with 3 ml of triacetin, IPM, olive oil and camellia oil in 10 ml test tubes. Then, the two phases were mixed by vortex mixer for 1 min and the miscibility of the two phases was examined visually. Then, each of mixture was filled into 10 ml cylinders and allowed to set for 5 min. The volume miscibilities after setting for 5 min were recorded.

4.2.9 Antimicrobial study

The antimicrobial activities of five solvents were tested using a cylinder plate method. This technique was based on diffusion of the solvent from a stainless-steel cylinder (6 mm. in diameter) through agar gel inoculated with tested microorganism. Briefly, the 5.0 McFarland turbidity of studied bacterial strain was spread thorough on MullerHinton agar (MHA) (Oxoid, UK). The 100 μL of tested preparation diffuses from a cylinder placed on the surface of an inoculated MHA plate and incubated at 35°C for 18 hours, the inhibition zone against the tested organisms around cylinder were detected visually. All tests were carried out in triplicate. Standard strains such as *S. aureus* ATCC 25923, *S. aureus* DMST 6935, *K. pneumoniae* ATCC 700603, *E. faecium* UCLA192, *E. coli* ATCC25922, *P. aeruginosa* DMST 37166, *P. gingivalis* and *C. albicans* were tested.

4.2.10 Determination of cytotoxic activity

The cytotoxic activity was determined by MTT assay using colorectal HCT 116 cell line. To evaluate the cytotoxicity effect of different solvents on colorectal HCT 116 cell line, viability tests were applied using MTT colorimetric assay. Briefly cell line was seeded in 96-well plates at a density of 20 x 10^3 cells per well and then incubated at 37°C in 5% CO_2 to allow cell attachment. The medium was removed and

replaced with fresh medium containing various concentrations (0, 0.1%, 0.5%, 1%, 2%, 3% and 5% v/v) of solvents. After treatment for 24 hours, 10 μL MTT (5 mg/mL) was added to each well and the plate was further incubated. Four hours later, all remaining supernatant was removed and 100 μL of DMSO was added to each well to dissolve the resulting formazan crystals. Finally, absorbance was read at 570 nm using microplate reader (BioTek, USA).

The cell viability percentage was calculated using the equation:

$$\text{Cell viability percentage} = \frac{\text{mean OD of treated cells}}{\text{mean OD of control cells}} \text{x} 100 \quad (5)$$

4.2.11 Statistical analysis

The statistical significance of the obtained data was examined using one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) post hoc test. The significance level was set at $p < 0.05$. The analysis was performed using SPSS for Windows (version 11.5).

4.3 Results and discussion

4.3.1 Density

Density is one of the crucial factors related with the stability of an emulsion. The difference in density between two phases of an emulsion creates the rising up of oil. Then, the droplet of oil appears and coalesces to form a separate layer of oil on the top. In order to prevent this phase separation, the density gradient between the external and the internal phase should not be much different (Mieth, 1984). On the other hand, the ISG/ISM system transforms into gel or solid-like matrix when exposures to surrounding aqueous environment by solvent exchange process (Srichan and Phaechamud, 2017). The higher the density of the system than water might facilitate the solvent exchange process which could support for deposition it at the bottom of the target site.

The olive oil had a lower density while bleached shellac in different solvents showed a higher unique density depending on the type of solvents (Phaechamud and Setthajindalert, 2017). According to the experiment, IPM showed the lowest value of density and the oil samples such as camellia oil, olive oil also showed the lower density values which was followed by distilled water, NMP, glycofurol, DMSO, PYR and triacetin, respectively as shown in Table 5. The emulsion containing NMP, glycofurol and DMSO as injectable solvents could be more stable than other solvents.

4.3.2 pH

Typically, the amide compounds possessing coplanar structures are not basic but for NMP and PYR, their carbonyl groups and lone pair electrons of nitrogen atoms are not coplanar and the resonance effect did not occur. Therefore, the water molecules can protonate the hydrogen atoms with those electrons that lead to an increase in acidity (Deruiter, 2005). Therefore, solvents such as NMP, PYR showed the high pH or alkalinity. DMSO also showed high pH whereas the others exhibited pH of lower than 7 (Table 5). Some polymers with acidic structure such as bleached shellac are practically soluble in these high alkaline solutions for fabrication into the ISG and ISM (Phaechamud and Setthajindalert, 2017). Olive oil is mainly composed with the triglycerides and the amount of free fatty acids; therefore, it showed the lowest pH value whereas the pH of the IPM was slightly lower but higher than that of olive oil. The trend for pH of various solvents was olive oil< IPM< triacetin <

camellia oil < glycofurol < distilled water< DMSO< PYR< NMP, respectively which are shown in Figure 32.

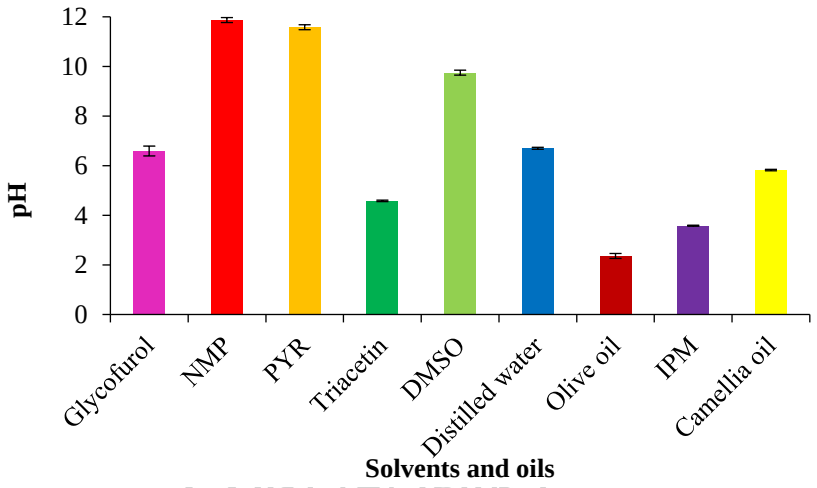


Figure 32 pH values for various solvents and oils (n=3)

Table 5 The physicochemical properties of various solvents and oils (Mean ± SD) (n=3)

Solvent	Density (g/ml)	pH	Viscosity (cPs)	Flow time (min)	Injectability (N.m)	Contact angle (degree) at 5 sec	Surface Tension (mM/m)
Glycofurol	1.0800 ± 0.0003	6.59 ± 0.20	17.20 ± 0.20	91.42 ± 3.70	17.74 ± 0.50	16.65 ± 0.50	41.74 ± 3.02
NMP	1.0300 ± 0.0004	11.87 ± 0.10	4.88 ± 0.20	9.17 ± 0.03	6.11 ± 0.30	16.84 ± 0.90	49.68 ± 0.30
PYR	1.1100 ± 0.0002	11.58 ± 0.10	13.76 ± 0.20	65.39 ± 1.50	14.28 ± 0.70	21.66 ± 1.80	49.06 ± 1.80
Triacetin	1.1500 ± 0.0005	4.58 ± 0.03	15.75 ± 0.20	72.21 ± 0.80	13.92 ± 0.03	19.92 ± 1.90	37.48 ± 0.60
DMSO	1.0900 ± 0.0003	9.75 ± 0.10	4.52 ± 0.01	10.32 ± 0.10	5.42 ± 0.30	29.96 ± 6.80	51.63 ± 1.10
Distilled water	0.9900 ± 0.0001	6.70 ± 0.04	1.57 ± 0.03	5.23 ± 0.03	3.07 ± 0.20	44.79 ± 1.60	72.62± 0.70
Olive oil	0.9100 ± 0.0001	2.36 ± 0.10	69.98 ± 0.20	375.64 ± 5.13	84.03 ± 3.02	21.74 ± 0.80	32.81 ± 0.20
IPM	0.8500 ± 0.0001	3.58 ± 0.02	8.47 ± 0.01	244.9 ± 6.13	76.23 ± 5.40	8.97 ± 1.30	30.73 ± 0.90
Camellia oil	0.9110 ± 0.0002	5.822 ± 0.03	70.403 ± 5.90	387.33 ± 2.52	64.94 ± 7.80	35.68 ± 0.49	21.67 ± 1.60

4.3.3 Viscosity

The viscous nature of the solvent could directly affect not only the viscosity of the polymer solution but also the formulation (Rungseevijitprapa and Bodmeier, 2009), but also the diffusion rate and the character of the solvent exchange and the injectability of *in situ* drug delivery system. Moreover, in non-aqueous ISM emulsion, the oils are used as the external phase and the results revealed that the most

viscous one was the camellia oil which was followed by olive oil which were significantly different from others ($p<0.05$). The choice of oil for the external phase is also important for the drug release property of the system because the oil could retard the immediate drug release from the system thereby the burst effect in the ISF system can be reduced. It had been reported that the oil with medium viscosity was selected for the preparation of non-aqueous/oil emulsion (Rungseevijitprapa and Bodmeier, 2009). Glycofurol showed the highest viscosity which was followed by the triacetin while DMSO gave the lowest value. The trend of viscosity of solvents was glycofurol >triacetin > PYR > IPM > NMP > DMSO which are shown in Table 5 and Figure 33. PYR gave the highest viscosity among three solvents such as PYR, NMP and DMSO. It may be due to the stronger interaction of PYR via H-bonding of H atom in C-H. Typically, a good solvent should have the strongest interaction with the polymer. Moreover, the replacement of some solvents by the drug could lead to the higher viscosity of the system in the drug-loaded formula (Maravajhala et al., 2009). The previous study reported that the lower amount of solvent in the doxycycline hyclate (DH)-loaded ISG increased the viscosity of the system than the drug free formula (Phaechamud and Setthajindalert, 2017). DMSO and NMP have a more positive charge than N-H in PYR which is liable to interact with oxygen atom. The distilled water with the smaller molecules gave the lowest viscosity. The longer chains of large hydrocarbon molecules are easily entangled than smaller molecules with shorter chains. Typically, the tighter the molecules are linked, the more the substance will resist the stress-assisted deformation (Mezger, 2011).

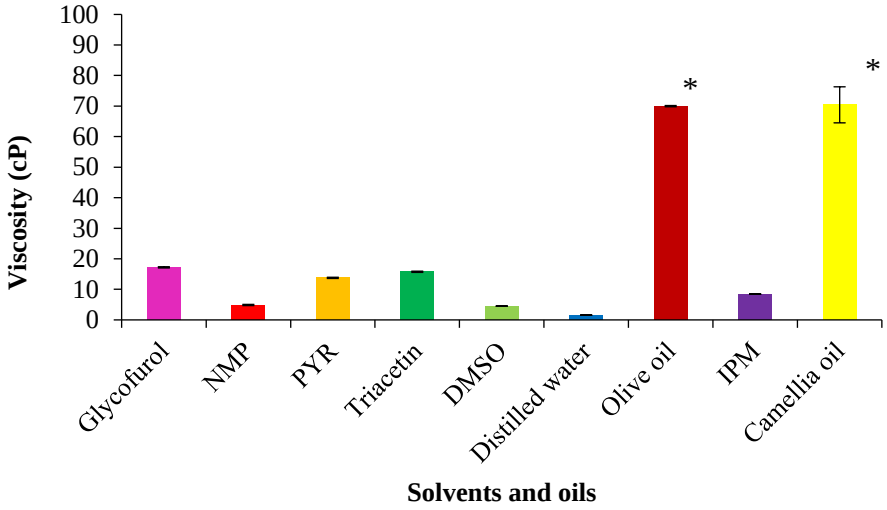


Figure 33 Viscosity of various solvents (n=3)
The esterics (*) represent the significant differences ($p < 0.05$)

4.3.4 Flow time

Among the various solvents, the most viscous camellia oil gave the highest flow time which was followed by the olive oil while the distilled water showed the lowest value due to its low viscosity. This proved that the increased viscosity retarded the flow time of the solvents. In addition, the high flow time supported the phase separation rate of the *in situ* forming emulsion composition. The most viscous solvent retarded the flow time which could also resist the separation flow from the oil in the system. The trend for the flow times were camellia oil > olive oil > IPM > glycofurol > triacetin > PYR > DMSO > NMP > distilled water, respectively (Figure 34).

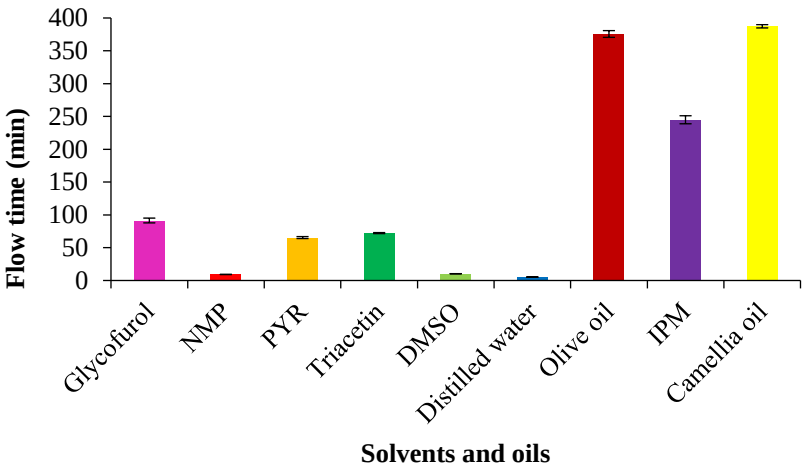


Figure 34 Flow time for various solvents (n=3)

4.3.5 Injectability

For an effective and pharmaceutically acceptable injectable formulation, the administration of the ISF system with a proper needle and syringe should be easily and rapidly carried out without pain (Xiong et al., 2011). Determination of injectability indicates the force required to expel the product (Phaechamud et al., 2016). All the solvents, except the olive oil, IPM and camellia oil, were acceptable as injectable products because the forces were lower than 50 N (Rungseevijitprapa and Bodmeier, 2009). Owing to the most viscous nature of the oil, the work force required for expulsions of the olive oil, camellia oil and IPM was apparently high ($p < 0.05$) indicating lower injectability while the distilled water with the lowest viscosity showed the lowest work force which was followed by DMSO, NMP, triacetin, PYR and glycofurol, respectively (Table 5). There may be a linear relationship between viscosity and force of injectability force as the force required to inject was dependent on the viscosity (Rungseevijitprapa and Bodmeier, 2009). The comparison between the viscosity and the injectability of solvents and oils are shown in Figure.35. The amount of polymer used in the ISF system could affect the injectability of the solvent and eventually on the final formula as the increased polymer content increased the work force to expel indicating a lower injectability (Kim and Lee, 2015). However, the injectability is facilitated by the solvent affinity to the polymer. For a good solvent, the polymer-solvent interaction should be dominant over the polymer-polymer thus lowering the viscosity (Parent et al., 2013).

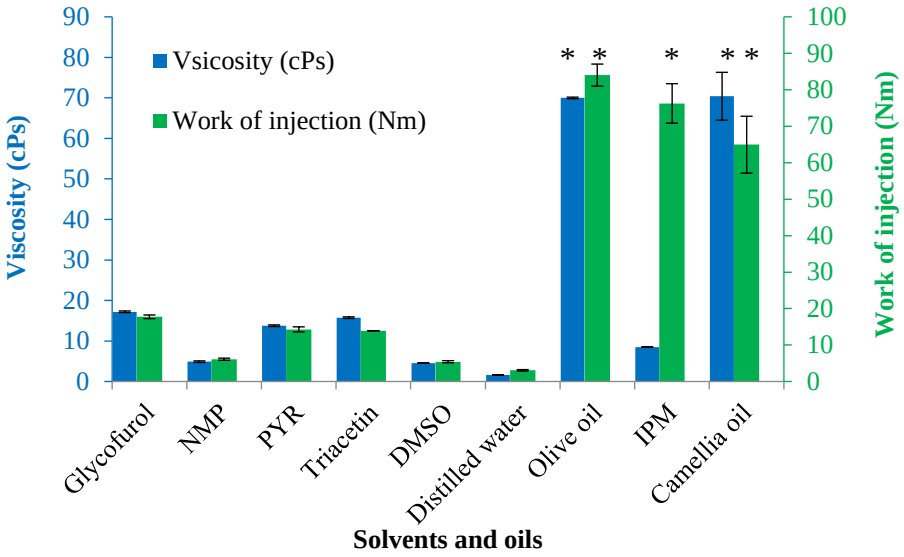


Figure 35 Comparison of viscosity and work of injectability of solvents and oils (n=3) The esterics (*)represent the significant differences ($p < 0.05$)

4.3.6 Contact angle and surface tension

The wettability data are the indicative interfacial interaction and the compatibility among formula excipients which are described by the value of a contact angle of a liquid on a solid surface. Typically, the wetting ability is described by the value of a contact angle of a liquid on a solid surface. Lower the contact angle and surface tension lead to better wettability (Phaechamud and Savedkairop, 2012). However, the contact angle between liquids decreased with time (Figure 36). The distilled water exhibited the highest contact angle due to the high surface tension whereas the lowest surface energy at air/liquid of IPM gave the lowest contact angle (Table 5). In addition, the oil and solvents with higher viscosity such as olive oil and PYR showed low contact angle values indicating the decreased wettability which in turn reduced the spreading rate. Although the surface tension of NMP was high, the lower viscosity behavior of NMP possessed the lower contact angle indicating the high degree of wettability. The comparison of the contact and surface tension of the respective solvents and oils are shown in Figuer 37. The higher contact angle of DMSO with its high surface tension contributed to the difficulty in wettability of this solvent due to the strong intermolecular interaction of DMSO.

The surface tension of water was high owing to the strong hydrogen bond formation of water molecules (Phaechamud and Savedkairop, 2012), whereas the surface tension of three solvents such as NMP, PYR and DMSO showed

comparatively low which ensured the weaker molecular interaction between molecules than that of water. The DMSO showed the lowest surface tension. Owing to the low surface tension of DMSO, it would minimize the free energy thereafter the stable ISM system prepared with this solvent could be obtained (Table 5).

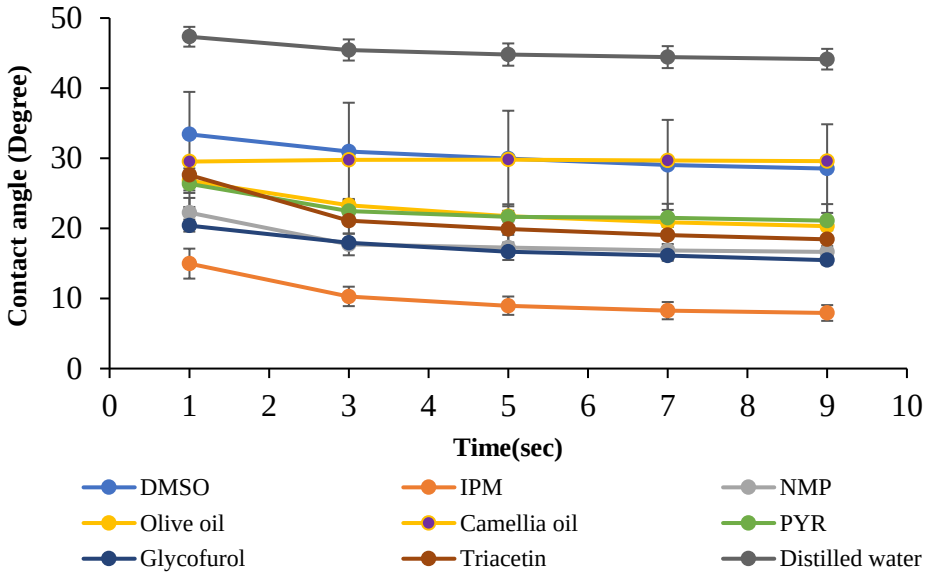


Figure.36 Contact angle of various solvents and oils with time (n=3)

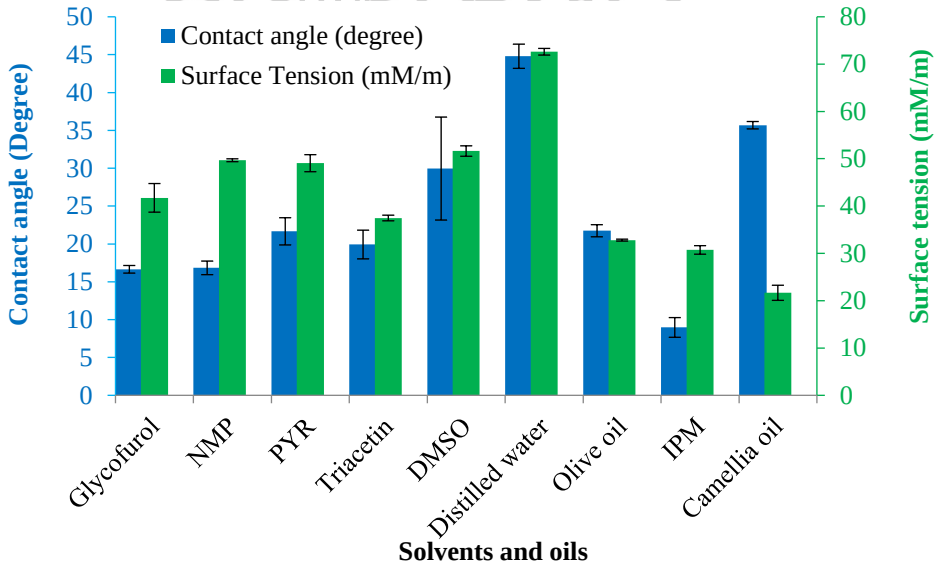


Figure.37 Comparison of contact angle and surface tension of solvents and oils (n=3)

4.3.7 Phase separation study

Emulsion may comprise one or more emulsifying agents to stabilize the system. Therefore, the role of emulsifier in the emulsion is crucial for stability of emulsion whereas the phase separation of emulsion leads to the unstable emulsion. In the present study, the emulsifiers, GMS and polyaqual, were used in the preparation of external phase of ISM system and investigated the phase separation of the system containing DMSO as the solvent in the internal phase. All systems containing IPM showed phase separation (Figure 38). It may be due to the density differences between DMSO and IPM. When GMS was used as the emulsifier, the ISM preparation comprising olive oil showed no separation till longer than 12 hr and with camellia oil, partial mixing was occurred. It may be due to the low density gradient between the external and internal phase. In addition, the GMS, the mixture of monoacylglyceride and some di- or triacylglycerols can form the gel network to the vegetable oils (Chen and Terentjev, 2009). In previous research, the GMS showed no signs of phase separation at 1 h independent of whether medium chain triglycerides or sesame oil was used as continuous phase (Voigt, 2011.). When polyaquol was used as emulsifier, ISM system containing camellial oil gave better stability till longer than 12 h.

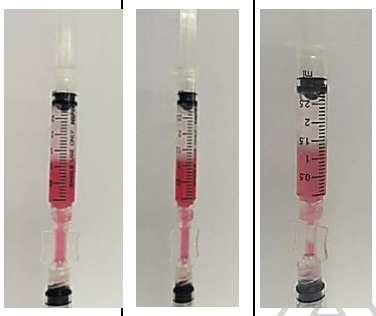
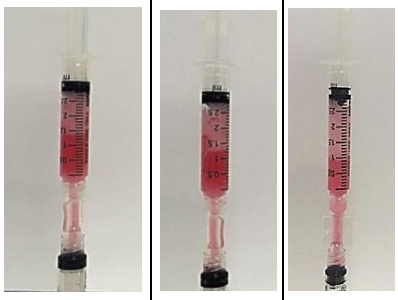
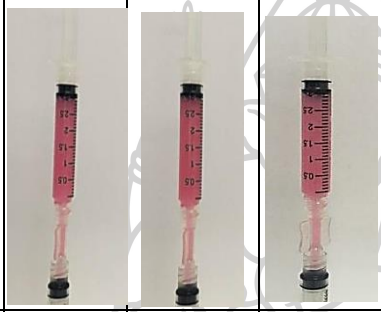
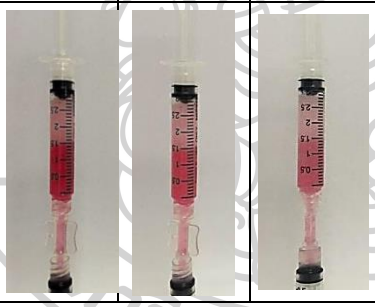
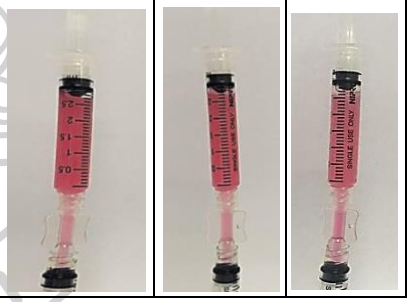
GMS (in external phase)	**Time intervals**			**Polyaqoul (External)**	**Time intervals**		
	30 min	60 min	12 h		30 min	60 min	12 h
IPM				**IPM**			
Olive oil				**Olive oil**			
Camellia oil				**Camellia oil**			

Figure 38 Phase separation study of drug free emulsion containing internal phase and external phase comprising GMS and polyaquol as emulsifier

4.3.8 Measurement of color diffusion pattern and solvent/oil miscibility

Typically, the movement of molecules for the diffusion process occurred from the higher concentration to the lower concentration area. The water soluble dye, amaranth, freely diffused into the distilled water with Brownian motion (Figure 39 A). The diffusion of the coloring agent happened till it spread out evenly throughout the distilled water until uniform colored water was obtained. The amaranth also diffused in DMSO in the same manner. Another factor of diffusion of dye was the density and viscosity of the diffusion medium. Amaranth diffused and dispersed easily into distilled water and DMSO due to their low viscosity manner. The high viscosity

dissipation of PYR and triacetin slowed the diffusion of the amaranth. Because glycofurol exhibited the higher viscosity, the differences in the density gradient caused the amaranth solution to be precipitated. While the amaranth solution was immiscible with the oils.

The nile red, a lipophilic stain, gives a fluorescent manner depending on solvent polarity. Nile red is almost non-fluorescent in water and also in most polar solvents. However, in a lipid-rich environment, it becomes fluorescent with varying shades from deep red to a strong yellow-gold emission. Therefore, the color of nile red in distilled water disappeared (Figur 39 B). In addition, owing to the lipophilic nature of the nile red, only small diffusion were occurred in the polar solvents such as DMSO, NMP, PYR and glycofurol. The nile red solution in IPM, olive oil and camellia oil gave a yellow color because of the lipid nature but it could not diffuse into the olive oil and camellia oil while the slight diffusion occurred in IPM because the viscosity of the olive oil and camellia oil was higher than that of the IPM. The oil soluble colors did not diffuse but floated on the surface of the distilled water (Figure 40). All the oil soluble colors apparently diffused into DMSO greater than that in NMP. However, the little diffusion of dyes in PYR was due to the higher viscous nature of PYR. Although the oil soluble colors were used in this study, the high viscosity of the oil retarded the diffusion of the oil soluble colors. Nevertheless, the three oil soluble colors diffused freely into all solvents and oil except distilled water after mixing with a vortex mixer (Figure 41). Owing to the apparently high viscosity nature among tested solvents and oils, the camellia and olive oils took about 20 min to obtain a complete diffusion.

The type of solvent used in the emulsion should have an appreciable polarity to make it immiscible with oil. Thus, the miscibility and phase separation behavior of solvents such as DMSO, NMP, PYR, glycofurol and water with the oil phases such as the olive oil, camellia oil, IPM and triacetin were investigated. Generally, a polar solvent dissolves or mixes in other polar components to comply with the ‘like dissolves like’ rule. Polar solvents can form a single phase mixture with other polar substances whereas no interaction will be formed between polar and non-polar compounds; thus, there is a clearly defined surface that appears between these two immiscible phases. The density gradient, the polar/non-polar interaction, solubility,

miscibility are the critical factors for the phase separation and miscibility. The miscibility and phase separation behavior of the solvents with the oil phase were studied. The experiment showed that all the solvents except distilled water mixed well with the triacetin and no separation occurred (Figure 42). The more similar the densities between the two phases were, the longer the time it took for phase separation. When the density gradient between triacetin and solvents was not much different, the phase separation was not observed untill 12 h. It has been reported that the water affinity of solvents was reduced by water-immiscible or partially water miscible components (Graham et al., 1999). Thus, the phase separation occurred between triacetin and distilled water. Because the density of distilled water was lower than the triacetin, the distilled water layer appeared at the upper part. When polar and non-polar compounds are mixed, they tend to separate with minimal surface area between them. The solvents and oils (camellia oil, olive oil and IPM) exhibited the phase separation which oil appeared at the upper layer. Significantly, when the oils were mixed with glycofurol, the olive oil and camellia oil layer were slightly cloudy at first whereas the clear separation of the olive oil phase was obtained after 12 h. The results indicated that the separation was occurred between DMSO and camellia oil, olive oil and IPM. Thus, DMSO could be employed as the internal phase and these three oils could be used as the external phase of ISM preparation.

Color	Solvents								
	DMSO	Glycofurol	Distilled water	NMP	PYR	Triacetin	Camellia oil	IPM	Olive oil
A									
B									

Figure 39 Diffusion pattern of 0.3% w/w amaranth solution (A) and 0.3%w/w nile red solution (B)

Color	Solvents								
	DMSO	Glycofurol	Distilled water	NMP	PYR	Triacetin	Camellia oil	IPM	Olive oil
Green									
Blue									
Yellow									

Figure 40 Diffusion pattern of oil soluble dyes in different solvents and oil after 24 h

Color	Solvents								
	DMSO	Glycofurol	Distilled water	NMP	PYR	Triacetin	Camellia oil	IPM	Olive oil
Green									
Blue									
Yellow									

Figure 41 Diffusion pattern of oil soluble colors in different solvents and oil after vortex mixing

DMSO aganist	A			Glycofurol aganist	B		
	Time intervals				Time intervals		
	30 min	60 min	12 h		30 min	60 min	12 h
Triacetin				**Triacetin**			
IPM				**IPM**			
Olive oil				**Olive oil**			
Camellia oil				**Camellia oil**			

PYR aganist	C			NMP aganist	D		
	Time intervals				Time intervals		
	30 min	60 min	12 h		30 min	60 min	12 h
Triacetin				Triacetin			
IPM				IPM			
Olive oil				Olive oil			
Camellia oil				Camellia oil			

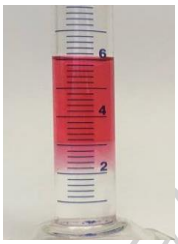
Distilled water aganist	E		
	Time intervals		
	30 min	60 min	12 h
Triacetin			
IPM			
Olive oil			
Camellia oil			

Figure 42 Miscibility study of dyed DMSO (A), Glycofurol (B), Distilled water (C), NMP (D) and PYR (E) with different oils

4.3.9 Antimicrobial study

The inhibition zone of tested solvents against different microbes is shown in Table 6. *S. aureous* could be isolated from periodontitis (Souto et al., 2006) and it has the ability to form a biofilm which can lead to antibacterial drug resistance (Carpentier and Cerf, 1993; Costerton et al., 1995). In addition, *E. coli* is the microorganism which was found in patients with periodontitis (Amel Y, 2010;). It has been reported that *S. aureus* and *P. aeruginosa* occurred in high numbers within

the buccal/gingival crevice cells from periodontitis patients (Colombo et al., 2013). Staphylococcal colonization can be found not only in tongue, saliva, mucosal surfaces, supragingival tooth surfaces but also in periodontal pocket (Rams et al., 1990). In addition, *S. aureus* may lead to aggressive periodontitis (Amel Y, 2010;). The difficulty of treatment may also occur due to the formation of a biofilm on dental plaque that can cause periodontitis (Ezeamagu et al., 2018). Although *E. faecium* is rarely found in a healthy human oral cavity, it has emerged as an increasingly important cause of nosocomial infections (Sood et al., 2008). Moreover, it has been reported that refractory periodontitis could be provoked with an infection with *C. Albican*s (Seymour and Heasman, 1995). Thus, these standard microbes were used in this study. NMP and PYR exhibited outstanding antibacterial activities against all bacterial strains (Table 6). Because NMP and PYR are the dipole aprotic, solvents could dissolve many substances including polymers and drugs as well as they can solubilize the lipids in microbial cell membranes and consequently enhance the drug penetration (Sanghvi et al., 2008; Srichan and Phaechamud, 2017) Thus, NMP as well as PYR showed a concordant potency based on the similar clear zone on tested microorganisms. The aqueous NMP-loaded lutrol® theremosensitive gel inhibited *E. coli* and *C. albican*s with the dose-dependent manner (Phaechamud et al., 2012). Glycofural, NMP and PYR inhibited a methicillin-resistant *S. aureus* (MRSA) such as *S. aureus* ATCC 43300 in this study. PYR inhibited this MRSA strain more effectively than NMP and glycofural, respectively. The use of antibiotics to treat periodontal disease may lead to the increase of *Staphylococcus spp.* in the orcal cavity (Kim and Lee, 2015) as MRSA strain (*S. aureus* ATCC 43300) which has multi-drug resistant challenges (Ezeamagu et al., 2018). This condition creates the resistance to many antibiotics which has multi-drug resistant challenges (Kim and Lee, 2015). Thus, these solvents were unique for using as the solvent for the ISM for delivery of antimicrobial active compound for treatment of infectious diseases because they could promote the efficacy of antimicrobial activities. DMSO did not show antibacterial activities except against *E. coli* and *C. albicans*. DMSO exhibited a high permeability through the cell membrane of *E.coli* and C. albicans (Stanley JC and W., 2017.). All the solvents inhibited the larger inhibition zones against *C. albican*s than bacteria. Glycofurol showed the smaller zone diameter against the test microorganisms than NMP and PYR whereas triacetin showed the smallest zone diameter. However, triacetin showed more inhibitory effect on *P. aeruginosa* DMST 37166 and *S.*

malttophilia ATCC 19079 than glycofurol. The smaller zone diameter of triacetin and glycofurol was owing to the viscous nature of these solvents that retarded their diffusion into the medium.

Table 6 Inhibition zone diameter (mm) of tested formulations against different microbes (n=3)

Sample	Clear zone diameter (mean ± S.D.) (mm)				
	Glycofurol	NMP	PYR	Triacetin	DMSO
Gram positive bacteria					
S. aureus ATCC 25923	13.33 ± 0.94	20.67 ± 1.15	21.33 ± 1.15	7.00 ± 1.00	-
S. aureus DMST 6935	12.67 ± 0.47	14.33 ± 0.58	12.33 ± 0.58	7.00 ± 0.00	-
S. aureus ATCC 43300	11.50 ± 0.50	17.20 ± 0.30	21.00 ± 1.00	-	-
E. faecium 192	15.33 ± 0.47	15.33 ± 0.58	18.33 ± 0.58	-	-
Gram negative bacteria					
E. coli ATCC 25922	15.00 ± 0.00	18.33 ± 0.58	18.67 ± 1.15	7.00 ± 0.00	-
E. coli	13.00 ± 1.00	20.00 ± 0.50	20.00 ± 0.00	-	13.20 ± 1.26
K. pneumoniae ATCC 700063	14.33 ± 0.94	17.33 ± 1.15	18.00 ± 0.00	-	-
P. aeruginosa DMST 37166	8.67 ± 0.47	20.33 ± 0.58	20.33 ± 0.58	12.00 ± 1.00	-
S. malttophilia ATCC 19079	18.67 ± 0.94	23.33 ± 1.15	25.33 ± 1.15	19.33 ± 1.15	-
Yeast					
C. albicans	19.3 ± 0.60	30.70 ± 1.80	32.20 ± 2.80	11.70 ± 0.60	17.80 ± 1.80

- = No clear zone

4.3.10 Determination of cytotoxic activity of solvents

Typically, the cell culture systems are used to assess the bioactivity of compounds whereas the organic solvent is used to dissolve these compounds (Timm et al., 2013; Vandhana et al., 2010). The cytotoxic effect of solvents was investigated against colorectal HCT116 cells (Figure 43). The different concentrations (0.8-200 µg/ml) of the individual solvents exhibited the inhibitory effect on cell growth in HCT

116 cells with concentration-dependent fashion whereas the effect of cytotoxicity increased with dose dependently. It has been reported that the cytotoxic activity of the solvents are dose dependent. The trend for viability of cells was Glycofurol < triacetin < NMP < PYR < DMSO. The previous study stated that DMSO, at concentrations < 0.5% (v/v) was a compatible solvent vehicle towards the examined cells (Jamalzadeh et al., 2016). The 80% viability were observed at 0.8 µg/ml for glycofurol and triacetin, 1.6 µg/ml for NMP, 3.1 µg/ml for PYR and 25 µg/ml for DMSO, respectively. Therefore, glycofurol showed the highest cytotoxic activity among solvents. DMSO displayed the least cytotoxicity against HCT 116 cell line which was consistent with Q3C solvent classification in which DMSO was claimed to be in the safest category, class 3 solvents (Timm et al., 2013). DMSO showed the lowest cytotoxicity among the tested solvents which was followed by PYR, NMP, triacetin and glycofurol, respectively.

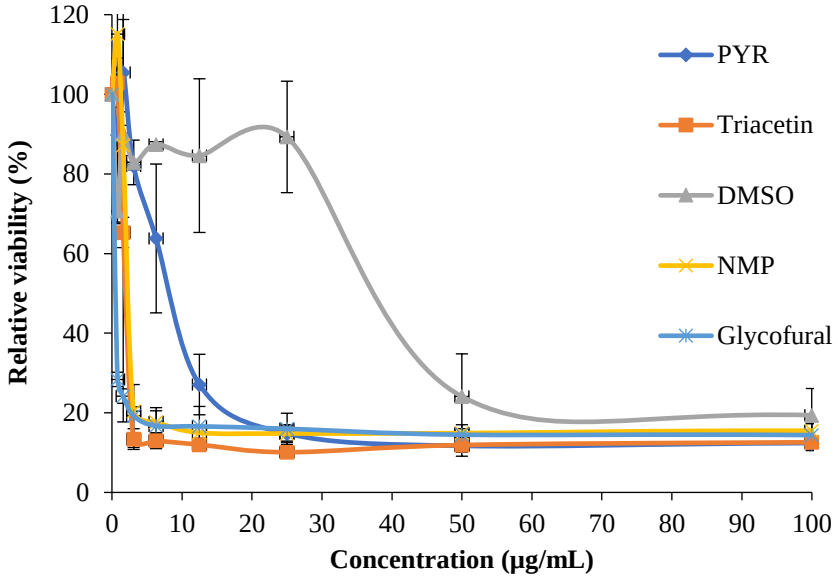
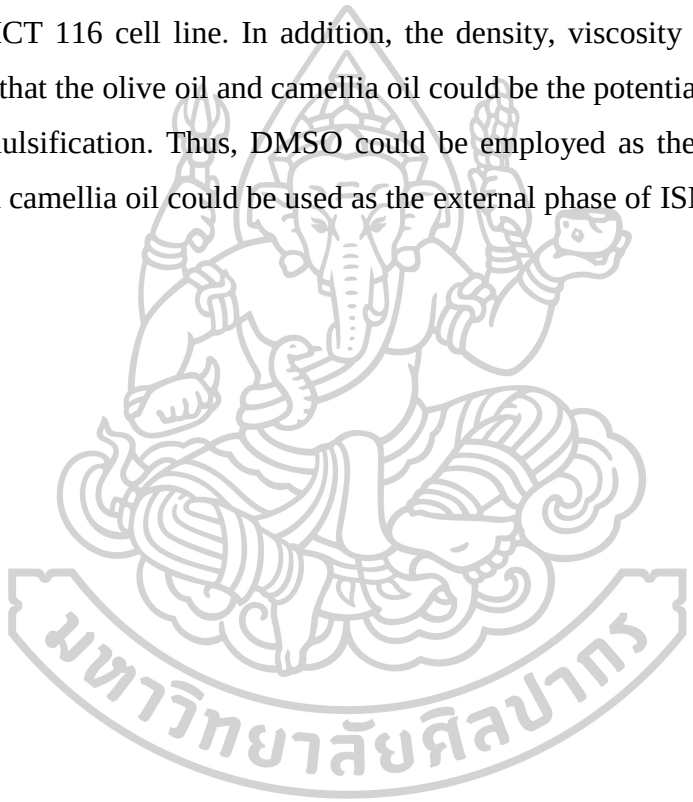


Figure 43 Viability percentage of colorectal HCT 116 cells exposed to different solvents (n=3)

4.4 Conclusion

The physicochemical properties such as pH, density, viscosities, surface tension and contact angle measurements provide useful information of various solvents and oils used in ISM. Moreover, the water soluble dye could dissolve in DMSO which this diffusion behavior proved that DMSO can be used as the solvent of choice. Furthermore, the low viscosity of the DMSO provided the ease of injectability. Moreover, DMSO showed the lowest cytotoxic activity against colorectal HCT 116 cell line. In addition, the density, viscosity and surface tension data proved that the olive oil and camellia oil could be the potential use as the external phase in emulsification. Thus, DMSO could be employed as the internal phase and olive oil and camellia oil could be used as the external phase of ISM preparation.



CHAPTER 5
Matrix formation of β-CD loaded ISG and ISM

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5.3.1.5.1. Transformation of sol into microparticle study

5.4. Conclusion

5.1. Introduction

Periodontitis, the chronic inflammation of gum and tissues that surround and support the teeth (Belstrom et al., 2012), is an inflammatory response to the overgrowth of anaerobic organisms. If untreated, periodontal disease results in the destruction of the bone and soft tissue supporting the tooth which causes tooth loss. According to the severity of the disease, gingivitis is an early stage of periodontal disease characterized by inflammation of the gum tissues which is followed by the periodontitis, an advanced stage characterized by progression of gingivitis, can lead to loss of teeth and erosion of supporting tissues (Shaddox and Walker, 2010). In more complicated condition of periodontitis, the bacteria in the infected gums penetrate the blood stream and develop systemic disease such as rheumatoid arthritis and cardiovascular disease (Foster et al., 1994). The common treatment strategies to treat periodontitis are scaling and root planning, enhancement of patient hygiene, antibiotic administration, and surgical procedures (Belstrom et al., 2012). The periodontal gap is the ideal conditions for proliferation of microbes. In periodontal disease, the depth of the gap usually exceeds 5 mm in a periodontal disease (Friedman and Steinberg, 1990). Although systemic antimicrobial agents have been used for periodontitis treatment, the low therapeutic concentrations in gingival crevicular fluid and tend to be associated with strong side effects limits its usefulness. On contrary, a local intra-pocket delivery system promotes a high drug concentration in the gingival crevicular fluid, lowering the side effects and providing advantages in the improvement of drug efficacy and patient compliance (Jain et al., 2008).

In situ forming microparticles (ISM) system is an injectable emulsion which of polymer solution droplets were dispersed in a continuous phase of oil and stabilizer (Luan and Bodmeier, 2006b). The emulsion is achieved by pushing two syringes coupled with a connector before administration (Voigt et al., 2012). After injection into the body, the exposure of the emulsion to the environment by solvent exchange mechanism hardens the inner polymer droplets which form into the microparticles *in situ* (Kranz et al., 2001).The ISM system induced lower viscosity of the emulsion when compared to the pure polymer solution. This leads to better injectability and less painful during injection. In addition, the initial rapid release is reduced because of the

presence of an external oil phase. The multiparticulate systems minimize the variation of single unit implant morphology and provide more consistent and reproducible drug release (Luan and Bodmeier, 2006b). Distinguishing form the numerous local drug delivery systems for the treatment of periodontitis, it is interesting of using intra-pocket drug delivery system for treatment of periodontitis which promotes high drug concentration in the gingival crevicular fluid, lower side effects, improved drug efficacy and enhancement of patient compliance (Jain et al., 2008). The ISM system comprising the poly (lactide-co-glycolide) in NMP as the internal phase and the peanut oil with 2% w/w span 80 as the external phase found that the easier to inject with smaller needle size (Rungseevijitprapa and Bodmeier, 2009). The previous study proved that the initial burst release of leuprolide acetate loaded-ISM system containing aluminum monostearate as the emulsion stabilizer was decreased as the polymer concentration was increased (Luan and Bodmeier, 2006b; Yapar et al., 2012). In addition to the specific periodontal pathogens which cause the periodontitis, the host immune-inflammatory mediators such as prostaglandin E2 correlates to the inflammation and bone resorption of the teeth are also take part in important role of disease progression (Krayer et al., 2010). NSAIDs can inhibit these host immune-inflammatory mediators, thereby reducing tissue destruction and bone loss (Farahmand et al., 2016; Howell and Williams, 1993). In addition, they reduce swelling and acute inflammation of periodontitis, and decrease pain (Rutger 2012). Thus, anti-inflammatory agents such as NSAIDs have been recently used as an adjunctive therapy to periodontitis (Agossa K et al., 2015).

The meloxicam (Mex), selective cyclooxygenase-2 inhibitor (COX-2 inhibitor), is a highly potent NSAID (Engelhardt, 1996). It could prevent the alveolar bone loss in an experimental periodontitis in rats when administered subcutaneously (Bezerra et al., 2000). Moreover, Mex is a potent inhibitor for acute exudation in periodontal tissues and bone and cartilage destruction (Engelhardt, 1996). When the adjunct to scaling and root planning, the oral administration of Mex can reduce the gingival crevicular fluid (GCF) level matrix metalloproteinase 8 (MMP8) which is a main factor in the connective tissue destruction (Buduneli et al., 2002). The ISG formulations containing 3% Mex comprising thermosensitive Pluronic® gels showed significant improvement in chronic periodontitis patients and it was manifested by

decrease in pocket depth and gingival index and increase in bone density (Kassem et al., 2014). The previous study stated that the plaque index, gingival index and probing depth decreased significantly ($p < 0.05$) in Mex-loaded gel at first month when comparing the initial levels (Toker et al., 2006).

When the ISM system is exposed to the aqueous environment, the influx of water and efflux of solvent from the system leads to the precipitation of the polymer as the matrix from which the drug is released in a controlled manner. When the ISM system comprising the PLGA solution dispersed in oil phase is injected into the body, the polymer droplets solidify and form microparticles *in situ* (Luan and Bodmeier, 2006b). The previous study prepared the internal phase using polymer (such as poly(D,L-lactide-co-glycolide), poly(D,L lactide)) which was dissolved in a biocompatible solvent such as N-methyl-2-pyrrolidone (NMP), 2-pyrolidone (PYR), dimethyl sulfoxide (DMSO), triacetin or low molecular weight polyethylene glycol (Rungseevijitprapa and Bodmeier, 2009). Doxycycline hyclate-loaded ISM systems were also fabricated using bleached shellac as the polymer in internal phase and olive oil comprising GMS as the external phase (Phaechamud et al., 2016). In the present study, β-cyclodextrin (β-CD) was used as the matrix former. Regarding to the limited aqueous solubility of β-CD (Loftsson and Brewester, 1996; Magnúsdóttir et al., 2002), the complexes formed by CD exhibit the limited solubility which resulting in precipitation of solid β-CD complexes from water and other aqueous systems. Thus, the low aqueous solubility character of β-CD is interesting as matrix former in solvent exchange-induced ISM.

In the periodontal pocket, the precipitation of polymer occurred after exposure the aqueous at injection site inducing the formation of a depot for consequent entrapping the drug with the controlled release manner (Phaechamud and Mahadlek, 2015). In the present study, the ISM systems were prepared by dissolving the β-CD in the DMSO. DMSO was applied as a polymer vehicle in ISG and ISM. The objective of the present study is to investigate physicochemical properties of Mex-loaded ISM comprising β-CD dissolved in DMSO which have not been reported before.

5.2. Materials and methods

5.2.1. Preparation of the injectable meloxicam (Mex) loaded *in situ* forming systems containing β-CD as matrix former

5.2.1.1. Preparation of Mex-loaded ISG systems containing β-CD dissolved in DMSO (β-MexDMSO ISG)

In the present study, the 30-60% of β-CD were used and the ISG system was prepared using 1% of Mex. The Mex and β-CD were mixed in a mortar for 1 min. Then, 1 mL of ethanol was added to each mixture and kneaded for 3 min. After mixing, mixtures were dried in the hot air oven at 50°C for 12 hr and the solvent, DMSO was added to the mixtures. The preparations were stirred, and vortex mixed until clear solutions were obtained. The formula compositions containing different concentrations of β-CD are shown in Table 7.

Table 7 Formula composition of ISG containing Mex with β-CD as matrix former (β-Mex DMSO ISG)

Ingredients	**Amount of components (%w/w)**						
Mex	1	1	1	1	1	1	1
β -CD	30	35	40	45	50	55	60
DMSO	69	64	59	54	49	44	39

5.2.1.2. Preparation of Mex-loaded ISM systems containing β-CD dissolved in DMSO (β MexDMSO ISM)

The external phase of ISM was prepared by dissolving GMS (7.5% w/w or 5% w/w of external phase) in camellia oil at 80°C under continuous mixing until obtaining a clear solution. Then it was cooled to convert into the milky gel under ambient condition. The ISG prepared as described in 5.2.1.1 was used as the internal phase for ISM preparation. The ISM emulsion was prepared in 2 syringes coupled with a connector. The constant volume ratio of the external phase and the internal phases at 1:1 was used. The emulsification was achieved by back-and-forth movement of the syringe plungers for 50 mixing cycles for 1-2 min in 3 mL single-use syringe as previously described (Kranz and Bodmeier, 2008; Rungseevijitprapa and Bodmeier, 2009). The compositions of formula of ISM containing different concnetrations of β-CD as matrix former with 7.5% GMS and 5% GMS are shown in Table 8 and Table 9, respectively.

Table 8 Formula composition of ISM containing Mex with β-CD as matrix former (β-MexDMSO ISG) with 7.5% w/w GMS

Phase	Ingredients	Amount of components (%w/w)				
Internal phase (%w/w)	Mex (1%)	0.5	0.5	0.5	0.5	0.5
	β-CD	15	17.5	20	22.5	25
	DMSO	34.5	32	29.5	27	24.5
External phase (%w/w)	GMS (7.5%)	3.75	3.75	3.75	3.75	3.75
	Camellia oil	46.25	46.25	46.25	46.25	46.25

Table 9 Formula composition of ISM containing meloxicam with β-CD as matrix former (β-MexDMSO ISG) with 5% w/w GMS

Phase	Ingredients	Amount of components (%w/w)				
Internal phase (%w/w)	Mex (1%)	0.5	0.5	0.5	0.5	0.5
	β-CD	15	17.5	20	22.5	25
	DMSO	34.5	32	29.5	27	24.5
External phase (%w/w)	GMS (5%)	2.5	2.5	2.5	2.5	2.5
	Camellia oil	47.5	47.5	47.5	47.5	47.5

5.2.2. Evaluation of ISG and ISM properties

5.2.2.1.Evaluation of ISG and ISM systems before exposure to solvent exchange

5.2.2.1.1. Appearant viscosity and rheological behavior studies

The appearant viscosity and rheological behavior studies were carried out according to the method described in 3.3.1.2.

5.2.2.1.2. Injectability test

This procedure was applied as method described in 3.3.1.8.

5.2.2.2.Evaluation of ISG and ISM systems during exposure to solvent exchange

5.2.2.2.1. *In vitro* gel formation (ISG)

This method was applied as described in 3.3.1.7.

5.2.2.2.2. *In vitro* gel formation (ISM)

This method was applied as described in 3.3.1.7.

5.2.2.2.3. *In vitro* drug release studies

5.2.2.2.3.1. Calibration curve of drug in PBS pH 6.8

The Mex (50 mg) was accurately weighted, dissolved in methanol and diluted with PBS pH 6.8 to 50 mL to obtain the concentration of 1 mg/mL. Then the stock solutions were diluted with PBS pH 6.8 to 10 mL to obtain concentration of 10, 20, 30, 40, 50 and 60 µg/mL, respectively .The relationship between concentration and absorbance was measured at 360 nm using UV-vis spectrophotometer (Cary 60 UV-Vis, Model G6860A, Agilent, Malaysia). The calibration curve was constructed by plotting the absorbance versus concentration.

5.2.2.2.3.2. Drug release test with dialysis membrane method

About 1 g formulation was placed into a dialysis tube (Spectrapor, MW cutoff 6,000-8,000) and then the dialysis tube was immersed in 100 ml of PBS buffer solution pH 6.8 at 37°C (Priyanka and Meenakshi, 2011). The rotational speed was maintained at 50 rpm. The aliquot of about 10 ml each was withdrawn from the release medium at time intervals of 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120, 144, 168 h and each withdrawal volume was replaced by the fresh medium. Then, the withdrawal samples were measured by UV-Vis spectrophotometer at 360 nm. All of the experiments were carried out six times and the mean cumulative drug release ± S.D were calculated.

5.2.2.2.3.3. Drug release test with direct contact method

For direct contact method, 1 g of sample was added directly into the glass bottle containing 100 mL of phosphate buffer solution with a pH of 6.8 at 37°C and rotational speed of 50 rpm was maintained. Another method for direct contact was to investigate the drug release using small cup method in which the sample 1 g was added to a ceramic cup (10 mm x 12 mm) and then this cup was placed into a glass bottle containing 100 mL of phosphate buffer solution with a pH of 6.8 at 37°C and rotational speed of 50 rpm was maintained. The determination of drug release was conducted using the same method as described in the dialysis membrane method.

5.2.2.2.3.4. Analysis of drug release data

The data from *in vitro* drug release were analyzed by Microsoft Office Excel 2010. Different mathematical release equations are the essential tool for fitting the cumulative percentage of drug release profiles in range of 10% to 80%. Considerable models of mathematic equation such as power law, zero order, first order, and Higuchi's were applied in this experiment.

5.2.2.2.4. *In vitro* degradability study

To determine the mass loss, 0.5 g in-situ forming gel and microparticles containing 40**%** w/w β-CD (M_0) were injected into a small cup and then the cups were placed into the glass bottles containing 5 mL PBS pH 6.8 and incubated in a shaking thermostatically controlled bath at 37 °C with a rotational speed of 50 rpm**.** Fresh PBS pH 6.8 was replaced evey day for one week to minic oral cavity conditions**.** the sample was then dried in a hot air oven at 65 °C for 24 h and kept in a desiccator for 72 h before weighing (Final weight) (n=3). The percentage of weight loss was calculated as follows**:**

$$\%Total\ mass\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} x100 \quad \text{.....} (6)$$

$$\%\ \beta - CD\ mass\ loss = \frac{Mo - Mnet}{Mo} x100 \quad \text{.....} (7)$$

Where M_{net} is the weight of dry sample without residual solvent and Mex (Boimvaser et al., 2012).

5.2.2.2.5. Rate of matrix formation

The evaluation method was performed as described in 3.3.1.5.

5.2.2.2.6. Solvent diffusion

This study was carried out as described in 3.3.1.6.

5.2.2.3.Evaluation of ISG and ISM systems after exposure to solvent exchange

5.2.2.3.1. Mechanical property studies

The mechanical properties of the investigated ISG were determined with a texture analyzer) TA.XT plus, Charpa Techcenter, Godalming, Stable micro Systems Ltd., UK (using agarose gel with cylindrical hole as an artificial periodontal pocket. The preparation of agarose gel has been described previously in section 3.3.1.5. At the center of the agrose gel, the cylindrical holes (diameter 6 mm) were made and the liquid formulations were filled with standard syringe. With the process of solvent exchange mechanism, the ISG/ISM was formed which was completely in 1 week. At the end of gel transformation, a cylindrical probe with diameter of 3 mm was driven downwards at a speed of 0.5 mm/s. Once in contract with ISG, the applied force and displacement of the probe were recorded as a function of time .The position was held for 60 sec since penetration depth was 1.5 mm. Subsequently, the probe was driven upwards at a speed of 10 mm/s.

The force measured at maximum probe penetration into ISG/ISM is called the maximum deformation force ($F_{max\ deformation}$). The force measured after 60s holding time is called remaining force ($F_{remaining}$). The ratio "$F_{remaining}/F_{max\ deformation}$" used as a measure for the elasticity/plasticity of ISG. High values indicate high elasticity while low values indicate high plasticity. Each experiment is repeated for 6 times and results are presented as mean values ± standard deviation (Do et al., 2014).

5.2.2.3.2. Determination of surface morphology by SEM

After incubation in PBS pH 6.8 at 7 days, the surface morphology was determined with scanning electron microscope (Maxim 200 Camscan, Cambridge, England). The dried samples were coated under an argon atmosphere with gold-palladium and then surface morphology was investigated according to the method as mentioned in 3.3.2.2.

5.2.2.4.Evaluation of ISM properties

5.2.2.4.1. Evaluation of ISM systems before exposure to solvent exchange

5.2.2.4.1.1.Morphology studies of o/o emulsion

The transition of emulsion after contact with distilled water into microparticle as morphological change was determined by using an inverted microscope by dropping 60 µL of samples onto slide and same amount of distilled water was dropped on emulsion and visually recorded the results every 10 sec for 3 min**.** The average droplet size was analyzed by image frame work software (Nikon DXM 1200, 17 Japan) (n=150; 3 regimens, 50 droplets per regimen). Emulsion without β-CD was used as the control group**.**

5.2.2.5.Evaluation of ISM systems during exposure to solvent exchange

5.2.2.5.1. Transformation of emulsion into microparticle study

The transformation was observed under the same condition as described in section 5.2.2.4.1**.** where PBS pH 6.8 approximately 60 µL was dropped to sample with the same amount on the glass slide and recorded every 10 sec for 3 min**.**

5.2.3. Statistical analysis

All the measurements were carried out in tirplicate and the results were described in mean ± SD**.** One-way analysis of variance (ANOVA) followed by the least significant difference post hoc test was used for statistical significance which the significance level was set at $p < 0.05$**.**

5.3. Results and discussion

5.3.1.Evaluation of ISG and ISM properties

5.3.1.1.Evaluation of ISG and ISM systems before exposure to solvent exchange

5.3.1.1.1. Appearance viscosity and rheological behavior studies

5.3.1.1.1.1. Apparent viscosity of ISG and ISM

The viscosity is one of the important parameters for ISF system which governs the character of solvent exchange and diffusion rate (Phaechamud and Setthajindalert, 2017). The drug loaded ISG system showed the increased viscosity when the concentration of β-CD was increased whereas the ISG system comprising 45% β-CD exhibited a significantly higher viscosity among other drug-loaded ISG systems ($p<0.05$). In the polymer-solvent interaction, the polymer extension and stretching occur while polymer–polymer interaction causes the polymer coiling up, thus the more increase in viscosity of the polymer solution occurs in poor solvent. On the other hand, the good solvent favors the polymer-solvent interaction instead of polymer-polymer ones (Camargo et al., 2013).

The drug free and drug-loaded ISG systems showed the same trend which an increased β-CD concentration enhanced the viscosity of the system (Figure.44). By comparison, the drug-loaded ISG system showed significantly higher in viscosity especially Mex-loaded ISG comprising 45% β-CD ($p < 0.05$) due to the replacement of solvent with higher amount of drug in system (Jones, 2004) (Figure.45). In addition, the drug–polymer interaction could increase a viscosity of the system (Mayol et al., 2008; Phaechamud and Mahadlek, 2015). The increase in viscosity offers the resistance of flow (Phaechamud and Setthajindalert, 2017).

Typically, the ISM system should have the lower viscosity than that of the ISG system owing to predominant lubricating effect of oil in the external phase on the ISM (Rungseevijitprapa and Bodmeier, 2009). But, in the present study, the ISM system comprising 7.5% GMS showed the higher viscosity than ISG system. The increased amount of GMS in the external phase indicated that the GMS ratio in the external phase was large. In addition, the inherent increase in viscosity of oil presented in the external phase increased the viscosity of ISM.

When the amount of GMS was reduced to 5% w/w, the viscosity of the ISM was reduced (Figure 46). However, the viscosity was still higher than that of the ISG

owing to the inherent increase in viscosity of the camellia oil which was accounted for 70.403 cPs (Figure 4.3).

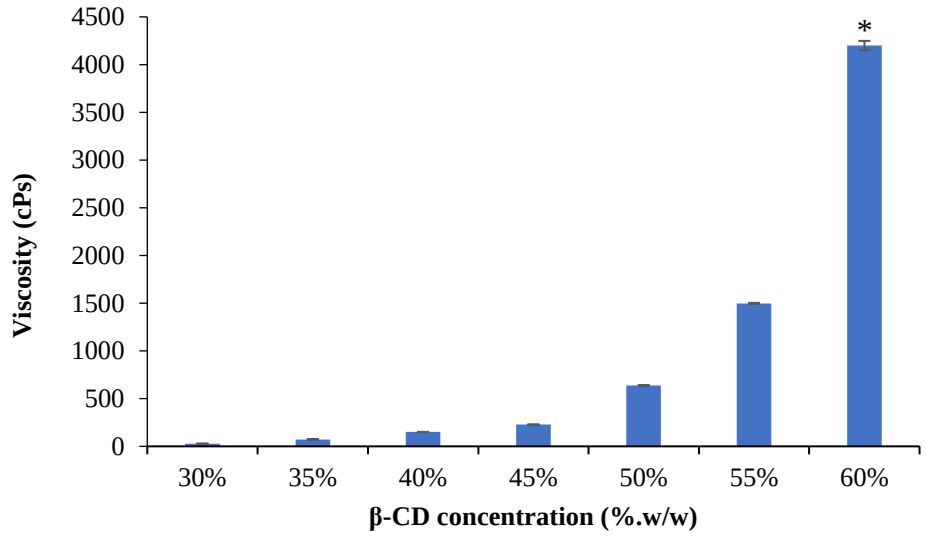


Figure 44 Apparent viscosity of β-CD in DMSO (N=3)
The asterisk (*) represents a significant difference ($p < 0.05$)

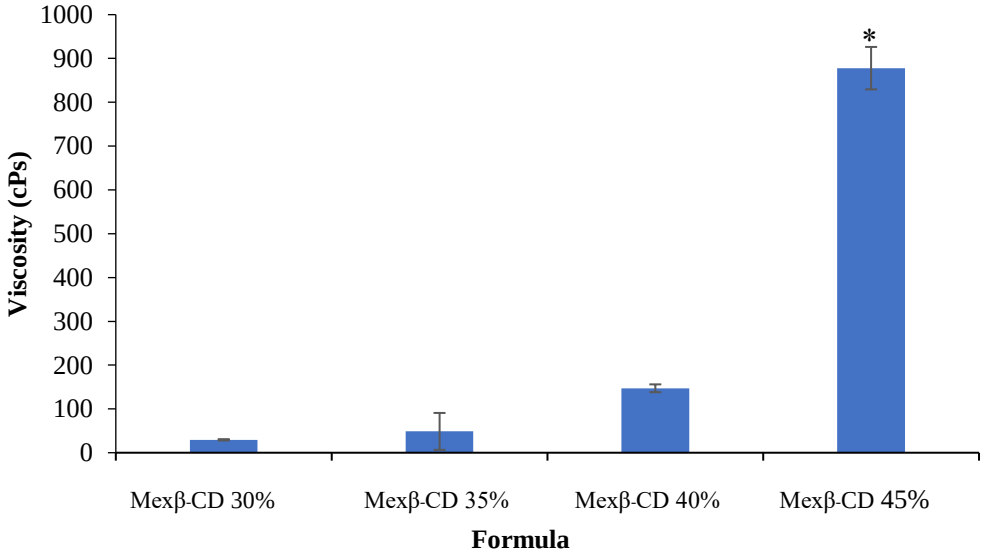


Figure 45 Apparent viscosity of Mexβ-CD ISG
The asterisk (*) represents a significant difference ($p < 0.05$)

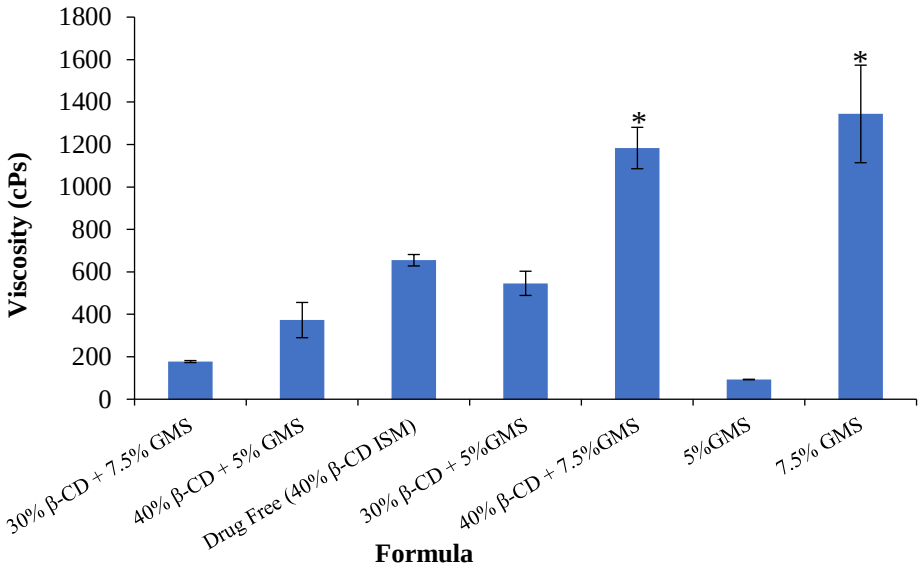


Figure 46 Apparent viscosity of Mexβ-CD ISM (n=3)
The asterisk (*) represents a significant difference < 0.05)

5.3.1.1.1.2. Rheological behavior of Mexβ-CD ISG

Although the shear rate was increased, the viscosity of drug free ISG did not change indicating the Newtonian flow (Bjorn et al., 2012). The viscosity of both drug free and drug-loaded ISG and ISM systems increased with increasing of β-CD concentration. Rheological behavior of system containing different concentrations of β-CD showed a Newtonian flow except the ISG comprising the high concentration of β-CD (Figure 47 and 48). The ISG with 45% and 50%w/w β-CD exhibited the decreased in viscosity as the shear rate increased indicating that the shear thinning and their viscosity was dependent on the shear rate due to the structural rearrangement of the fluid molecules (Figure 48). Another reason was the segregation of the different phases in the flow owing to the high concentration of β-CD.

The shear stress versus shear rate showed the linear increase (Figure 50 and 51). The shear stress of formulas comprising β-CD was increased as the shear rate and the β-CD amount were increased whereas the curve moved to a high shear stress value signifying the compact structure of ISG system.

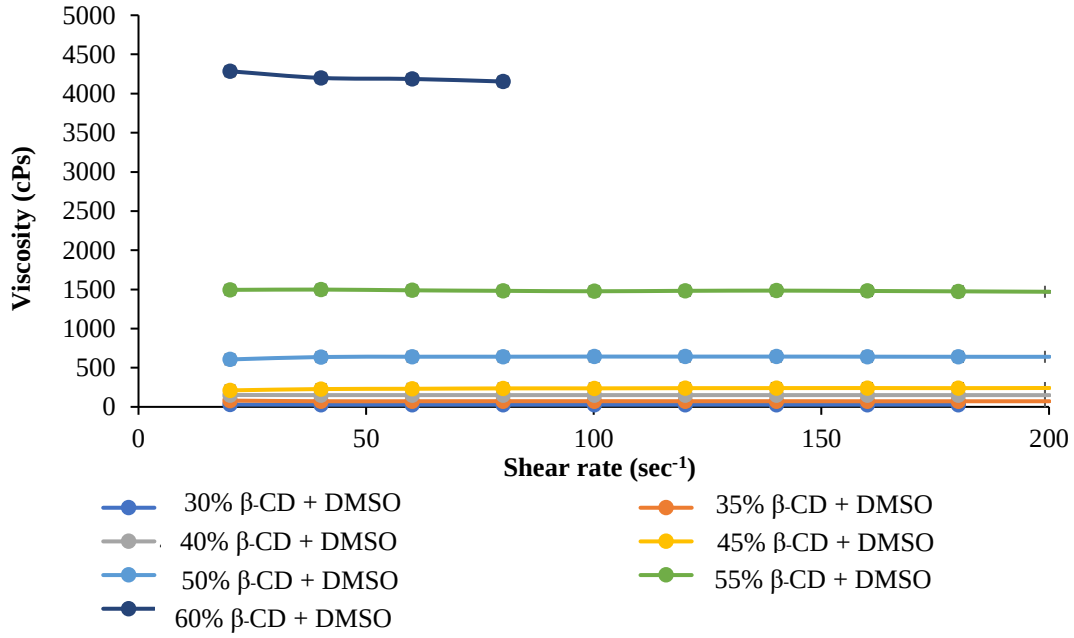


Figure.47 Relationship between viscosity and shear rate of drug free ISG system containing different amounts of β-CD dissolved in DMSO

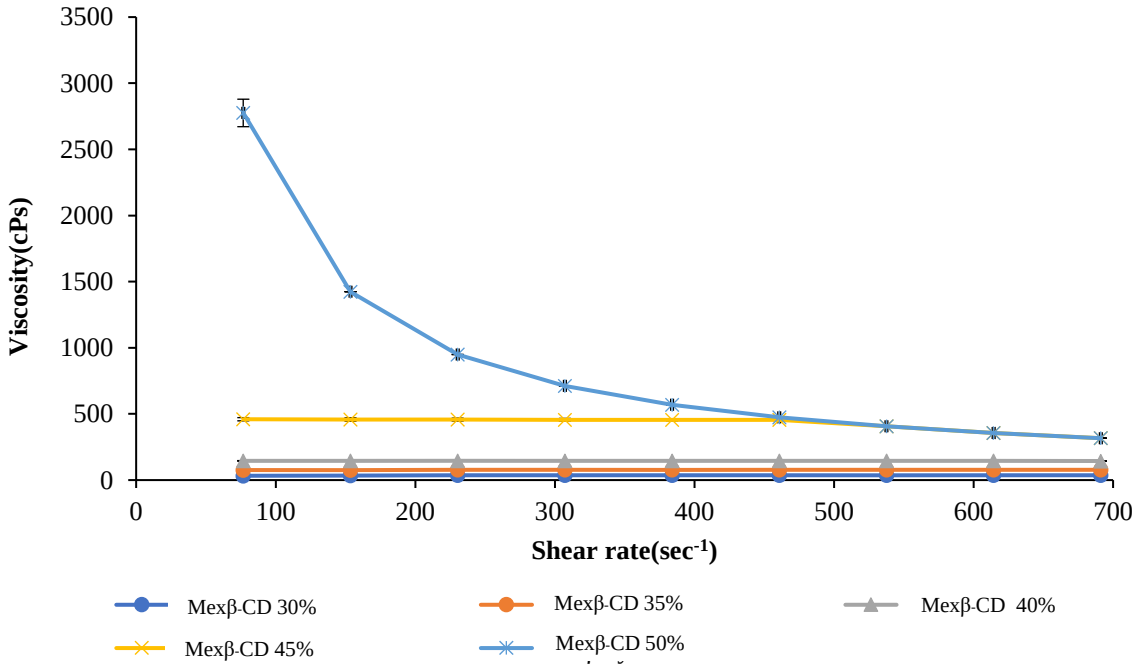


Figure.48 Relationship between viscosity and shear rate of Mex-loaded ISG system containing different amounts of β-CD dissolved in DMSO (Mexβ-CD ISG)

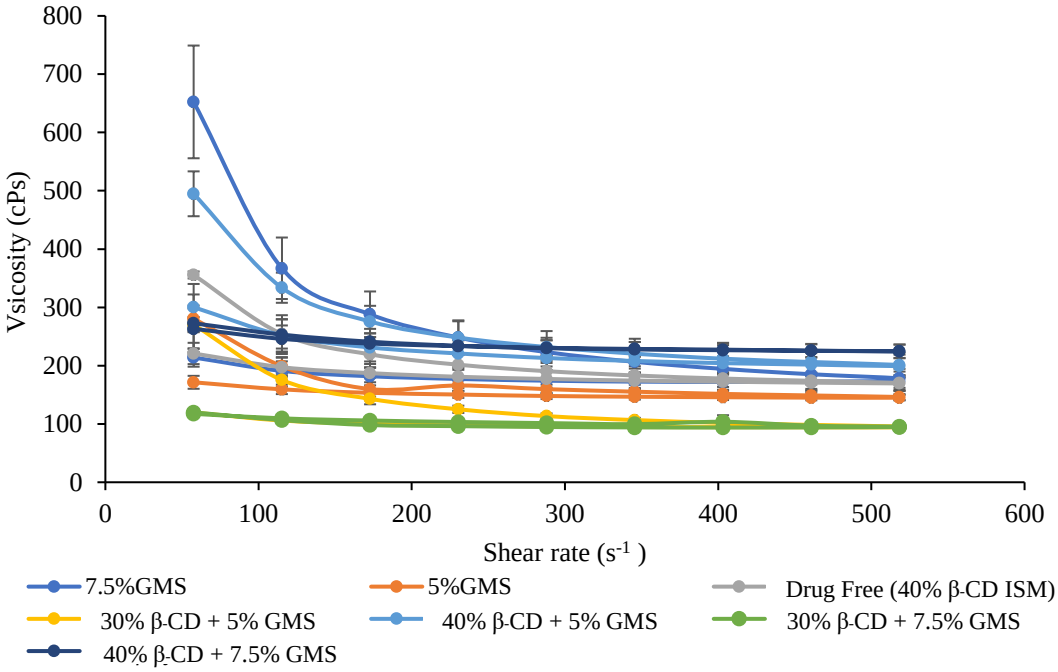


Figure.49 Relationship between viscosity and shear rate of Mex-loaded ISM system

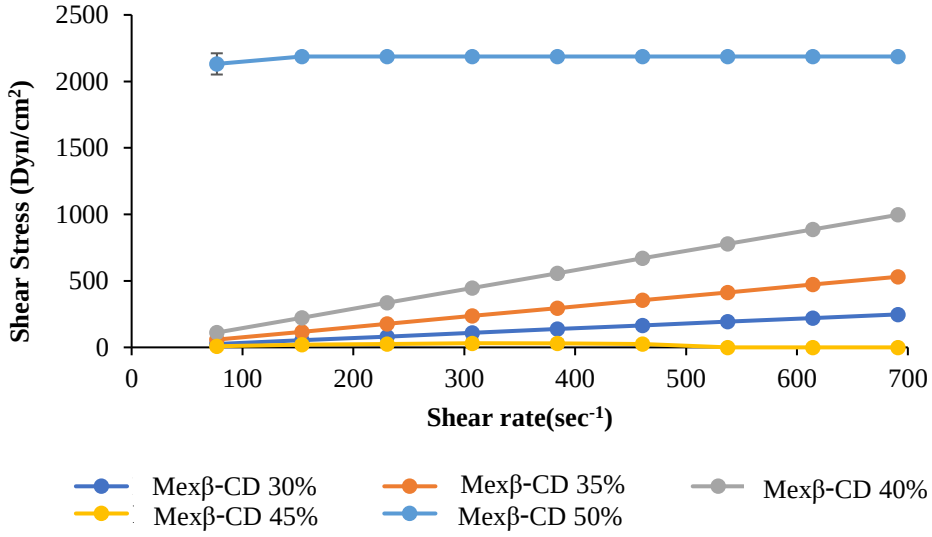


Figure 50 Flow curve of Mex-loaded ISG system containing β-CD dissolved in DMSO

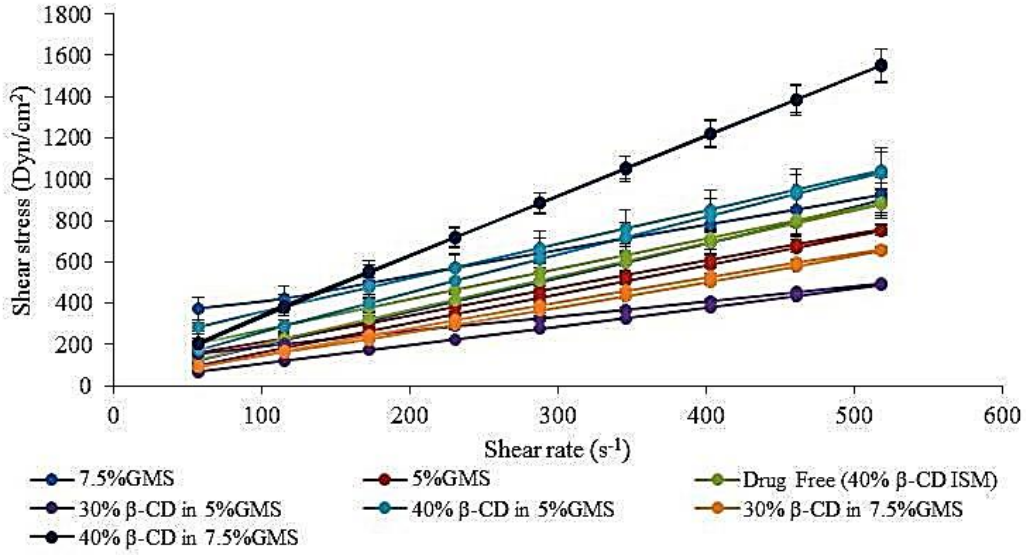


Figure 51 Flow curve of Mex-loaded ISM (n=3)

5.3.1.1.2. Injectability test

Typically, as a parenteral dosage form, the ISF systems should be more easily injectable with smaller needle size for less painfulness with better patient comfort/compliance. Therefore, the injectability study is a necessary parameter to be determined. It is the ability of product to pass through a needle easily which is determined by measuring the force required to expel the product through the needle. The injectability study was carried out for ISG system comprising different concentrations of β-CD whereas the increase in work force was observed with increasing β-CD concentration (Figure 52). The viscosity of the formulation directly influences the injectability (Rungseevijitprapa and Bodmeier, 2009). Owing to evidence of high viscosity nature for high concentrated β-CD solution, the increased β-CD content caused an increase in the work required for expulsion through needle indicating lower injectability. By comparison, the ISG comprising 30- 40% w/w β-CD exhibited the lower work force than ISG with 40-45% w/w β-CD and the work of injectability for ISG systems comprising 30-40% w/w β-CD were lower than 5 Nm. However, ISG with higher concentration of β-CD (50 % and 55% w/w) could not be expelled through the 27-gauge needle (data not shown).

Mex-loaded ISM systems showed the significantly higher work force for expulsion through the 18-gauge needle than ISG systems ($p < 0.05$). The present research employed GMS in the camellia oil as the external phase of ISM. The thickening ability of GMS controlled the oil migration upon mixing with the internal phase (Daniel and Marangoni, 2012). When tested with the syringe 18 G and 21 G, the 5% GMS in camellia oil had a higher work force than 40% β-CD Mex ISG as shown in Figure 53. Thus, 5%GMS dispersed in camellia oil was one of the factors which lowered the injectabilityof 40% β-CDMex ISM than that of corresponded ISG. The force required to inject the ISM system was determined via different needle sizes (Figure.53). The injection force required to expel the Mex β-CD 40% ISM and Mex β-CD 30% ISM through the 18 G was 4.225, 2.216 N, respectively. The reducing the needle diameter by increasing the needle gauge number led to an increase in injection force as reported previously (Rungseevijitprapa and Bodmeier, 2009). Thus, when assessing the work force of ISM with different needles, the smaller the needle size needed the higher the work force indicating the lower injectability (Figure 53). The injection of a solution via a needle 20–21G with a force of 100N or more ranked as very difficult to inject and a force approximately 50 N or below remarked as slightly difficult. The previous study proved that O/W ISM system could be easily injected via a needle 23 G or even smaller size with the force of 25 N or below (Rungseevijitprapa and Bodmeier, 2009). The injection force needed to expel the Mex β-CD 40% ISM and Mex β-CD 30% ISM through the 24 G was 8.523 N and 6.525 N, respectively. Although the work of syringeability of the prepared ISM was higher than the prepared ISG, it could be easily injected via 24 G needle.

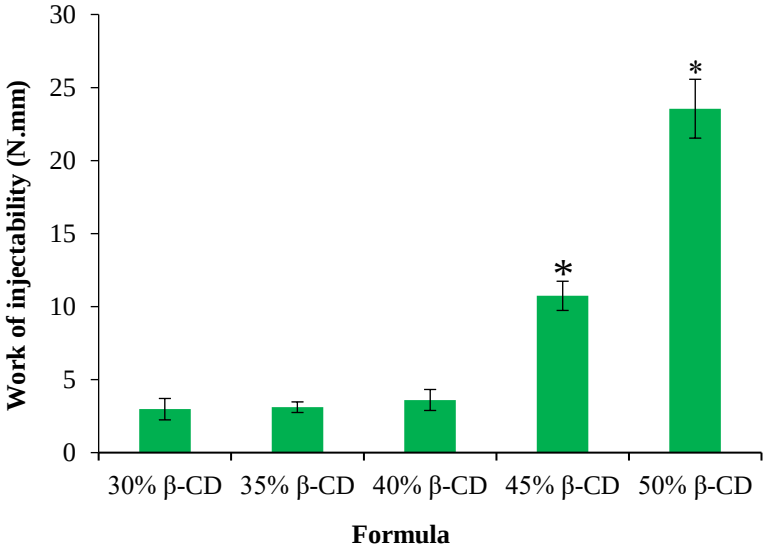


Figure 52 Injectability of drug free ISG system (n = 3)
The asterisk **(*)** represents a significant difference ($p < 0.05$)

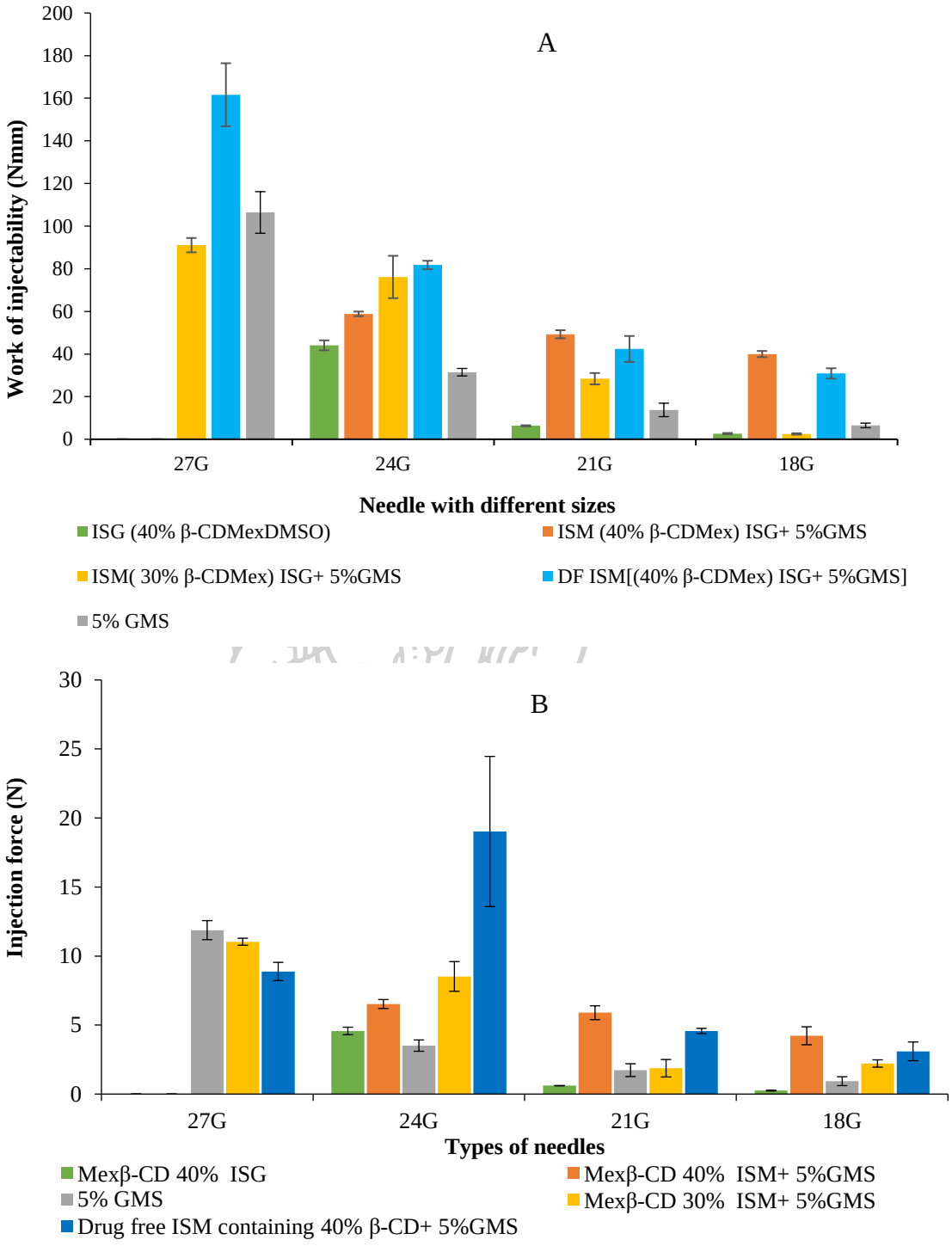


Figure 53 Comparison of (A) work of injectability and (B) injection force for ISG and ISM systems through needles with different gauges

5.3.1.2. Evaluation of ISG and ISM systems during exposure to solvent exchange

5.3.1.2.1. *In vitro* gel formation of ISG

The ISG system could transform into gel or solid-like matrix by solvent exchange mechanism when exposed to the surrounding PBS liquid. The present study β-CD was employed as the matrix former. Thus, the present experiment was to investigate the ability of the transformation of drug-loaded ISG comprising β-CD into solid-like matrix by solvent exchange mechanism. The low amount of β-CD showed the discontinuation of the precipitated network. Although the transformation was occurred when the formula contacted with PBS, the complete solidification depended on concentration of β-CD. The matrix of 35% w/w β-CD formulation was denser and more opaque than those of 30%w/w β-CD formulation which proved that the higher β-CD concentration promoted the apparent precipitation of β-CD. Thus, the high concentration of β-CD showed the rapid phase separation which drug-loaded ISG system comprising 50% w/w β-CD exhibited a denser matrix than others (Figure 54). Therefore, the contact of drug-loaded ISG solution into the GCF initiated a phase separation by exchange of DMSO and water from GCF which promoted the precipitation of matrix-like depot.

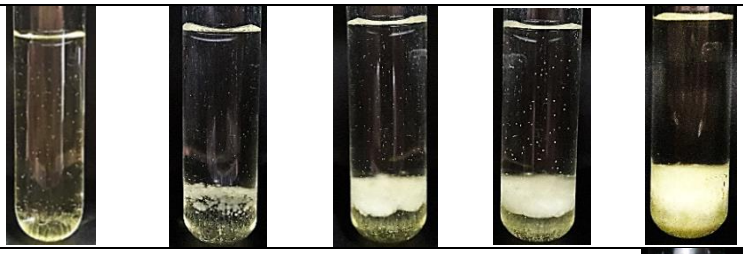
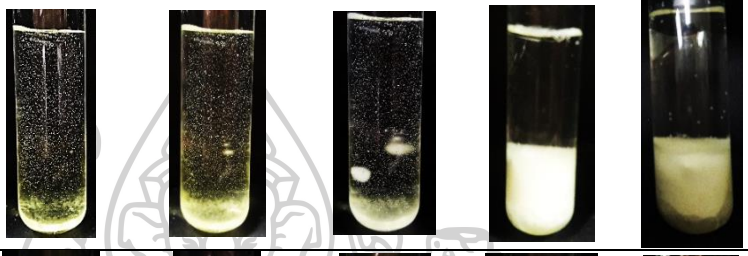
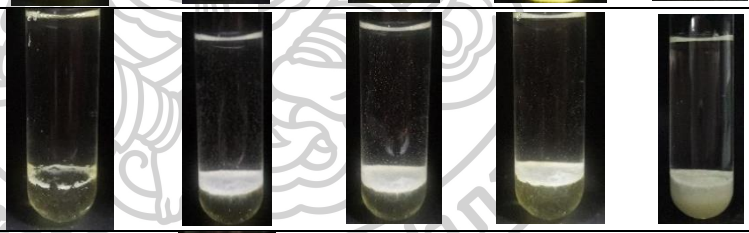
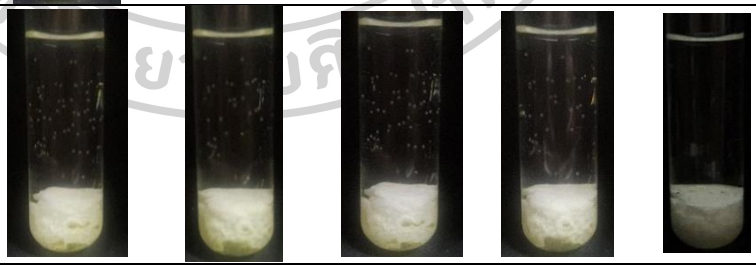
ISG					
Formulations	Time (min)				
	0	1	5	30	1440
30%					
35%					
40%					
45%					
50%					

Figure 54 *In vitro* gel formation of Mex-loaded ISG using different concentration of β-CD as matrix former

5.3.1.2.2. *In vitro* gel formation of ISM

The exposure of the surrounding liquid provoked the gelation of the ISM systems by solvent exchange process which provided the matrix-like depot of the system**.** The rapid gelation was evident for the prepared drug-loaded ISM systems after exposure to the PBS pH 6**.**8**.** When compared with the drug-loaded ISG system, the formed matrix from the ISM floated onto the top instead of precipitation to the bottom due to the low density of the oil in ISM system (Figure 55). ISM comprising a high concentration of β-CD (50%w/w) formed into matrix at the bottom. The appearance of obtained matrix of ISM was more compact and elastic than those of ISG**.** The matrix formation was increased and more evident with time**.**

5.3.1.2.3. *In vitro* drug release studies

5.3.1.2.3.1. Drug release test with dialysis membrane method

Solvent-exchanged ISF system is based on phase separation of polymer by solvent movement which the organic solvent (dissolving the polymer) flows out from the system and the water inflows via diffusion inducing polymer precipitation (Graham et al., 1999). This process related to the sol-to-gel transformation causing polymer precipitation or solidification and forming into polymer matrix to control the drug release (Nirmal et al., 2010; Parent et al., 2013). Four major diffusional motions have to be considered for the formation of ISG or ISM: solvent diffusion out, non-solvent diffusion in, drug out and probably fractions of polymer out (Kranz and Bodmeier, 2008).

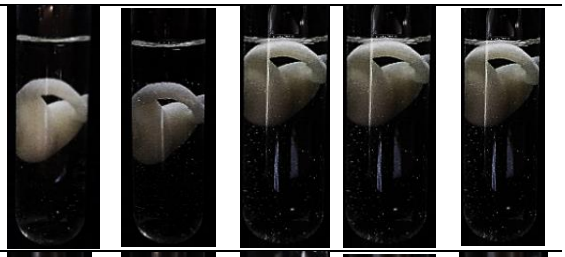
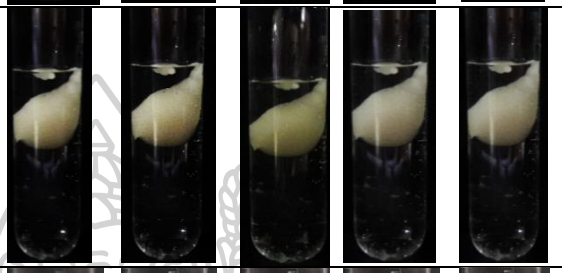
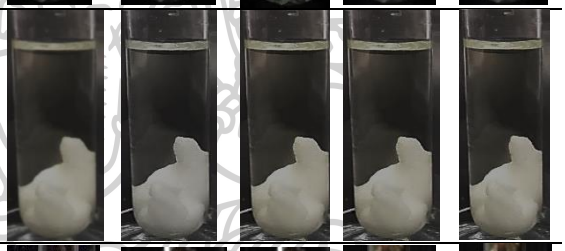
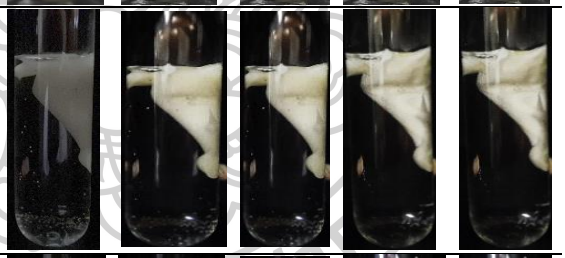
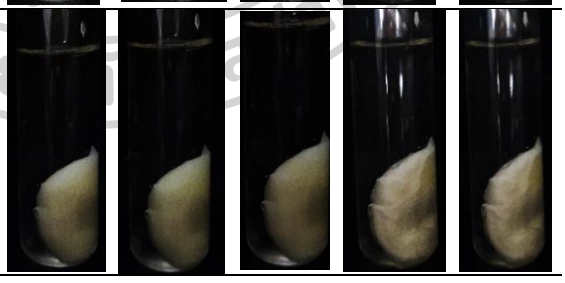
Formulations	Time (min)				
	0	1	5	30	1440
30%					
35%					
40%					
45%					
50%					

Figure 55 *In vitro* matrix formation of Mex-loaded ISM using different concentration of β-CD as matrix former

The release of Mex from system was studied in PBS pH 6.8 as the simulated environment for periodontitis. The drug release from Mex in DMSO without β-CD (control) and ISM comprising 40% β-CD are shown in the Figure.56. Initially, the

drug release from β-CD free system (control) was less than that of ISM comprising 40% β-CD within 1 h in which system without β-CD (control) was accounted for 0, 1.61, 1.76 2.59, 4.16, 6.45% respectively whereas ISM comprising 40%β-CD showed drug release of 0, 3.76, 5.78, 7.44, 9.28 and 10.76%, respectively, for 0, 5, 15, 30, 45 and 60 min. The hydrophilic outer surface of β-CD contributed to the higher polarity of the ISM system at the initial phase of drug release than system without β-CD (control). When the DMSO exchanged with the surrounding liquid of β-CD ISM, the matrix like microparticles were occurred which could retard the release of drug. Thus, β-CD free system (control) exhibited the higher drug release in later stage which proved that the matrix formation of microparticles retarded the drug release. The systems without β-CD free ISM (control) showed the 68.64% at 24 h, whereas ISM comprising 40%β-CD gave the drug release of 41.67%. The multiparticulates system of ISM provided the minimum variations in implant morphology which provided more consistent and reproducible drug release profiles (Luan and Bodmeier, 2006a). At 3.5 days , the release of the system without β-CD was 99.1% while the release of ISM containing 40%β-CD showed the gradual release until 6 days which the drug release of 55.30% were attained at 6 days.

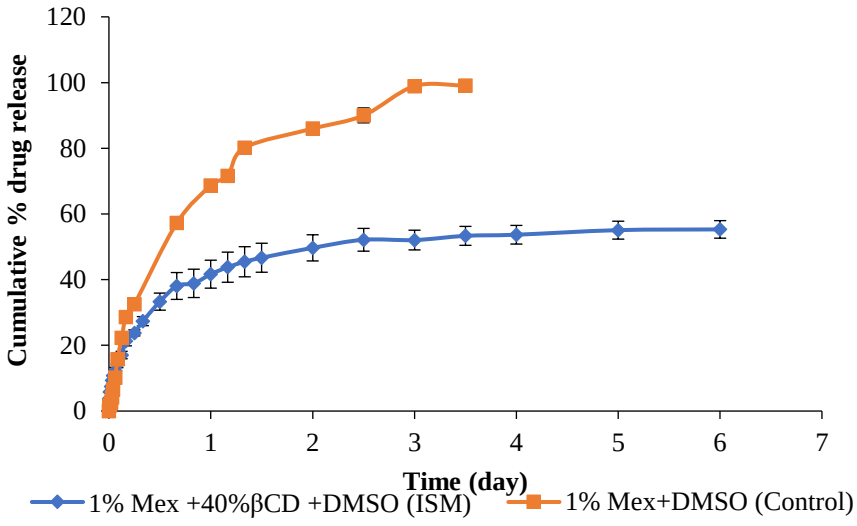


Figure 56 Mex released from ISG and ISM systems into PBS pH 6.8 using dialysis membrane method (n=3)

5.3.1.2.3.2. Drug release test with direct contact method

After exposure of the ISF system with the environment, the phase separation by solvent exchange provoked the matrix-like depot of the system from which the drug was released in a controlled manner**.** In the persent study, the PBS pH 6**.**8 was used as the simulated GCF and allowed the ISF system directly contact with this medium solution **.** The drug release was extended up to 2**.**5 days for ISM whereas the drug release from system without β-CD were occurred up to 16 h. The Mex was released after ISF system directly contacted with the meidum solution whereas the drug release profile of 1% Mex in DMSO without β-CD showed the fastest release rate (Figure 58). Initially, the drug release from 1% Mex in DMSO without β-CD was the highest within the initial release of 15 min because the absence of β-CD in the system led to the rapid changes of solvent and PBS pH 6.8. The drug release of Mex from the system without β-CD was more than 95% till 16 h.

The extent of drug release from Mex-loaded ISG and ISM comprising 40% β-CD system were 78.6% and 49.04%, respectively at 4 h while the extent of drug release from ISF system without β-CD was 96.55% (Figure 57). Although, there is some tendency of free CD molecules to self-assemble to form aggregates, their tendency to self-assemble increases on formation of inclusion complexes (Messner et al., 2011). Upon contacting with the PBS pH 6.8, the diffusion out of DMSO from the system that exchanged with the PBS pH 6.8 provoked the drug/β-CD partial complex aggregates. These aggregates behaved like a matrix from which drug could be released in a controlled manner. Thus, by comparison, the drug release of ISF system comprising β-CD showed significantly a decrease in drug release. In addition, the dynamic polymer phase inversion and high polymer concentration could reduce the burst release (Liu and Venkatraman, 2012). Thus, when 40% w/w β-CD was employed, the initial release of *in situ* system comprising β-CD was apparently reduced. The hydrophobicity of the oil used as external phase retarded the diffusion of solvent and drug out of the system (Ahmed et al., 2014). Thus, the initial drug release from ISM system was lower than that of ISG system whereas the camellia oil as the external phase of ISM behaved as the barrier for drug release (Figure 58).

The direct contact method was allowed the gel for direct contact with the simulated GCF whereas the large amount of simulated fluid entered without delaying. Although drug released in a controlled manner from the drug/β-CD partial inclusion complex aggregates matrix, influx of huge amount of simulated fluid promoted a large amount of drug dissolved and diffused out from the system. By comparison, in dialysis method, the simulated GCF gradually diffused through the dialysis membrane. Therefore, the extent of drug release from direct contact of ISF system with PBS pH 6.8 was higher than from dialysis membrane method.

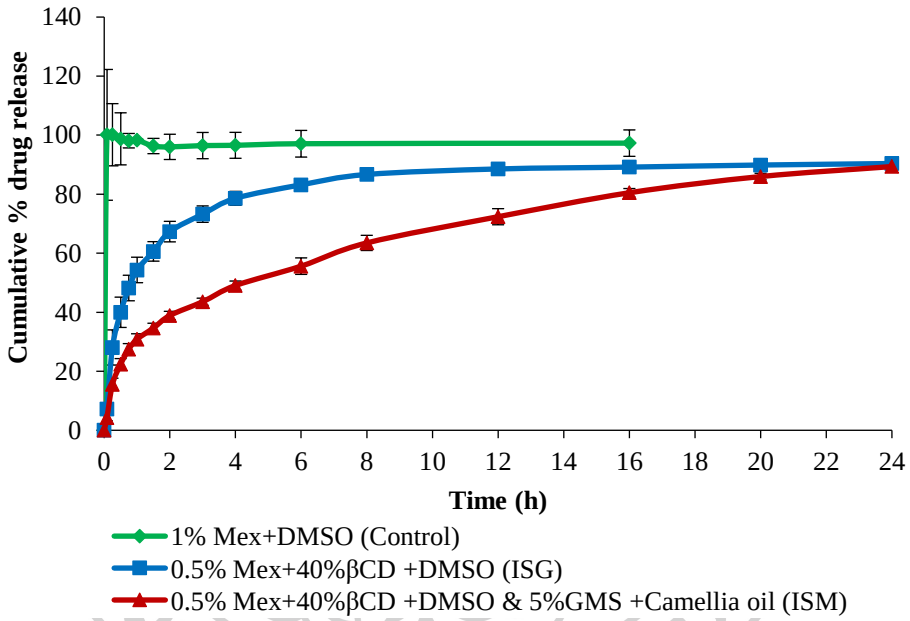


Figure 57 Mex released from ISG and ISM systems into PBS pH 6.8 using direct contact method within 24h (n=3)

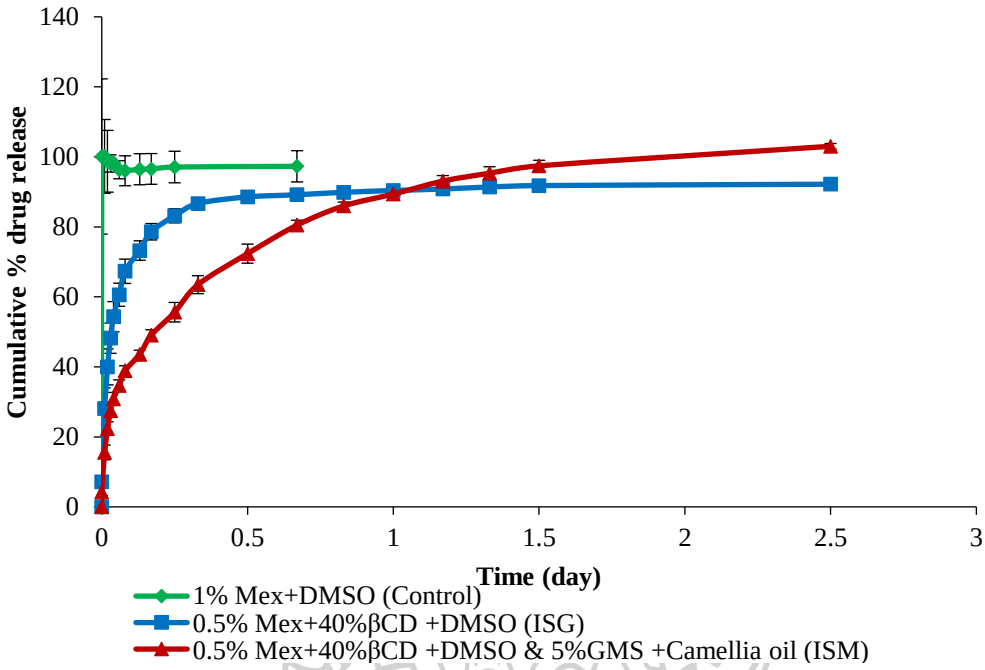


Figure 58 Meloxicam (Mex) released from ISG and ISM systems into PBS pH 6.8 using direct contact method (n=3)

5.3.1.2.3.3. Drug release test with small cup method

The drug release of the prepared ISF systems was investigated by small cup method. The drug release was extended up to 7 days (Figure 60). The drug release profile comprised of two phases with high initial release followed by almost constant and slower release (Luan and Bodmeier, 2006b). In the present study, the initial burst release occurred in the system without β-CD which showed the fastest release rate which 105.3% were attained at 4 h (Figure 59). Meanwhile, the amount of drug release of ISG and ISM containing 40% β-CD depicted 23.6% and 15.5%, respectively, at 4 h. Upon contacting the gel directly with the simulated medium, the rapid exchange of solvent and simulated medium created the rapid precipitation of drug/β-CD aggregates which acted as the matrix. The specific properties of chain length, their flexibility and mobility, their water uptake and swelling behavior, extent of drug potentially affected the diffusion rates of solvent in the polymer matrix and the drug release (Wischke and Schwendeman, 2008). In the present study, β-CD used as the matrix former had some tendency to self-assemble in the presence of aqueous medium and these tendencies gained more when drug/ β-CD complexes was occurred (Messner et al., 2011). The more concentration of β-CD proned to the formation of larger aggregates (Szente and Szejtli, 2004). These aggregates could be precipitated

as the matrix like when the gel was exposed to the simulated medium by exchange between solvent and simulated medium from which drug was released in a controlled manner. Thus, the increased amount of β-CD (40%) could provoke the larger aggregates which acted as the thicker barrier for drug release. The present study showed that the extent of drug release of ISF system comprising 40% β-CD exhibited the significantly lower release profile than that of the system without β-CD. Although, the drug linked by hydrogen bond of OH presented on the outer surface of β-CD could liberate freely, most of drugs could be trapped in the β-CD aggregates from which the drug was hardly diffused passing this matrix. Thus, the liberation of the Mex from the ISG and ISM system comprising 40% β-CD were gradually observed until 7 days where the extent of drug release were 80% and 43.9%, respectively (Figure. 60). The diffusion out of the drug and solvent from the system were hampered by hydrophobic property of the camellia oil presented as the external phase (Ahmed et al., 2014). In addition, the viscosity is one of the factors which contributes to the slow release of drug. The ISM system showed higher viscosity than that of the ISG system; thus, the drug release of ISM was lower than that of the ISG system. Moreover, the hydroxyl group on the outer surface of β-CD molecules (Brewster and Loftsson, 2007) behaved as the hydrophilic in nature. Thus, the initial release of ISF system comprising β-CD exhibited the significantly lower release profile when compared with the system without β-CD. The hydrophobic poly (dl-lactide co-caprolactone) exhibited the slowest drug release whereas the hydrophilic poly (dl-lactide-co-glycolide) exhibited the low initial release of drug followed by a more rapid release once the polymer became hydrated (Malik K et al., 2010).

The extent of drug release by small cup method exhibited the lowest release profile when compared with dialysis membrane method and direct contact method. By comparison, the dialysis method required the gradual diffusion of simulated medium through the membrane into the gel whereas the small cup method allowed the direct contact of gel with the medium. Thus, upon contacting the gel with medium, the rapid phase inversion of the system provoked the rapid transformation of the β-CD partial inclusion complex aggregates matrix. These aggregates matrix behaved like a barrier for entering of the simulated medium which comparatively lowered the extent of drug release than the other two methods discussed above.

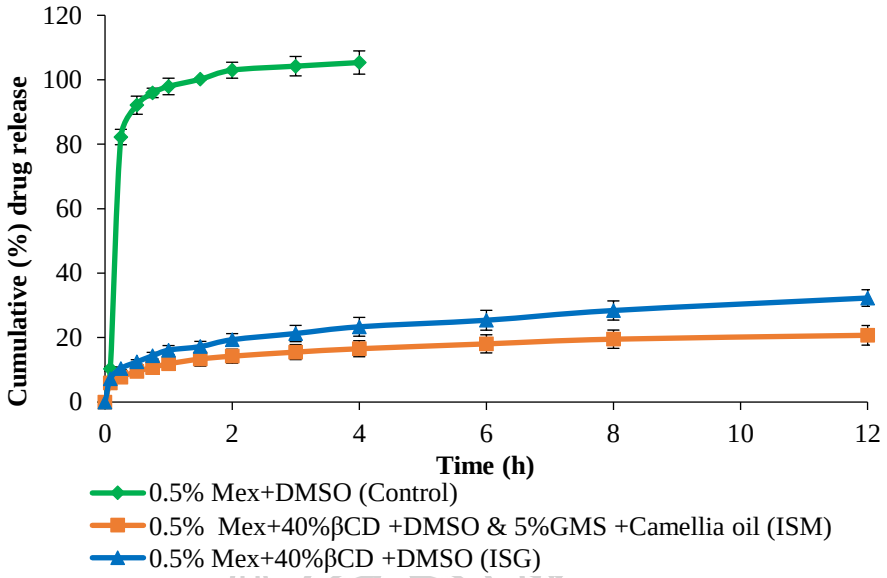


Figure 59 Mex released form ISG and ISM systems into PBS pH 6.8 using small cup method within 12 h (n=3)

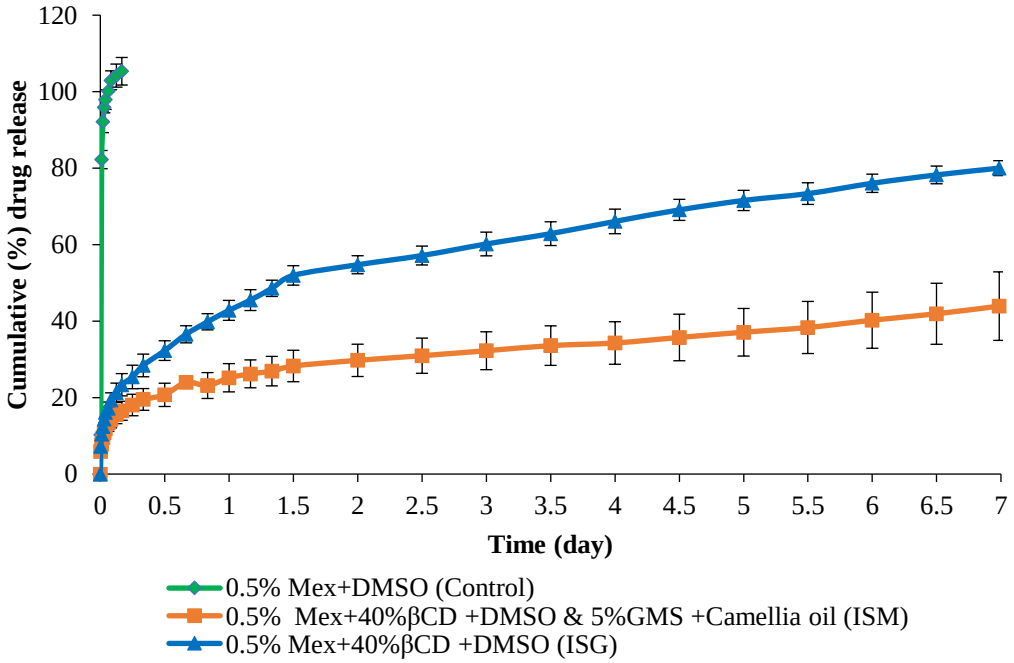


Figure 60 Mex released from ISG and ISM systems into PBS pH 6.8 using small cup method (n=3)

5.3.1.2.3.4. Analysis of drug release data

For characterization of mechanism of drug release, the release data of all formula were fitted to four different mathematical models such as zero order, first order, Higuchi's and power law (Korsmeyer-Peppas). The comparison of degrees of goodness-of-fit from curve fittings of the release profiles of the prepared formulas after drug release studies using dialysis membrane method, direct method and small cup method are shown in Table 10. The (r^2) with high value of coefficient indicated a superiority of the release profile fitting to mathematical equations (Bruschi, 2015).

The drug release of Mex-loaded system without β-CD (Control) studied by dialysis method fitted well with Higuchi‟s model whereas the drug release for system without β-CD (Control) using direct method and small cup method were not observed the fitted drug release model fitted well with power law model except drug release by dialysis method. For both Mex-loaded ISG and ISM system comprising 40%β-CD were best explained by power law where r2 from curve fitting were higher than Higuchi‟s model and zero order curve fitting but closed relation were occurred with Higuchi‟s model. Generally, the drug release from polymeric systems are described by power law equation (Bruschi, 2015). The release exponent values (n) for power law of all formulations from the release studies using dialysis membrane method, direct method and small cup method are shown in Table 11. The n value obtained from power law equation of all ISG and ISM formula using 40%β-CD with dialysis membrane method, direct method and small cup method were less than 0.45. The results indicated these formulas showed the drug release by Fickian diffusion mechanism. Whereas, the drug release from the system without 40%β-CD also exhibited the Fickian (n=0.464). The liquid didffusion rate and polymeric chain relaxation rate are important factors for drug release kinetics (Perioli et al., 2004). In Fickian model, the drug releases are governed by the diffusion and the solvent transport rate or diffusion rate is much greater than the process of polymeric chain relaxation (Bruschi, 2015).

Table 10 Comparison of degrees of goodness-of-fit from curve fittings of the release profiles of Mex-loaded ISF systems containing 40%β-CD in PBS pH 6.8 using the dialysis membrane method, direct method and small cup method for different release models

Models	r^2							
	Dialysis membrane method		**Direct method**			**Small cup method**		
	Control	**Mex - loaded ISM**	**Control**	**Mex - loaded ISG**	**Mex - loaded ISM**	**Control**	**Mex - loaded ISG**	**Mex - loaded ISM**
Zero order	0.8445	0.6577	0.0719	0.405	0.7476	0.3103	0.8666	0.8498
1st order	0.952	0.7266	0.0063	0.6165	0.9067	0.1098	0.9529	0.8633
Higuchi's model	0.9702	0.8824	0.1612	0.6373	0.9354	0.4818	0.982	0.9598
Korsmeyer–Peppas model (power law)	0.9569	0.976	0.2939	0.7344	0.9256	0.6007	0.9967	0.9969

Table 11 Estimate parameters form curve fittings of Mex-loaded ISF systems containing 40% β-CD in PBS pH 6.8 using the dialysis membrane method, direct method and small cup method to power law expression

Drug release method	**Formula**	**n**	**Release mechanism**
Dialysis membrane method	Control	0.6775	Fickian
	ISG	0.3826	Fickian
Direct method	Control	-0.027	
	ISG	0.2853	Fickian
	ISM	0.4136	Fickian
Small cup method	Control	0.4721	Fickian
	ISG	0.3176	Fickian
	ISM	0.2467	Fickian

5.3.1.2.4. *In vitro* degradability

The degradable ISF systems should be gradually eroded or dissolved in the gingival crevice because the removal after treatment is not required (Medlicott et al., 1994). The degradation could be described by weight loss or molecular weight loss (Ren et al., 2006). During exposure to solvent exchange, the influx of water and efflux of DMSO occurred which promoted the matrix-like from which the drug released gradually. Thus, the main weight loss in this system was due to the diffusion of DMSO, erosion of polymer and release of drug. The percentage weight loss of ISF systems and %β-CD are shown in Table 12. The Mex-loaded ISG system comprising 30%β-CD showed a percent weight loss of 93.324 ± 1.270% which proved that almost all of the solvent diffused out of the gel after one week. However, the percent weight loss of Mex-loaded ISG system comprising 40% β-CD was 78.538 ± 7.954%. The weight loss of the ISG system was significantly decreased with increased amount of β-CD ($p < 0.05$). When compared between ISG systems, the low viscosity in 30% β-CDMex ISG caused the higher outflow of DMSO with exchange with water leding to the higher weight loss of the ISG. Therefore, the results indicated that the weight loss of the prepared gel occurred by the diffusion of drug and solvent after exchange with medium.

The total mass loss of 30% β-CDMex ISM was apparently lower than that of ISG because the external oil phase acted as a diffusion barrier (Frelichowska et al., 2009; Kranz and Bodmeier, 2008) which minimized the aqueous diffusion. When compared between the prepared ISM systems, however, the weight loss of Mex-loaded ISM comprising β-CD was higher when the amount of β-CD was increased. In case of the ISM systems, the exchange of solvent with water promoted the precipitation of β-CD as the matrix, but instead of gel matrix, causing the formation of microparticles. The higher concentration of β-CD increased the formation of microparticles which was discussed in section 5.3.1.5.1. The in vitro degradation could associate with the number of microparticles whereas the degradation occured from these small microparticles. The increased weight loss of ISM system comprising 40% β-CDMex proved the high degradability of the system. Thus, the developed ISM exhibited the potential use to deliver Mex with biodegradable characteristics.

Upon contacting with the simulated medium, the medium entered into the system and eroded the precipitated β-CD aggregates matrix. Since the residual weight of solvent and drug were excluded, the present weight loss could be the net weight loss of β-CD. In the present study,% β-CD mass loss was calculated from the Mex-loaded ISG and ISM systems comprising only 40% β-CD which were used for durg release. According to the results, percentage weight loss of β-CD from Mex-loaded ISG and ISM systems comprising 40% β-CD were 55.7 ± 13.6 and 80.56 ± 8.5, respectively. When compared, %β-CD mass loss form Mex-loaded ISM was significantly different from that of the ISG ($p < 0.05$). Thus, at the end of one week, the high percentage β-CD mass loss of Mex-loaded ISM proved the erosion of precipitated β-CD aggregates.

Table 12 *In vitro* biodegradability test (n=3)

Formula	%Total mass loss	% β-CD mass loss
	Mean ± SD	Mean ± SD
30**%** β-CDMex ISG	93.324 ± 1.270*****	-
40**%** β-CDMex ISG	78.538 ± 7.954*****	55.7 ± 13.591*****
30**%** β-CDMex ISM	81.413 ± 5.144	-
40**%** β-CDMex ISM	83.694 ± 6.966	80.56 ± 8.5*****

The asterisk **(*)** represents the significant difference ($p < 0.05$)
(-) indicates not determined

5.3.1.2.5. Rate of water diffusion/Matrix formation into the systems

When water diffuses into the system by exchange with solvent, the apparent system is changed from transparent to opaque which is important for solvent-exchanged induced ISG or ISM (Sanghvi et al., 2008). The pattern of water influx in term of water diffusion is illustrated in Figure 61. The solvent diffusion rate of drug free ISG and Mex-loaded ISG (40% β-CDMex) showed the higher diffusion rate than the drug free ISM and Mex-loaded ISM **(**40% β-CDMex). Whereas the distance of matrix formation of drug free ISG was similar and coincided with ISM comprsing 40% β-CDMex.The camellia oil included in the external phase of ISM retarded the diffusion of water into the system. In addition, the high viscosity of the ISM was the one of factors for lowering in diffusion rate of water. The rapid diffusion rate of water into Mex-loaded ISG (40% β-CDMex) systems initiated the rapid change of solution

to opaque and matrix which made the diffusion rate slower. Thus, at 5 min, the diffusion rate of water into Mex-loaded ISG was lower than that of Mex-loaded ISM (40%β-CDMex). On the other hand, after penetration of water into the oil barrier, the aqueous diffusion rate of Mex-loaded ISM was higher than that of ISG. However, the rate of diffusion of water into both ISG and ISM systems were slower with time and exhibited not much difference at 30 min. This indicated that the diffusion of water into the systems altered the front of surface systems to be opaque and harder with time which created like a barrier to diffuse water anymore. Typically, the low aqueous diffusion rate is associated with the high retention which attained a prolongation in drug release (Wang et al., 2012).

When the water diffused into the β-CD solution, the opaque-ring occurred by solvent-exchange which represented the process of matrix formation from outer area to inner area. As the opaque ring was gradually increased, the enlargement of matrix formation occurred in all systems (Table 13) which created the longer distance matrix formation with time. The longer distance of thick matrix formation acted like a barrier for aqueous penetration into the system. Thus, the aqueous diffusion rate of all systems slowed with constant rate (Figure 61). As the enlargement of the opaque ring occurred, the radius of inner solution well disappeared at 30 min in all systems except that of the Mex-loaded ISG (Table 13). When compared, the matrix formation in Mex-loaded ISG was increased gradually and the distance was shorter than that of the other systems. The highest distance of matrix formation of Mex-loaded ISM notably occurred which the trend was similar with drug free ISG. The enlargement of matrix formation could entrap the Mex from which the Mex released in a controlled manner.

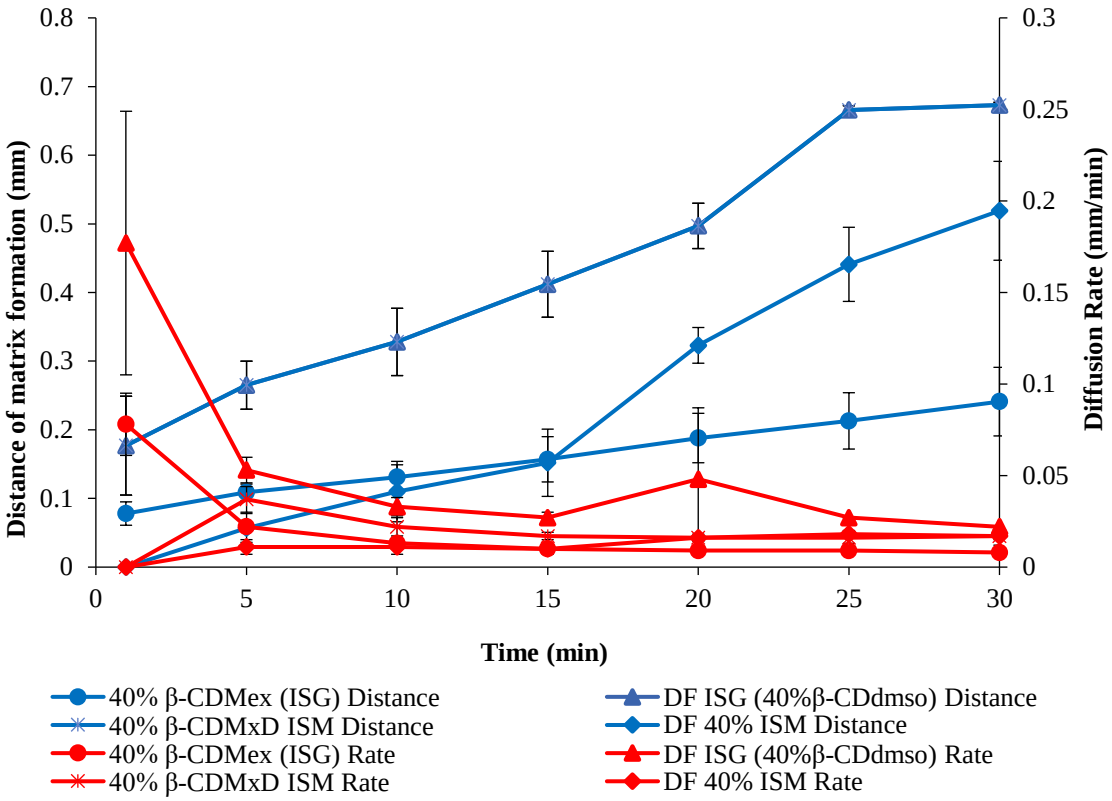
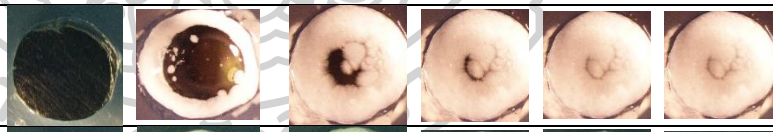
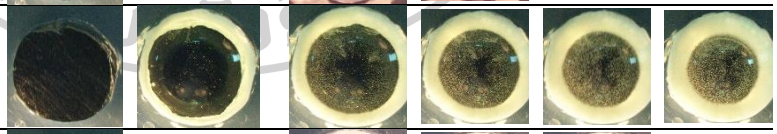
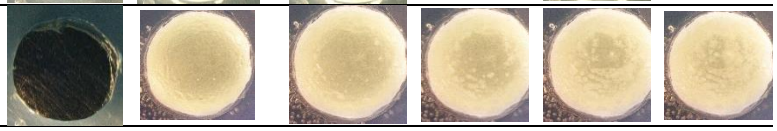
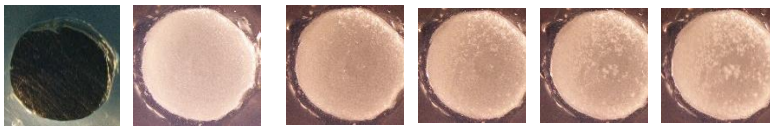


Figure 61 Water diffusion and matrix formation of prepared ISG and ISM (n=3)

Table 13 The visual image of matrix forming behaviour of ISG and ISM system

Formula	Time (min)					
	0	5	15	20	25	30
Drug Free ISG (40%β-CD DMSO)						
40% β-CD in Mex-loaded ISG						
40% β-CD in Mex-loaded ISM						
Drug Free 40% ISM						

5.3.1.3. Evaluation of ISG and ISM systems after exposure to solvent exchange

5.3.1.3.1. Mechanical property studies

Since the ISF system was aimed for the treatment of periodontitis, the mechanical property of the ISF system after deposition as matrix like could provide not only the deformability of the sample but capacity of it to reside in the artificial periodontal pocket. In addition, the deformability is related to the drug release, degradation and porosity under SEM. The mechanical properties such as hardness and ratio of elasticity/plasticity were measured as a basic indication for the deformability and the results are depicted in Figure 62 and Figure 63, respectively. The hardness of ISM system was significantly higher than ISG system ($p < 0.05$). This result proved that ISM system was more resistant to compression than ISG system. The mechanical property could relate with the porosity of the matrices which the large pores was exclusively responsible for crack initiation after compression (Yi et al., 2003). The previous study stated that the slow phase separation of solvent exchange decreased the number of porosity (He et al., 2013; Kranz and Bodmeier, 2008) whereas a fast phase separation created the large pores (Yi et al., 2003).

ISF system for periodontitis was formulated for local delivery to the periodontal pockets whereas the residence time of ISF system in the patient's periodontal pocket is necessary. The ratio of elasticity/plasticity plays a major role for determination of residence time in periodontal pocket (Do et al., 2014). A system with difficult to deform plastically is unlikely to adapt its geometry to dynamic changes in the periodontal pocket's size and shape with time. A value "1" indicates the system is ideally elastic (Do et al., 2014). The result of ratio of elasticity/plasticity for ISG and ISM are shown in Figure 5.20. The $F_{remaining}/F_{max\ deformation}$ ratios of ISM was significantly higher than that of ISG systems which were accounted for 0.981 and 0.881, respectively ($p < 0.05$). The low value of ISG indicates as the system with plastic nature which at least partially changes in a permanent manner during the holding time (Do et al., 2014). The porosity could relate to the elasticity because the regions of high porosity generally reduced the elasticity (Carr et al., 2015). Thus, the prepared ISG system with low value of ratio of elasticity/plasticity proved for the system with high porosity. Thus, the ISG system with reduced elasticity could provoke more deformability than ISM system. On the other hand, the Mex-loaded

ISM comprising 40% β-CD showed more resistant to compression which could reduced the pores and in turn could decrease in phase separation and initial release when compared with that of ISG.

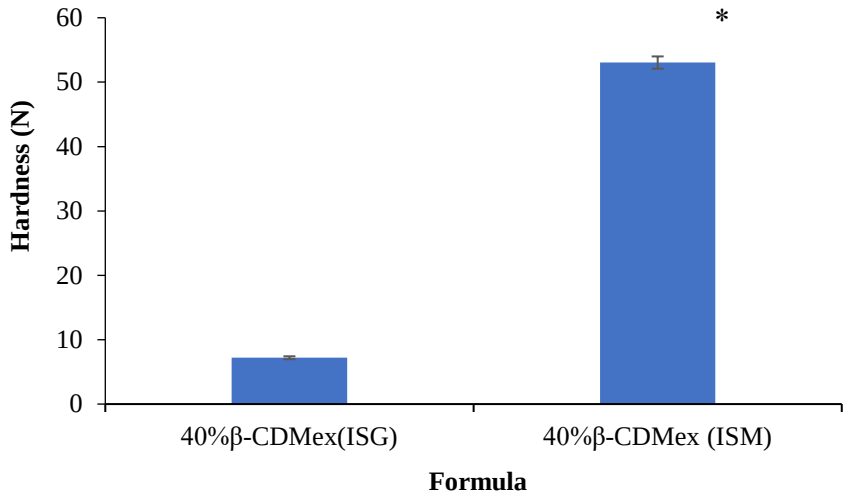


Figure 62 Maximum deformation force or hardness of ISG system (n=3)
The asterisk (*) represents a significant difference ($p < 0.05$)

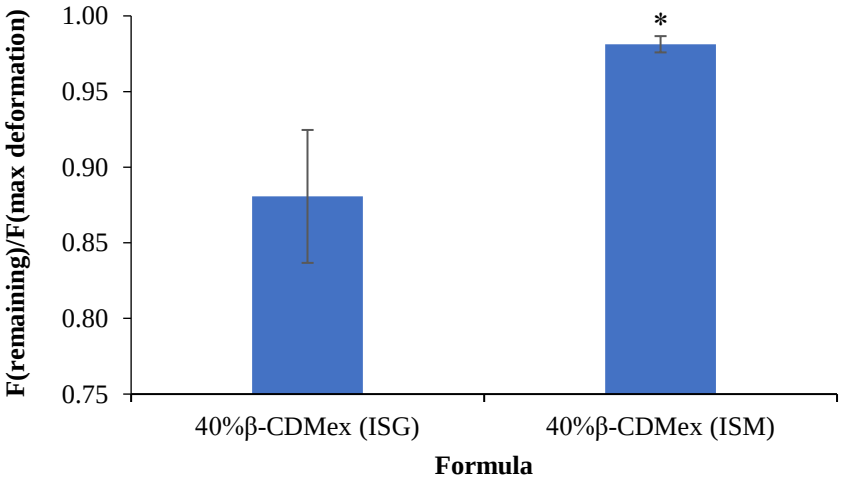


Figure 63 Ratio of remaining force/maximum deformation force or ratio of elasticity/plasticity of ISF system (n=3)
The asterisk (*) represents a significant difference ($p < 0.05$)

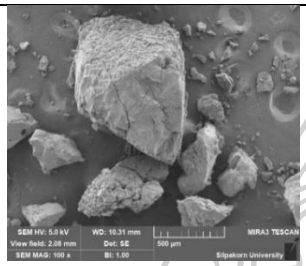
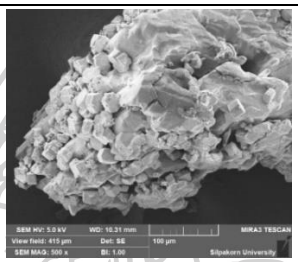
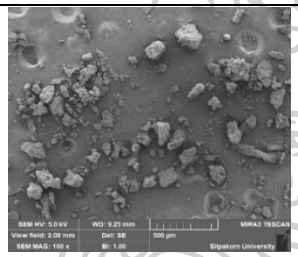
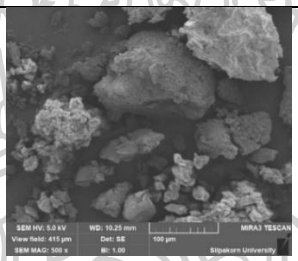
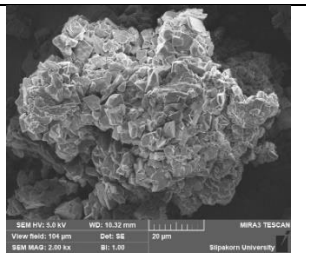
5.3.1.3.2. Determination of surface morphology by SEM

Upon contact with aqueous medium, the ISF system initiated the diffusion out of solvent by exchange with water whereas the polymer was progressively solidified and triggered phase separation and porous matrix (Parent et al., 2013). The rate of solvent release could affect the surface morphology of matices**.** Thus, SEM was used to investigate the porosity character of the precipitated gels or microparticles which could relate to the drug release (Kranz and Bodmeier, 2008).

The morphology of both ISG and ISM systems showed the irregular surface similar to the intact β-CD which the intact β-CD exhibited the large well-defined flake-type crystalline particles as previously discussed in chapter 3, section 3.4.2.2, (Figure 27). Typically, the diffusion of solvent and penetration of water promoted the porous structures (Phaechamud et al., 2016). However, in this system, no distinct pores were observed, insteadly, the crystal-like irregular particles were occurred. This may be due to the strong interaction between drugs and CDs caused no more possible for differentiation of the crystals of both components (Mura, 2015). In addition, the property of the guest/host inclusion complexes of β-CD could lead to the tendency of β-CD molecules to form aggregates (Messner et al., 2011). In addition, the increased concentration of the β-CD promoted the larger aggregates (Szejtli, 1988). The entering of the solvent into the system was difficult to pass through these dense matrices. Although the penetration of water and formation of matrix occurred as discussed above, the high maximum deformation force or hardness confirmed this structure of the system. The high viscous character of the system initiated the slow diffusion rate of solvent which conducted the less porous matrix surface (Kranz and Bodmeier, 2008). However, upon addition of high concentration of β-CD, this promoted to the high viscous nature of the system containing DMSO which minimized pores of the surface. The slower the solvent release into the aqueous medium, the less porous was evident for microparticles which provoked the reduced in initial drug release from the system (Kranz and Bodmeier, 2008). Thus, the decreased initial drug release of the system as discussed above supported the less porous nature of the microparticles. According to the surface morphology, the prepared ISM systems conveyed an extended drug release

profiles but higher degradation (Table 14) which are the essential requirement for injectable local drug delivery systems for dentistry (Kaplish et al., 2013).

Table 14 SEM of Mex-loaded ISG and ISM comprising 40% β-CD after release test for 7 days and dried at 60°C with different magnifications

Formula	100x	500x	2000X
40%ISG			-
40%ISM (5%GMS)			

5.3.1.4. Evaluation of ISM systems before exposure to solvent exchange

5.3.1.4.1. Morphology of o/o emulsion

Upon exposure with the medium, the DMSO diffused rapidly into the aqueous environment, resulting in precipitation of Mex/β-CD aggregates and formation of microparticles. The particle size of oil droplets in emulsion and the droplet size of o/o microparticles after exposure with the PBS pH 6.8 are shown in Table 15. The adequate area formation of GMS on droplet prevented the droplet accumulation or coalescence which tended to decrease the droplets size of microparticles (Phaechamud et al., 2018). In the present study, the droplet size of microparticles was 180.207 ± 26.065 μm which was statistically larger than the droplet size of oil globules in emulsion ($p < 0.05$). It had been reported that droplet size was influenced by stabilizer concentration and phase ratio (Phaechamud and Savedkairop, 2012). In the present study, the 5% GMS was used as stabilizer with the internal to external phase ratio of

1:1. GMS was able to gel the vegetable oils by forming its network (Chen and Terentjev, 2009) and prevented the coalescence.

In the emulsion states, the oil droplets are surrounded by the continuous phase. Upon injection of emulsion into PBS pH 6.8, DMSO diffused out through the external oil phase by exchange with PBS solution, thus, Mex-loaded β-CD aggregates were harden to form Mex entrapped matrix like microparticles. But during the solvent exchange process, certain amount of oil could enter and entrap in the hydrophobic inner cavity of β-CD and formed complexation. Otherwise, after exposure to the PBS solution, the interfacial thin film between oil droplets could be ruptured because of the low concentration of GMS which formed some coalescences. This will increase the surface area or could change the porosity of the polymer matrix which was confirmed by the surface morphology of the microparticles investigated by SEM as shown in Table 14. The previous study also stated that the initial release increased with increasing surfactant (Span 80) concentration in the oil phase could possibly be the smaller particle size (Luan and Bodmeier, 2006a). The previous study stated that ISM emulsion with 5% GMS gave the formation of microparticles larger than 100 μm with some extent of formation of polymer lumps (>1mm) (Voigt, 2011.).

Table 15 Particle size of oil droplets (internal phase) in emulsion (n=100)

Formulation	Particle size (mean ± SD) (μm)	
	oil droplet	microparticles
40% β-CD ISM	89.15 ± 24.732	180.207 ± 26.065 *

The asterisk (*) represents a significant difference ($p < 0.05$)

5.3.1.5. Evaluation of ISM systems during exposure to solvent exchange

5.3.1.5.1. Transformation of emulsion into microparticle study

Upon exposure to the surrounding environment, the ISG system transforms from solution into matrix by solvent exchange (Parent et al., 2013). The transformation of ISG system as the internal phase of ISM into the solid like is depicted in Table 16. The opaque like appearance occurred on the sample surface proved the transformation of into matrix-like**.**

ISM systems transformed from the emulsion to microparticles due to the phase separation triggered by solvent exchange mechanisms**.** The present study used GMS

as stabilizer because it has been used for decreasing a droplet coalescence of emulsion (Hodge and Rousseau, 2005). GMS exhibited the efficient emulsifying property for o/o emulsion of ISM (Phaechamud et al., 2016). In addition, after emulsification, there was a bi-refringent layer at the interface between the oil phase and PLGA solution droplets (Voigt, 2011.). Furthermore, the lower amount of GMS promoted the stable emulsion containing DMSO (Phaechamud et al., 2016). The camellia oil which was immiscible with the DMSO (Figure. 42) was used as the external phase of ISM. The phase ratio dominantly affected the transformation into microparticles. The higher quantity of the internal phase proned to merge together the particles resulting the coalescence (Tcholakova et al., 2002) while the lower ratio of the external phase also did not form microparticles in PBS (Phaechamud et al., 2016). Moreover, the previous research stated that the average droplet size of emulsion was significantly decreased when the phase ratio of internal and external was 1:1 ($p < 0.05$) (Phaechamud and Setthajindalert, 2017). Thus, ISM emulsion was prepared from β-CD as matrix former using GMS dispersed in camellia oil with the external and external phase ratio of 1:1. This study explored the solidifying droplets of the internal phase from emulsion upon contact with environmental fluids under inverted microscope**.**

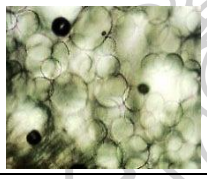
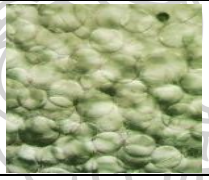
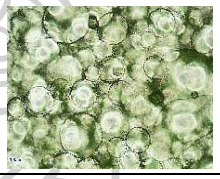
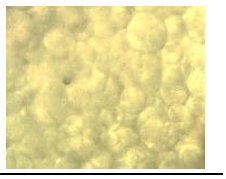
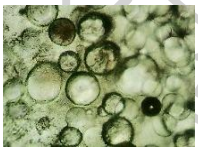
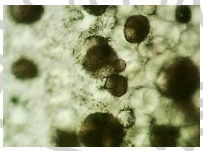
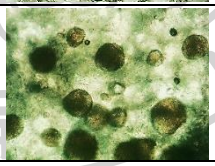
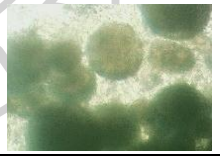
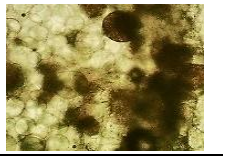
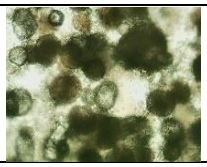
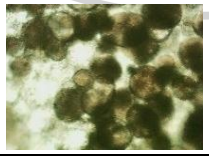
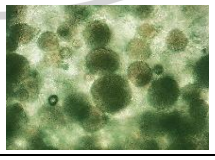
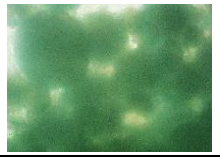
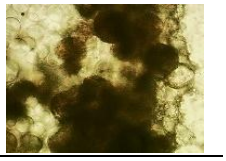
The influx of aqueous medium and outflow of solvent from the internal phase was the main parameter for phase inversion dynamics (Parent et al., 2013). DMSO with high polarity (George and Sastry, 2004) allowed the rapid diffusion of water into the system and the transformation rate emulsion into microparticles was initiated. DMSO with low viscosity value promoted a rapid phase inversion than PYR (Parent et al., 2013). Thus, from dynamic transformation study under an inverted microscope, ISM system transformed into microparticle suddenly after contacting with PBS (Table 17). 5% GMS of external phase could initiate the system transforming to the microparticles after exposure to phosphate buffer pH 6.8. GMS was able to gel the vegetable oils by forming its network remaining intact upon emulsification in non-aqueous ISM emulsion containing 5% MS (Chen and Terentjev, 2009). The ISM system comprising 40%w/w β-CD showed the numerous microparticles**.** At high concentration of β-CD **(**45% and 50% w/w), the coalescence of particles were occurred (Table 17). In stabilization of the emulsion, the GMS should have to prevent

the particles contact and coalescence**.** However, at relatively high concentrations, CDs can spontaneously self-assemble to form visible microparticles (Ryzhakov et al., 2016). Thus, the coalescence of particles may be due to the self-assemble of β**-**CD or the increased viscosity of the system with high concentration of β**-**CD**.** The schematic diagram of Figure 64 represents the transformation of o/o emulsion into microparticles**.** Upon exposure to the PBS pH 6**.**8, the diffusion of DMSO and entering of PBS caused the formation of β**-**CD matrix by solvent exchange mechanism (ISG) (Figure 64, A).Upon exposure to PBS pH 6**.**8, the solvent diffused out through the external oil phase into the environment and PBS solution diffused through the external oil phase into the internal phase (Figure 64, B)**.** As a result, the transformation of emulsion into micropaticles was occurred in the case of SM**.**

Table 16 Transformation of Mex-loaded ISG system comprising different concentration of β-CD into microparticles (x4) & (x10) after 60 sec

Magnification	30% β-CDMex	35% β-CDMex	40% β-CDMex	45% β-CDMex	50% β-CDMex	Mex + DMSO
x4						
x10						

Table 17. Transformation of o/o emulsion of Mex-loaded ISM comprising different concentration of β-CD into microparticles (x10)

Time (sec)	30% β-CDMex	35% β-CDMex	40% β-CDMex	45% β-CDMex	50% β-CDMex
0					
30					
120					

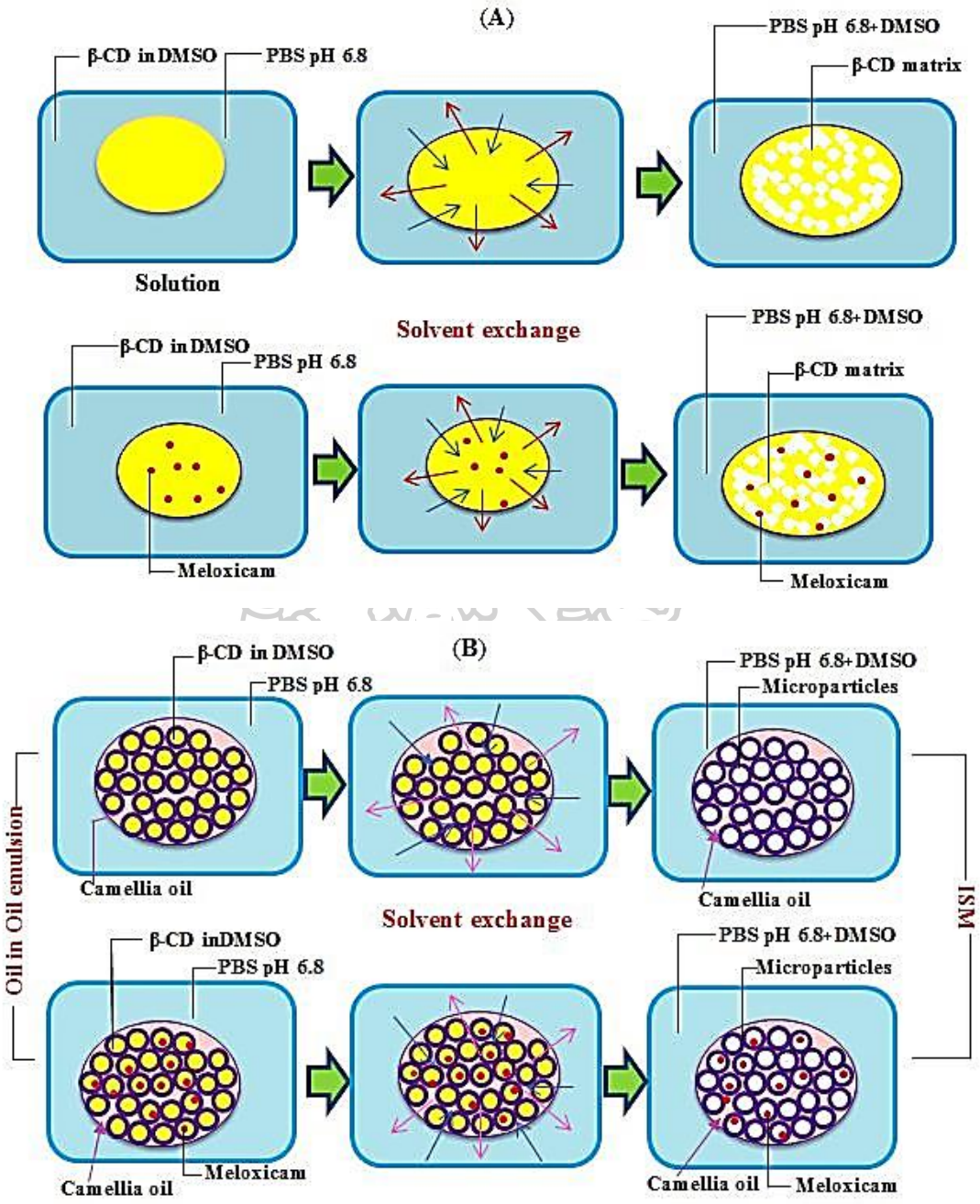


Figure 64 Schematic diagrams of ISG (A) *and* transformation for o/o emulsion into microparticles (ISM) (B)

5.4. Conclusion

Mex-loaded ISF system was developed using β-CD as the matrix former in which the 1:1 external and internal phase ratio. The external phase comprising camellia oil and 5%GMS as the stabilizer was used in ISM system. The viscosity of the system was increased when the concentration of β-CD increased with Newtonian flow. But, the lower injection force to expel the system proved that the Mex-loaded β-CD ISM comprising 40% β-CD could be easily injected via 24 G needle. Upon contact with PBS pH 6.8, the precipitation of drug/ β-CD aggregates as the matrix like occurred which was concentration dependent whereas Mex-loaded ISM comprising 40% β-CD formed into a matrix floating on the upper part of medium. The *in vitro* drug release studied by dialysis membrane, direct contact and small cup method revealed that both systems released the drug in a controlled liberation manner from the β-CD aggregate matrix with reduced burst release whereas the drug release obeyed diffusion control as Fickian diffusion mechanism. In addition, Mex-loaded ISM comprising 40% β-CD extended the drug release to 7 days with release mechanism of Fickian diffusion. The increased weight loss of both ISG and ISM systems comprising 40%β-CDMex proved the potential degradability of the system. The hydrophobicity of camellia oil included in the external phase with its high viscosity retarded the diffusion of water into the Mex-loaded ISM system. In addition, the high maximum deformation force or hardness with high viscous character initiated the slow diffusion rate of solvent which conducted the less porous matrix surface of prepared ISF system. The ISM system comprising 40% β-CD with 5% GMS of external phase could initiate the system transforming into the microparticles after exposure to PBS pH 6.8**.** Therefore, the prepared Mex-loaded ISG and ISM systems comprising 40%β-CD could be the potential controlled release delivery system for periodontitis.

CHAPTER 6

SUMMARY AND GENERAL CONCLUSION

Regarding to its limited aqueous solubility, it depicted the precipitation of β-CD aggregates as matrix like from water and other aqueous system. The usefulness of β-CD as the matrix former has been interested in solvent exchange-induced ISM. Therefore, the present study, β-CD was used as the matrix former. The investigation of the β-CD solutions reveals the β-CD solutions dissolved in PYR exhibited the highest viscosity because of its apparent high viscosity which was followed by the β-CD solution in DMSO and NMP, respectively. When β-CD was added, the apparent viscosity of solvents were noticeably increased which proved the strong interaction of respective solvent with β-CD molecule and all the β-CD solutions showed the Newtonian flow which is the required characteristics for the ISF system. The investigation of the intrinsic viscosity showed that β-CD solution in DMSO exhibited the lowest value of 0.07 dL/g which indicated that DMSO was more suitable solvent for β-CD for controlled drug release system with good injectability. Upon contact with PBS solution, diffusion out of solvents and influx of water provoked β-CD matrix as opaque-ring which the process of matrix formation was observed from outer area to inner area and the enlargement of this band was increased by time. This study indicated that β-CD solution containing DMSO promoted a rapid phase inversion. But, the transformed solid-like matrix behaved like a thicker barrier with time for the aqueous penetration into inner system. By comparison, β-CD solution in PYR showed the slower gelation rate than β-CD solution in NMP and DMSO, respectively. Among three solvent, DMSO presented the achievement of highest β-CD loading which further reduced the injection volume and provoked the dense matrix for controlling the drug release whereas it also showed the proper viscosity with Newtonian flow and lower work of injectability. From DSC, TGA, XRD and FT-IR data, β-CD precipitates prepared from these solvents showed the partial complexation between β-CD and solvent molecules. In addition, intrinsic viscosity, apparent and relative viscosities, density and surface and interfacial tension, contact angle measurements provided the useful information of β-CD solution dissolved in DMSO as a potential internal phase of emulsion. In addition, study of the phase separation, miscibility with DMSO, the camellia oil could have the potential for further preparing oil in oil emulsion of ISM. The study of physicochemical properties such as pH, density,

viscosities, surface tension and contact angle measurements provided useful information of various solvents and oils for preparation of ISM. Moreover, the miscibility and phase separation studies indicated that water soluble dye (amaranth) was dissolved and easily dispersed in DMSO. Furthermore, the low viscosity of the DMSO provided the ease of injectability. DMSO showed inhibitory zone against *E.coli* and *C. albicans* whereas all the tested solvents exhibited the larger inhibition zones against *C. albican*s than bacteria. Owing to supporting action on antimicrobial active compounds, the tested solvents were unique for using as the solvent for the ISM for treatment of infectious diseases. Moreover, DMSO showed its lowest cytotoxic activity against colorectal HCT 116 cell line. According to the above data, DMSO was used as the choice of solvent and the Mex-loaded ISF system was developed using β-CD as the matrix former in which the 1:1 external and internal phase ratio comprising 5% GMS as the stabilizer and camellia oil as the external phase were used in ISM system. Owing to its high loading in DMSO, 40%β-CD was used as the matrix former. The rheology of both ISG and ISM showed the Newtonian flow. The viscosity of prepared ISM was higher than that of ISG due to inherent high viscoisty of camellia oil added as the external phase. Although, the viscosity of the ISM was higher than ISG, the lower injection force to expel the system proved that the Mex-loaded β-CD ISM comprising 40% β-CD could be easily injected via 24 G needle. Although, the camellia oil as the external phase of ISM retarded the diffusion of water into the system, after penetration of water into the oil barrier, the aqueous diffusion rate of Mex-loaded ISM was higher than that of ISG. The solvent diffusion rate of both ISG and ISM systems as matrix formation were slower with time which indicated that the diffusion of water into the systems altered the front of surface systems to be opaque and harder with time. As the enlargement of the opaque ring occurred, the radius of inner solution well disappeared at 30 min in all systems except that of the Mex-loaded ISG. From this enlarged matrix, the trapped Mex could be released in a controlled manner. In the present study, the β-CD with self-assembled property in aqueous solution was used as the matrix former. Thus, upon contact with PBS pH 6.8, the Mex/β-CD aggregates precipitated as the matrix-like which was concentration dependent. The study of drug release showed the system without β-CD exhibited the fastest drug release whereas the Mex-loaded ISG and ISM system comprising 40%β-CD extended the drug release. The drug release by direct method was allowed to contact the system directly with the PBS solution. Thus, in

comparison, the drug release by direct method showed the higher drug release of ISG and ISM than those using dialysis membrane method and small cup method. The lower initial burst and slow release of Mex-loaded ISG and ISM comprising 40% β-CD were observed using small cup method with the best curve fitting of power law. The *in vitro* drug release of ISM extended up to 7 days with Fickian diffusion. The hydrophobicity of the oil presented in the external phase provoked the slower release of ISM than ISG systems. The high maximum deformation force or hardness initiated the slow diffusion rate of solvent which conducted the less porous matrix surface of prepared ISF system. The increase in viscosity retarded the diffusion of solvent and drug, thus, the weight loss of Mex-loaded ISG comprising 30% β-CD was significantly higher than those of ISG comprising 40% β-CD ($p < 0.05$). In addition, % β-CD mass loss in ISM showed the significantly increased weight loss than that of ISG ($p < 0.05$). The increased in total mass loss and % β-CD loss after 7 days proved the higher degradability of the ISG and ISM system. The ISM system comprising 40% β-CD with 5% GMS of external phase initiated the system into the microparticles after exposure to PBS pH 6.8**.** Therefore, the prepared Mex-loaded ISM system comprising 40% β-CD could be the potential use as the controlled release delivery system for periodontitis.

Future direction of the research

The self-asseble nature of β-CD and its limited solubility in water creates the β-CD into the formation of matrix former. Thus, in addition to the solubility enhancer, β-CD is useful advantage for new drug delivery formulation. The self-asseble aggregates of β-CD could control the release of the drug, although the viscosity of it should be necessary to reduce. In addition to the hydrophobic drug, the hydrophilic drugs could be formed partial inclusion complex because of the hydroxyl group on the surface of β-CD which may be useful in controlled drug delivery system. In future, it should be co-formulated with plasticizer such as PEG 400 for ISG. Moreover, in addition to o/o emulsion, o/w emulsion could be formulated using β-CD since it possesses both hydrophobic inner cavity and outer hydrophilic moiety. In conclusion, because of its complexation potential and matrix forming ability, β-CD may be useful for controlled release targeted drug delivery system.

Appendices

Appendix I

Viscosity and Rheology of Mex-loaded ISM

Table 18. Apparent viscosity of Mexβ-CD ISG (n=3)

Formula	Viscosity (cPs)	
	Mean	SD
Mexβ-CD 30% ISG	29.43	1.51
Mexβ-CD 35%ISG	48.67	42.15
Mexβ-CD 40%ISG	147.1	8.92
Mexβ-CD 45%ISG	877.9	48.42

Table 19. Apparent viscosity of ISM (n=3)

Formula	Viscosity (cPs)	
	mean	SD
30% β-CD in 7.5%GMS	177.53	4.74
40% β-CD in 5%GMS	372.93	83.03
Drug Free (40% β-CD ISM)	654.73	27.02
30% β-CD in 5%GMS	546.03	57.09
40% β-CD in 7.5%GMS	1183.33	97.52
5%GMS	92.90	1.23
7.5% GMS	1344.00	229.81

Appendix II

1. Study of injectability test

Table 20 **.** Work of injectability for ISG and ISM with different needles size **(**n=3**)**

Formula	Work of injectability (N.mm)							
	27G		24G		21G		18G	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mexβ-CD 40% ISG	ND	ND	44.06	2.36	6.30	0.25	2.66	0.29
Mexβ-CD 40% ISM+ 5%GMS	ND	ND	58.85	1.071	49.28	1.89	39.98	1.44
5% GMS	106.41	9.76	31.48	1.74	13.76	3.15	6.49	1.09
Mexβ-CD 30% ISM + 5%GMS	91.07	3.36	76.14	9.94	28.41	2.673	2.50	0.32
Drug free ISM containing 40% β-CD with 5%GMS	161.64	14.78	81.76	1.98	42.37	6.08	30.88	2.42

Table 21 **.** Injection force for ISG and ISM with different needles size **(**n=3**)**

Formula	Injection force (N)							
	Needle size							
	27G		24G		21G		18G	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mexβ-CD 40% ISG	-	-	4.57	0.27	0.61	0.01	0.22	0.03
Mexβ-CD 40% ISM+ 5%GMS	-	-	6.52	0.32	5.89	0.50	4.22	0.65
Mexβ-CD 30% ISM+ 5%GMS	11.04	0.26	8.52	1.08	1.87	0.63	2.22	0.27
Drug free ISM containing 40% β-CD+ 5%GMS	8.88	0.66	19.02	5.44	4.57	0.19	3.1	0.68
5% GMS	11.88	0.69	3.51	0.41	1.73	0.47	0.94	0.32

Appendix III

Table 22**.** Study of mechanical property **(**n=3**)**

Formula	Hadrness (N)		F(remaining)/F(max deformation)	
	Mean	SD	Mean	SD
40%βMexD (ISG)	7.19	0.22	0.88	0.04
40%βMexD ISM)	53.04	0.96	0.98	0.01

Appendix IV

1. Calibration curve of Mex in phosphate buffer pH 6.8 for *in vitro* release study

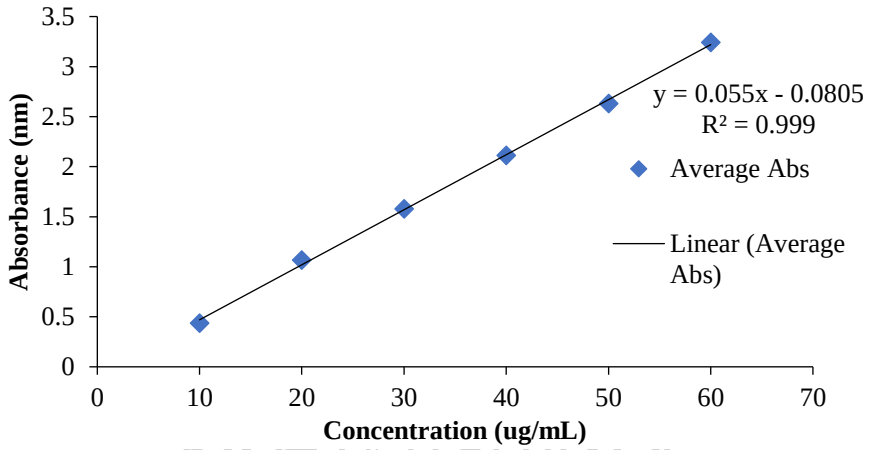


Figure**.** 65 Calibration curve of drugs in phosphate buffer pH 6**.**8 using UV-Vis spectrophotomerter at 360 nm

2. ***In vitro* Mex release using dialysis membrane method**

Table 23**.** Release of drug from Mex-loaded ISG and ISM comprising 40% β-CD in PBS pH 6.8 (n=3)

Cumulative drug release (%)					
Time (Day)	Control		Time (Day)	Mex-loaded ISM with 40% β-CD	
	Mean	SD		Mean	SD
0.00	0.00	0.00	0.00	0.00	0.00
0.07	1.61	0.00	0.00	3.76	0.23
0.07	1.76	0.00	0.01	5.78	0.36
0.11	2.58	0.00	0.02	7.44	0.48
0.17	4.16	0.00	0.03	9.28	0.78
0.27	6.45	0.00	0.04	10.75	0.86
0.43	10.22	0.01	0.06	12.27	1.04
0.65	15.72	0.01	0.08	14.26	1.07
0.93	22.26	0.01	0.13	17.05	1.13
1.19	28.66	0.01	0.17	21.31	1.45
1.35	32.47	0.02	0.25	23.78	0.91
2.39	57.27	0.28	0.33	27.37	1.37
2.86	68.64	0.28	0.50	33.32	2.60
2.98	71.60	0.39	0.67	38.08	4.08
3.34	80.21	0.44	0.83	38.87	4.31
3.58	85.97	0.87	1.00	41.67	4.25
3.75	89.99	2.26	1.17	43.80	4.59
4.12	98.92	1.53	1.33	45.45	4.50
4.13	99.09	0.86	1.50	46.68	4.41
			2.00	49.67	3.99
			2.50	52.14	3.46
			3.00	52.05	3.01
			3.50	53.33	2.88
			4.00	53.68	2.84
			5.00	55.07	2.72
			6.00	55.29	2.67

3. ***In vitro* solvent release using direct method**

Table 24**.** Release of drug from Mex-loaded ISG and ISM comprising 40% β-CD in PBS pH 6.8 (n=3)

Cumulative drug release (%)							
Time (day)	Control		Time (day)	Mex-loaded ISG with 40% β-CD		Mex-loaded ISM with 40% β-CD	
	Mean	SD		Mean	SD	Mean	SD
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	102.49	22.16	0.00	7.20	10.07	4.27	1.10
0.01	124.13	10.54	0.01	28.06	5.94	15.55	2.10
0.02	98.74	8.82	0.02	39.98	5.14	22.36	1.93
0.03	98.11	2.45	0.03	48.20	4.33	27.49	1.88
0.04	98.32	0.37	0.04	54.32	4.32	30.85	1.84
0.06	96.31	2.59	0.06	60.61	3.30	34.66	1.61
0.08	96.02	4.28	0.08	67.31	3.48	38.89	1.41
0.13	96.46	4.45	0.13	73.23	2.77	43.56	1.21
0.17	96.55	4.39	0.17	78.60	2.35	49.05	1.53
0.25	97.09	4.51	0.25	83.16	2.03	55.61	2.79
0.67	97.29	4.47	0.33	86.67	0.95	63.48	2.57
			0.50	88.54	0.36	72.35	2.73
			0.67	89.17	0.35	80.48	1.43
			0.83	89.87	0.40	85.97	1.11
			1.00	90.38	0.31	89.36	1.35
			1.17	90.81	0.36	93.07	1.57
			1.33	91.39	0.50	95.32	1.85
			1.50	91.79	0.64	97.43	1.59
			2.50	92.17	0.56	103.02	0.79

4. ***In vitro* Mex release using small cup method**

Table 25**.** Release of drug from Mex-loaded ISG and ISM comprising 40% β-CD in PBS pH 6.8 (n=3)

Cumulative drug release (%)						
Time (day)	Control (Meloxicam (0.5%) + DMSO)		Mex-loaded ISG with 40% β-CD		Mex-loaded ISM with 40% β-CD	
	Mean	SD	Mean	SD	Mean	SD
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	10.26	0.65	7.21	0.47	5.89	0.07
0.01	82.23	2.39	10.44	0.45	7.75	0.71
0.02	92.11	2.81	12.46	0.69	9.50	1.35
0.03	95.92	1.47	14.38	1.04	10.74	1.44
0.04	97.93	2.56	16.11	1.43	11.87	1.81
0.06	100.17	0.23	17.22	1.61	13.36	2.19
0.08	102.97	2.47	19.35	1.89	14.24	2.17
0.13	104.21	3.02	21.27	2.52	15.51	2.33
0.17	105.35	3.60	23.35	2.92	16.54	2.48
0.25			25.39	3.08	18.06	2.82
0.33			28.41	2.96	19.52	2.84
0.50			32.28	2.57	20.72	3.04
0.67			36.55	2.24	23.96	0.79
0.83			39.81	2.12	23.15	3.38
1.00			42.81	2.61	25.18	3.70
1.17			45.49	2.72	26.20	3.65
1.33			48.58	2.13	26.91	3.86
1.50			51.95	2.53	28.26	4.12
2.00			54.73	2.36	29.74	4.22
2.50			57.15	2.48	30.95	4.61
3.00			60.17	3.11	32.24	4.95
3.50			62.86	3.10	33.59	5.17
4.00			66.07	3.22	34.28	5.56
4.50			69.08	2.76	35.72	6.09
5.00			71.55	2.65	37.09	6.22
5.50			73.34	2.83	38.33	6.81
6.00			76.05	2.39	40.23	7.33
6.50			78.23	2.32	41.92	7.98
6.99			80.00	1.95	43.91	8.95

BIOGRAPHY

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Education

2016 (Jan) - 2018 (Dec): Doctor of Philosophy, Ph.D. in Pharmaceutical Engineering, Silpakorn University, Thailand

2010 (June) - 2012(Jan):Master of Pharmacy, University of Pharmacy, Yangon, Myanmar

2001(Jan) -2005(Jan):Bachelor of Pharmacy, University of Pharmacy, Mandalay, Myanmar

Presentation/Proceedings and Publication

Oral Presentation

1. Sia Myo Thu Rein (2017). Fluid properties of beta-cyclodextrin solutions in injectable solvents. International Conference of Pharmaceutical Sciences and Medicines 2017 (ICPAM 2017), Central laboratory, Faculty of Sciences, Burapha University, Chonburi, Thailand, 16 Jun 2017.
2. Sai Myo Thu Rein (2018). Physicochemical properties of β-cyclodextrin fluids and precipitated prepared from biocompatible vehicles (2018). International Conference and Exhibition on Pharmaceutical Sciences and Technology 2018)PST 2018, The Ambassador, Bangkok Hotel, Bangkok, Thailand, 24 – 25 Jan 2018.

Poster presentation

1. Sai Myo Thu Rein, Thawatchi Phaechamud (2018). Single phase and interfacial behavior of concentrated beta-cyclodextrin solution. The 34th International Annual Meeting in Pharmaceutical Sciences and 2nd CU FPhS -RIKEN CDB Symposium: Advances in Cellular and Molecular Biology, Arnoma Grand Hotel, Bangkok, Thailand, 8-9 March 2018.
2. Sai Myo Thu Rein, Torrsak Intaraphairotic, Wichai Santimaleeworagund, Tiraniti Chuenbarna, Takron Chantadeea, Thawatchai Phaechamud (2018). Fluid properties of solvents used in *in situ* forming microparticles preparation. International Conference on Materials Research and Innovation (ICMARI-2018), Emerald Hotel, Bangkok, Thailand, 17-21 Dec 2018.

Scientific Publication

3. Sai Myo Thu Rein, Nutdanai Lertsuphotvanit, Thawatchai Phaechamud, 2018. Physicochemical properties of β-cyclodextrin fluids and precipitates prepared from biocompatible vehicles. Asian Journal of Phamraceutical Sciences 18:438-449, https://doi.org/10.1016/j.ajps.2018.02.002.
4. Thawatchai Phaechamud, Sai Myo Thu Rein, Takron Jantadee, 2018. Role of clove oil in solvent exchange-induced doxycycline hyclate-loaded Eudragit RS *in situ* forming gel.Asian Journal of Pharmaceutical Sciences. 13: 131-142. 2017.09.004.
5. Sai Myo Thu Rein, Nutdanai Lertsuphotvanit, Thawatchi Phaechamud. Single phase and interfacial behavior of concentrated beta-cyclodextrin solution. The Thai Journal of Pharmaceutical Sciences (TJPS), 2018, Vol.42)Supplement Issue), page 193 -197 (Mini manuscript)
6. Sai Myo Thu Rein, Torsak Intaraphairot, Wichai Santimaleeworagun, Takron Chantadee, Tiraniti Chuenbarn, Thawatchai Phaechamud. Characterization of fluid properties of solvents and oils used in *in situ* forming microparticles. (Submitted)

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Ahmed, T. A., Ibrahim, H. M., Samy, A. M., Kaseem, A., Nutan, M. T., & Hussain, M. D. (2014). Biodegradable injectable in situ implants and microparticles for sustained release of montelukast: in vitro release, pharmacokinetics, and stability. AAPS PharmSciTech, 15(3), 772-780.

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