การพัฒนาสูตรตำรับและการศึกษาความคงตัวของแคปซูลที่บรรจุไซโครสปอริน เอ ในระบบนำส่งยาแบบไมโครอิมัลชันชนิดเกิดด้วยตัวเอง

นางสาว นิชธิมา แพ่งนคร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

Chulalongkorn University
จุฬาลงกรณ์มหาวิทยาลัย

FORMULATION DEVELOPMENT AND STABILITY STUDIES OF CAPSULES CONTAINING CYCLOSPORIN A SELF-MICROEMULSIFYING DRUG DELIVERYS SYSTEM

Miss Nichthima Paengnakorn

A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	FORMULATION DEVELOPMENT AND STABILITY STUDIES OF CAPSULE CONTAINING CYCLOSPORIN A SELF-MICROEMULSIFYING DRUG DELIVERYS SYSTEM
By	Miss Nichthima Paengnakorn
Field of Study	Industrial Pharmacy
Thesis Advisor	Professor Garnpimol C. Ritthidej, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master ’s Degree

…… Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor .Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

…… Chairman
(Assistant Professor Wichein Thanindratarn, M.Sc. in Pharm.)

…… Thesis Advisor
(Professor Garnpimol C. Ritthidej, Ph.D.)

…… Member
(Assistant Professor Waree Tiyaboonchai, Ph.D.)

…… Member
(Assistant Professor Warangkana Warisnoicharoen, Ph. D.)

…… Member
(Narueporn Sutanthavibul, Ph.D.)

นิชธิมา แพ่งนคร : การพัฒนาสูตรตำรับและการศึกษาความคงตัวของแคปซูลที่บรรจุไซโคลสปอริน เอ ในระบบนำส่งยาแบบไมโครอิมัลชันชนิดเกิดขึ้นด้วยตัวเอง (FORMULATION DEVELOPMENT AND STABILITY STUDIES OF CAPSULES CONTAINING CYCLOSPORIN A SELF-MICROEMULSIFYING DRUG DELIVERYS SYSTEM) อ.ที่ปรึกษา : ศ.ดร. กาญจน์พิมล ฤทธิเดช, 155 หน้า

ไซโคลสปอริน เอ มีลักษณะเป็นผงสีขาว ไม่ละลายในน้ำ แต่สามารถละลายได้ในน้ำมันและตัวทำละลายอินทรีย์ วัตถุประสงค์ของการทดลองนี้ เพื่อเตรียมตำรับไซโคลสปอริน เอ ในอยู่ในรูปแบบแคปซูลที่บรรจุรูปแบบการนำส่งยาแบบไมโครอิมัลชันชนิดเกิดได้ด้วยตัวเอง เพื่อให้มีการละลายที่ดีขึ้นและมีความคงตัวดี โดยการศึกษาผลของชนิดและปริมาณของ น้ำมัน สารทำอิมัลชัน และสารอิมัลชันร่วม ต่อการเกิดเป็นไมโครอิมัลชันชนิดเกิดได้ด้วยตัวเอง โดยสารที่เลือกใช้คือ ไตรกลีเซอร์ไรด์โมเลกุลขนาดกลาง เพื่อใช้เป็นส่วนวัฏภาคน้ำมัน สารลดแรงตึงผิวที่ใช้คือ ครีโมฟอร์ อีแอล, ทวีน 80 และ โซลูทอล เอชเอส 15 สารช่วยละลาย คือ โพรพิลีนไกลคอล, กลีเซอรีน, โพลีเอธิลีนไกลคอล 400 และ เอธานอล ระบบถูกเตรียมจากส่วนประกอบดังกล่าว และนำมาสร้างเป็นเฟสไดอะแกรม เพื่อศึกษาพื้นที่การเกิดไมโครอิมัลชัน จากผลการศึกษาพบว่าระบบที่ให้ ก่อให้เกิดไมโครอิมัลชัน มากที่สุด คือ ระบบของน้ำมัน และ ครีโมฟอร์ อีแอล หรือ ทวีน 80 ซึ่งสามารถให้พื้นที่ไมโครอิมัลชันได้เท่ากัน โซลูทอล เอชเอส 15 ก่อให้เกิดไมโครอิมัลชันได้น้อยที่สุดและทำให้เกิดการแยกชั้นระหว่างวัฏภาคมากที่สุด การใช้สารลดแรงตึงผิวสองตัวร่วมกันคือ ครีโมฟอร์ อีแอล และ ทวีน 80 ร่วมกันสามารถก่อให้เกิดพื้นที่ของไมโครอิมัลชันได้มากเช่นเดียวกับการใช้เพียงชนิดเดียว สารที่ช่วยเพิ่มการละลายของ ไซโคลสปอริน เอ ในน้ำมันได้มากที่สุด คือ โพรพิลีนไกลคอล การเพิ่มขึ้นของสารช่วยทำละลายทำให้การลายละลายยาเพิ่มมากขึ้นแต่ทำให้การเกิดไมโครอิมัลชันลดลง ระบบไมโครอิมัลชันชนิดเกิดขึ้นเองที่ความเหมาะสมต่อการเตรียมเป็นในรูปยาแคปซูล คือระบบของ ไตรกลีเซอร์ไรด์โมเลกุลขนาดกลาง : ครีโมฟอร์ อีแอล : ทวีน 80 ที่อัตราส่วน 35: 32.5:32.5 และ โพรพิลีนไกลคอล ร่วมกัน เอธานอล ที่ปริมาณอย่างละ 5%ของตำรับ ตำรับที่ได้นี้สามารถบรรจุตัวยา ไซโครสปอรินให้มีปริมาณ 25 และ 100 มิลลิกรัมต่อแคปซูลได้โดยมีขนาดของวัฏภาคภายในภายหลังผสมน้ำแล้วเท่ากับ 69.19±1.36 และ 74.96±1.71 นาโนเมตรตามลำดับ ระบบมีความหนืดเท่ากับ167±1.00 และ 250±0.00 เซนติพอยด์ ซึ่งสามารถบรรจุลงแคปซูลโดยใช้เครื่องบรรจุของเหลวลงในแคปซูลได้ จากการศึกษาความคงตัวพบว่าสูตรตำรับสามารถรักษาสภาพความคงตัวภายหลังการเก็บรักษาที่สภาวะเร่งเป็นเวลา 4 เดือนได้ โดยตัวยาไม่เปลี่ยนแปลงจากเริ่มผลิตอย่างมีนัยสำคัญ (p>0.05) อย่างไรก็ตามสภาวะของการเก็บรักษาแคปซูลมีผลต่อการละลายของยาออกมาจากเปลือกแคปซูล เนื่องจากเปลือกแคปซูลไม่สามารถทนต่อสภาวะความร้อน หรือความชื้นสูงซึ่งมีผลทำให้คุณสมบัติของแคปซูลเปลี่ยนไปไม่สามารถละลายและปลดปล่อยตัวยาได้ และจากการศึกษาการนำสูตรตำรับที่ได้ไปผสมไมโครคริสตัลลีน เซลลูโลส ที่ใช้เป็นสารดูดซับเพื่อทำให้อยู่ในรูปผงแห้งและพัฒนาเป็นแกรนูล สำหรับบรรจุแคปซูล พบว่าแคปซูลที่บรรจุไมโครอิมัลชันที่อยู่ในรูปแบบแกรนูลมีการละลายของตัวยาออกมาช้ากว่าระบบแคปซูลที่บรรจุไมโครอิมัลชันที่อยู่ในรูปแบบไมโครอิมัลชันชนิดเกิดขึ้นได้ด้วยตัวเองในรูปของเหลว

ภาควิชา.....เภสัชอุตสาหกรรม.....ลายมือชื่อนิสิต.....

สาขาวิชา.....เภสัชอุตสาหกรรม.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2550.....

477 65713 33 : MAJOR INDUSTRIAL PHARMACY

KEY WORD:CYCLOSPORIN A/SELF-MICROEMULSIFYING DRUG DELIVERY /CAPSULE /STABILITY

NICHTHIMA PAENGNAKORN : FORMULATION DEVELOPMENT AND STABILITY STUDIES OF CAPSULES CONTAINING CYCLOSPORIN A SELF-MICROEMULSIFYING DRUG DELIVERYS SYSTEM. THESIS ADVISOR : PROF. GARNPIMOL C. RITTHIDEJ, Ph.D.,155 pp.

Cyclosporin A is a white powder, insoluble in water but soluble in oil and organic solvent. The purpose of this study was to prepare capsules containing cyclosporin A self-microemulsifying drug delivery to improve solubility in water and provide good stability. The effect of type and quantity of oil, surfactant and co-surfactant to form self-microemulsion was investigated. Medium chain triglyceride was used as oil phase. The surfactants used were Cremophor® EL, Tween80 and Solutiol® HS 15. The co-surfactants used were propylene glycol, glycerine, polyethylene glycol400 and ethanol. Pseudoternary phase diagrams were constructed to evaluate the microemulsion existing area. From the results, it was found that the systems of Cremophor® El and Tween 80 provided the largest microemulsion area. Solutol® HS15 provided the smallest microemulsion area and the most phase are separation. Combined surfactants, Cremophor® El and Tween 80 yielded microemulsion regions similar to used single surfactant. Propylene glycol provided the highest solubility of cyclosporin A in oil. Increasing of co- solvent content the increased solubility but decreased the microemulsion area. The suitable system for incorporation into gelatin capsule was the system of oil : Cremophor® El : Tween80 at the ratio of 35:32.5:32.5 with propylene glycol and ethanol each 5% w/w of formulation. Each capsule contained cyclosporin A 25 mg and 100 mg. The droplets size after diluted in water were 69.19±1.36 and 74.69±1.71 nm respectively. Viscosity of formulation were 167±1.00 and 250±0.00 cP respectively which were suitable to be filled by liquid filling machine. The stability study at accelerated conditions for 4 months found that the content of drug were non significant difference from initial (p>0.05). However the storage condition had effect on dissolution of drug from capsule because capsule shell was intolerance to high temperature and high humidity that caused changing of capsule property leading to undissolved and unable to release drug.Formulation was absorbed onto microcrystallene cellulose to be dry powder and granule for filling into capsule. It was found that the dissolution rate of drug in dry power capsules was slower than that form of liquid preparation capsules.

Department : …Manufacturing Pharmacy....Student's Signature :…….

Field of study :..Industrial pharmacy……..Advisor's Signature :……..…

Academic Year :……..2007……

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I wish to thank my friends, colleagues, staffs of the Department of Manufacturing Pharmacy and other person whose names have not been mentioned here for their assistance and encouragement.

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LIST OF ABBREVIATIONS

ANOVA	=	analysis of variance
$^{\circ}C$	=	degree Celsius
C_{300}	=	medium chain triglyceride (captex®300)
C_{EL}	=	Cremophor EL
CV	=	coefficient of determination
CyA	=	cyclosporin A
cm	=	centimeter (s)
EtOH	=	ethanol
g	=	gram(s)
hr	=	hour(s)
L	=	liter (s)
mg	=	milligram (S)
min	=	minute(s)
ml	=	milliliter(s)
MW	=	molecular weight
OS	=	oil solution
OS CyA	=	oil solution containing cyclosporin A
OS CyA-DP	=	dry powder of oil solution containing cyclosporin A
PCS	=	photon correlation spectroscopy
PEG_{400}	=	polyethylene glycol 400
PG	=	propylene glycol
PVP	=	polyvinyl pyrrolidone
RH	=	relative humidity
rpm	=	revolutions per minute
S_{15}	=	solutol HS 15
SD	=	standard deviation
SEM	=	scanning electron microscopy
SMEDDs	=	self-microemulsifying drug delivery system
SMEDDs CyA	=	self-microemulsifying drug delivery system containing cyclosporine A

SMEDDs 25 CyA	=	self-microemulsifying drug delivery system containing 25 mg cyclosporine A
SMEDDs 100 CyA	=	self-microemulsifying drug delivery system containing 100 mg cyclosporine A
SMEDDs CyA-DP	=	Dry powder of microemulsifying drug delivery system containing cyclosporine A
T_{80}	=	Tween 80
TEM	=	transmission electron microscopy
µl	=	microliter(s)
W	=	water
w/ v	=	weight by volume
w/ w	=	weight by weight

CHAPTER I
INTRODUCTION

At present, the progress of medical technology could transfer an organ that called organ transplantation. Transplantation not only helps the patients to survive or to prolong their live but can upgrade quality of life and reduces the torment from treatment the process. There are many factors to the success in transplantation such as immunosuppression, compatibility of graft and receiver tissue. The main problem of transplantation is the graft rejection. Immunosuppressive agents are important to reduce risk of rejection. In the past, steroids especially prednisolone and azathioprine were used for preventing graft rejection. Until 1977, new immunosuppressive agent, cyclosporin A, was found by Jean Borel, a swiss chemist. Since then, This agent was used to treat transplanted patient by Sir Roy Clane from Cambrige University UK. Since then, the evidence of graft rejection and mortality of patients dramatically decreased. Until now several of immunosuppressants have discover such as FK 506, rabamycin, mycophenolate mofetil and monoclonal antibodies as OKT-3. The transplanted patient have to take immunosuppressant as long as they live (อุษณา และ คณะ,1995).

Cyclosporin is an example of poorly water soluble drug. It is a lipophilic cyclic undecapeptide that can be isolated from the fungus *Tolypoclodium inflatum* which produces calcium dependent, specific and reversible inhibition of transcription of interleukin-2 and several other cytokines, most notably in T helper lymphocytes. Because of its immunosuppressive properties, it is widely used as first line therapy in the prophylaxis and treatment of transplant rejection (e.g., allo-or xeno-transplant rejection such as in patients receiving heart, lung, combined heart-lung, liver, and kidney, pancreatic, skin or corneal transplants) and various autoimmune and inflammatory diseases. Cyclosporin A is used in the treatment of multi-drug resistance syndrome, for example, in patients undergoing chemotherapy or following organ transplantations (Kastrup, 2004; Dipiro, 1997). The first commercial product of cyclosporin was produced in oil solution dosage form and developed to emulsion form. In 1995, cyclosporin A was loaded into microemulsion dosage form which provided higher bioavailability than the existing dosage (Noble, 1995; Odeberg,

2003). Since 2003, Neoral®, the self emulsifying microemulsion filled in soft capsule was produced by Novatis company. After launched to drug market, it was reported to gain 1,020 million dollars and ranking in top sell products of this company (Humphreys, 2004).

Microemulsion is the drug delivery system which consists of water phase, oil, emulsifier and /or co-emulsifiers in the specific ratio. It could be spontaneously formed and has thermodynamic stability. Micoemulsions is transparent or translucent because small internal dispersed droplet size ≤ 100 nm. As emulsion, microemulsion can classified to w/o or o/w type (Kumar, 1999; Pouton, 1997). Medicine could be incorporated into microemulsion especially water insoluble substances to increase water solubility and bioavailability. According to the component of microemulsion, water in microemulsion can cause degradation of active ingredient. Moreover, water can dissolve gelatin capsule. Thus it is inappropriate to fill microemulsion in capsule hard gelatin as oral dosage form. Therefore self-microemulsifing system has been developed. The components of self-microemulsifing system are similar to microemulsion but without water. It could form microemulsion after mixed with water such as gastric water in stomach with or without gentle agitation as bowel movement. (Constaintinides, 1995; Gursoy, 2004; Kang, 2004; Araya, 2005; Hong, 2006)

The main component of microemulsion is oil phase. Natural oil is safe but unsuitable for preparing microemulsion because it usually has long fatty acid chain causing low solubility and difficulty to form microemulation. Favorable oils for microemulsion are shot chain or medium chain triglycerides because of their solubility property and ease to form microemulsion. (Constaintinides, 1994,1997)

Self-microemulsion system requires high level of emulsifiers up to 30 to 60 % of formulation. High HLB value emulsifiers are favorable to be used because they easily form oil droplets and provide good disperseion in water. Using combined emulsifies increase higher percentage of drug load in the microemulsion system than using single emulsifier. Since high level of emulsifiers that could irritate gastrointestinal tract, non-ionic emulsifiers should be used because of their low

irritation and low toxicity. Moreover for reduce total amount of emulsifiers in the system, co-emulsifer added in the self-microemulsion system. (Constaintinides, 1997; Pouton,2002)

Eventhough self-microemulsion system can increase solubility and bioavailability of cyclosporin A, there is a limitation of dosage form. Self-microemulsion system as a liquid system needs a special machine as filling and sealing machine to be filled into capsule. Absorbent has been used to absorb and transform self-microemulsion system them to a solid dosage form which is easily to be prepared and there is no need of special machine.

From the excellent clinical treatment outcomes of commercial cyclosporin A in self-microemulsion system for oral dosage form, thus; the cyclosporin A loaded self-microemulsion capsules were of interest. This investigation was aimed to develop cyclosporin A loaded self-microemulsion and cyclosporin A loaded self-microemulsion dry powder granule which were suitable to be filled into hard gelatin capsule had suitable physiochemical properties.

The objective of the present study were

1. To study develop cyclosporin A as self- microemusifying dosage form and studying effect of type and quantity of oil, emulsifier and co-emulsifier on physicochemical properties.
2. To develop capsule containing cyclosporin A self-microemulsifying drug delivery in liquid and dry powder dosage forms.
3. To study the stability of capsule containing liquid cyclosporin A self-microemulsifying drug delivery under accelerated condition (45±2°C and 75±5%RH).

CHAPTER II
LITERATURE REVIEW

1. Cyclosporin

Cyclosporin (CyA) is a cyclic polypeptide immunosuppressant of 11amino acid. It was produced as a metabolite by the fungus species Beauveria nivea (synonym Tolypocladium inflatum) The molecular of cyclosporin A is shown in Figure 1. It is a white or almost white crystalline powder, odourless and tasteless. Empirical formular is $C_{62}H_{111}N_{11}O_{12}$ with molecular weight 1202.6 dalton.The chemical name is :{R-(R*,R*-(E))}-cyclic-(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L- α-amino-butyric-N-methyl-glycinyl-N-methyl-L-leucyl-L-valyl-N-methyl-leucyl). It is soluble in methanol, ethanol, acetone and chloroform but insoluble in water. J. F. Borel, Swiss biochemistry, discovered its immunosuppressive activity in 1976. Cyclosporin A, the main form of the drug is a potent immunosuppressant widely used in post-allergenic organ transplant to reduce the activity of the patient's immune system and so the risk of organ rejection. It has been studied in transplants of skin, heart, kidney, lung, pancreas and bone marrow (Upton, 2005).

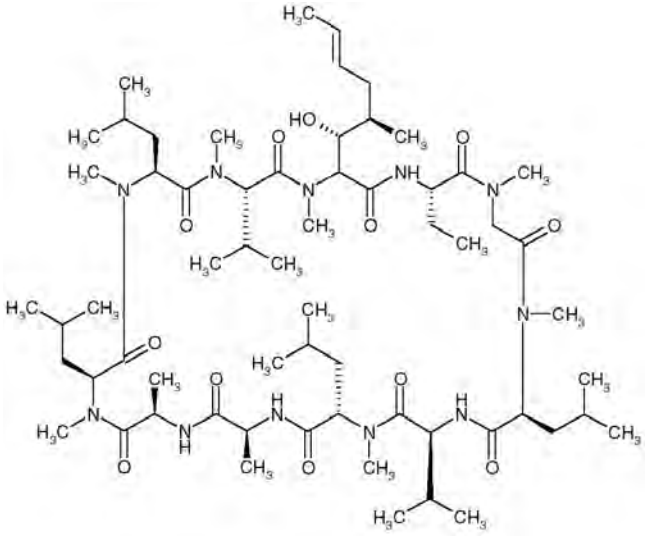


Figure 1. The molecular structure of cyclosporine A.

Indication

Cyclosporin is widely used as first line therapy in the prophylaxis and treatment of transplant rejection (e. g., allo-or xeno-transplant rejection such as in patients receiving heart, lung, combined heart-lung, liver, kidney, pancreatic, skin or corneal transplants) and various autoimmune and inflammatory diseases.

Cyclosporin A is used in the treatment of multi-drug resistance syndrome, for example in patients undergoing chemotherapy or following organ transplantations. In patients with severe disease refractory to standard treatment; cyclosporin A is an effective therapy in acute ocular Behcet's syndrome; endogenous uveitis; psoriasis; atopic dermatitis; arthritis, particularly rheumatoid arthritis; active Crohn's disease and nephrotic syndrome.

Other conditions include arthritis chronica progrediente and arthritis deformans, autoimmune hematological disorders including hemolytic anemia, aplastic anemia, pure red-cell anemia and idiopathic thrombocytopenia, systemic lupus erythematosus, polychondroitis, scleroderma, Wegener granulamtosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, Steven-John syndrome, idiopathic sprue, autoimmune inflammatory bowel disease, e. g., ulcerative colitis, endocrine ophthalmology, Graves disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes, keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis, juvenile dermatitis, asthma, tumors, hyperproliferative skin disorders and fungal infections. This drug has also been used to treat patients with moderate or severe aplastic anemia who are ineligible for bone marrow transplantation and those with primary biliary cirrhosis. Cyclosporin A may be effective in patients with intractable pyoderma gangrenosum, polymyositis/dermatomyositis or severe, corticosteroid-dependent asthma (Kastrup, 2004).

Pharmacology

The exact mechanism of action is unknown. Expermental evidence suggests it caused by specific, reversible inhibits T-lymphocyte proliferation by inhibiting the production of IL-2 and other cytokines by T cells. Cyclosporin produces this effect by binding to a cytoplasmic immunophilin called cyclophyllin. This drug-immunophilin complex can then block the action of a cytoplasmic phosphatase enzyme called

calcinurin. In vitro studies with cell lines deficient in immunophilin suggest that CyA is inactive in absence of this intracytoplasmic protein (Kastrup, 2004).

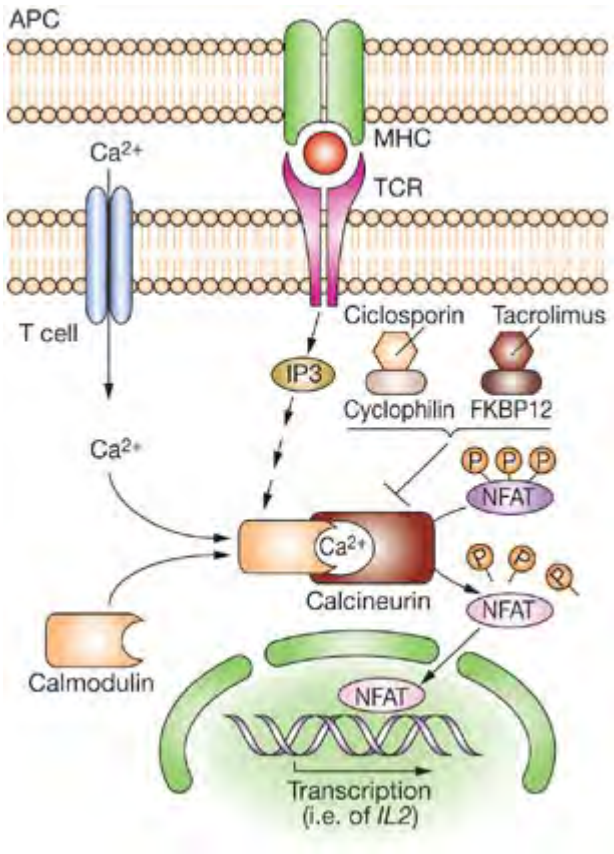


Figure 2. The model of Cyclosporin mechanism of action (Fantini et al, 2006).

Pharmacokinetic

The absorption of conventional cyclosporin form GI tract is incomplete and variable; the lipid microemulsion formulation has improved absorption characteristic and is more rapidly and completely absorbed. The extent of absorption is dependent on the individual patient, patient population and formulation (Kastrup, 2004).

Cyclosprorine may be administered orally or as an intravenous infusion. A wide range of does are used for CyA depending on the clinical status of patient (early versus late postoperative period, open versus triple or quadruple therapy, coadministration of drug that affect CyA metabolism). The normal does range for intravenous CyA is 2-5 mg/kg/day as a continuous infusion since rapid intravenous

administration of CyA has been associated with hypotension, tachycardia, severe headache, bronchospasm flushing and nausea. The oral bioavailability of CyA is about 30% and the initial doses are 8-17 mg/kg/day. Lower initial oral CyA does and doses reduction long term to less than 5 mg/kg/day are strategies used to minimize nephrotoxicity ,but dosage reductions below 5 mg/kg/day have been associated with chronic rejection.

Cyclosporin blood or plasma concentration monitoring is useful in making dosage adjustment for drug. While only a week correlation exists between high CyA concentrations and drug toxicity or between low concentration and allograft rejection, monitoring is a useful guide to dosing in patients with poor absorption, hepatic dysfunction, and drug interaction. Significantly higher incidences of graft rejection have been reported in patients who have poor absorption of CyA. Chromatographic assay and immunoassays for CyA are both useful for clinical monitoring, but therapeutic range depend on assay method and whether the biological specimen is blood or plasma. Newer immunoassay techniques using monoclonal antibodies yield result closer to those of high performance liquid chromatograpy assays. Methodologic problem will be encountered with assay for new immunosuppressant that has immunoreactive metabolites.

A new product formulation of CyA called Neoral was approved in 1995. Neoral is a microemulsion of cyclosporin and has improved and more reliable absorption of drug as shown in Table 1.

Table 1. Pharmacokinetic parameter of cyclosporin formulation (Dipiro, 1997).

Formulation	**Absolute bioavailability (%)**	**T max (hours)**	**C max (ng/ml/mg of dose)**	**t½(hours)**
Conventional (Sandimmune)	301	3.5	≈1 (2.7 to 1.4)2	19 (10 to 27)
Lipid microemulsion (Neoral)	60	1.5 to 2	↑ (40% to 106%)3	8.4 (5 to 18)

1 <10% in liver transplant and ≈ 89% in renal transplant patients.

2 Blood level for low to high doses,respectively.
3 In renal transplant patients treated with neoral, peak level were 40% to 1065 grater than those following sandimmune administration.

2 Transplantation

Transplantation, which is the transfer of organs, cells, and tissues from one location to another, began many centuries ago as a primitive practice and has since evolved into a modern reality. Modern medicine has triumphed over many challenges and overcome many hurdles to achieve successful organ transplantation. The contemporary practice of medicine includes transplantation of tissues, partial organs, and whole organs. In addition, successful bone, heart valve, cartilage, vein, and cornea transplantations are being performed on a daily basis.

Transplantation can be characterized according to either the genetic relationship between the donor and recipient or the anatomical site of the implantation. The genetic relationship is characterized into 4 classes. In an autograft, the donor and recipient is the same individual. In an isograft or syngeneic graft, the donor and recipient are genetically identical (eg, monozygotic twins). In an allograft or homograft, the donor and recipient are genetically unrelated but belong to the same species. In a xenograft or heterograft, the donor and recipient belong to different species

Based on the site of implantation, the transplantation can be described as orthotropic or heterotropic. Orthotopic transplantation refers to donor tissue implanted in the anatomically correct position in the recipient; heterotopic transplantation refers to the relocation of the implant in the recipient at a site different from the normal anatomy.

Role of cyclosporin in transplantation

Cyclosporin improved graft rejection in animals by inhibiting T-lymphocyte activity. Roy Calne investigated the effects of cyclosporin in dogs with renal allografts and pigs with orthotopic heart grafts. His work proved that cyclosporin was a much better immunosuppressive agent than corticosteroids, azathioprine, or a

combination of both. Calne also found that cyclosporin was nephrotoxic; work by other investigators on devising safe protocols for cyclosporin led to marked improvement not only in kidney transplantation, but also in successful transplantation of the lungs, heart, heart and lungs, pancreas, and liver.

In the late 1970s, cyclosporin increased the 1-year survival rate of liver allografts from 18% to 68%. Although cyclosporin is generally associated with significant adverse effects, administration of small doses in a controlled protocol results in minimal adverse events (Sharma, 2006; Pellegrino, 2007).

3 Microemulsion

The microemulsion concept was introduced early as the 1940s by Hoar and Schulman who generated a clear single-phase solution by titrating a milky emulsion with hexanol (Hoar, 1943). Schulman and coworkers (Schulman, 1956) subsequently coined the term microemulsion however, the microemulsion definition provided by Danielsson and Lindman in 1981 will be used as the point of reference (Daniielsson, 1981). Microemulsions are thus defined as ‘a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution. Microemulsions are spontaneously forming single-phase colloidal dispersions of either oilin- water (o/w) or water-in-oil (w/o) stabilized by an interfacial film of surfactant(s) and cosurfactant(s) (optional) Systems devoid of cosurfactants are the ‘‘ternary systems’’ and those requiring cosurfactants are the ‘‘pseudoternary’’ systems (where the surfactant and cosurfactant are together taken as a single-phase) The surfactants are amphphilic molecules with a polar head and a nonpolar (hydrophobic) tail, and the cosurfactants can be short chain alcohols, amines and similar substances. The dispersions are formed when oil, water, and surfactant/cosurfactant are mixed in appropriate proportions

These self-assembled dispersions have low viscosity, ultraslow interfacial tension, enormousinterfacial area, good shelf-life (stability with time), high solubilizing capacity, macroscopichomogeneity, and microscopic heterogeneity (microdomains). Depending on composition and type of amphiphiles, there may be dispersion of oil droplets in water continuum (o/w microemulsion) or vice versa (w/o microemulsion). Phase inversion of microemulsion upon addition of an excess of the

dispersed phase or in response to temperature variation is another interesting property1 when a transition from w/o to o/w microemulsion can occur through a bicontinuous state (Fig. 3) The differences between emulsions and microemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate. Another important difference concerns their appearance; emulsions are cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not.

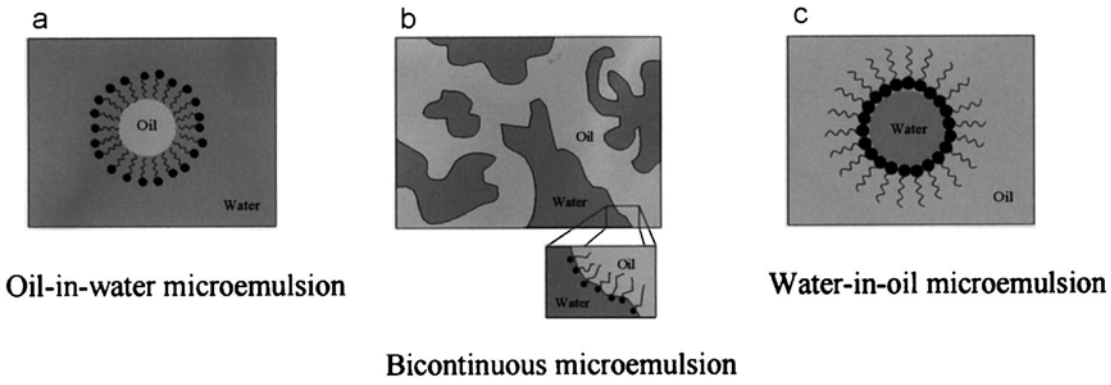


Figure 3. Schematic representation of the three most commonly encountered microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion (Lawrence and Rees, 2000).

Formulation

Emulsifying agent are used to promote emulsion at the time of manufacturing and to control stability during a shelf life that can vary from day for extemporaneously prepared emulsions to months or years for commercial preparations. The ideal emulsifying agents for pharmaceutical purpose should be stable, inert, non-toxic non irritant. It should be odorless, tasteless, colorless, effective and can be produce stable emulsions at low concentration of emulsifier (Lund, 1994).

The nonionic surfactants are normally used to produce o/w or w/o emulsions for both external and internal administration. The advantages of nonionic surfactants include their resistance to the effects of electrolytes, their compatibility with other

surfactants, unionization in acidic or basic condition, easily adjustment the value of hydrophilic and lipophilic balance (HLB) for emulsification efficiency, very low toxicity, antibacterial activity , less impurities. Disadvantage of nonionic surfactants is possibly their tendency to bind or inactivate preservatives containing phenolic or carboxylic groups in formulation (Attwood and Florence, 1983).

Microemulsions often include a cosurfactant. A cosurfactant is an amphiphilic molecule that substantially accumulates with the surfactant at the interfacial layer. Usually a very low HLB cosurfactant is used with a high HLB surfactant to modify the overall HLB of the system. Unlike surfactant, the cosurfactant may not be capable of forming self-associated structures like micelles on its own. Several kinds of molecules including nonionic surfactants and alcohols can function as cosurfactants in a given system. The quantity of a cosurfactant in a system is usually less than that of the surfactant and it often serves to modifythe overall HLB value of the system.

Co-solvents are often included in microemulsion formulations to increase drug solubility by cosolvency and to stabilize the dispersed phase. In addition to making the environment more hydrophobic by reducing the dielectric constant of water, cosolvents increase the amount of molecularly dispersed surfactant in the aqueous phase. Availability of free surfactant aids in drug solubilization by creating pockets of hydrophobic regions within the aqueous phase (Narang et al, 2007).

Several of oils, surfactants and co-surfactants were used from many previous studies. Especially the vegetable oil or biocompatible oil s as shown in Table 2.

Table 2. Components for Preparation of Biocompatible Microemulsions for Drug Delivery (Gupta and Moulik, 2007).

No.	Oil	Surfactant	Cosurfactant	Aqueous Phase
1	Corn oil, cottonseed oil, clove oil, orange oil, and peppermint oil	Tween-20, Brij-30, and Brij-92	Ethanol and isopropanol	PBS buffer (pH 7.2), Tris–HCl buffer (pH 7.4), Ringer lactate solution (sodium lactate injection i.p.), urea solution (30 mg mL^{-1}), glucose solution (100 mg mL^{-1}), and 0.9% saline
2	Ethyl oleate	Surfactant blend (sorbitan monolaurate + polyoxyethylene 20 sorbitan mono-oleate)	Four aliphatic alcohols (1-propanol; 1-butanol; 1-hexanol; and 1-octanol) and four 1,2 alkane diols (1, 2 propane diol; 1,2 pentane diol; 1,2 hexane diol; and 1,2 octane diol)	
3	Ethyl oleate	SbPC (Epikuron 200)	Alkane 1-2 diols	
4	Ethyl oleate, ethyl caprylate, and ethyl butyrate, soybean oil, Migyol 812 and ethyl oleate	Polyoxyethylene-based surfactants	—	
5	Ethyl oleate	Tween-20	—	Water
6	Ethyloleate	Lecithin + distearoylphosphatidyl -ethanolamine–*N*-poly (ethyleneglycol) 2000 (DSPE–PEG)	Ethanol	Water
7	Ethyl laurate	Tween-80 + Propylene glycol	Ethanol	Water
8	Eucalyptus oil	Tween-20	—	
9	Eucalyptol, coconut oil, and isopropyl myristate	Brij-30, Brij-52, and Brij-92	Ethanol and isopropanol	
10	Eucalyptus oil	AOT and Brij-35 in single or mixed condition	Isomers of butanol	
11	Heptane + cholesterol benzoate	Tx-100	Butanol	Brine, dextran, geltin, and BSA

Table 2. Components for Preparation of Biocompatible Microemulsions for Drug Delivery (continute)

No.	Oil	Surfactant	Cosurfactant	Aqueous Phase
12	IPM	Polysorbate 40, 60, and 80	Sorbitol	
13	IPM	Soy lecithin + polysorbate 80		
14	IPM	Egg lecithin and Soy lecithin	A series of short chain alcohols (*n*-propanol, isopropanol, *n*-butanol, sec-butanol isobutanol, *tert*-butanol, and *n*-pentanol)	
15	MCT or IPM	A series of modified phospholipids (m-PCs, possessing different acyl chains in position 2 butanoyl to hexadecanoyl) + SbPC	Ethanol	
16	IPM	Tween-80	Propylene glycol	Phosphate buffer (pH 7.4)
17	IPM	Lecithin, lysolecithin	Alcohol	Water
18	IPM	(PEG-8 caprylic/capric glycerides + polyglyceryl-6 dioleate)	—	Water
19	IPM	A mixture blend of a high HLB surfactant Tween-80 and low HLB surfactant Span-20	—	Water
20	IPM	AOT	—	Water
21	IPM	Tween-85	—	Water
22	IPM	SbPC + Brij-92	—	Water
23	IPP	Tween-80 + Span-20	—	Water
24	IPP, glyceryl oleate	Polyoxyl 40 fatty acid derivative	Tetraglycol	Water
25	IPP			
26	MCT and LCT	SbPC	1-Propanol	
27	MCT	SbPC and polyethylene glycol 660 (PEG660)-12 hydroxystearate (12HSA-EO_{15})	PEG 400 and ethanol	
28	MCT	/(SbPC + poly (ethylene glycol) (660))-12-hydroxystearate (Solutol HS-15) + PEG-400	Ethanol	Water
29	Miglyol 812	SbPC + HS-15	PEG 400 + Ethanol	Water
30	Oleic acid	Tween-20	Prpylene glycol	Water

Table 2. Components for Preparation of Biocompatible Microemulsions for Drug Delivery (continue)

No.	Oil	Surfactant	Cosurfactant	Aqueous Phase
31	Oleic Acid	Labrasol + diethyl glycol monoethyl ether (Transcutol P)	—	Water
32	Labrafil M 1944 CS	Cremophor RH 40	Ethanol	Water
33	Propylene glycol, isopropyl palmitate, oleic acid, and eutanol G	Blend of low and high HLB surfactants (Tagat-20, HLB = 15 and Ploxamar 331, HLB = 1).	—	
34	Polyoxyl 40 hydrgenated castor oil (Cremophor RH 40) and glyceryl monostearate and glycerol mono- and di-caprylate/ caprate	Imwitor 308 and Imwitor 7429, polyoxyethylene (10) oleyl ether (Brij-97)	Polyoxyethylene (20) sorbitan monostearate (Crillet 3) and sorbitol	Water
35	PEG-8-glyceryl caprylate/ caprate (Labrasol)	Isostearique/isostearyl isotearate (Plurol)	—	Water
36	Ricebran, saffola, soybean, sesme, palm and linseed oil	AOT	Cinnamic alcohol	
37	Ricebran, saffola, and clove oil	(TX-100) (Tween-20) AOT, Igepal and Na-oleate and	Ethanol and cinnamic alcohol	Urea, NaCl, cholesterol, glucose
38	Saffola 73% linoleic acid (v/v)	AOT	Hexylamine	Cholesterol, crown ether, urea, and brine
39	Tricaprylin	Tween-80 + Span-20	—	Water
40	Xylene + cholesteryl benzoate	NaDC	Butanol	

Microemulsion in pharmaceutical

microemulsions have been found to improve the drug bioavailability, e.g., in topical administration and in oral administration of peptide and protein drugs, sparingly soluble lipophilic drugs, and drugs labile at the conditions in the stomach. There are also other advantages with microemulsions compared to other drug

Table 3. Phamaceutical Advantages of Microemulsions (Kumar and Mittal**,** 1999)

General advantages
Ease of preparation
Clarity
Stability
Ability to be filtered
Vehicle for drugs of different lipophilicities in the same system
Low viscosity (no pain on injection)
Specific advantages
Water-in-oil (W/O)
Protection of water-soluble drugs
Sustained release of water-soluble material
Increased bioavailability
Oil-in-water (O/W)
Increased solubility of lipophilic drugs
Sustained release of oil-soluble material
Increased bioavailability
Bicontinuous
Concentrated formulation of both oil- and water-soluble drugs

4. Self-emulsifying drug delivery systems (SEDDS)

Self-emulsifying drug delivery systems (SEDDS) and Self-miroemulsifying drug delivery systems (SMEDDs) can be described as isotropic solutions of oil and surfactant, which form o/w (micro)emul- sions on mild agitation in the presence of water (Greiner and Evan, 1990; Shah, 1994). It is also useful to note that under the definition given, self-microemulsifying drug delivery systems (SMEDDs) are not microemulsions, although they maybe considered being a closely related system. A SMEDDs typically comprises a mixture of surfactant oil and drug (known as the concentrate) which when introduced into the body is rapidly dispersed to form droplets of approximately the same size range as those observed in microemulsion systems. Once dispersed such systems would be expected to behave in vivo much the same way as oil-in-water (o/w) microemulsions

Self-emulsifying drug delivery systems for improve bioavailability of medicine

The utility of SEDDS has been investigated by Charman and coworkers who, although unable to show enhanced bioavailability of an investigational lipophilic drug WIN 54954, were able to demonstrate greatly improved pharmacodynamics using systems based on medium chain triglyceride(MCT) and ethoxylated glyceryl trioleate (Tagat TO) (Charman, 1992). More recently, self-emulsifying w/o microemulsions based on MCTs such as Captex 355 and Captex 8000 have been reported. The systems contained a mixture of mono and diglycerides (Capmul MCM) in combination with Tween 80 as surfactant. The bioavailabilities of calcein, a water-soluble marker, and an RGD peptide were shown to be significantly increased using a microemulsion concentrate and preformulated w/o microemulsions compared to the control aqueous formulation (Constantinides, 1994, 1995, 1997) The bioavailability of a poorly water soluble 5a-reductase inhibitor has similarly been shown to be improved in Beagle dogs (Matuszewska, 1996). It is also notable that the presence of liquid crystalline phases in the pseudo binary oil / surfactant mixtures are claimed to be a feature of the most efficient SEDDS (Craig, 1995).

After administration, the microemulsion formulated with straight chain fatty acid esters will undergo rapid enzymatic hydrolysis being degraded in the gastrointestinal tract. The breakdown products are surface active and will stabilise any (micro)emulsion that may form, as well as acting as membrane permeation enhancers (Yeh, 1994). As a consequence of the important role played by metabolic processes in vivo, formulators should be aware that certain hydrophilic surfactants such as Brij 96/ Brij 97, Tween 80 and polyoxyethylene 40 hydrogenated castor oil (Cremophor RH40) have been shown to inhibit lipolysis in vitro. Clearly if this behaviors is mirrored in vivo one of the principal mechanisms facilitating drug uptake would be compromised

It is also notable that in the case of w/o microemulsion systems, there is no obvious correlation between droplet size and oral bioavailability. This contrasts with the known relationship between o/w emulsion droplet size and bioavailability (Myer, 1992; Karali, 1992)

Examples of commercialized SMEDDS formulations include cyclosporin (Neoral®), ritonavir (Norvir®), and saquinavir (Fortovase®) (Cooney et al., 1998; Porter and Charman, 2001). Very few SEDDS and SMEDDS formulations have been commercialized because of limitations in the usage level of excipients, e.g., surfactants and cosolvents, and the unpredictable improvement of oral bioavailability due to possibility of drug precipitation upon aqueous dilution *in vivo*.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Cyclosporin A (Lot NO.R0993/01, India)
2. Acetronitrile HPLC grade (Burdick & Jackson, USA)
3. Activated charcoal (Lot NO.DO72/1607/1503/51, Sd fine.Chem Limited, India)
4. Anhydrous lactose (Lot NO.R1 45/00614, Wyndale, Newzealand)
5. Cremophor® EL (Lot NO.04517856PO, BASF, Germany)
6. Dicalcium phosphate (Lot NO.A84071A, Budenheim, Germany)
7. Ethanol (The Liquor distillery organization excise department of Thailand, Thailand)
8. Ethanol HPLC grade (Merck, Germany)
9. Glycerin (Lot & Control NO.504568, Distributed from Srichand United Dispensary Co., Ltd., Thailand)
10. Medium chain triglyceride (Captex300®, Lot NO. 0604046, Abitec corporation, USA)
11. Microcrystalline cellulose (Avecel PH 101®, Lot NO.1396,AsahiKasei Coperation, Japan)
12. Polyethylene glycol 400 (Lot & Control NO.567835, Distributed from Srichand United Dispensary Co., Ltd., Thailand)
13. Propylene glycol (Lot & Control NO.78998, Distributed from Srichand United Dispensary Co., Ltd., Thailand)
14. Polyvinylpyrrolidone K-90 (Fluka Chemie GmbH, Switzerland)
15. Solutol HS® 15 (Lot NO.59-1768, BASF, Germany)
16. Silicon Dioxide (Aerosil®; Lot NO.VA70093, Wacher Chemie GMBH, Germany)
17. Tween 80 (Lot & Control NO.405854, Distributed from Srichand United Dispensary Co., Ltd., Thailand)

Equipment

1. Anlytical balance (Sartorius, A200S, Germany)
2. Centrifuge (Model 5810, Eppendorf, Germany)
3. Differential scanning calorimeter (Model DSC 822c, Mettler Toledo, Germany)
4. Dissolution apparatus (Model VK 7000, Vankel, USA)
5. High performance liquid chromatography instrument equipped with
    a. Liquid chromatograph pump (LC-10AD,Shimadzu corporations, Japan)
    b. UV-VIS detector (SPD-10A, Shimadzu corporations, Japan)
    c. Recorder (C-R6A Chromatopac, Shimadzu corporations, Japan)
    d. Column oven (CTO-10ASvp, Shimadzu corporations, Japan)
6. Modified Franz Diffusion Cell
7. Particle analyzer
    a. Leser diffraction Spectroscopy (Model Mastersizer2000, Marvern Intrument, UK)
    b. Photon Correlation Spectroscopy (Model Zetasizer ZS, Marvern Intrument, UK)
8. pH meter (Model 210A, Orion Research, USA)
9. Polarized light Microscope (Model elipse E2000,Nikkon,Japan)
10. Transmission Electron Microscopy (Model JEM-200CX, Jeol® ,Japan)
11. Shaking incubator (Labtech International LTD , USA)
12. Viscometer (Model LVDVI+, Brookfield Engineering Laboratories, Inc. USA)
13. Vortex mixer (Model Genie2, Scientific Industies Inc, USA)

Miscellaneous

1. Dialysis membrane (MW. Cut off 12,000 Dalton, Sigma, USA)
2. Nylon membrane filte (47mm, 0.45µm)
3. Phenyl-hexyl column (Model Luna, 5µm, 250 x 4.6 mm, Phenomenex, USA)
4. Phenyl-hexyl guard column (phenomenex, USA)

Methods

1. Formulation of Microemulsion

1.1 Physical appearance

The visual grading was used to determine the appearances of the microemulsion. The physical appearances of all microemulsion formulas were collected and presented as pseudo ternary phase diagram

1.2 Psudo-ternary phase diagram study

The pseudo-ternary phase diagrams were constructed to examine the formation of microemulsions using 3 components of oil, surfactant and water. A series of sequential studies were done by varying compositions and ratios of ingredients as shown in Table 4. The study included only one type of oil: medium-chain triglycerides; Captex 300(C_{300}) and varying type of surfactants as Cremophor EL (C_{EL}),Tween 80 (T_{80}), Solutol HS 15 (S_{15}). The components were weighed (quantity of each component per one formula shown in pseudo-ternary phase formulation sheet in appendix part B) into glass vials and mixed using vortex mixer until the components were perfectly dissolved. The data were collected to construct ternary phase diagrams.

Furthermore, two surfactants which provided large area of microemulsion were chosen to mix together to be used as combined surfactants. The selected surfactants were mixed in various weight ratios as 4:1, 2:1, 1:1, 1:2 and 1:4 before mixing together with oil and water. The formulation of combined emulsifier is shown in Table 5. The data were also collected to construct ternary phase diagrams.

Table 4. Formulation of microemulsion with single surfactant.

Formula.	Oil	Surfactants			Water
	C_{300}	C_{EL}	T_{80}	S_{15}	
1	√	√			√
2	√		√		√
3	√			√	√

C_{300} : medium-chain triglycerides, **C_{EL}** : Cremophor EL, **T_{80}** : Tween80, **S_{15}** : Solutol HS15

√ = The substance was selected in that formula.

Table 5. The Formulation of microemulsion with combined surfactants.

Formula.	Oil	Ratio of Surfactants		Water
	C_{300}	S1	S2	√
1	√	1	1	√
2	√	1	2	√
3	√	1	4	√
4	√	2	1	√
5	√	4	1	√

C_{300} : medium-chain triglycerides

S1 ,S2 = The selected surfactant.

√ = The substance was selected in that formula

1.3 Polarized light microscopy

A microscope with polarized lens and analyzer was employed to examine the birefringent property of formulation at room temperature. Microscopic pattern of selected SMEDDs was verified under cross polarized light. A small amount of sample was placed between a cover slip and glass slide and then examined under polarized light by turning polarized lens at 90 to cross polarizing angle. The sample that appeared dark field or exhibited non-birefringent property would be classified as microemulsion. The sample that exhibited birefringent property would be classified as liquid crystal.

2. The self-microemulsifying drug delivery system (SMEDDs)

2.1 Solubility of cyclosporin A.

An excess amount of cyclosporin A (CyA) was added to oils [Captex®300 (C_{300})] and various of co-solvent [glycerin (Gly), polyethylene 400 (PEG_{400}), polyethylene glycol (PG), ethanol (EtOH)], and mixed by vortexing as the formulation shown in Table 6. The mixtures were continuously shaken by shaking incubator at 80 strokes per minute at room temperature for 7 days to get to equilibrium. The equilibrated sample was centrifuged at 4000 rpm for 10 min to remove the undissolved cyclosporin A. The supernatant was taken and diluted with ethanol. The amount of cyclosporin A in various systems was quantified using an HPLC system (Ran et al, 2001; Hong et al., 2006). Cyclosporin is a the stable molecule.The previous study of evaluated for stability of cyclosporin A in oral solution. It was treated with acid, alkali, hydrogen peroxide, heat and light for 3 days. It was found that cyclosporin A in oral solution was stable under the treated conditions exception of acidic conditions (Kumar et al, 2000). Thus in this study we could be assumed that cyclosporin A did not degradation in the oil and co-solvent formulations. The results of solubility were used for estimate the quantity of co-solvent used in the formulation of SMEDDs.

Table 6. The formulation of cyclosporin A for solubility test

Formula	0il	% of Co-solvent in oil phase											
		5%				10%				20%			
	C_{300}	Gly	PEG 400	PG	EtOH	Gly	PEG 400	PG	EtOH	Gly	PEG 4000	PG	EtOH
1	√												
2	√	√											
3	√		√										
4	√			√									
5	√				√								
6	√					√							
7	√						√						
8	√							√					
9	√								√				
10	√									√			
11	√										√		
12	√											√	
13	√												√

C_{300} : medium-chain triglycerides, **EtOH** : Ethanol, **PG** : propylene glycol,

PEG_{400} : polyethylene 400, **Gly** : glycerin

√ = The substance was selected in that formula.

2.2 Preparation of self-microemulsifying drug delivery system (SMEDDs) / Pseudo-ternary phase diagram study

A series of mixtures were prepared with individually ratio of oil, surfactant and co-solvent. The components in each formula are shown in Table 7. The componants were weighed (quantity of each component per one formula shown in pseudo-ternary phase formulation sheet in appendix part A) into glass vials and mixed using vortex mixer until the components were perfectly dissolved. Only the mono-phasic mixture was obtained after storage at room temperature for 3 days should be examined in the further study. The mixtures were characterized. The data was

collected to construct ternary phase diagrams. The suitable formulations were chosen for further study.

Table 7. Formulation of self-microemulsifying drug delivery systems**.**

Formulation.	**Oil**	**Surfactants**			**% of Cosolvent**			
	C_{300}	**C_{EL}**	**T_{80}**	**S_{15}**	**EtOH**	**PE_{400}**	**PG**	**Gly**
1	√	√	√		10			
2	√	√	√			10		
3	√	√	√				10	
4	√	√	√					10
5	√	√	√		5	5		
6	√	√	√		5		5	
7	√	√	√		5			5
8	√	√		√	5	5		
9	√	√		√	5		5	
10	√	√		√	5			5
11	√		√	√	5	5		
12	√		√	√	5		5	
13	√		√	√	5			5

C_{300} : medium-chain triglycerides, **C_{El}** : cremophor EL, **T_{80}** : Tween 80, **S_{15}** : solutol HS15, **EtOH** : Ethanol, **PG** : propylene glycol, **PEG_{400}** : polyethylene 400, **Gly** : glycerin

√ = The substance was selected in that formula.

2.3 Effect of dilution ratio study

The aim of this study was to determine a suitable ratio of water and SMEDDs to obtain a microemulsion before characterization and clarify if ratio of dilution had an effect on the size of droplet. A selected SMEDDs formula was performed in this study by 1:50, 1:100, 1:200 and 1:500 dilution of SMEDDS with deionized water under gentle agitation of 50 rpm. Microemulsion were characterized for physical appearance by visual observation. The particle size was determined by photon correlation spectroscopy (PCS) and transmission electron microscope (TEM).

2.4 Characterization of SMEDDS

2.4.1. Physical appearance

Physical appearance of after dilution.

The selected SMEDDs were mixed with water at the suitable ratio of dilution under gentle agitation of 50 rpm. The mixtures were examined by eye. The appearance as color or turbidity was recorded.

2.4.2. Particle size determination

a) Photon correlation spectroscopy (PCS)

The sample was performed by mixing the selected SMEDDs with water at the suitable ratio of dilution under gentle agitation of 50 rpm until it become microemulsion. The droplet size of microemulsion was determined by the photon correlation spectroscopy (PCS) method using Zetasizer ZS (Malvern Instruments, UK). There were 15 times metered per 1 cycle of run and triplicate runs per sample. In case that particle size larger than 100 nm the droplet size was measured by leser diffraction spectroscopy method using Mastersizer 2000 (Malvern Instruments, UK).

b) Transmission Electron Microscopy (TEM)

Microemulsion samples were viewed using JEM-200CX by negative staining technique. The sample prepared by placing a drop of specimen on a formvar coated 400 mesh copper grid for 15 seconds and wiped away excess sample, placing a drop of 2% phosphotungstic acid on the grid for 1 minute, wiped away and letting the specimen dry completely. Pictures were then taken on various fields of interest at various magnifications.

c) Scanning Electron Microscopy (SEM)

Microemulsion samples were viewed using JSM-5800LV by gold coating techniques. A drop of sample was placed on cover slid glass and storage at room temperature until dried. The cover slid glass was kept in the

chamber fumigated with the osmiumtretroxide vapor for 1 hour. After that, it was soaked 3 minutes in absolute ethanol for 3 times. The slide was dried by critical point dryer. When the slide was absolutely dried, then it was placed on stub and coated with gold by using sputter coater. Finally the slide was examined by scanning electron microscope.

2.4.3. Polarized light microscopy

A microscope with polarized lens and analyzer was employed to examine the birefringent property of formulation at room temperature. Microscopic pattern of selected SMEDDs was verified under cross polarized light. A small amount of sample was placed between a cover slip and glass slide and then examined under polarized light by turning polarized lens at 90° to cross polarizing angle. The sample that appeared dark filed or exhibited non-birefringent property would be classified as microemulsion. The sample that exhibited birefringent property would be classified as liquid crystal.

2.4.4. Viscosity determinations

The rheological measurement was performed with a viscometer (Brookfield LVDV-II+, USA) equipped with spindle NO 31 and metering at room temperature. The resulting of shear stress was performed by increasing the shear rate from 10 to 100 rpm. The relationship of shear stress of sample as function of shear rate was plotted.

2.4.5. pH determination

The pH values of micoemulsions were determined in triplicate at room temperature by Thermo Orion 210 pH meter. The equipment was calibrated at pH 4, 7 and 10 using Beckman standard buffer solutions.

3. Self-microemulsifying drug delivery system containing cyclosporine A (SMEDDsCyA)

3.1 Preparation of Self-microemulsifying drug delivery system containing cyclosporine A (SMEDDsCyA)

The suitable formulations of SMEDDs were loaded with cyclosporin A. The SMEDDsCy were prepared in the same manner as SMEDDs. Cyclosporin A were loaded to the blank SMEDDs, which were selected before. Twenty five milligrams of cyclosporine A would be mixed with 0.475 g of SMEDDs to make 0.5 g of SMEDDs25Cy and the 100 mg of cyclosporine A would be mixed with 0.8 g of SMEDDs to make 0.9 g of SMEDDs100Cy. Cyclosporin A was dissolved with oil and co-solvent before mixing with surfactant.

3.2 Characterization of SMEDDsCyA

The SMEDDsCy were characterized by the same procedure as in the aforementioned SMEDDs.

3.3 *In vitro* drug release studies

a) Kesshary-Chien diffusion apparatus method

The *in vitro* drug release study of microemulsions was carried out using modified Kesshary-Chien diffusion apparatus. The apparatus consisted of two glass compartment, donor and receptor compartments. The internal diameter of each cell was 1.8 cm, corresponding to an effective permeable surface area of 2.55 cm^2. The receptor compartment contained 12-16 ml of deionized water as release medium. Two compartments were separated by dialysis membrane that had a molecular weight cut-off 12,000-14,000 dalton. Before placing on a diffusion cell, the dialysis membrane was cut into a circular shape and soaked in deionized water for 12 hours and then rinsed with

boiling water to wash off any water soluble contaminants. The membrane was soaked for 30 minutes in deionized water before using.

The cell was allowed to equilibrate at temperature 37°C before and throughout the experiment. After equilibration, 3 ml of SEDDs100 CyA, which was mixed with water to form micoemulsion at ratio 1:10, were filled into the donor part. The two components were clamped with treated membrane between them. The release medium was carefully filled into the receptor part to ensure no air bubble. Then the cell was stirred by magnetic bar at 850 rpm. A 5 ml aliquot of receptor medium was withdrawn at appropriate time interval and replaced immediately with an equal volume of fresh medium. A portion of solution under test was diluted and determined for the amount of drug release using HPLC technique. The amount of drug release was calculated from calibration curve. The diffusion experiment was performed in triplicate for each formulation.

b) Dialysis tube method

The purpose of this method was same as the as Kesshary-Chien diffusion apparatus method but this method needed to enlarged the surfaced area of dialysis tube. The apparatus is shown in Figure 4. The Twenty milliliters of SEDDs100CyA, which was mixed with water to form micoemulsion at ratio 1:10, were filled into dialysis tube ,which was soaked in deionized water for 12 hours and then rinsed with boiling water to wash off any water soluble contaminants. The membrane was soaked for 30 minutes in deionized water before using, and tightened at the end of tube with thread. Then dialysis tube was placed the into a small dissolution tube which filled with 100 milliliters of deionized water, as a dissolution medium and maintained at temperature 37±0.5°C along the process. A small paddle was set at a speed of 50 rpm. A portion of dissolution sample was with drawn at 30, 60 minutes and 12 hours and assay by HPLC as HPLC assay procedure as described. Three samples of each formulation were determined.

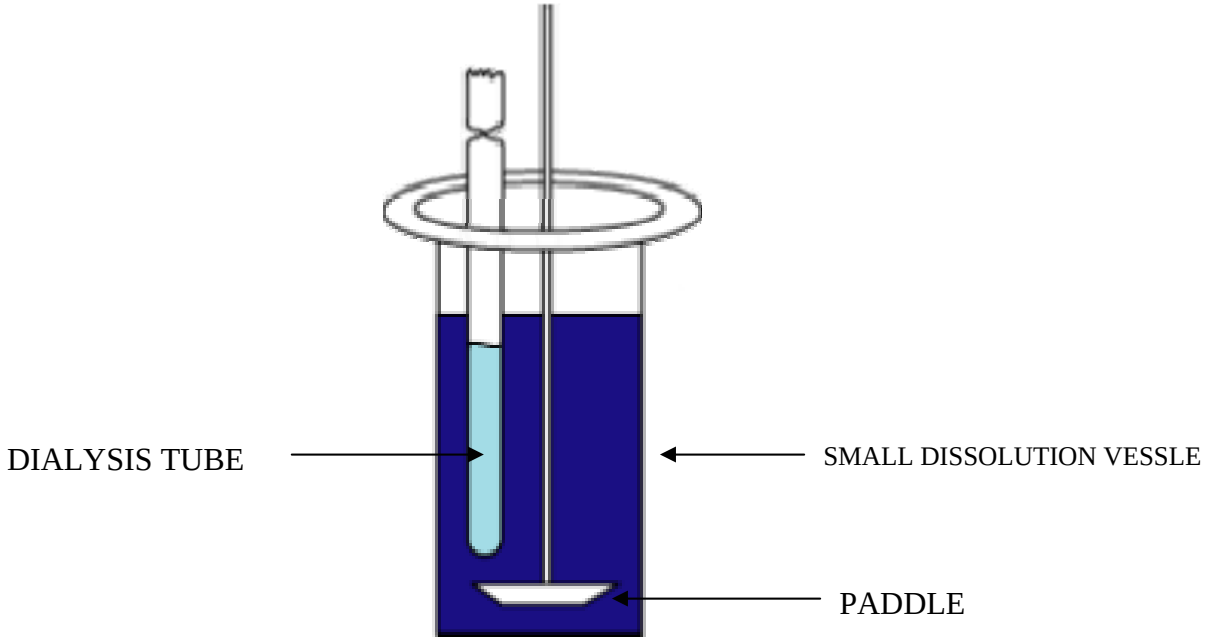


Figure 4. Schematic diagram of the Dialysis tube diffusion apparatus for *in vitro* diffusion studies

3.4 Determination of drug content by HPLC method

3.4.1 Validation characteristic for determination of Cyclosporin A content by HPLC method

The parameters evaluated to ensure the acceptability of performance of the selected analytical method were specificity, precission, accuracy and linearity

HPLC condition

Column :	Phynyl-hexyl (Model Luna, 5µm, 250 x 4.6 mm, Phenomenex, USA)
Mobile phase :	Acetonitrile : water (70 : 30 v/v) was freshly prepared and filtered through a 0.45 µm membrane filter. It was degassed by sonication for 30 minutes.
Flow rate :	1.0 ml/min
Detection wavelength :	210 nm

Injection volume : 10 µm

Temperature : 70 ˚C

Retention time : 9.5 – 10.5 minutes

Validation procedure

Specificity

Under the chromatographic condition, determination of cyclosporin A quantity was evaluated. Solvents and all drug-free SMEDDs formulations that had the same component as cyclosporin A loaded formulations were dertermined.

Precision

a) Within run precision

The within run precision was determined by analyzing three sets of five standard solution of cyclosporin A in the same day. The coefficients of the peak area response (%CV) for each concentration were determined.

b) Between run precision

The between run precisions was determined by comparing each concentration of cyclosporin A standard solution prepared and injected on different days. The percentage coefficient of variation (%CV) of cyclosporin A peak area from the three sets of standard solutions on different days was calculated.

Accuracy and recovery

The recoveries of Cyclosporin A from placebo were assessed by spiking placebo with Cyclosporin A and following the extraction procedures

Linearity

Linearity was evaluated with various amount of appropriately diluted stock standard solution to form working solutions containing 0.001-0.1 mg /ml of cyclosporin A. For each concentration three measurement were performed

and calibration curves were obtained by plotting the peak area versus nominal concentration expressed in mg /ml of cyclosporin A. The slope, intercept and correlation (r^2) of each calibration curve were determined.

System suitability

System suitability was evaluated by making 6 replicate injection of the standard and recording the peak responses. It was used to verify that the resolution and reproducibility of the chromatographic system were adequate for analysis to be done.

3.4.2 Calibration curve of cyclosporin A

A stock solution was prepared by accurately weighing cyclosporin A reference standard 25 mg into 25 ml volumetric flask diluting to volume with ethanol. The stock solution was diluted to reach concentrations of cyclosporin A between 0.001-0.1 mg /ml. Each solution was subjected to HPLC in triplicate. Peak areas were recorded for all solutions. The equation was calculated form the relationship between peak area responses of cyclosporin A and their concentrations.

3.5 Preparation of SMEDDsCyA capsules

The SMEDDsCyA was filled into capsule #.0 or 00 in order that each capsule contained 25 and 100 mg of cyclosporine A respectively. The filled-capsules were sealed by Capsule Filling and Sealing Machine (CFS 1200, Capsugel)

3.6 Determination of drug content in SMEDDsCyA capsules

The six capsules of SMEDDsCyA were accurately weighed by analytical weight balance. The capsules shell were cut by sharp blade and wash out SMEDDsCyA by ethanol. The elute was collected and assay by HPLC procedure. The samples were duplicate run. The mean standard deviation of percent labels amount were calculated.

3.7 The release assays

The release assays of SMEDDsCyA capsule were performed with apparatus equipped with paddle as USP doissolution apparatus II. Five hundred milliliters deionized water was used as a release medium, maintained at temperature 37±0.5°C along the process. The paddle was set at a speed of 50 rpm. A portion of dissolution sample was with drawn at 10, 20, 30, 40, 50 and 60 minutes and assay by HPLC as previously described. Three capsules of each formulation were determined. The release profiles were then constructed by plotting percent of cyclosporin dissolved versus time.

4 .Preparation of SMEDDs CyA granule

4.1 Determination of absorbability SMEDDs of various absorbents

The most suitable absorbent for SMEDDs must require smallest and still be able to form granules to be filled in a capsule. Four kinds of absorbents were chosen for this study such as anhydrous lactose, dicalcium phosphate, microcrystalline cellulose (Avicel ® PH101) and activated charcoal. Each absorber was carefully poured and mixed with 3 milliliters of SMEDDs in glass mortar until became damp mass. The mass was sieved through hand sieve NO.20. The quantity of absorbent and degree of sieving difficulty were recorded.

4.2 Formulation of dry powder adsorbed SMEDDsCyA (SMEDDsCyA-DP)

The absorbents which had suitable property were chosen to obtain SMEDDsCyA –DP appropriate ratio with SMEDDsCyA in order to make granules. The 10% (w/w) PVP K90 in ethanol was selected as a binder. The wet granulation process proceeded by using glass pestle and mortar. The wet-granules were sieved through hand-sieve NO 20 and dried in a hot air convection oven at 60°C until

constant weight was obtained. The dry-granules were sieved again through hand-sieve NO 16.

4.3 Preparation of oil solution containing 100 mg cyclosporin A (OSCyA)

This study was also compared between SMEDDsCyA-DP and oil solution loaded cyclosporin A (OSCyA) which was used as traditional dosage form. The oil solution loaded with 100 mg cyclosporin A was prepared by dissolving 100 mg of cyclosporin A with 736 mg Captex ®300 and added 10% of ethanol (8g) as co-solvent, the final weight and cyclosporine A concentration (w/w) would be similar to SMEDDsCyA formula.

4.4 Preparation of dry powder adsorbed oil solution containing 100 mg cyclosporin A (OSCyA-DP)

The OSCyA was also prepared to be dry power dosage form as the same method as SMEDDsCyA-DP by replacing SMEDDsCyA with OSCyA in the formula.

4.5 Determination of granules

1. Compressibility assay

Ten grams of granular powder was poured lightly into a 25 ml graduated cylinder. The powder was tapped until no further change in volume was observed. Powder bulk density, ρb (g/cm3), and powder tapped density, ρp (g/cm3) were calculated as the weight of the powder divided by its volume before and after tapping, respectively. Percent compressibility was computed from the following equation:

$$\% \text{ compressibility} = [\ 100\ x\ (\rho p - \rho b)\ ] / \rho p \quad \text{(eq 1)}$$

2. Determination of Angle of repose

The dynamic angle of repose for powder was determined by funnel method. Angle of repose was measured by using a protractor for the heap of

granules formed by passing 10 g of the sample through a funnel at a height of 8 cm from the horizontal surface. The angle of repose was averaged from three determinations. The angle of repose was computed from the following equation.

$\tan\theta = h/r$ …………………. ……………………………………..…(eq 2)

(h = Height of heap, r = Radius of heap)

3. Determination of flow rate

Ten gram of granular powder, accurately weighed, was filled in 1.5 cm internal orifice diameter paper funnel that fixed on the clamp. The time was recorded when the granule started to flow until finished. The flow rate was averaged from three determinations and reported in term of g/sec.

4. Disintegration test

The disintegration test was determined on three compressed granular powder without capsule shell. Fifteen grams of granular powder was compressed by the hand-pressed mould to become a cylindrical shape of 0.2 centimeters height and 0.5 centimeters in diameter. Each compressed granular tube was placed into a dissolution apparatus I equipped with Basket, 40 Mesh USP. Five hundred milliliters of purified water at 37 ◦C was added under gentle stirring of 50 rpm. The time was recorded until entire granules passed through basket sieve. The samples were triplicate.

4.6 Preparation of SMEDDsCyA-DP and OSCyA-DP capsules

The SMEDDsCyA-DP and OSCyA-DP granule were filled in capsule NO.00 in order that each capsule contained 25 mg of cyclosporine A. The granular powder was compressed by hand-press mould to become a cylindrical shape of 0.5 centimeter in diameter and inserted into hard gelatin capsule.

4.7 Determination of drug content by HPLC method

The SMEDDsCyA-DP capsules and OSCyA-DP capsules were determined drug content and dissolution with HPLC procedure as described before. Three capsule of each formulation were determined

4.8 The dissolution assays

The dissolution assays of SMEDDsCyA-DP capsules and OSCyA-DP capsules capsule were performed with apparatus equipped with paddle as USP dissolution apparatus II. Five hundred milliliters deionized water was used as a release medium, maintained at temperature 37±0.5°C along the process. The paddle was set at a speed of 50 rpm. A portion of dissolution sample was with drawn at 10, 20, 30, 40, 50 and 60 minutes and assay by HPLC procedure. Three capsules of each formulation were determined. The disolution profiles were then constructed by plotting percent of cyclosporin A dissolved versus time.

5. Stability study

Stability study of capsule was performed according to Thai FDA guideline on stability testing of drug product (จุไรรัตน์, 2547).

The capsules, packed in close amber glass container, were stored under accelerated (45±2°C, 75±2 %RH) and ambient condition for 4 months and randomly sampled every 2 months interval to observe the physical appearances of the tablets. Moreover, the percent remaining of drug contents and dissolution profile were analyzed by HPLC. The sample preparations were prepared as described in determination of drug content by HPLC method.

CHAPTER IV

RESULTS AND DISCUSSION

1. The Microemulsion

1.1 Physical appearance

The visual grading was used to determine the appearances of the mixture. The physical appearances of all preparation are present as pseudo ternary phase diagram in Figure 5 -6. Figure 5 presented pseudo ternary phase diagram of 3 components. Oil phase ,represented by C symbol, was on the top of every diagram, water (W) was on the low left angle and surfactant was on the low right angle represented by their individual symbol as Cremophor EL (C_{EL}) (Figure 5A), Tween 80(T_{80}) (Figure 5B) and Solutol HS 15 (S_{15})(Figure 5C). Similar to Figure 5, Figure 6 presents pseudo ternary phase diagram of 3 components but the surfactant was a mixture of Cremophor EL and Tween 80 (C_{EL}: T_{80}) in various ratio. The red dot represented area of microemulsion (mono-phasic translucent to transparent mixture), the green dot represented where macro emulsion (mono-phasic white opaque mixture) formed and blue dot represented the separation of two phases, and **B** symbol represented as liquid crystal or having birefringent property.

1.2 Formulation of microemulsion/ pseudo-ternary phase diagram study

The purpose of this part was to evaluate the capability of a single surfactant to form microemulsion. The microemulsions were prepared by only oil phase mixed with various ratios of surfactant and water to make an emulsion. The results are present as pseudo-ternary phase diagram in Figure 5. The results revealed that Cremophor EL (Figure 5 A) and Tween 80 (Figure 5 C) could form larger area of mono-phasic and transparent mixture; which assumed to be a microemulsion, than Solutol HS 15 (Figure 5 A). Cremophor EL and Tween 80 formed the microemulsion area at equal percent (25%). However Cremophor EL was formed microemulsionin at

higher ratio of oil:surfactant than Tween 80. Solutol HS 15 provided the largest area of separation phase as shown in Figure 5B. Furthermore, higher ratio of Solutol HS 15: oil showed clearly separation phase as the white gel-like Solutol HS15 sank at the bottom of vial. These results could be due to its physical appearance as gel-like and its molecular structure. Solutol HS 15 is gel-like at room temperature and becomes liquid at 30 $^{\circ}$C (Solutol HS15 Technical Information sheet ,BASF company). Thus, it could be assumed that Solutol HS 15 preferably formed gel and was easily separated from other liquid ingredient. Moreover due to HLB value of each surfactant; Cremophor = 12-14, Tween80 = 14, Solutol HS15 = 15, it could be assumed that the required HLB of medium chain triglyceride system was about 12-14 as the HLB values of Cremophor El and Tween 80 while HLB value of Solutol HS15 was too high that caused immiscibility of system, It could be concluded that Cremophor EL and Tween 80 were preferably selected as surfactants for SMEDDs formulations than Solutol HS 15.

Although Cremophor EL was the surfactant that provided the largest area of microemulsion, it was expensive. Moreover, high content of Cremophor EL could reduc oral bioavailability in beagle dogs (Cuine et al., 2007). Li et al (2005) was found that combination of Cremophor EL with Tween 20 could generate clear microemulsions of small particle size. In addition increasing the drug loading seemed to have little effect on particle size. This finding was consistent with Moreno et al (2003) who reported that the combined use of Tween 80 and soybean lecitin would greatly increased the oil content in microemulsion and increase the drug loading. Therefore in this studies Tween 80 was chosen to be mixed with Cremophor EL as combined surfactants.

The results of combined surfactants are present as pseudo-ternary phase diagram in Figure 6A-E. The results revealed that combined surfactants could increase area of the microemulsion. At ratio of Cremophor EL : Tween 80 at 1:1, 1:2, 1:4, 2:1 and 4:1, microemulsion areas were 28%,28%,25% 26% and 28% respectively. This finding was consistent with previous study by Li et al (2005) That combination of nonionic surfactant between Tween 20 and Cremophor EL was greatly increased the microemulsion ragion in phasediagram. Moreover, they found that combind surfactants might provide a better surfactant' hydrophilic-lipophilic balance.

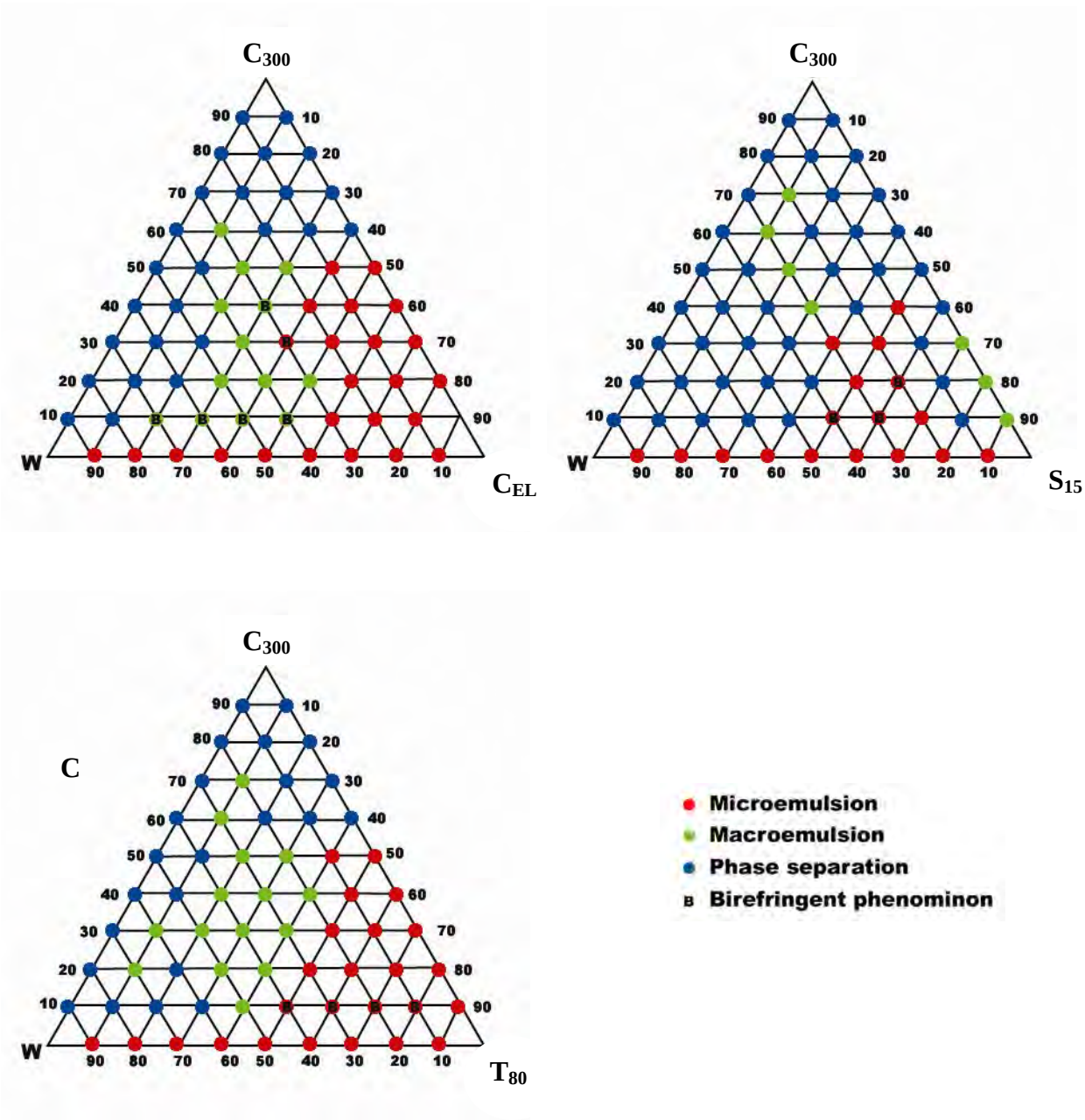


Figure 5. Pseudo-ternary phase diagram from the system of oil, surfactant and water
A: System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Water (W)
B: System of Captex 300 (C_{300}) : Solutol HS 15 (S_{15}) : Water (W)
C: System of Captex 300 (C_{300}) : Tween 80 (T_{80}) : Water (W)

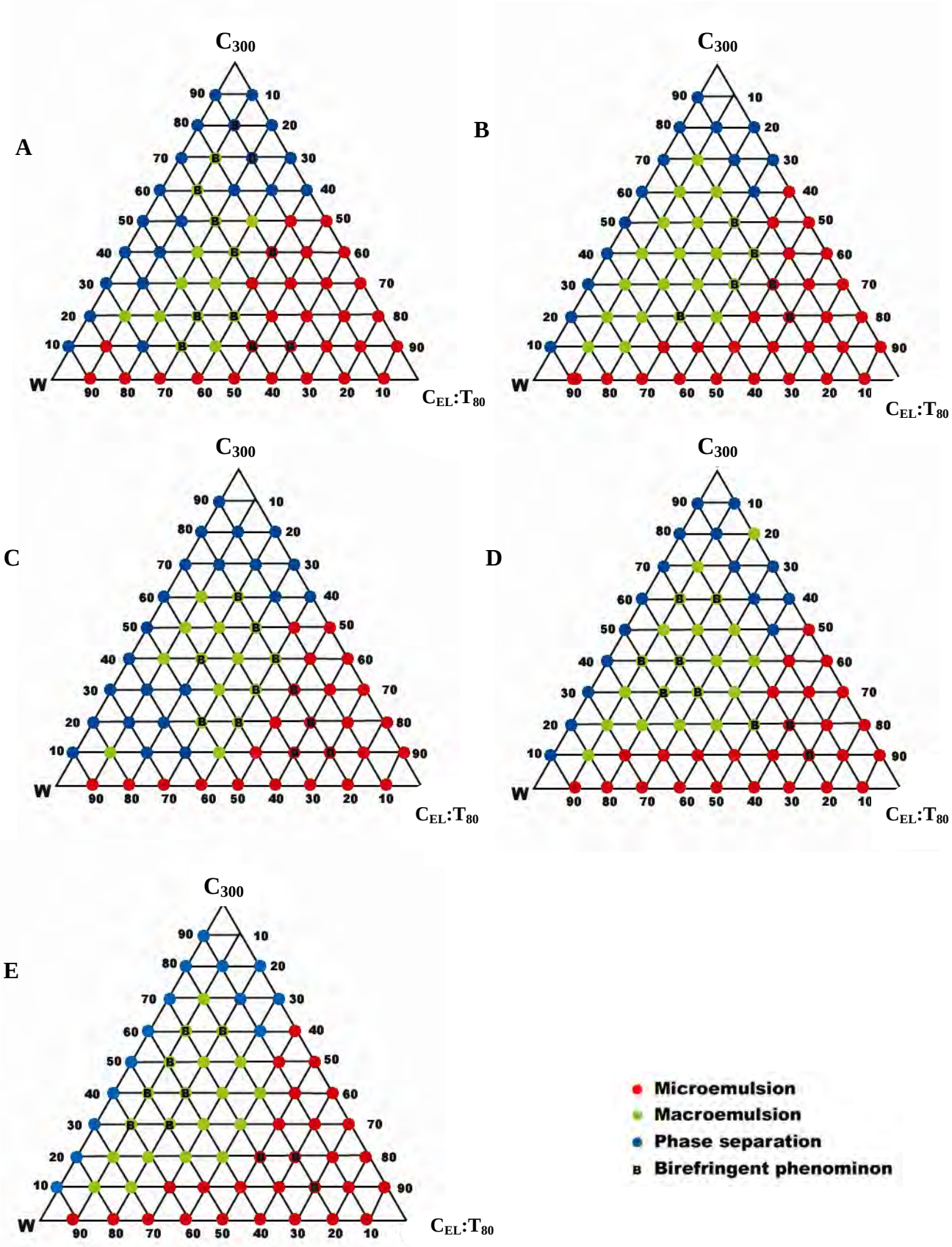


Figure 6. Pseudo-ternary phase diagram from the system of oil and various ratio of Cremophor EL : Tween 80 and water

A : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) ratio of C_{EL}: T_{80} = (1:1) and Water (W)

B : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) ratio of C_{EL}: T_{80} = (2:1) and Water (W)

C : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) ratio of C_{EL}: T_{80} = (4:1) and Water (W)

D : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) ratio of C_{EL}: T_{80} = (1:2) and Water (W)

E : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80})ratio of C_{EL}: T_{80} = (1:4) and Water (W)

1.3 Polarized light microscopy

Only mono-phasic mixtures were selected to examine the birefringent property by microscopy equipped with polarized lens as shown in Figure 7. The sample which showed birefingent property would be classified as liquid crystals. In the mono-phasic transparent or translucent mixtures which appeared dark field when viewed under cross polarizer were classified as microemulsion such as system of $40C_{300}50C_{EL}10W$ presented in Figure7A. The birefingent phenomena in this study presented as white streaks on the black field as presented in Figure7B-D and were referred as a lamellar phase structure. Makai et al (1999) and Fehér et al (2005) used polarized light microscopy to identify formation and structure of various liquid crystal and microemulsion.

Low ratio of oil especially more than 20% of system or on other hand high ratio of water and surfactant showed birefringent property in various areas. Most of them were shown as lamellar pattern as from system of $10C_{300}60s_{15}30W$ and $10C_{300}$ $60T_{80}30W$ (Figure 7A and 7B). Liquid crystal of system were resulted from water and surfactant molecules rearrangement. High ratio or high amount of surfactant to water caused molecular attachment or rearrangement to be lamellar phase structure(Fehér et al, 2005).

Figure 7C presents the lamella microscopic pattern under cross-polarized light microscope form system of $20C_{300}$ 30 (C_{EL}:T_{80}= 2:1) 50W. The results presented that Cremophor El and Tween80 combined together increased the area of liquid crystal phase as presented in pseudo-ternary phase diagram at Figure 6A-D. This finding is consistent with a previous study by Trotta et al (1999) that combination of surfactants caused more occurrences of liquid crystal. The second hydrophilic surfactant could adjust the packing properties of the lecithin–alcohol systems, and/or to increase the fluidity of the surfactant film, increased the region of existence of the isotropic systems led to more liquid crystal and microemulsion occurred. According to the microscopic pattern of each system, the formation and structure of liquid crystalline phase depended on the characteristic of the amphiphilic compound, the other component of system, the type and ratio of to components and time. Moreover, Kunieda et al (1999) found that using mixed type of polyoxyethylene nonionic

surfactant with different of side chain caused surfactant molecules to paked in the aggregateas and reduction repulsion force

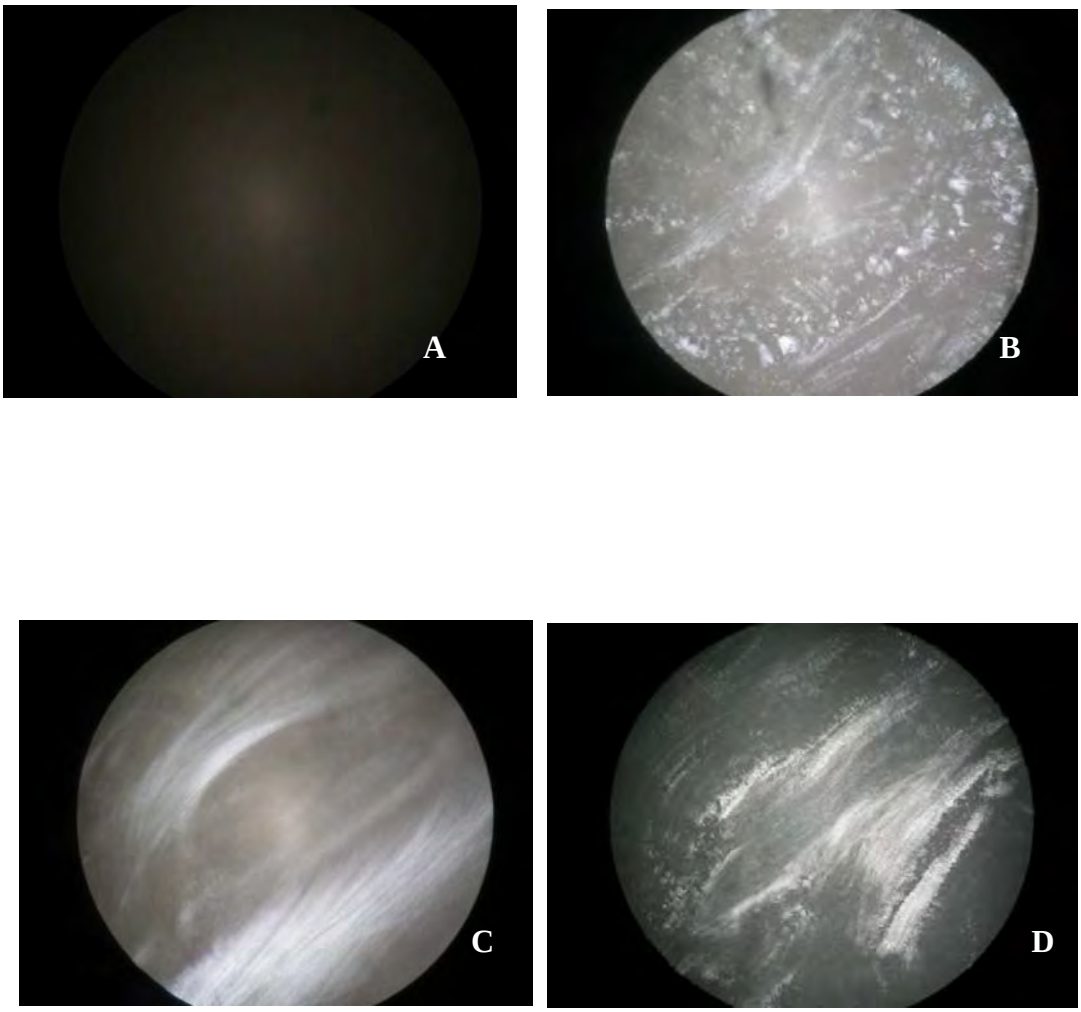


Figure 7. The macroscopic pattern under cross-polarized light microscope (40 X)
A. The non-birefringent property of system C_{300}:C_{EL}:W = 40:50:10
B. The birefringent property from the system C_{300}:S_{15}:W = 10:60:30
C. The birefringent property from the system C_{300}:T_{80}:W = 10:60:30
D. The birefringent property from the system C_{300}:2C_{EL}/1T_{80}:W = 20:(C_{EL}:T_{80} =2:1)30:50

2. The Self -Microemulsifying Drug Delivery system (SMEDDs)

2.1 Solubility of drug in oils with various co-solvents.

It is notable that an increasing proportion of new studies recognizes the benefit associated with employing pharmaceutically acceptable surfactants, co-surfactants or co-solvent and oils. To develop a microemulsion system for oral delivery of poorly water-soluble cyclosporin A, selection of co-solvent and surfactant is essential to increase drug silobility. The surfactants used in SMEDDs formulations are known to improve the bioavailability by various mechanisms (Wei et al, 2005; Petel et al, 2007).

The results of solubility of cyclosporin A is shown in Figure 8. The solubility of cyclosporin A in oil, and surfactant as Cremophor EL, Tween80 and Solutol HS15 were 25.29±0.14, 23.54±0.09, 26.29±0.15 and 22.94±0.12 mg/g. Propylene glycol(Pg) provided the highest solubility of cyclosporin A followed by ethanol(EtOH) glycerin(Gly) and polyethylene400(PEG_{400}) respectively Low molecular weight co-solvents as ethanol (MW = 46.07) and propylene glycol (MW= 79.09) presented higher solubility than high molecular weight as polyethylene400 (MW = 400) due to its small molecules could easily penetrated around cyclosporin molecules. For glycerin, even its molecular weight is 92.90 but its high viscosity of 1420 mPa·s caused separation from low viscosity oil; viscosity of medium chain triglycerides (25-33 mPa s). Thus solubility of cyclosporine A in glycerin was low.

These results were correlated wih the previous study by Ran et al (2001) that the less polar co-solvent was more effective to increase solubility of cyclosporine A. Ethanol was more non polar than propylene glycol, polyethylene glycol400 and glycerin. Thus the solubility in ethanol was the highest and in glycerin was the lowest. In present study, propylene glycol provided the highest solubility. These might be explained by the evaporation of ethanol while shaking in room temperature for 7 days. Moreover, Ran et al (2001) only studied cyclosporine with pure co-solvent. Thus there was no report on phase separation of oil and co-solvent.

Increase percentage of co-solvent enhanced drug dissolved especially propylene glycol. Propylene glycol 20 % provided the highest solubility of cyclosporin A of (98.82±0.34 mg/g). Although 20 % of propylene glycol,

polyethylene 400 and glycerin were able to increase solubility drug more than 10% but it could not be used because of the separation of oil phase after storage at room temperature for 2 months as described in Table 8. From these results, propylene glycol and ethanol were selected to be co-solvents for the preparation of a microemulsion system of cyclosporin A.

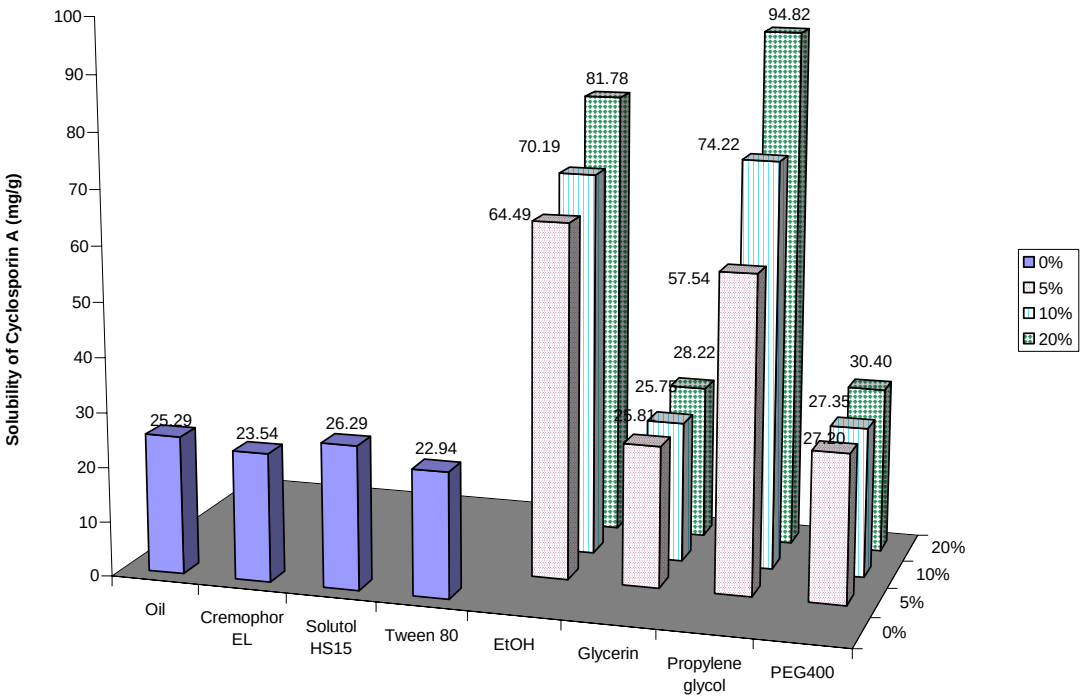


Figure 8. Solubility of Cyclosporin A in various type of ingredient and different percentage of co-solvent in oil.

Table 8. The physical appearance of oil, surfactant and co-solvent mixture.

Formulation	Macroscopic observation		Formulation	Macroscopic observation	
	initial	After storage for 2 months		initial	After storage for 2 months
Oil	-	-	Oil + 10%EtOH	-	-
Cremophor EL	-	-	Oil + 10%Gly	-	-
Tween 80	-	-	Oil + 10%PG	-	-
Solutol HS15	-	-	Oil + 10%PEG_{400}	-	+
Oil + 5%EtOH	-	-	Oil + 20% EtOH	-	-
Oil + 5%Gly	-	-	Oil + 20%Gly	-	+
Oil + 5%PG	-	-	Oil + 20%PG	-	+
Oil + 5% PEG_{400}	-	-	Oil + 20%PEG_{400}	-	+

- Mono-phasic mixture , + phase separation

C_{300} : medium-chain triglycerides, **C_{El}** : cremophor EL, **T_{80}** : Tween 80, **S_{15}** : solutol HS15, **EtOH** : Ethanol, **PG** : propylene glycol, **PEG_{400}** : polyethylene 400, **Gly** : glycerin

2.2 Formulation of Self -Microemulsifying Drug Delivery system (SMEDDs) / Pseudo-ternary phase diagram study

Similar to microemulsion, SMEDDs contained oil phase and surfactant but without water. When SMEDDs was mixed with water it became microemulsion. Figure 9,10,11 and 12 present the pseudo-ternary phase of SMEDDs system which consisted of oil, surfactants as Cremophor EL (C_{EL}), Tween80 (T_{80}) and Solutol HS15 (S_{15})and co-solvent as ethanol (EtOH), propylene glycol (PG), glycerin (Gly) and polyethylene400 (PEG_{400}). Co-solvents were chosen to be added in SMEDDs in medium-chain triglyceride as previously described. in order to formulate as 2 doses of cyclosporin A capsules, each contained 25 mg and 100 mg of drug because of poor solubility of cyclosporin A. According to the results solubility study, propylene glycol had shown the highest drug solubility. However, from the preliminary study co-solvents showed the high incompatibility with gelatin capsule shell.

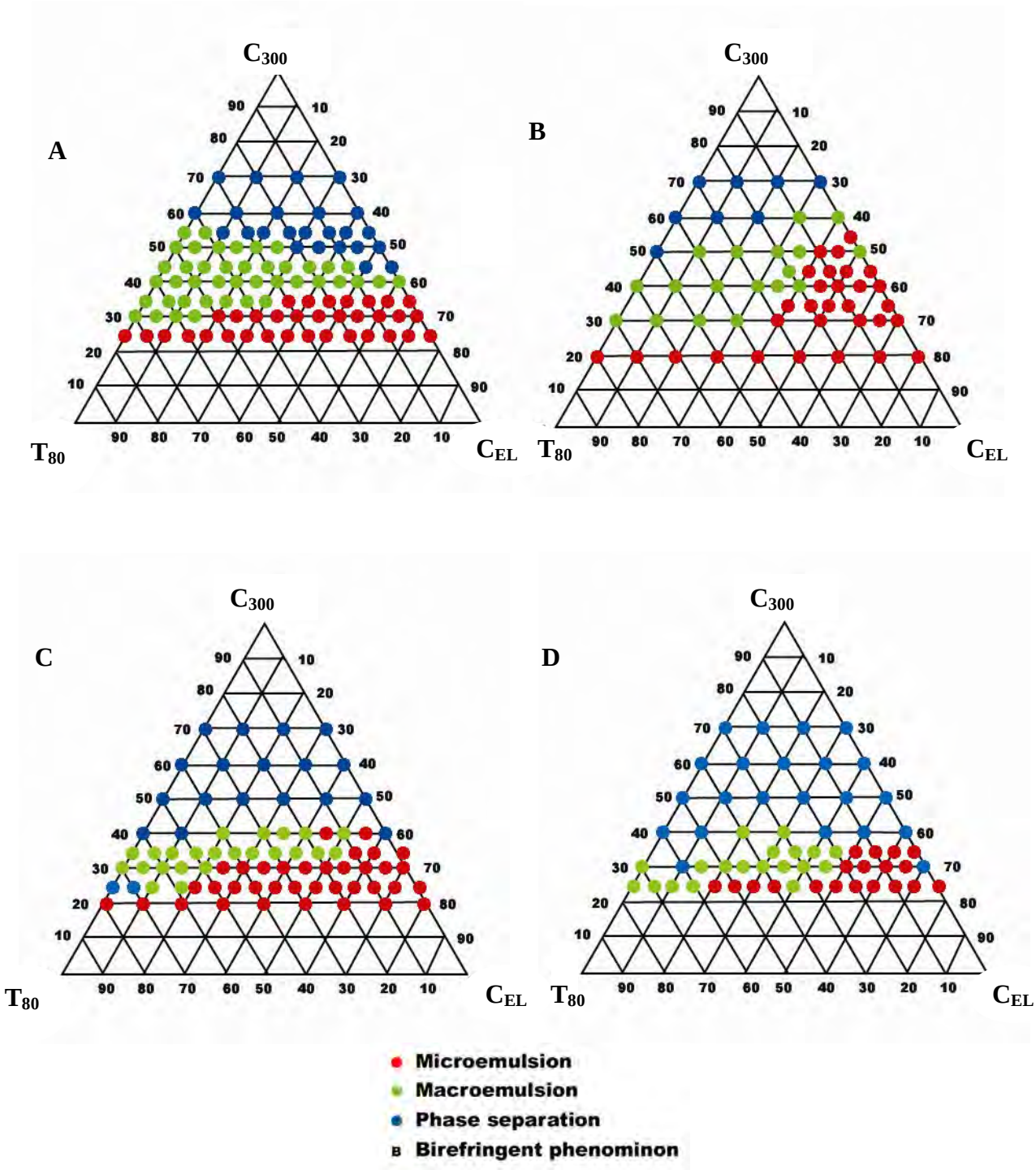


Figure 9. Pseudo-ternary phase diagram from the SMEDDs system of oil : Cremophor EL : Tween 80 and 10% co-solvent

A : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Ethanol 10%
B : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Glycerin 10%
C : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Propylene glycol 10%
D : System of Captex 300 (C_{300}) : Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Polyethylene 400 10%

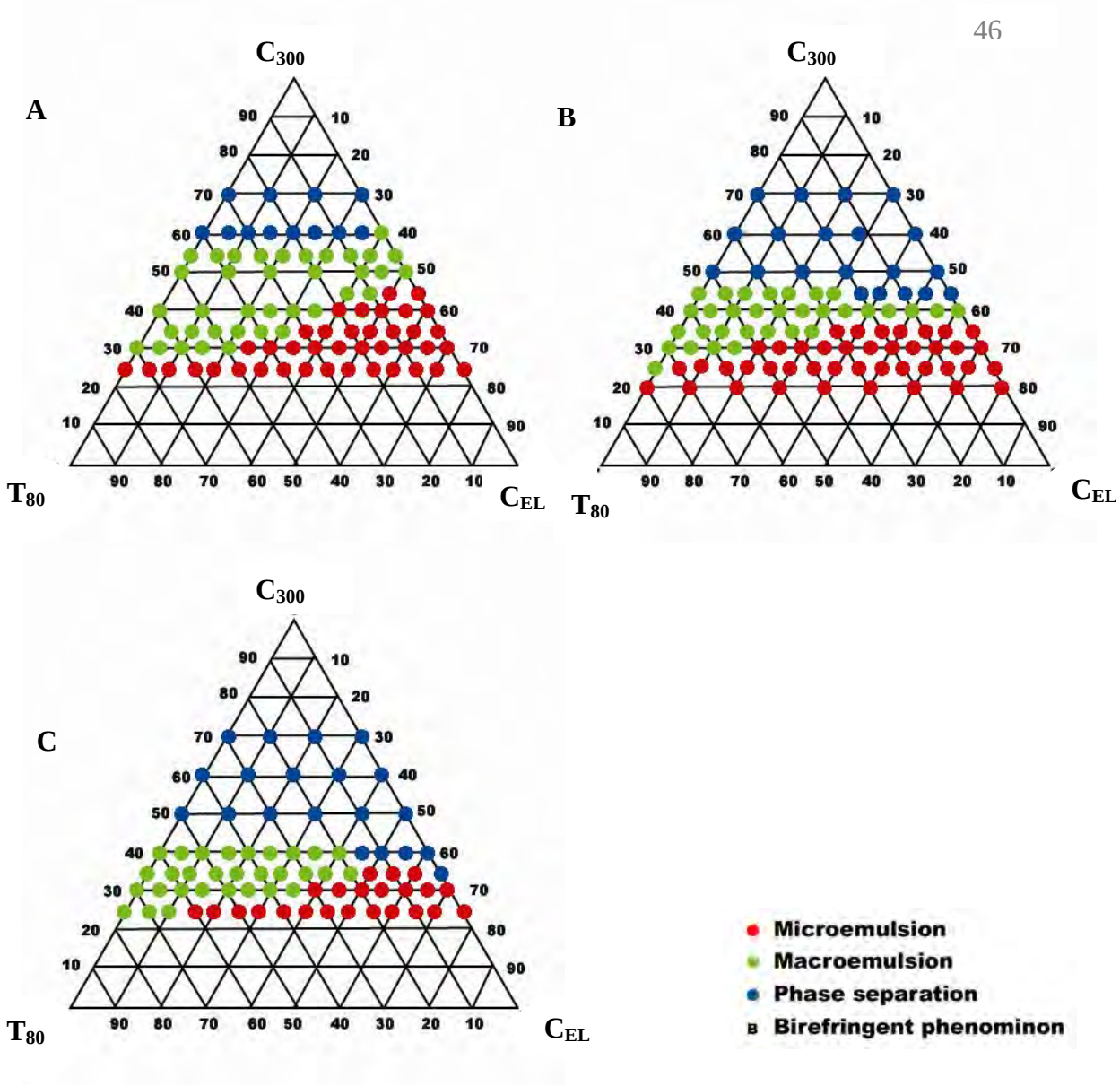


Figure 10. Pseudo-ternary phase diagram from the SMEDDs system of oil : Cremophor EL : Tween 80 and combined co-solvent

A : System of Captex 300 (C_{300}): Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Glycerine 5% + Ethanol 5%

B : System of Captex 300 (C_{300}): Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Propylene glycol 5% + Ethanol 5%

C : System of Captex 300 (C_{300}): Cremophor EL (C_{EL}) : Tween80 (T_{80}) and Polyethylene 400 5% + Ethanol 5%

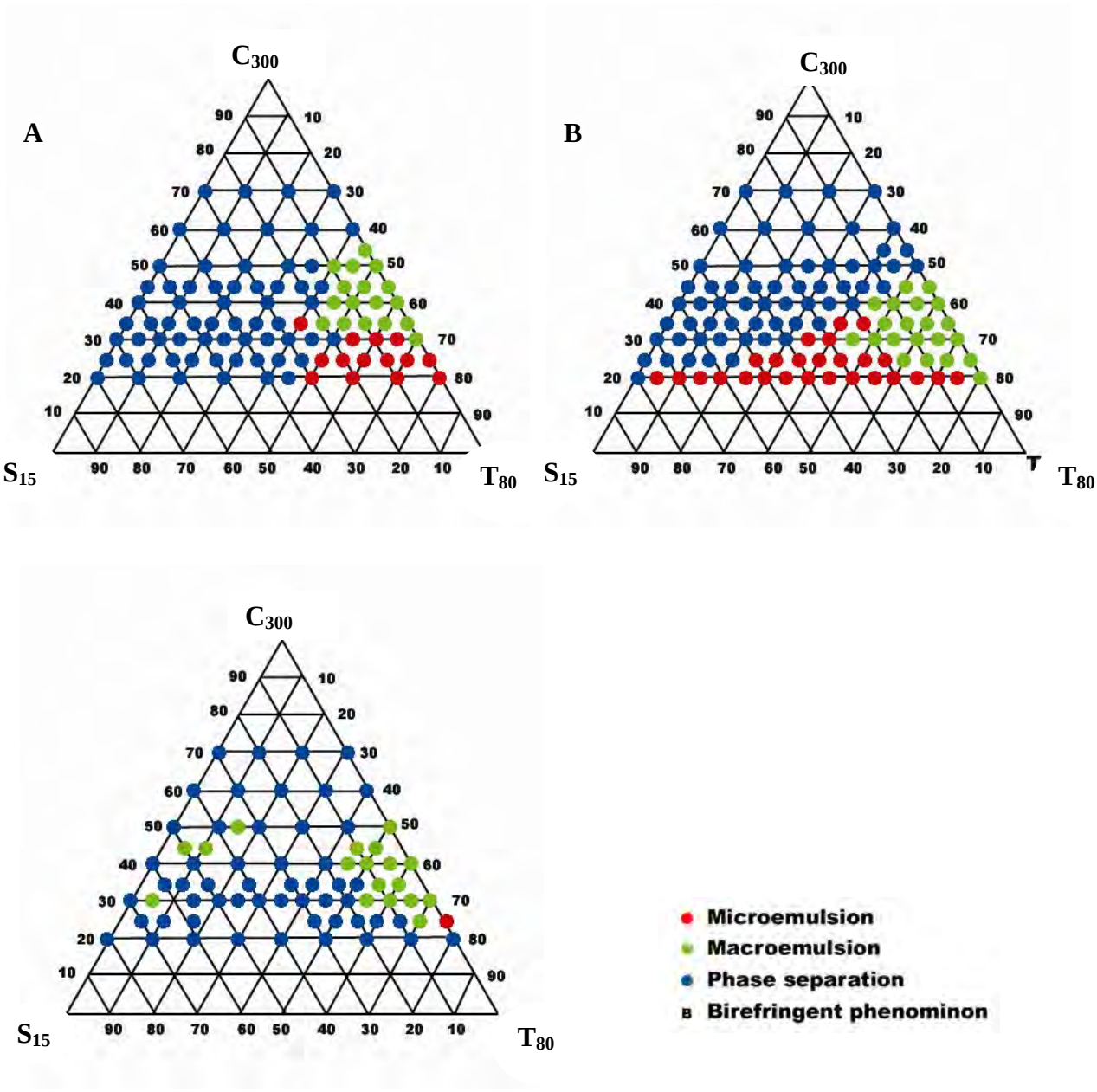


Figure 11. Pseudo-ternary phase diagram from the SMEDDs system of oil :Tween 80 : Solutol HS15 and combined co-solvent

A : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Tween80 (T_{80}) and Glycerin 5%+ Ethanol 5%

B : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Tween80 (T_{80}) and Proplylene glycol 5% + Ethanol 5%

C : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Tween80 (T_{80}) and Polyethylene 400 5% + Ethanol 5%

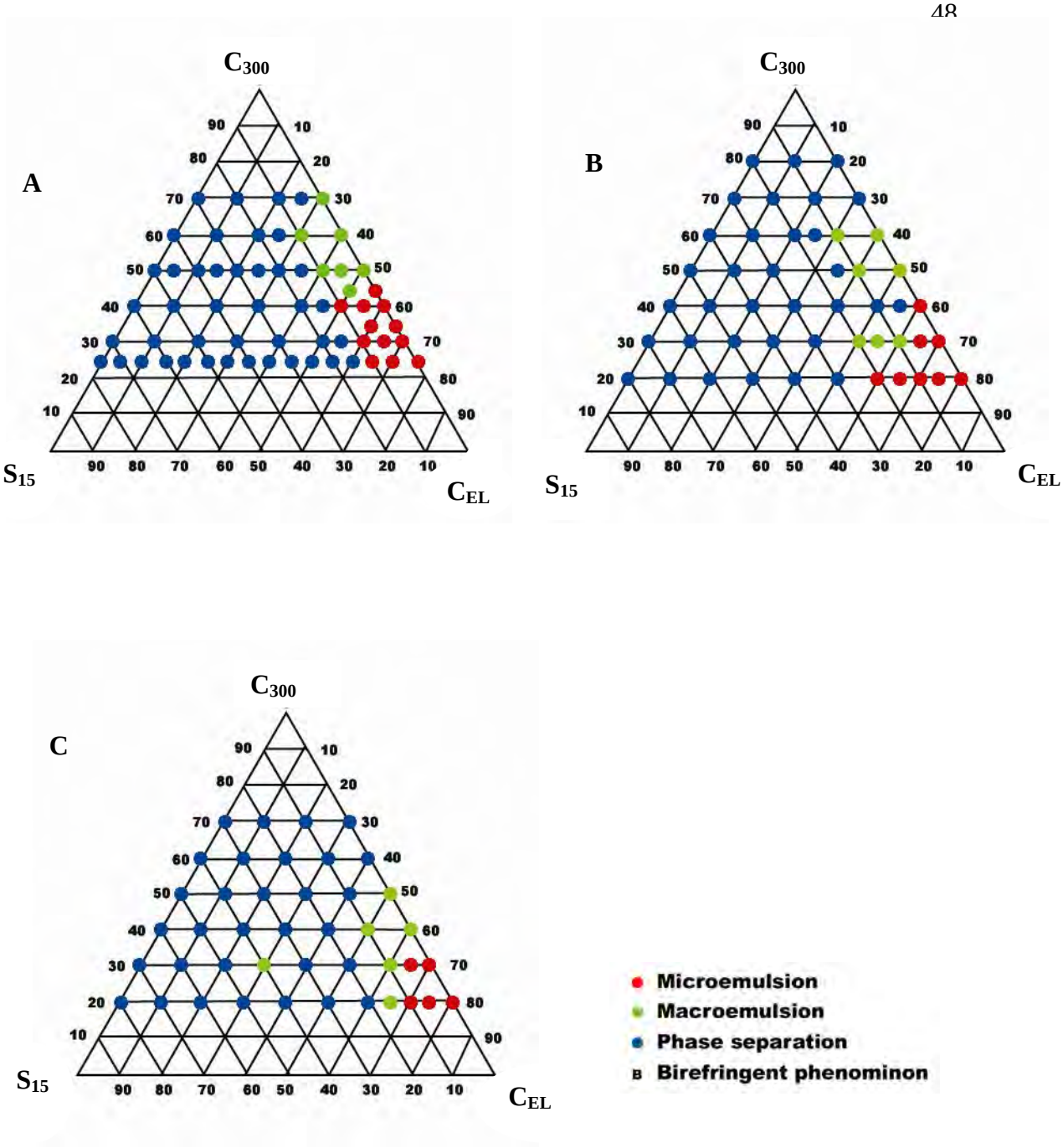


Figure 12. Pseudo-ternary phase diagram from the SMEDDs system of oil and Solutol 15 : Cremophor EL combined co-solvent

A : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Cremophor EL (C_{EL}) and Glycerin 5 % and Ethanol 5%

B : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Cremophor EL (C_{EL}) and Propylene glycol 5% + Ethanol 5%

C : System of Captex 300 (C_{300}) : Solutol HS15 (S_{15}) : Cremophor EL (C_{EL}) and Polyethylene 400 5% + Ethanol 5%

The physical appearance of capsule was changed. Ethanol dissolved gelatin which caused capsules brittle and easily leakage. Propylene glycol and glycerin could penetrate through gelatin matrix thus caused capsule to have distorted shape and also soften the shell (Moreton, 1997). Although co-solvents were necessary to dissolved cyclosporin A they had to be used as smallest adequate amount to obtain 25mg and 100 mg cyclosporin A per each capsule. Since commercial SMEDDs product contains10% of cosolvent (Neoral®, United state patent NO. 5342625). This amount of co- solvent was also incorporated into the system. According to the solubility and capability to form microemulsion of surfactant, Cremophor EL and Tween80 were chosen to be used as combined surfactants.

The physical appearances which examined by visual observation of all SMEDDs formulas were presented as pseudo ternary phase diagram in Figure 9-12. The red dot represents area of microemulsion(mono-phasic translucent to transparent mixture) formed, the green dot represents where macro emulsion (mono-phasic white opaque mixture) formed. On the other hand, if the mixture was separated to double layers, it would be marked as blue dot and not bring to dilution test. Most preparations had yellowish color and become darker when the amount of surfactant such as Cremophor EL and Tween80 increased due to the color for surfactant. When increasing the amount of Soltutol HS15, the mixture was clearly translucent. After the series of mixture were mixed together, they were kept at room temperature for 3 days. The mono-phasic mixtures would be diluted with water at the ratio of 1:100 and the appearance was examined after dilution.

Figure 9 illustrates pseudo-ternary phase diagram from the SMEDDs system contained 10% of co-solvent, Glycerin (Figure 9 B) provided the largest area of microemultion and formed microemulsion at higher ratio of oil : surfactant than ethanol (Figure 9 A), propylene glycol (Figure 9 C) and polyethylene glycol 400 (Figure 9 D)respectively. This result could be explained by the different hydrophilic head group and short hydrophobic chain length of co-surfactants. Due to glycerin had appropriat structure, its hydrophilic head group and short alkyl chain were of sufficient size and length to ensure that it resided in the interfacial layer; resulting in altering the rigidity of the interface. Thus the interfacial layer could be curve enough to form fine droplet and provided large microemulsion area. On the other hand,

polyethylene glycol 400 had high molecular weight and long chain hydrophobic that seemed insufficient size and length so its gave a smallest of microemulsion area (Lawrence and Rees, 2000).

After mixing with co-surfactant the ratio of oil:surfactant which provided micreoemulsion was lower than the system without co-surfactant. This might be explained by the effect of surfactant and co-surfactant mixing ratio even the opposing effect of surfactant and the co-surfactant. Theoretically, co-surfactants increase the size of polar head group of surfactant and influence the curvature of surfactant film to form microemulsion. However, increasing or presence the amount of co-surfactant decreased the surfactant: co-surfactant ratio. Hence, the amount of surfactant in the systems may not be enough to form microemulsion. The result of this study suggested that ethanol, glycerin, propylene glycol and polyethylene 400 which was added to SMEDDs formulas rather functioned as co-solvent than as co-surfactant.

However after keeping SMEDDs containing 10% co-solvent for 2 months, some mono-phasic mixures turned to bi-phasic mixture especially the formulas which contained polyethylene 400 confirming that these formulas were unstable. Moreover the 10 % of single co-solvent in SMEDDs seemed to be high due to large phase separation area as described in Figure 9.

Figure 10 showed the pseudo-ternary phase diagrams of combination of surfactant in SMEDDs systems. Cremophor EL and Tween 80 mixed with several combined co-solvent presented the largest area of microemulsion especially the combination of 5%Et +5%Gy and 5%Et +5%PG as showed in Figure 10A, B. Whereas the SMEDDs formulas consisted of combination of surfactant between Cremophor EL and Solutol HS15 or Tween80 and Solutol HS15 presented two layer separation mixture area as showed in Figure 11 and 12. These results corresponded with the study of the aforementioned microemulsion system that Solutol HS 15 provided the smallest area of microemulsion due to its physical appearance as gel-like and the molecular structured. It could form rigid lameller structure that could not be altered by type and ratio of co-sovent.

After keeping the SMEDDs formulas which consisted of Cremophor EL and Tween80 with 5%EtOH +5%Gly ($C_{300}C_{EL}T_{80}$+5EtOH5Gly) and Cremophor EL and Tween80 with 5% EtOH +5%PG ($C_{300}C_{EL}T_{80}$+5EtOH5PG) for 2 month the appearances of these preparations were still the same as mono-phasic mixture.

The formulation of C_{300} : C_{EL} : T_{80} at ratio 40 : 40 : 20 and 40 : 50 : 10 with 5%EtOH +5%Gly ($40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly and $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly) and formulation of C_{300} : C_{EL} : T_{80} at ratio 35 : 32.5 : 32.5 and 35 : 40 : 25 with 5%EtOH +5%PG ($35C_{300}32.5C_{EL}32.5T_{80}$+5EtOH5PG and $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG) were chosen because they contained highest ratio of oil : surfactant to load cyclosporin A in the preparation of cyclosporin capsule. The physical appearances of the selected SMEDDs are shown in Table 8. According to the solubility study although provided the low solubility of cyclosporine A in oil. Previous it provided the highest ratio of oil : surfactant to form microemulsion. In addition, as system also contained 5 % ethanol which could be increase drug solubility. Thus formulas with glycerin were chosen for further studies.

2.3 Effect of dilution ratio study

The aim of the effect of dilution study is to find a suitable ratio of water and SMEDDs to produce microemulsion before characterization methods and to clarify if ratio of dilution had an effect on the size of droplet. In general, there was a good correlation between visual observations and particle size measurement. The SMEDDs usually formed microemulsions where the particle size were less than 50 nm and appeared clear or slightly bluish. In the previous study many ratios of dilution used to prepared microemulsion such as 1:200 (Khoo et al, 1998) or 2.3:500(Wei et al, 2005).

Only one system was chosen to study as the system of $40C_{300}40C_{EL}20T_{80}$ +5EtOH5Gly. This system was chosen because it resided between boundary of microemulsion and macroemulsion phase. Figure 13 presented picture of $40C_{300}40C_{EL}20T_{80}$ +5EtOH5Gly diluted in water at ratio of 1:50, 1:100, 1:200 and 1:500 from left to right respectively. The difference of turbidity was visually indicated. The results presented that the visual turbidity had changed when large amount of water was added that the ratio of 1:500 seemed to be clearer than ratio 1:50. However at less clear solution the at 1:50 dilution ratio, the system still appeared

as transparent solution. These results might be explained by the fusion of droplets at high ratio of dilution. High concentration led droplets to become close together caused which could fuse together and became larger droplet.

After SMEDDs was diluted with water at different ratio, the drolet sizes were determined by the photon correlation spectroscopy (PCS) method. The results of droplet size determination at dilution ratio 1:50, 1:100, 1:200 and 1:500 were 85.4±0.89, 78.6±0.95, 72.7±0.2 and 79.8±2.01 nm respectively as shown in Figure 14. The analysis of variance (ANOVA) of particle size diameter showed that the mean particle diameter was significantly different among the different dilution ratios ($P < 0.05$). The multiple comparisons test revealed in particle size at 1:50 dilution was different from those of 1:100 and 1:200 dilution ratio but not different from1: 500. Moreover these was non different among groups of 1:100,1:200 and 1: 500 dilution ratio.

Because particle diameter at ratio 1:50 was different from other dilutions, possibly due to droplet fused together, this may lead to inaccurate determination. Thus dilution ratio of 1:100 was chosen, although this may not correlate to the quantity of eater in gastrointestinal tract which varied among individual person.

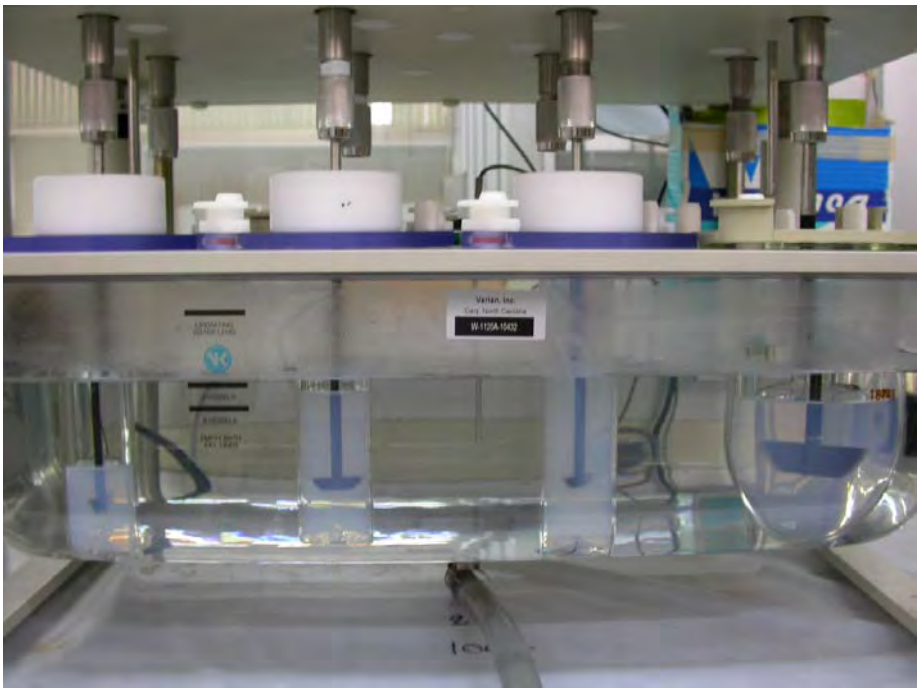


Figure 13. The effect of dilution study determined by visual observation.(From left to right ratio of SMEDDs : water ; 1:50, 1:100,1:200 and 1: 500.respectively)

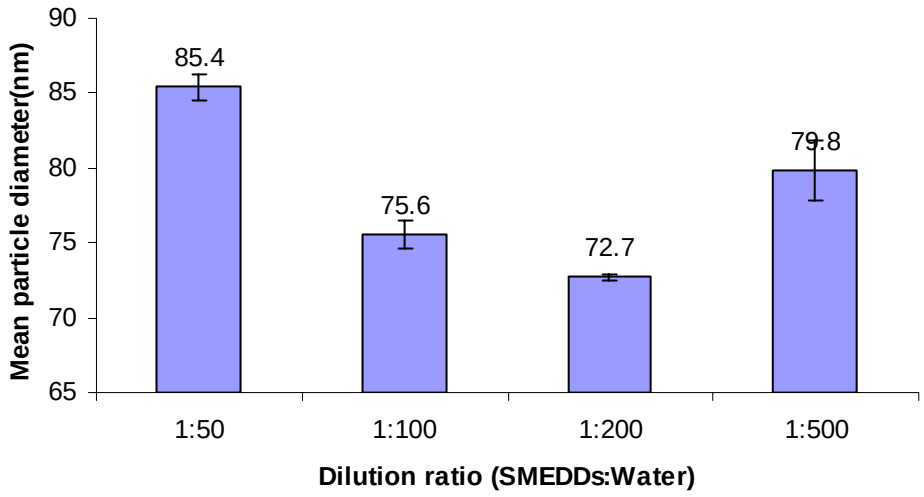


Figure 14. Particle size of $40C_{300}40C_{EL}20T_{80}$ +5EtOH5Gly diluted at various ratio of water.

2.4 Characterization of SMEDDs

2.4.1 Physical appearance

a) Physical appearance of mixture before dilution

Table 9 shows physical appearance of selected SEMDDs. Every formulation was yellowish clearly and slightly viscous. There was no change in visual appearance after 2 months storage

Table 9. The physical appearances of selected SMEDDs.

Formulation		**Physical appearance**	
Formula name	**% of ingredient in formula**	**After 3 day**	**After storage for 2 month**
	C_{300}:C_{EL}:T_{80}:Co-solvent		
$40C_{300}40C_{EL}20T_{8o}$ +5EtOH5Gly	32.73 **:** 32.73 **:** 16.36 **:** 9.1EtOH **:** 9.1Gly	- yellowish,slightly viscous	- yellowish,slightly viscous
$40C_{300}50C_{EL}10T_{80}$ +5EtOH5Gly	32.73 **:** 40.91 **:** 8.18 **:** 9.1EtOH **:** 9.1Gly	- yellowish,slightly viscous	- yellowish,slightly viscous
$35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG	28.64 **:** 26.59 **:** 26.59 **:** 9.1EtOH **:** 9.1PG	- yellowish,slightly viscous	- yellowish,slightly viscous
$35C_{300}40C_{EL}25T_{80}$ +5EtOH5PG	28.63 **:** 32.73 **:** 20.45 **:** 9.1EtOH **:** 9.1PG	- yellowish,slightly viscous	- yellowish,slightly viscous

- Mono-phasic mixture , + Separation phase

C_{300} : medium-chain triglycerides, **C_{El}** : cremophor EL, **T_{80}** : Tween 80, **S_{15}** : solutol HS15, **EtOH** : Ethanol, **PG** : propylene glycol, **PEG_{400}** : polyethylene 400, **Gly** : glycerin

b) Physical appearance of mixture after dilution

Mixtures of selected SMEDDs;$40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly, $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly, $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG and $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG with water at the ratio of1:100 appeared bluelish transparent. These results concluded that selected SMEDDs could form microemulsion .at dilution ratio of 1:100.

2.4.2 Particle size determination

a) Photon correlation spectroscopy (PCS)

After selected SMEDDs ware diluted with water at ratio 1:100, the particle sizes of; $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly, $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly, $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG and $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG, were 97.5±0.79, 75.6±0.96, 59.0±0.02 and 40.0±0.00 nm respectively as shown in Figure

15. All preparations had particle size less than 100 nm. The largest size was $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly (97.5±0.79 nm) which correlated with the result from pseudo-ternary phase diagrame that this system was also at the boundary of microemulsion and macroemulsion. These result was also consistent with other reports (Wei et al, 2005), That decrease in droplet size might be the result of more surfactant being available to stabilize the oil-water inferface. Furthermore the decrease in the droplet size reflected the formation of better close-pack film of surfactant at the oil-water interface, there by stabilizing the oil droplets.

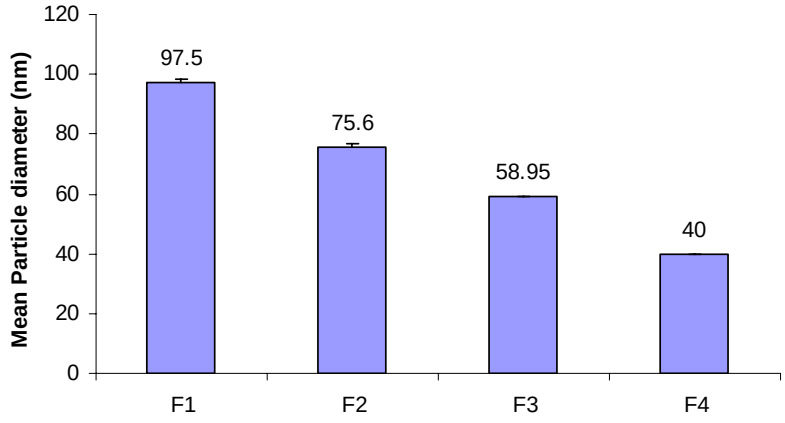


Figure 15. Particle size of selected SMEDDs.

F1 = $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly, **F2** = $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly

F3 = $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG, **F4** = $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG

b) Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is the one of important technique for the study of microemulsiom because it directly produces images at high resolution and it can capture any coexistence of structures and microstructural transitions. Thus this technique was used to determine the droplet size of microemulsion systems.

Figure 16 compares the photomicrographs among systems of $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly after dilute with water at ratios 1:50, 1:100, 1:200 and 1:500. Figures 17A, B and C show the photomicrographs of $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly, $35C_{300}32.5C_{EL}32.5T_{80}$+5EtOH5PG and $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG after dilute with water at ratios 1:100. The results from pictures presented that all produced spherical particles and their droplet size

were correlated with droplet size determined by PCS method. Most systems had droplet size in the range of microemulsion which was under 100 nm. However, the microemulsion from SMEDDs could not be accurately imaged with TEM due to the drying of sample during process of sample preparation caused fusion of droplet.

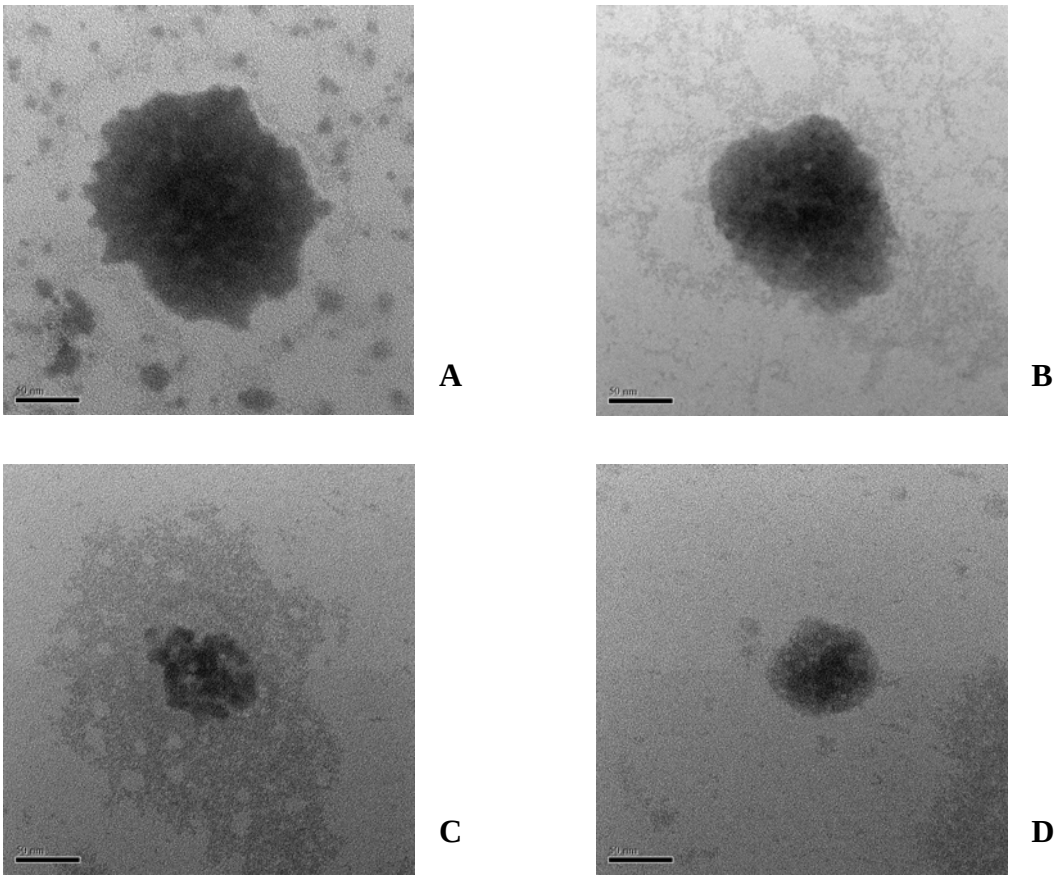


Figure 16. Comparison of TEM photomicrographs of $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly diluted in water at various ratios.

A : Dilution ratio 1:50 **B** : Dilution ratio 1:100

C : Dilution ratio 1:200 **D** : Dilution ratio 1:500

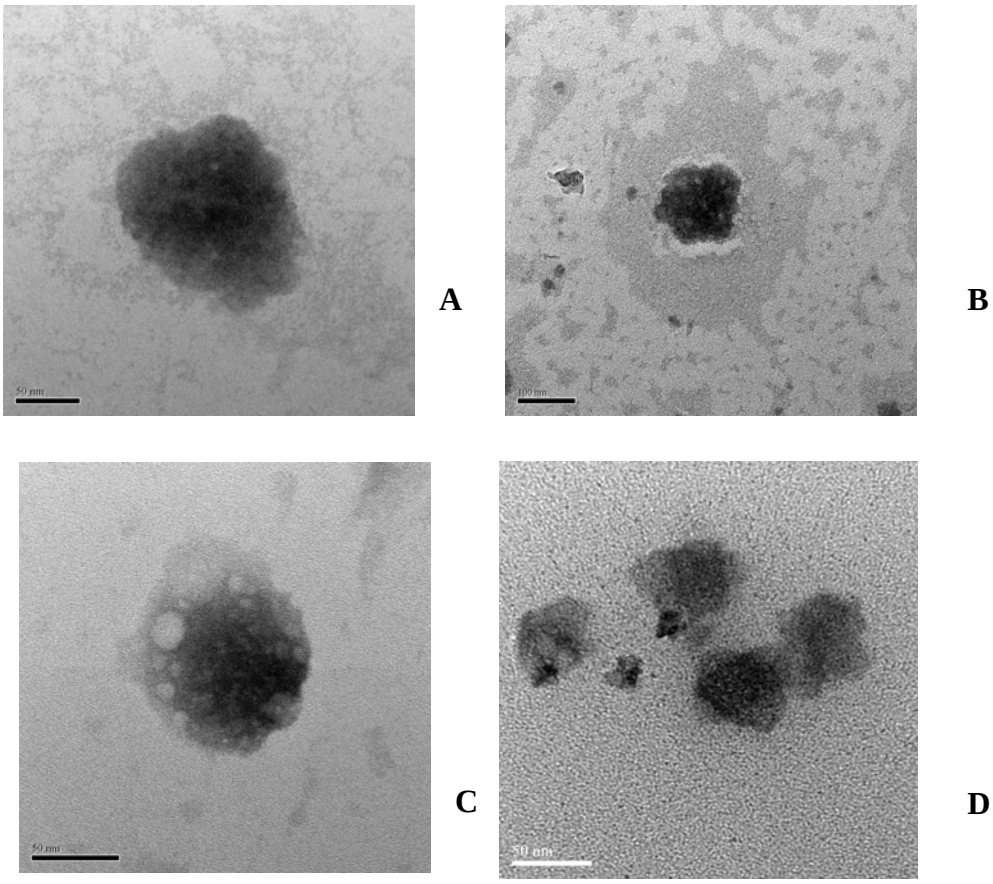


Figure. 17. TEM photomicrographs of
A : $40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly diluted in water ratio 1:100

B : $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly diluted in water ratio 1:100

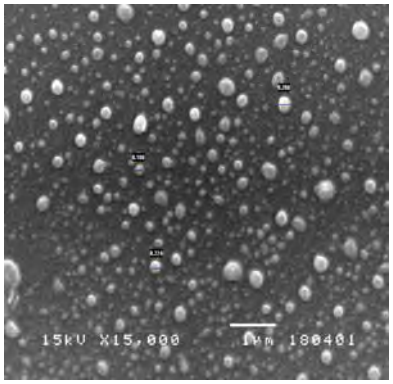
C : $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG diluted in water ratio 1:100

D : $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG diluted in water ratio 1:100

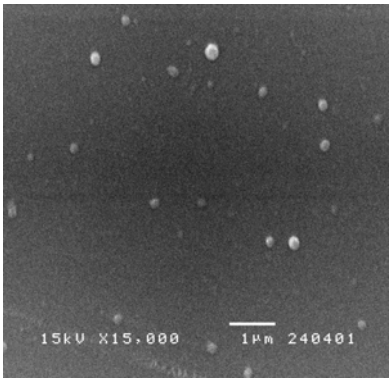
c) Scanning electron microscopy (SEM)

Figures 18 A and B present SEM photomicrographs of microemulsion droplet from system $C_{40}E_{40}T_{200}$+5Et5Gy diluted with water at ratio1:50. It was shown that microemulsion were spherical particles of less than 100 nm with wide particle size distribution. The Figure 18C presents SEM photomicrographs of microemulsion droplet from system $C_{40}E_{40}T_{200}$+5Et5Gy diluted with water at ratio1:50.

The SMEDDs sample showed few droplet. This was possibly to droplets of microemulsion were unable to attach with a slide glass or were washed off from glass surface especially when sample slide was soaked in ethanol.



A



B

Figure 18. SEM photomicrographs

A :. $40C_{300}40C_{EL}20T_{8o}$+5EtOH5Gly diluted in water in ratio 1:50

B : $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG diluted in water in ratio 1:50

2.4.3 Polarized light microscopy

There was non birefringent phenomenon appeared in all SMEDDs due to to all prepared SMEDDs contained co-solvent. This finding was supported by a theory, in that co-surfactant could penetrate to surfactant causeing interrupt arrangement of molecule and also decreased viscosity of formula. This finding was consistent with a previous study by Alany R.et al (2001) that addition of 1-butanol increased the area of microemulsions and eliminated the formation of any liquid crystalline phases.

2.4.4 Viscosity determination

The viscosity is necessary factor of SMEDDs because the system led to be filled into capsule. High viscosity mixture may not pass through the filling tip of capsule filling machine. The viscosity of SMEDDs formulas metering by spindle NO 31 at room temperature are shown at Table10. In addition, the relationship between shear rate and shear stress of SMEDDs was plotted as shown in Figure 19. The result of dynamic viscosity test reduced that the SMEDDs formulas were Newtonian fluid due to straight graph line and pointed to origin; which meant that the fluid continued to flow, regardless of the forces. The $40C_{300}50C_{EL}10T_{8o}$+5EtOH5Gly provided the

highest viscosity 316.1±0.34 cP. The viscosity of $35C_{300}32.5C_{EL}$ $32.5T_{80}$+5EtOH5PG was 269.0±0.34 cP.

Their viscosities were affected by each component. The viscosity of Captax 300, Cremophor EL and Tween80 are 21.6, 538.1 and 337.1 cP respectively. And viscosity of co-solvent, ethanol, propylene glycol and glycerin at 20°C are 1.1, 40.4 and 1420cP. Thus, the formulation which contained propylene glycol should have lower viscosity than formula containing glycerin. The suitable fill viscosity range for filling and sealing machine is 50-3000 cP (CFS 1200 Capsule filling and sealing machine's handbook). Therefore the viscosity of selected SMEDDs were appropriate for filling by machine.

Table 10. Viscosity of selected SMEDDs at 50 rpm

SMEDDs formula	**Viscosity (cP)**
$C_{40}E_{40}T_{20}$+5Et 5Gy	311.7±0.34
$C_{40}E_{50}T_{10}$+5Et 5Gy	316.1±0.34
$C_{35}E_{32.5}T_{32.5}$+5Et 5Pg	269.0±0.34
$C_{35}E_{40}T_{25}$+5Et 5Pg	290±0.58

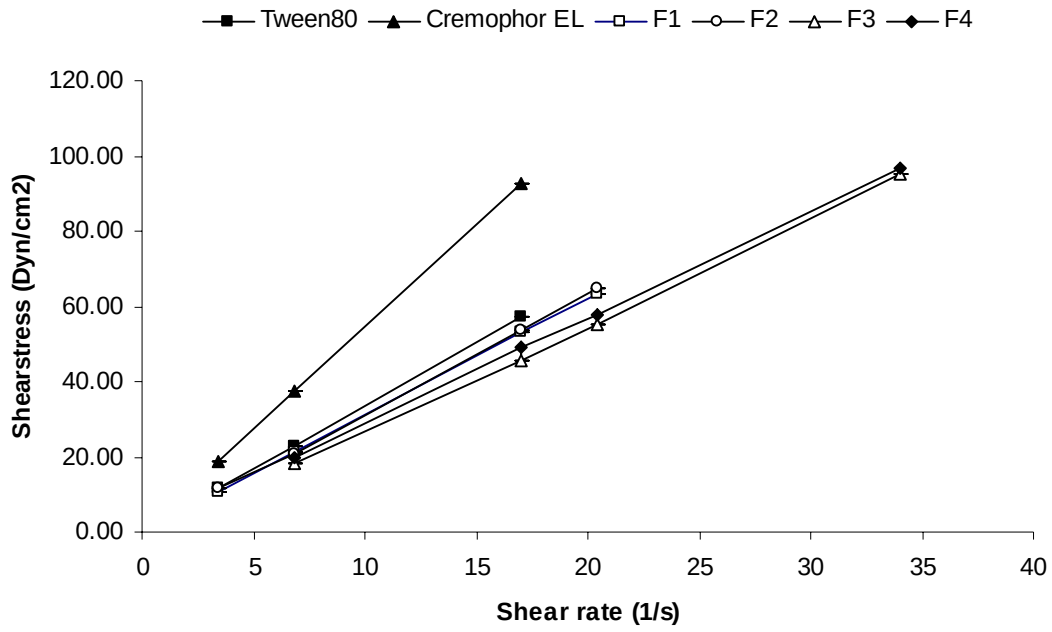


Figure 19. Shear rate-Shear stress relationships of selected SMEDDs.

F1 = $40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly

F2 = $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly

F3 = $35C_{300}32.5\ C_{EL}32.5T_{80}$+5EtOH5PG

F4 = $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG

2.4.5 pH determination

Because the pH of SMEDDs can not be measured directly, thus the measurement was undertaken after mixed with water at ratio 1:100. The pH of microemulsion systems were about 6.8 as shown in Table 11. The results of this study were not difference because their formulation ingredient were non acidic or basic and the system were quite dilute.

Table 11. The pH determination of the microemulsion systems.

SMEDDs formula	**pH value**	
	Water	**microemulsion**
$40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly	6.82±0.00	6.84±0.01
$40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly	6.84±0.01	6.83±0.01
$35C_{300}32.5\ C_{EL}32.5T_{80}$+5EtOH5PG	6.85±0.01	6.85±0.02
$35C_{300}40C_{EL}25T_{80}$+5EtOH5PG	6.83±0.01	6.84±0.02

3. The Cyclosporin A loaded self -Microemulsifying Drug Delivery system (SMEDDs CyA)

3.1. Formulation of Cyclosporin A loaded Self -Microemulsifying Drug Delivery system (SMEDDsCyA)

The aim of this study was to prepare the SMEDDs containing 25 mg and 100 mg of cyclosporin A to be filled in hard gelatin capsule. The 25 mg SMEDDsCyA (SMEDDs25CyA) would be filled into capsule number 0 which had volume of 0.5 ml (approximately 0.5 g of SMEDDs) and the 100 mg of SMEDDsCyA (SMEDDs100CyA) would be filled into capsule number 00 which had approximately volumn 0.9 ml (approximately 0.9 g of SMEDDs). Cyclosporin A were loaded to the previously selected SMEDDs.

The results of physical appearances of SMEDDsCyA after 7 days, 2 months and 4 months at room temperature are shown at Table 12. All 100 mg cyclosporin A loaded SMEDDs which contained glycerin ($40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly and $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly) were separated into two layers and cyclosporin A crystal was precipitated as shown at Figure 20. While 100 mg cyclosporin A loaded SMEDDs which contained glycerin showed no change in visual appearance even after 4 months. This findings was consistent with previous study on cyclosporin solubility that glycerin could dissolve less amount of cyclosporin A. The ability of oil and glycerin in these formulas were insufficient for loading100 mg cyclosporin A. For formula containing propylene glycol, $35C_{300}32.5$ $C_{EL}32.5T_{80}$+5EtOH5PG had lower amount of Cremophor EL than $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG but still provided an acceptable droplet size. Therefore, the formula was selected to loaded drug and filled into capsules in order to obtain SMEDDs25CyA and SMEDDs100CyA capsule. The percentage of each component in each capsule formulation are present at Table12.

Table 12. The components of SMEDDsCy capsule

Formulation	***Ingredient***	***Quantity per 1 capsule(mg)***	% in the formulation
Cyclosporin A 25 mg (SMEDDs 25Cy)	Cyclosporin A	25.00	5
	Oil	149.63	29.93
	Cremophor EL	138.94	27.79
	Tween80	138.94	27.79
	Propylene glycol	23.75	4.75
	Ethanol	23.75	4.75
Total		500	100
Cyclosporin A 100 mg (SMEDDs 100Cy)	Cyclosporin A	100	11.11
	Oil	252	28
	Cremophor EL	234	26
	Tween80	234	26
	Propylene glycol	40	4.44
	Ethanol	40	4.44
Total		900	100

3.2 Characterization of SMEDDs

3.2.1 Physical appearances

a) Physical appearances of mixture before dilution

The physical appearance of all SMEDDsCyA formulas are listed in Table 13. There were yellowish color**.** After first 7 days, all preparation were still mono-phasic mixture, but after storage at room temperature for 2 month the formulation of 100 mg cyclosporin A glycerin-contained SMEDDs formulas ($40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly and $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly) turned to phase showed drug precipitation as showed in Figure 20. After storage for 4 months, the appearances were similar to those at 2 months.

Table 13. The physical appearance of SMEDDs CyA before dilute.

Formulation		**Physical appearance**		
SMEDDs	**Cyclosporin A(mg)**	**After 7 days**	**After storage for 2 months**	**After storage for 4 months**
$40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly	25	M, -	S, -	S, -
	100	M, -	S, +	S, +
$40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly	25	M, -	S, -	S, -
	100	M, -	S, +	S, +
$35C_{300}32.5$ $C_{EL}32.5T_{80}$+5EtOH5PG	25	M, -	M, -	M, -
	100	M, -	M, -	M, -
$35C_{300}40C_{EL}25T_{80}$+5EtOH5PG	25	M, -	M, -	M, -
	100	M, -	M, -	M, -

S : Separation phase , M : monophasic mixture, + : precipitation, - :non precipitation

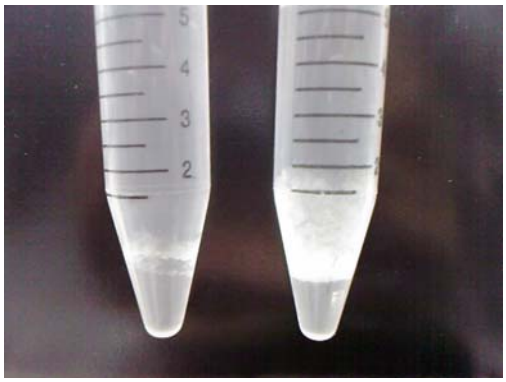


Figure 20. Precipitation of cyclosporin A from $40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly and $40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly loaded with 100 mg cyclosporin A (from left to right) after storage for 2 months.

b) Physical appearances of mixture before dilution

The microemulsion after diluting SMEDDs25CyA and SMEDDs100CyA with water 1:100 was translucent. These result coulded be concluded that after loaded cyclosporine 25 or 100 mg to selected SMEDDs microemulsion could still be obtained.

3.2.2 Particle size determination

a)Photon correlation spectroscopy (PCS)

The particle size after dilution 1: 100 of SMEDDs25Cy and SMEDDs 100 Cy with water were 67.87±0.62 nm and 73.25±0.53 nm respectively. Figure 21 compares the size of cyclosporine A loaded SMEDDs droplet after drug loading. There were significant difference in droplet size between cyclosporin A loaded SMEDDs and non drug loaded SMEDDs ($p< 0.05$). And also difference in droplet size between 25 mg and 100 mg cyclosporin A loaded SMEDDs.

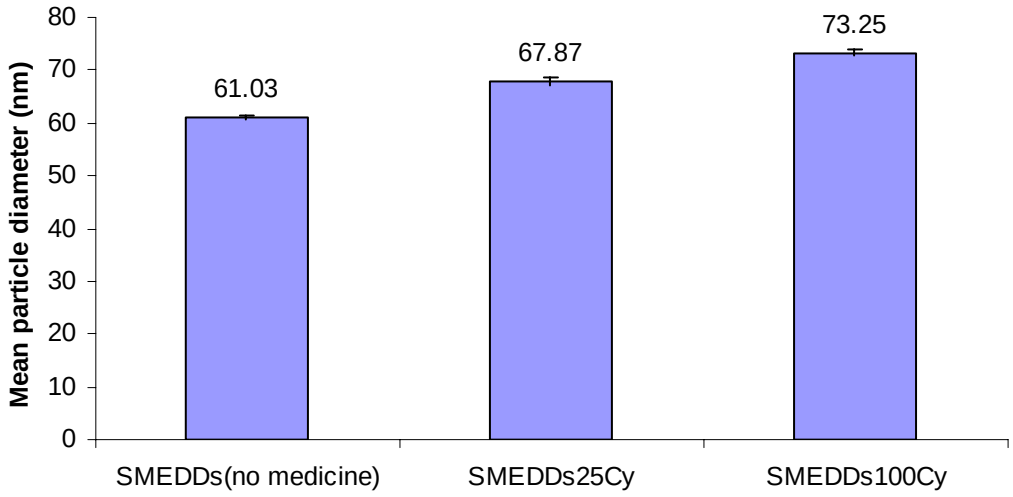


Figure 21 Particle sizes of cyclosporin A loaded SMEDDs

SMEDDs = $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG.

SMEDDs 25 Cy $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG Loaded 25 Cyclosporin A

SMEDDs100 Cy = $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG Loaded 100 Cyclosporin A

b)Transmission electron microscopy (TEM)

Figure 22 showsTEM photomicrographs SMEDDs 25CyA (Figure 27A) and SMEDDs 100Cy(Figure 22B) after dilute with water at the ratio of 1:100. Spherical particles droplet size in the range 200 nm could be seen. These was different form droplet size of SMEDDs 100CyA determined by PCS method which was not more 100 nm. These might be explained that the droplets would be flatten or fused together when dried.

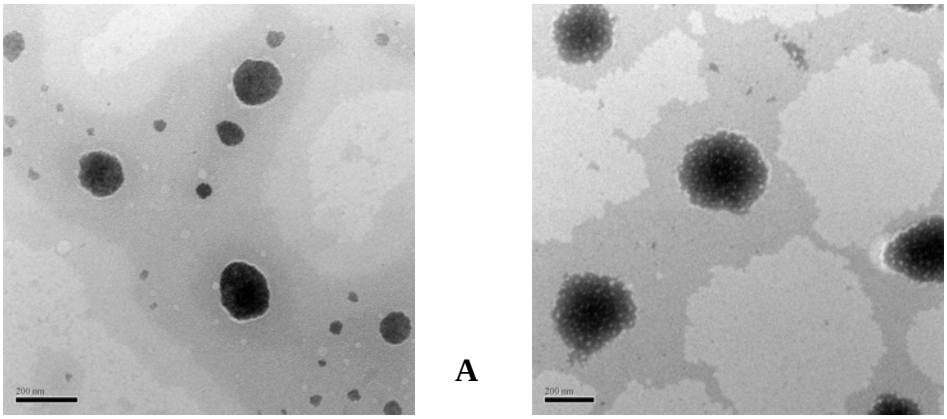


Figure 22. TEM photomicrographs of
A : $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG Loaded 25 Cyclosporin A
B : $35C_{300}40C_{EL}25T_{80}$+5EtOH5PG Loaded 100 Cyclosporin A

3.2.3 Polarrized light microscopy

There was no birefringent phenomenon appeared for cyclosporin A loaded SMEDDs formulas similar to blank SMEDDs**.**

3.2.4 Viscosity determination

The viscosity of blank SMEDDs and after load 25 and 100 mg of cyclosporin were 269.02±0.39, 167.25±0.34 and 250.00±0.00 cP respectively as shown in Table 14. The result of dynamic viscosity test shoed that drug loaded SMEDDs formulas were Newtonian fluid similar to blank SMEDDs. The viscosity of SMEDDs was appropriate for filling by machine. Normally viscosity would increase when solid was added in liquid system. However, in this study the viscosity of blank SMEDDs was higher than that of cyclosporin loaded SMEDDs. This might due to storage condition. The cyclosporin loaded SMEDDs was freshly prepared and kept in the well closed condition, plastic vial with cap, while blank SMEDDs, due to bulk prepared, were kept in beaker and wrapped around by paraflim that ethanol might easily evaporated out of systems that resulted in increased viscosity of mixture. However, 100 mg Cyclosporin loaded SMEDDs exhibited higher viscosity than 25 mg loaded. The

relationship between shear rate and shear stress of SMEDDs before and after loaded cyclosporin A were plotted as shown in Figure 23.

Table 14. Viscosity of selected SMEDDs at 50 rpm

SMEDDs formula	Viscosity (cP)
$35C_{300}40C_{EL}25T_{80}$+5EtOH5PG (blank SMEDDs)	269.02±0.39
SMEDDs 25CyA	167.25±0.34
SMEDDs 100CyA	250.00±0.00

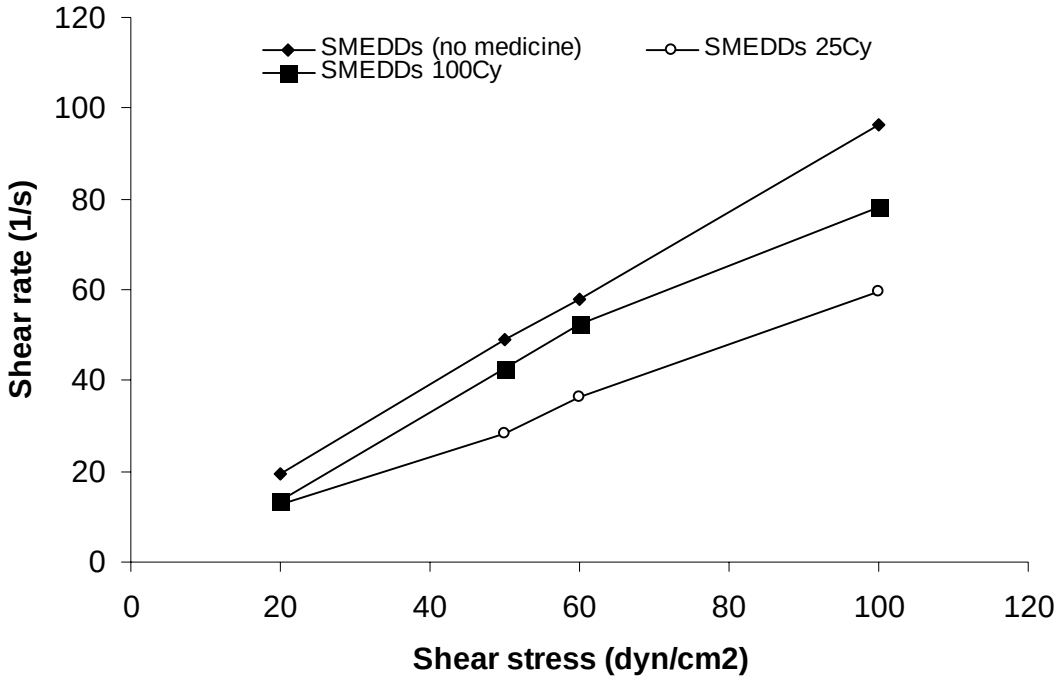


Figure 23. Shear rate-Shear stress relationships of SMEDDsCy by using spindle NO. 31.

3.2.5 pH determination

The pH of microemulsion form cyclosporin A loaded microemulsion did not changed from before drug loaded as shown in Table 15. This result was in agreement with previous study of blank SMEDDs.

Table 15. The pH determination of the loaded cyclosporin microemulsion systems.

SMEDDs	pH value	
	Water	**microemulsion**
$35C_{300}40C_{EL}25T_{80}$+5EtOH5PG (blank SMEDDs)	6.82±0.01	6.83±0.02
SMEDDs 25CyA	6.96±0.05	6.95±0.01
SMEDDs 100CyA	6.88±0.02	6.88±0.01

3.3 *In vitro* drug release studies

Cyclosporin which passed through the dialysis membrane into the receivers part after 24 hours drug release study could not be detect. This was due to the molecular size of the active drug in the particles. The cut off molecular weight of dialysis bag is 12000 dalton while molecular weight of cyclosporin A is 1202.6 dalton. Thus, cyclosporin molecule should be able to pass through the membrane pores. Aliabadi et al (2005) reported that the 70% of cyclosporin A in ethanolic solution was transferred through dialysis membrane MW cut off 12000 to BSA solution with in 2 hours at 37°C. The previous study (Ugozio et al, 2002) found that only 4% cyclosporine A was release from solid lipid nanoparticle through the dialysis bag with MW cut off 12000 dalton after 12 hours. Italia et al (2007) report that cyclosporine A slowed release from PLGA nanoparticles up to 23 days. Thus the present study assumed that cyclosporine A were entrapped in the microemulsion droplet and not released to the solution which was water. However for the absorption of lipid base delivery system, the droplets of oil or triglyceride would be emulsified by bile salt and digested by lipase or absorb via lipid absorption part way as the whole droplet of emulsion.(Christopher et al, 2007). Moreover this study also investigate the release of cyclosporine A from microemulsuion form Neoral® capsules, the results were similar to the SMEDDs CyA .

3.6 Determination of drug content

Uniformity of dosage unit

The content uniformity of freshly prepared cyclosporin A loaded SMEDDs capsules is shown in Table16. For 25 mg cyclosporin A loaded SMEDDs capsules, the content was in the range of 100.16- 103.48 % of the label amount and percentage of coefficicient variation (%CV) was 1.15. For 100 mg cyclosporin A loaded SMEDDs capsules the content was in the range of 102.85- 106.01 % of the label amount and percentage of coefficicient variation (%CV) was 1.55 (Table17). The results passed the specification of general monograph of USP 26, That the content had to be range within the of 90.0- 110 % of label amount and percentage of coefficient variation had to be less than 6.

Table 16. Content uniformity of freshly prepared 25 mg Cyclosporin A loaded SMEDDs.

Capsule NO.	**Weight of 25Cy SMEDDs (mg)**	**Total (mg)**	**% labeled amount**
1	501.2	25.79	103.17
2	502.3	25.87	103.48
3	501.3	25.04	100.16
4	504.2	25.70	102.80
5	502.7	25.57	102.29
6	501.2	25.61	102.45
Average	502.15	0.26	102.39
SD	1.19	0.0029	1.18
%CV	0.24	1.15	1.15

Table 17. Content uniformity of freshly prepared 100 mg Cyclosporin A loaded SMEDDs.

Capsule NO.	Weight of 100 Cy SMEDDs (mg)	Total (mg)	% labeled amount
1	900.0	106.01	106.01
2	900.1	101.85	101.85
3	900.5	102.85	102.85
4	900.5	103.83	103.83
5	900.0	102.34	102.34
6	900.8	105.01	105.01
Average	900.48	103.65	103.65
SD	0.59	1.61	1.61
%CV	0.06	1.55	1.55

3.7 Releases of cyclosporin A loaded SMEDDs capsules

Figure 24 illustrates the release profile of 25mg cyclosporin A loaded SMEDDs capsules after 7 days and after storage for 4 months at room temperature that percent cumulative reached to 97.25±3.00% and 97.35±3.00% in 60 minutes respectively. However release profile showed that after storage the cyclosporin A loaded SMEDDs capsules at ambient condition for 4 months, the release profile were slightly decreased. These results might be due to the change of capsule shell property after storage. However in 60 minutes all capsule were completely dissolved .

Figure 25 illustrates the release profile of 100 mg cyclosporin A loaded SMEDDs capsules after 7 days and after storage for 4 months at room temperature The release profile showed that percent cumulative can reach to 105.70 ±0.87% and 104.91±2.65% in 60 minutes after storage at ambient condition and 4 months respectively. This result was similar to 25mg cyclosporin A loaded SMEDDs capsules.

The moisture in capsule shell functions as a plasticizer to impart flexibility in hard gelatin capsule. Variation on moisture content of gelatin capsule shell which was

changed after storage condition or moisture transfer between the capsule shell and its content may lead to undesired physical properties, such as brittleness and stickiness (Chang R.K. et al). This effect seemed clearly after capsules were storage in accelerated condition. All of capsules which stored at 40 °C 75% RH for 4 months were undissolved in 30 to 60 minute in release study. Heat was enhancing factor of moisture loss. Moreover ethanol in SMEDDs formulation could be diffused though gelatin shells leaded to changed property of gelatin capsule and the rate of diffusion increased due to high temperature (Moteton et al, 1998). The lost of ethanol also owing to slower release rate after as shown in Figures 24 and 25. The slow release rate might be explained by the effect of increasing viscosity, ethanol reduced viscosity of formulation when it lost viscosity could be increased and leaded slow release of SMEDDs.

The capsules package was also important part to keep capsule for remained in good condition. This study, capsules were kept in the glass bottle with cap and wrapped by Parafilm®. It was insufficiency to protected moisture loss from the capsule shell, aluminum foil seemed suitable package for this dosage form. As seen in commercial products, Neoral® was packed in aluminum foil. However, every the capsule which storage at ambient condition for 4 months clouded ruptured in 15 minutes after release test. That passed the specification of general monograph of USP 30.

In additional, according to dissolution profile 100 mg cyclosporin A loaded SMEDDs capsules provided slower rate of dissolution than 25 mg cyclosporin A loaded SMEDDs capsules. These results were explained by the ratios of oil contained in the formulation. Due to 100 mg of cyclosporin A were added in formulation by substituted the other ingredients thus the ratio of cyclosporin A : other ingredients was higher than the ratio of cyclosporine A : other ingredients of 25 mg cyclosporin A loaded SMEDDs. The higher % drug loaded caused the higher dissolution rate.

Figures 26 and 27 showed the release profiles of 25 and 100mg cyclosporin A loaded SMEDDs capsules after storage at 40 °C 75% RH when cut the capsules before dissolution test compared with the dissolution profile of 25 mg and 100 mg cyclosporin A loaded SMEDDs at 7 days at ambient condition. The results revealed

that cyclosporin A released from SMEDDs and reach to 101.30±0.35% and 102.08±1.07% in 60 minutes. Thus, there were concluded that the effect of storage condition did not change SMEDDs system releases property but the outer capsule shell could not tolerated the high temperature and become a barrier against drug release to the medium solution.

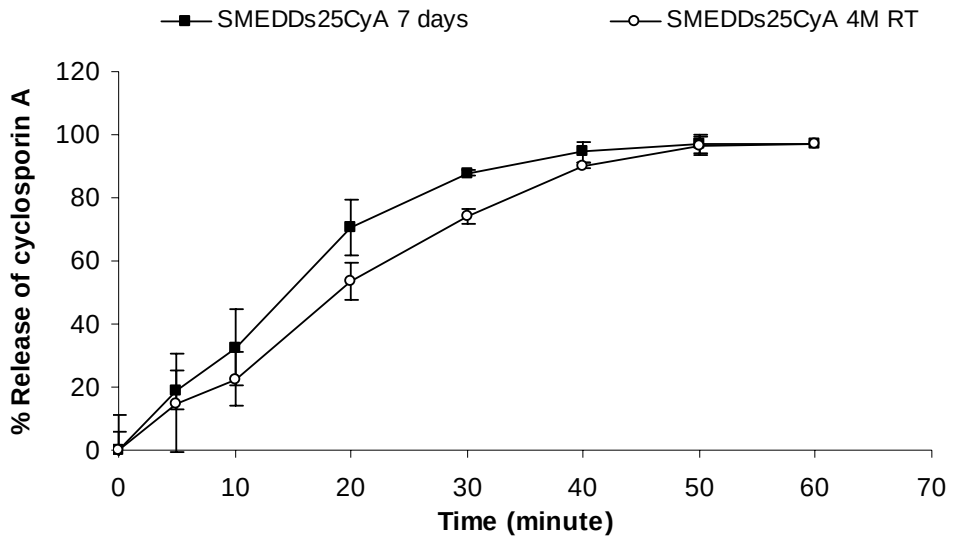


Figure 24. The comparison of dissolution profiles of 25 mg Cyclosporin A loaded SMEDDs at initial and after kept for 4 months at ambient condition

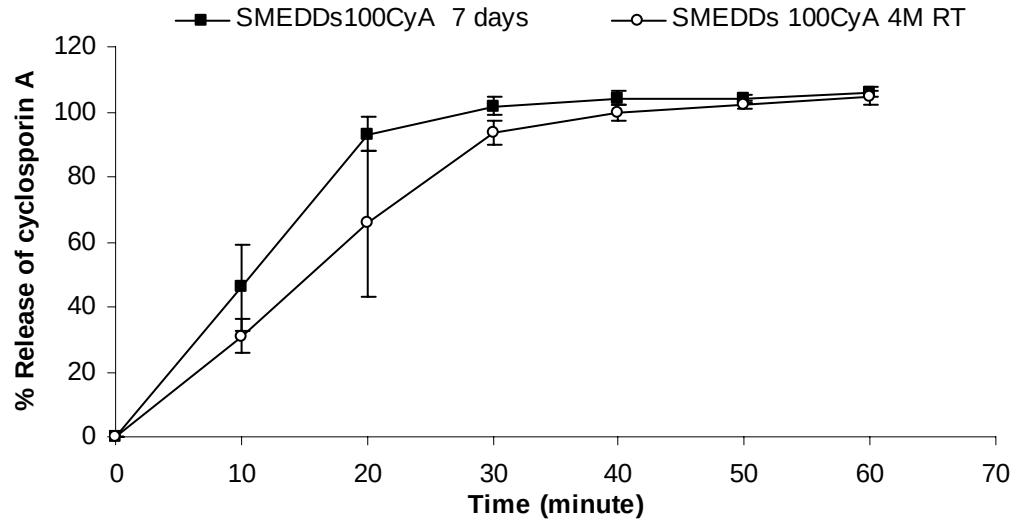


Figure 25. The comparison of release profile of 100 mg Cyclosporin A loaded SMEDDs at initial and after 4 months at ambient condition

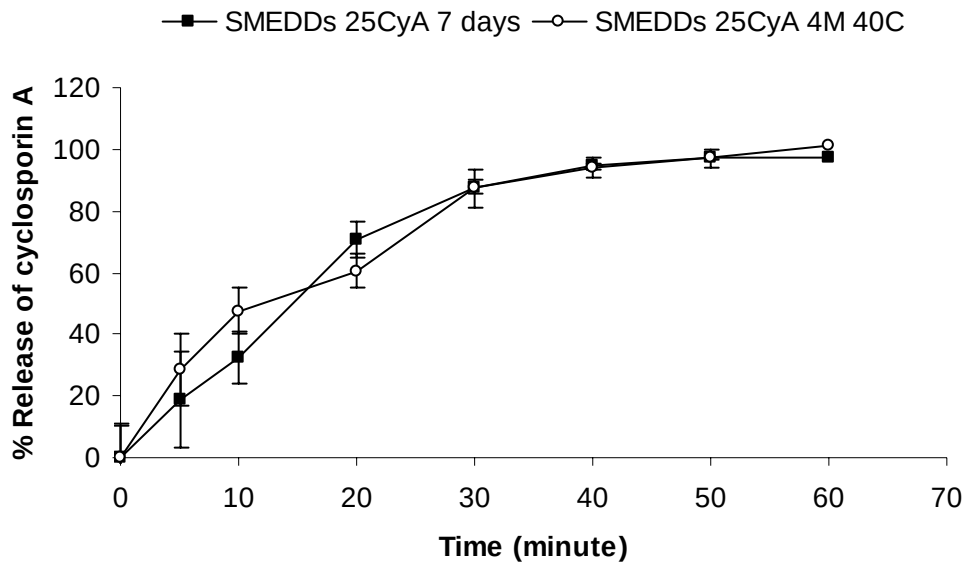


Figure 26. The comparisonsof release profile of 25 mg Cyclosporin A loaded SMEDDs after storage at 7days at ambient condition and 40 °C 75% RH for 4 months

* The capsules shell were cut before release testing

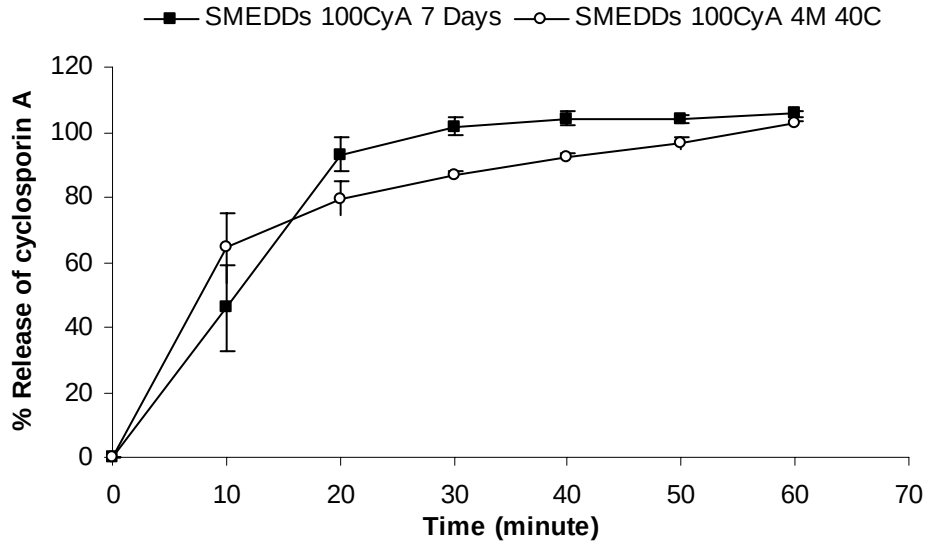


Figure 27. The comparisons of release profile of 100 mg Cyclosporin A loaded SMEDDs after storage at 7days at ambient condition and 40 ºC for 4 months.

* The capsules shell were cut before release testing

3.8 The Stability study

Physical appearances

In the stability study, cyclosporin A loaded SMEDDs capsule were stored at 40 ºC 75% RH for 4 months. After storage, the color of capsule seemed to be darker in both of SMEDDs25CyA and SMEDDs100CyA capsules as shown in Figure 28. This was due to the oxidation of double bond of Cremophor El chain. Cremophor EL was produced from castor oil had an alkyl group in the side chain. The oxidation of alkyl group resulted in darker colored capsule. The capsule shells also looked dull in color. It was noticed that some oil covered at the outer shells of some capsules indicating of SMEDDs leakage. This leakage could be occurred because the capsules were filled and sealed by manual process.

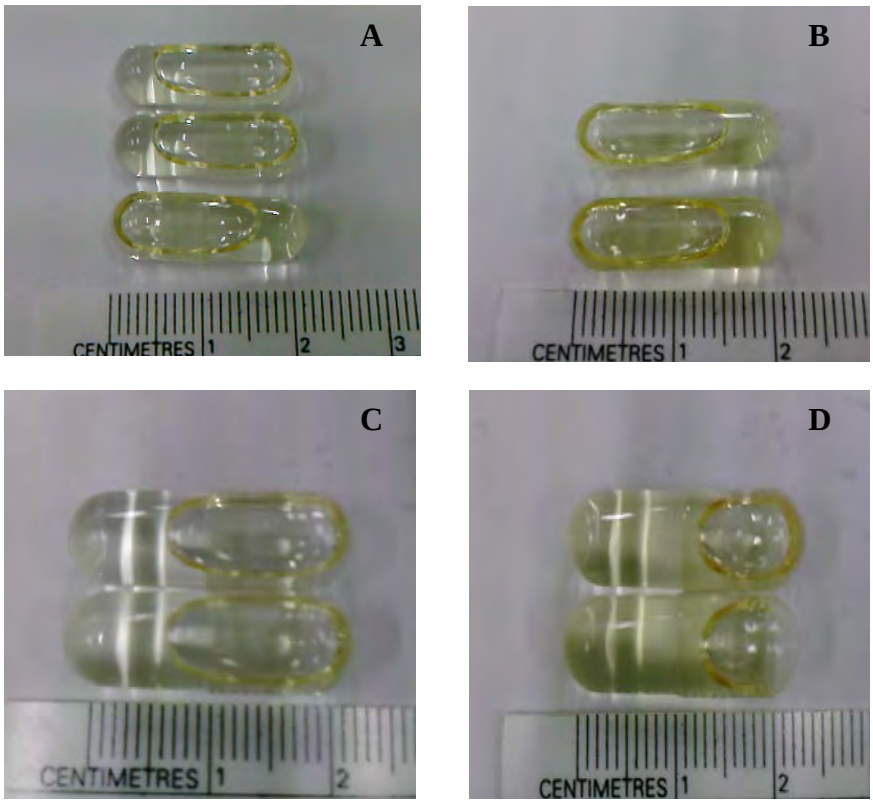


Figure 28. The physical appearance of cyclosporin A loaded SMEDDs capsules
A : 25 mg cyclosporin A loaded SMEDDs capsule after 7 days at ambient condition.
B : 25 mg cyclosporin A loaded SMEDDs capsule after 40 days at 40°C 75RH.
C : 100 mg cyclosporin A loaded SMEDDs capsule after 7 days at ambient condition.
D : 100 mg cyclosporin A loaded SMEDDs capsule after 40 days at 40°C 75RH.

Chemical stability study

The percent content of cyclosporin A loaded SMEDDs capsule is presented at Figure 29. The result showed that there. The analysis of variance (ANOVA) of was no difference in residual percent content of cyclosporin loaded SMEDDs capsule at before and after storage in accelerate condition for 2 months and 4 months ($P < 0.05$). These result agreed with a previous study that investigated the stability of cyclosporin A in stress condition. The cyclosporin is a stable molecule. It degradation pathways had been reported to be dehydration and loss of side chain in strong acidcondition(Manish et al, 2001). In this case, it could assume that the prepared cyclosporin A SMEDDs were stable in long time storage.

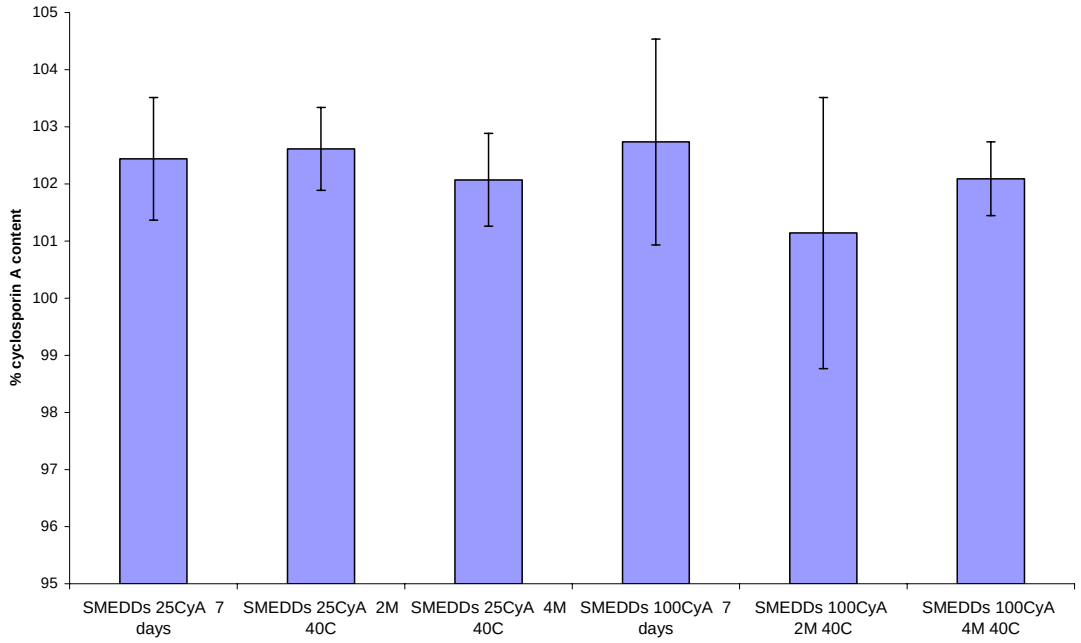


Figure 29. The comparisons of Cyclosporin A content of SMEDDs 25Cy capsule and SMEDDs 100 Cy capsule at 7 days at ambient and 2 months and 4 months at 40 ^{o}C 75% RH.

4. The Dry powder of Self -Microemulsifying Drug Delivery system containing cyclosporin A (SMEDDs Cy-DP)

4.1 Determination of SMEDDs absorbability of absorbent materials

Avicel® PH101, anhydrous lactose, dicalcium phosphate and activated charcoal were selected to test ability of the absortion to SMEDDs. The % carr's index of Avicel® PH101, anhydrous lactose, dicalcium phosphate and activated charcoal could be classified as poor, fair, poor and extremely poor, respectively as presented in Figure 30. These result indicated that the absorbents were poor flow but it could be compressed. The results of amount of absorbent required to absorb 3 g of SMEDDs are presented at Table 18. It was shown that activated charcoal and Avicel® PH101 required small quantity for absorbtion as 2 and 3 grams respectively. After absorption, activated charcoal appeared as dry granule while Avicel® PH101 appeared as loose damp mass which was appropriate to be sieved and made granule. The reason of activated charcoal was the best absorbent due to its physical character. Activated charcoal is the porous carbon only one gram of activated carbon can have a surface area in excess of 500 m^2, with 1500 m^2 being readily achievable. On other hand, anhydrous lactose and dicalcium phosphate required large amount to absorb and became hard sticky damp mass which was unable to be sieved.

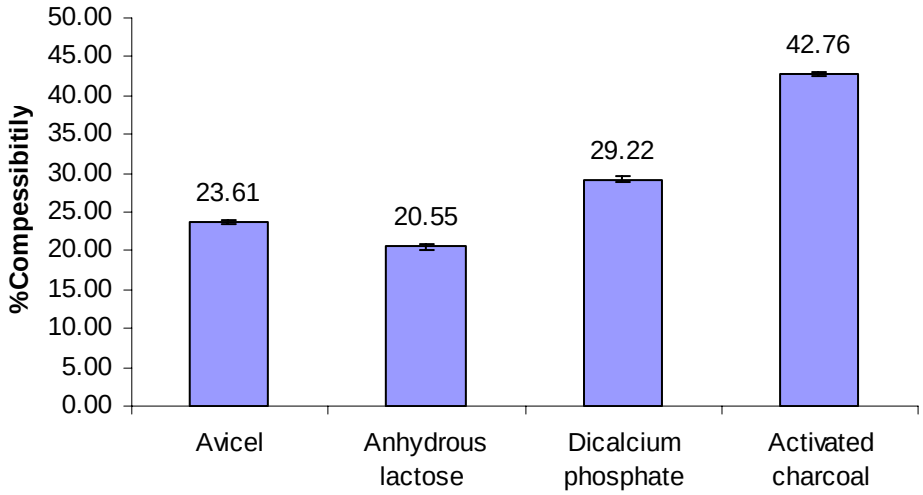


Figure 30. The comparison of %compressibility of absorbent

Table 18. The quantity of absorbent used to absorb 3 g of SMEDDs

Absorbent	**Quantity (g)**	**Physical appearance**
Avicel® PH 101	3	Loose damp mass,+
Anhydrous lactose	9	Thick paste, -
Dicalcium phosphate	8	Thick paste,-
Activated charcoal	2	Dry small granule,+

+ = Easy to be sieved through hand sieve NO.20

- = Difficult to be sieved through hand sieve NO.20

4.2 Preparation of dry powder self-microemulsifying drug delivery system cyclosporin A (SMEDDs CyA-DP) granule and dry powder of oil solution containing cyclosporin (OSCyA-DP) granule.

Although the SMEDDs systems could increase the solubility of cyclosporin A, they had a limitation on a dosage form production was limited due to be liquid system which needed special machine such as filling and sealing machine to be filled into capsule. Absorbent would be used to absorbed SMEDDs and transform into a solid dosage form which would be easily prepared with no requirement a special machine.

From the results of the absorption study, Avicel® PH101 was chosen as absorbent. Activated charcoal was excluded even it had the best absorption ability to SMEDDs because it could absorb other medicine or other nutrition. In addition it was possible that the absorbed SMEDDs was unable to be released.

One capsule of SMEDDs 100Cy (0.9 g) was divided to 4 capsules of SMEDDs 25Cy-DP. The ingredients of SMEDDs Cy-DP were formulated as described in Table 18. The Cyclosporin A in oil solution as conventional dosage form was selected to compare with SMEDDs. Similar to SMEDDs, cyclosporin A in Oil solution was prepared with the same excipients and method as SMEDDs. The formulation of dry powder cyclosporin in oil solution is also shown at Table 18.

Table19. Formulation of SMEDDs25CyA-DP and OSs25CyA-DP

Formulation	***Ingredient***	***Quantity per 1 capsule(mg)***	% in the formulation
Cyclosporin A 25 mg SMEDDs Dry powder (SMEDDs25Cy-DP)	SMEDDs100Cy	0.225	43.25
	Avicel PH101	0.281	54.05
	PVP K90	0.014	2.7
Total		0.520	100
Cyclosporin A 25 mg Oil solution Dry powder (OSs25Cy-DP)	OS 100Cy	0.225	43.25
	Avicel PH101	0.281	54.05
	PVP K90	0.014	2.7
Total		0.520	100

4.3. Determination of granule

The results of granule preparation are shown at the Table 20. Percentage of car's index of SMEDDs CyA-DP and OSCyA-DP were 19.23± 0.2 %and 18.34± 0.32 % that could be classified as fair flow ability. The angle of repose were 28.5 ± 0.12 and 26.6 ± 0.24 which could be classified as good flow. These results of granules were better than those powder form of absorbent due to the agglomeration to larger size, thus improved the flow property. Those results were correlated with the results of flow rate, 1.34 ± 0.02 (g/sec) and 1.52 ± 0.03(g/sec) for SMEDDs CyA-DP and OSCyA-DP respectively. The Relationship between flows ability, angle of repose, Carr's index are shown in Table 21. It could be predicted that the both prepared granules were able to flow during the process of filling into capsules. These granules were compressed by hand press mold to form a cylinder shape before disintegration test. r. The SMEDDs CyA-DP and OSCyA-DP granule disintegration time were 20 ± 1 minuets and 24.4± 1.53 minutes, respectively, which were compendially acceptabl.

Table20. The determination of prepared granules

Fomulation	**%carr's index**	**Angle of repose**	**Flow rate(g/sec)**	**Disintegration time(min)**
SMEDDs25 CY-DP	19.23± 0.2	28.5 ± 0.12	1.34 ± 0.02	20 ± 1
OS25Cy-DP	18.34± 0.32	26.6 ± 0.24	1.52 ± 0.03	24.4± 1.53

Table 21. Relationship between flow, angle of repose, Carr's index fee power flow

Flow	**Angle of repose**	**Carr's index (%)**
Excellent	<25	5-15
Good	25-30	12-16
Fair to passable	30-40	18-21
Poor	> 40	23-35
Very Poor		33-38
Extremely Poor		>40

4.4 Determination of drug content

Content uniformity

The content uniformity of freshly prepared SMEDDs Cy-DP and OSCy-DP capsules is shown in Table 22. The content SMEDDs CyA-DP capsules, was 92.74 % of the label amount and percentage of coefficicient variation (%CV) was 0.54 (Table 22). For OSCyA-DP capsules, the content was 92.80 % of the label amount and percentage of coefficicient variation (%CV) was 0.85 (Table 23). The results passed the specification of general monograph of USP 30, that the content should be range of 90.0- 110 % of label amount and percentage of coefficient variation was less than 6.

Table 22. Content uniformity of 25 mg freshly prepared cyclosporin A loaded SMEDDs Dry powder.

Capsule NO.	Weight of 25 Cy SMEDDs -DP(mg)	Total (mg)	% label amount
1	523.2	23.30	93.21
2	524	23.06	92.22
3	522.7	23.19	92.77
Average	523.3	23.18	92.74
SD	0.656	0.124	0.496
%CV	0.12	0.54	0.54

Table 23. Content uniformity of freshly prepared 25 mg Cyclosporin A loaded Oil solution Dry powder

Capsule NO.	Weight of 25 Cy SMEDDs -OP(mg)	Total (mg)	% label amount
1	520.1	23.06	92.23
2	521.5	23.12	92.59
3	523.0	23.43	93.70
Average	521.53	23.20	92.80
SD	1.45	0.20	0.785
%CV	0.28	0.84	0.85

4.5 Dissolution of dry powder of SMEDDs cyclosporin A containing capsules and dry powder of oil solution containing cyclosporin A capsules.

Figures 31 and 32 illustrate the dissolution profiles of SMEDDs25CyA-DP capsules after 7 days and after storage at heat-cool condition at -4 °C and 40 °C with 5 cycles. The dissolution profile showed that percent cumulative can reach to 86.49±1.40% and 84.63±1.53% in 60 minutes after 7 days and after storage at 5 cycles under freeze thaw condition respectively. The percent cumulative at 60 minutes rather low after compared with % content at initial (94.61±2.87mg). The results might be explained that the SMEDDs could not completely dissolved, some still absorbed on the surfaced of Avicel® PH 101 moreover the binder, PVP K90, could be a barrier to sustain it's released. It might required longer time of more than 60 minutes for completely released due to the dissolution profile graph it showed that % cumulative at 60 minute was not completely smooth.

For OS25CyA-DP, figures 31 and 32 illustrate the dissolution profiles of OS25CyA-DP capsules after 7 days and after storage at heat-cool condition at -4 °C and 40 °C with 5 cycles. The dissolution profile could not show the accurate value. The results might be explained that when cyclosporin in oil solution dissolved in water, oil droplet would be rapidly floated to the water surface it did not suspended to be homogenous mixture like microemulsion. The tip of the sample collector was placed at the center of dissolution vessel thus it could not collect the oil droplets.

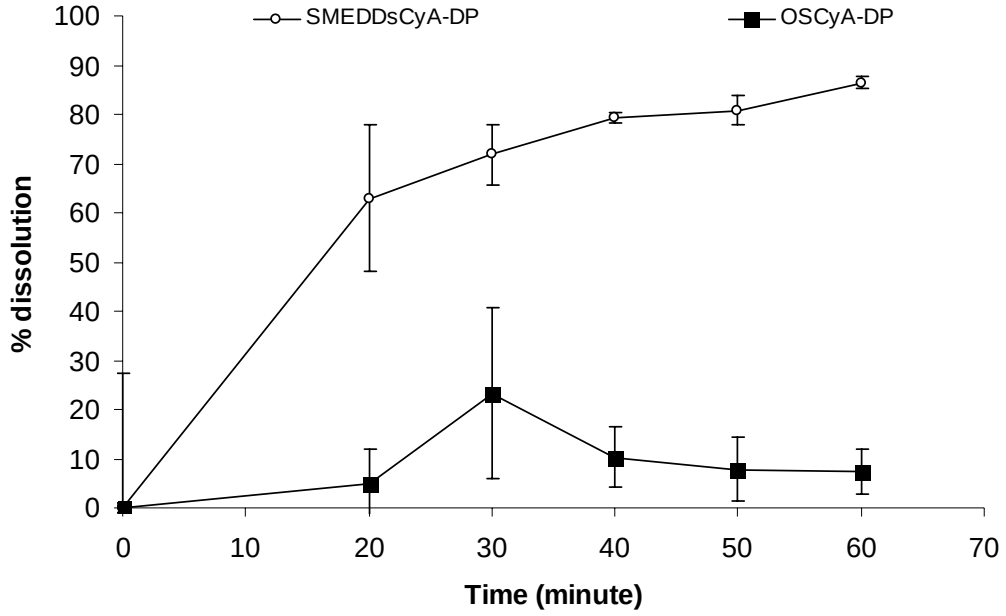


Figure 31. %Dissolution of SMEDDs 25 Cy-DP and OS 25 Cy-DP after storage 7 days at ambient condition.

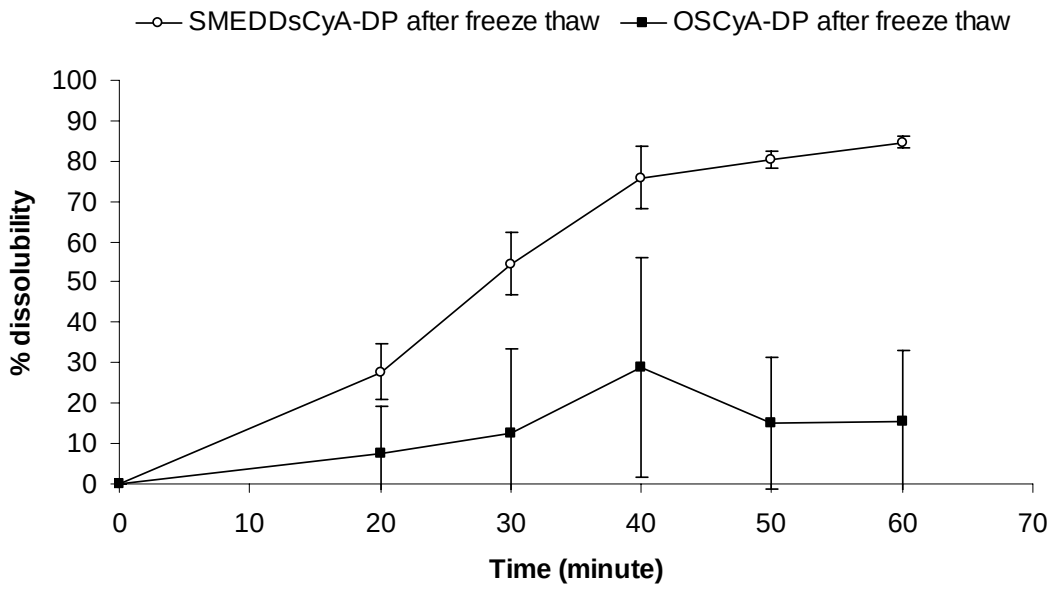


Figure 32. %Dissolution of SMEDDs 25 CyA-DP and OS25 CyA-DP after freeze-thaw condition.

4.6 Stability study

Physical appearances

This study SMEDDsCyA-DP and OSCyA-DP capsules were stored at freeze thaw condition at -4 ºC and 40 ºC with 5 cycles (Ashok and Pradeep, 2007). After storage at freeze thaw condition, the physical appearances of capsules were not changed. Figure 33 shown physical appearances of SMEDDs25CyA-DP capsules and OS25CyA -DP capsules after freshly prepared and after storage at -4 ºC and 40 ºC 5 cycles.

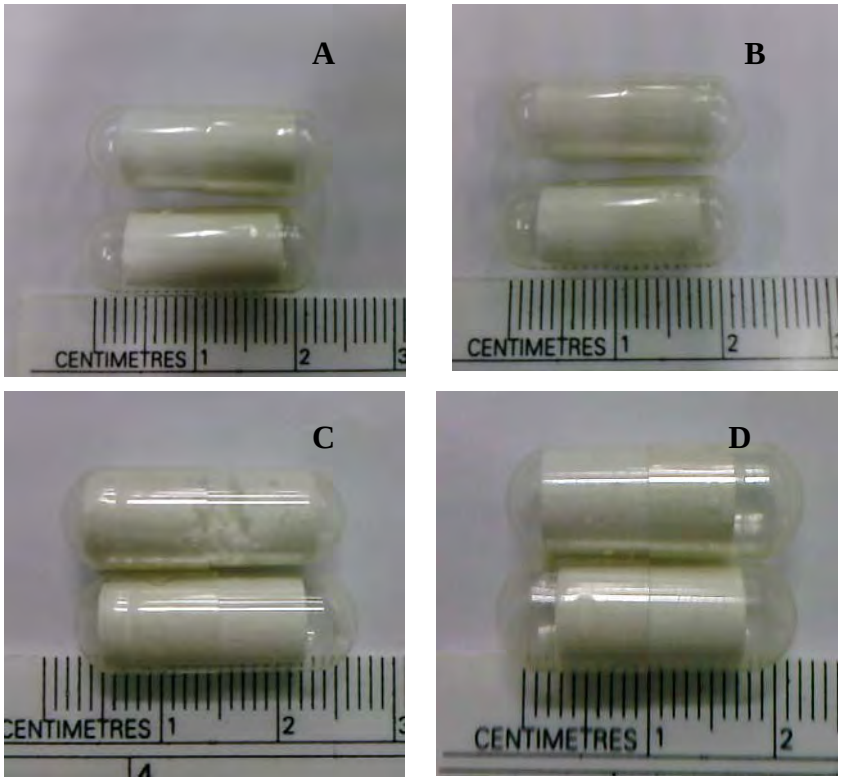


Figure 33. The physical appearance of SMEDDs25CyA-DP and OS25CyA-DP capsules
A : Freshly prepared SMEDDs25CyA-DP capsules.
B : Freshly prepared OS25CyA -DP capsules.
C : SMEDDs25CyA-DP capsules after storage at -4 °C and 40 °C 5 cycles.
D : OS25CyA -DP capsules after storage at -4 °C and 40 °C 5 cycles.

Chemical stability study

The percent content of cyclosporin A loaded SMEDDsCyA-DP capsule and OSCyCyA-DP are presented at Figure 34. The comparedT-test of was no difference in residual percent content in each group of SMEDDsCyA-DP capsule and OSCyA-DP capsule at before and after storage in freeze-thaw condition for 5 cycles ($P > 0.05$). This result was similar with a SMEDDs Cy capsules stability study. In this case, it could be assumed that the prepared cyclosporin A SMEDDs as dry granule in capsule were stable in long time storage.

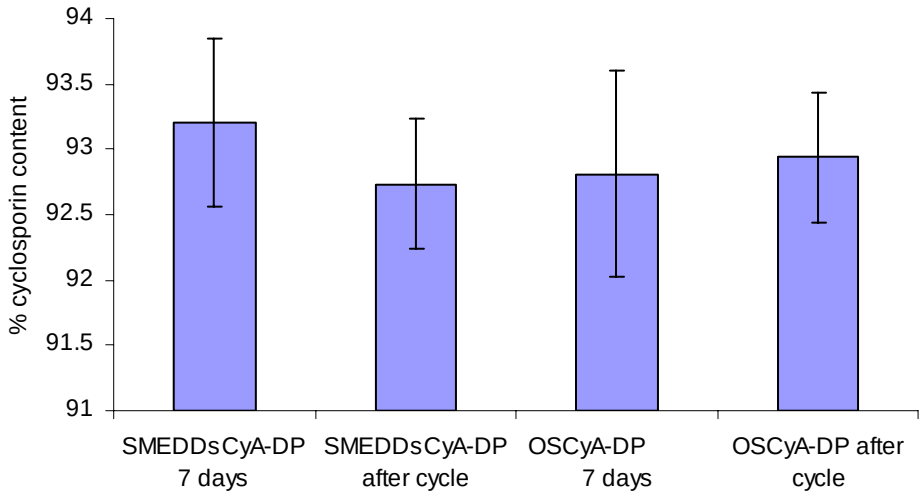


Figure 34. The comparisons of Cyclosporin A content of SMEDDs 25CyA-DP capsule and OS25CyA-DP capsule at 7 days at ambient and after heat-cool condition at -4 °C and 40 °C with 5 cycles (17days).

CHAPTER V
CONCLUSIONS

The study showed that micoemulsion could be prepared using commercially available and pharmaceutically acceptable excipients.

Overall results were as follows :

1. Preparation of microemulsion
    1. Types and ratios of surfactant affected the existing region of microemulsion. Cremophor EL and Tween 80 provided the largest area of microemulsion.
    2. Combined surfactant of Cremophor EL and Tween 80 provided the area of microemulsion similar to single surfactant.

2. Formulation of Self -Microemulsifying Drug Delivery system (SMEDDs)
    1. The physical appearance of SMEDDs formula depends on the ingredients of formula.
    2. Dilution ratio had an effect on the droplet size that the size at ratio 1:50 size of was larger than the size measured at dilution ratio 1:100, 1:200.
    3. The microemulsions were spherical droplet when viewed under TEM and SEM.
    4. As co-solvent, propylene glycol and ethanol provided high cyclosporin solubility in oil, while glycerin provided the largest area of microemulsion. .
    5. The formulation of C_{300} : C_{EL} : T_{80} at ratio 40:40:20 and 40:50:10 with 5%EtOH +5%GLy ($40C_{300}40C_{EL}20T_{80}$ +5EtOH5Gly and $40C_{300}50C_{EL}10T_{80}$ +5EtOH5Gly) and formulation of C_{300} : C_{EL} : T_{80} at ratio 35:32.5:32.5 and 35:40:25 with 5%EtOH +5%PgG($35C_{300}32.5C_{EL}32.5T_{80}$ +5EtOH5PG and $35C_{300}40C_{EL}25T_{80}$ +5EtOH5PG) were chosen because they contained highest ratio of oil : surfactant.

6. SMEDDs are Newtonian liquid. All ingredients incorporate also had effect on the viscosity of SMEDDs. Increasing the amount of surfactant would increase the viscosity of the system.
7. The pH of water did not change when water was added to SMEDDs ratio 1:100.

3. The Self -Microemulsifying Drug Delivery system containing Cyclosporin A (SEMDDs CyA)

1. SMEDDs containing propylene glycol was the suitable formulation for loaded cyclosporin while SMEDDs containing glycerin was precipitation after loaded cyclosporin.
2. Droplet size of SMEDDs was larger after loaded drug.
3. The % content of prepared SEMDDs CyA capsule before and after storage at 40°C 75% RH for 4 months passed the specification of general monograph of USP 30 and % dissolution at 60 minutes were 97.25±3.00% and 97.35±3.00%.
4. Color of SMEDDs was darken after storage at 40ºC 75% RH for 4 months.
5. The outer capsule shell became brittle and lost elastic property after storage at 40ºC 75% RH for 4 months.

4. The Dry powder of Self -Microemulsifying Drug Delivery system containing cyclosporin A (SMEDDs Cy-DPA)

1 Avicel®PH101 was the suitable absorbent for preparing the granule.
2. The % content was within the limit of compendium and unchanged before and after freeze thaw condition at -4 °C and 40 °C for 5 cycles.
3. The dissolution rate of SMEDDs CyA-DP was slower than SMEDDs.

5. *In vitro* drug release studies

Microemulsion was unable to pass the dialysis membrane.

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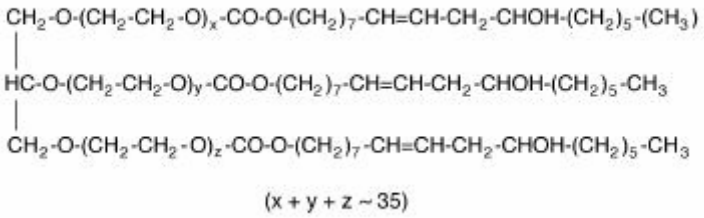
APPENDICES

APPENDIX A

Physicochemical Properties of Microemulsion compositions

1. Cremophor® EL (Kibbe, 2000) (Cremophor EL, Technical Information sheet, BASF company)

Chemical structure



Chemical name	Polyoxyethylenglyceroltriricinoleat 35 (DeutscherArzneimittelcodex), Polyoxyl 35 Castor Oil(USP/NF).
Molecular formular	variable composition, with the major component identified as oxylated triglycerides of ricinoleic acid (polyoxyethylene glycerol triricinoleate 35)
Molecular weight	≈ 3 k Dalton

General properties

Appearance	white to off-white viscous liquid, faint specific odour. The hydrophilic-lipophilic balance (HLB) lies between 12 and 14.
Solubility	forms clear solutions in water. It is also soluble in ethyl alcohol, n-propyl alcohol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene.

Melting point : 26 °C

Relative density : 1.05–1.06g /cm3 at 25°C

Viscosity : 700 – 850 mPa· s

Safety

LD 50 (7 days follow-up period):

Rat oral > 6.4 ml/kg

Rabbit oral > 10.0 ml/kg

Cat oral > 10.0 ml/kg

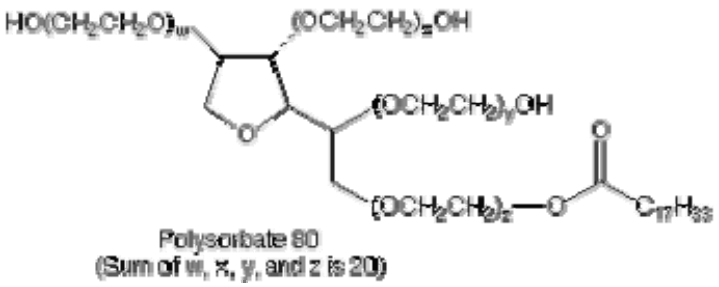
Mouse i. v. 2.5 – 4 ml/kg

Rat percutaneous > 4.0 ml/kg (maximum applicable dose)

No characteristic toxic symptoms were observed after oral doses or application to the skin, and no pathological changes of the inner organs were discernible with the naked eye during autopsy.

2. **Tween 80** (Wade and Weller, 1994)

Chemical Structure



Chemical name Polyoxyethylene 20 sorbitan monooleate

Molecular formular $C_{65}H_{120}O_{26}$

Molecular weight: 1310 g/mole

General properties

Appearance Tween 80 is a clear yellowish or brownish-yellow oily liquid with a faint characteristic odor, somewhat bitter taste. It has a HLB value of 15.0.

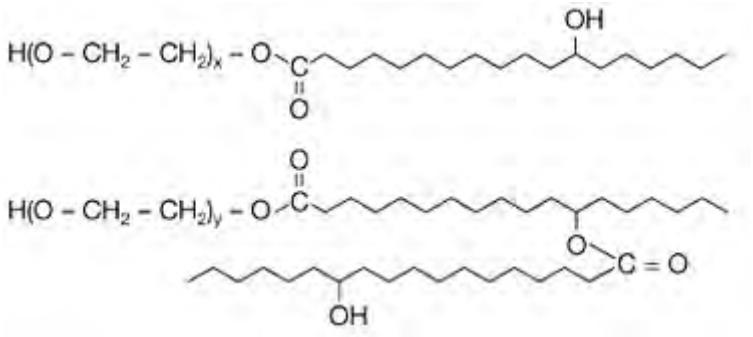
Solubility Tween 80 is miscible with water. alcohol, dehydrate alcohol, ethylacetate, and methyl alcohol; practically insoluble in liquid paraffin and fixed oils.

Safety

Tween 80 is widely used in cosmetics, food products and oral, parenteral and topical pharmaceutical formulations and is generally regarded as nontoxic and nonirritant material. The WHO has set an estimated acceptable daily intake for tween 80, calculated as total polysorbate esters, at up to 25 mg/kg.

3. Solutol® HS 15 (Wade and Weller, 1994) (Solutol HS15, Technical Information sheet ,BASF company)

Chemical structure



Chemical name Macrogol 15 Hydroxystearate, 12-Hydroxystearic acid-polyethylene glycol copolymer

Molecular formular: Consists of polyglycol mono- and di-esters of 12-hydroxystearic acid (= lipophilic part) and of about 30% of free polyethylene glycol (= hydrophilic part).

General properties

Appearance	Yellowish white paste at room temperature that becomes liquid at approx. 30°C. The hydrophilic-lipophilic balance lies between 14 and 16.
Solubility	dissolves in water, ethanol and 2-propanol to form clear solutions. Its solubility in water decreases with increasing temperature. It is insoluble in liquid paraffin.
Melting point	: 30 °C
Viscosity	: 73 mPa.s (60 °C),12 mPas 30% in water (25°C)
Density	: 1.03 g/cm3 (60 °C)

Safety

Acute toxicity

Oral:

LD50/rat/female: > 20,600 mg/kg

Skin irritation:

rabbit: non-irritant

Eye irritation :

rabbit: non-irritant

Sensitization:

Guinea pig maximization test/guinea pig: sensitizing

Open epicutaneous test /guinea pig: sensitizing

Chronic toxicity

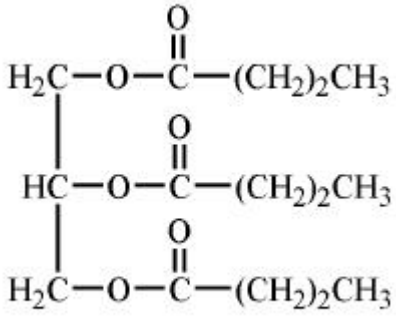
Genetic toxicity:

The substance was not mutagenic in bacteria.

No mutagenic effect was found in various tests with mammalian cell culture and mammals.

4 Medium Chain Triglycerides (Wade and Weller, 1994)

Chemical Formula



Chemical name Medium Chain Triglycerides

Empirical Formula Described in the PhEur 1993, Medium Chain Triglycerides are the fixed oil extracted from the hard, dried fraction of endrosperm of Cocos nucifera L. by hydrolysis, fractionation of the fatty acids were obtained by hydrolysis and then resterification to triglycerides. It consists of a mixture of exclusively short or medium chain triglycerides of fatty acid, of which less than 95% are the saturated fatty acids octanoic (caprylic) acid and decanoic (capric) acid.

Compositions

MCT oil is a lipid fraction of coconut oil and consists primarily of the triglycerides of C8 and C10 saturated fatty acids. Approximate percentages are

Fatty Acid	%
Shorter than C8	<6
C8 (caprylic)	67
C10 (capric)	23
Longer than C10	<4

General properties

Apprearance MCT is a clear, odorless or almost odorless liquid. It solidifies at about °0C and has a low viscosity even at temperatures near its solidification point.

Solibility

MCT is almost insoluble in water, miscible with alcohol, ether and chloroform.

Density : 0.940 to 0.960 g at 20°C

Energy provide : 8.3 Cal/g

Refractive index : 1.450 to 1.453

Surface tension : 31-32 mN/m at 25°C

Viscosity : 25-33 mPa s

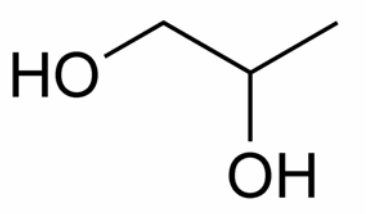
Purity MCT is consist of a mixture of triglycerides having medium acyl chain length of fatty acid (C8 and C10): shorter than C8 (<6%), C8 or octanoic (67%); C10 or decanoic (23%); and larger than C10 (<4%)

Safety

MCT is widely used as a component of lipid emulsion for parenteral nutrition regiments; it is also consumed as an edible oil.

5. Propylene Glycol (Kibbe, 2000)

Structural Formular



Nonproprietary names	BP: Propylene glycol; JP: Propylene glycol; PhEur: Propylenglycolum; USP: Propylene glycol
Synonyms	1,2-Dihydroxypropane; 2-hydroxypropanol; methyl ethylene glycol; methyl glycol; propane-1,2 –diol.
Chemical Name	1,2 propanediol
Chemical Formula	$C_3H_8O_2$
Moleccular Weight	76.1

General Properties

Density:	1.038 g/cm3 at20 °C
Osmolarity:	2.0% v/v aqueous solution is iso-osmotic with serum.
Solubility	Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils, but will dissolve some essential oils.
Surface tension:	40.1 mN/m (40.1 dynes/cm) at 25°C
Viscosity (dynamic):	58.1 mPa s (0.581 P) at 20°C

Safety

Propylene glycol is used in a wide variety of pharmaceutical formulations and is generally regarded as a nontoxic material. Probably as a consequence of its metabolism and excretion, propylene glycol is less toxic than other glycols.

Parenteral administration may cause pain or irritation when used in high concentration.

Propylene glycol is estimated to be one third as intoxicating as ethernol, with administration of large volumes being associate with adverse effects most commonly on the central nervous reactions reported, though

generally isolated. include: ototoxicity; cardiovascular effects; seizures; hyperomolarity and lactic acidosis, both of which occur most frequently in patients with renal impairment.

Based on metabolic and toxicological data, the WHO has set an acceptable daily intake of propylene glycol at up to 25 mg/kg body-weight. Formulations containing 35% propylene glycol can cause hemolysis in humans.

In animal studies, there has been no evidence that propylene glycol is teratoginic or mutagenic. Rats can tolerate a repeated oral daily does of up to 30 mL/kg in the diet over 6 months,while the dog is unaffected by a repeated oral daily does of 2 g/kg in the diet for 2 years.

LD50 (dog, IV): 25.9 g/kg; LD50 (guinea pig, SC): 13-15.5 g/kg

LD50 (mouse, IV): 7.6-8.3 g/kg; LD50 (mouse pig, SC): 15.5-19.2 g/kg

LD50 (rabbit, IV): 6 g/kg; LD50 (rabbit, IM): 5-6.5 g/kg

LD50 (rat, IM): 13-20.7 g/kg; LD50 (rat, IV): 6.2-12.7 g/kg

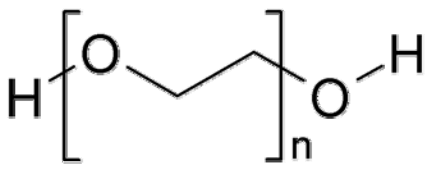
LD50 (rat, SC): 21.7-29 g/kg

Regulatory Status

Included in the FDA Inactive Ingredients Guide (dental preparations, IM and IV injections, inhalations, ophthalmic, oral, otic, percutaneous, rectal, topical, and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in the UK.

6. Polyethylene Glycol 400 (Kibbe, 2000)

Structural Formular



Nonproprietary names	BP:Macrogol 400, JP: Macrogel 400, PhEur: Macrogolum 400, US:Polyethylene glycol
Synonyms	Breox PEG; Hodag PEG; Lutrol E; PEG; polyoxyethylene glycol.
Chemical Names	-Hydro- -hydroxy-poly(oxy-1,2-ethanediyl)
Chemical Formular:	HOCH2(CH2OCH2)MCH2OH

General Properties

Soubility	All grades of polyethylene glycol are soluble in water. Liquid Polyethylene glycols are soluble in acetone. Alcohols, benzene, glycerin, and glycols.
Surface tension	approximately 44 mN/m (44 dynes/cm) for liquid polyethylene glycols;
Density	1.11-1.14 g/cm3 at 25°C for liquid PEGs;
Flash point	238°C for PEG 400;
Freezing point	4-8°C for PEG 400;
Moisture content	Liquid polyethylene glycols are very hydroscopic, although hydroscopic decreases with increasing molecular weight.

Safety

Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials. However, adverse reactions to polyethylene glycols have been reported and relatively low toxicity

Oral administration of large of polyethylene glycols can have a laxative. Therapeutically, up to 4 L of an aqueous mixture of electrolytes and high molecular weight polyethylene glycol is consumed by patients undergoing bowel cleansing. Liquid polyethylene glycols maybe absorbed when taken orally, but the higher molecular weight polyethylene glycols are not significantly absorbed from the gastrointestinal tract. Absorbed polyethylene glycol is excreted largely unchanged in the urine although polyethylene glycols of low molecular weight may be partially metabolized.

The WHO has set an estimated acceptable daily intake of polyethylene glycols at up to 10 mg/kg body-weight.

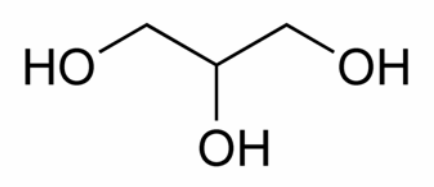
In parenteral products, the maximum recommended concentration of PEG 400 is approximately 30% v/v since hemolytic effects have been observed at concentrations greater than about 40% v/v.

Regulatory Status

Included in the FDA Inactive Ingredients Guide (dental preparation, IM and IV injections, ophthalmic preparations, oral capsules, solutions, syrups and tablets, rectal, topical, and vaginal preparations).

7. Glycerin (Wade and Weer, 1994; Kibbe, 2000)

Chemical structure



Chemical name	Glycerol, 1,2,3-propane-1,23-triohydroxypropane
Molecular formular	C3H8O3
Molecular weight:	92.09 g/mole

General properties

Appearance	Glycerin is a clear, colorless, odorless, syrupy and hygroscopic liquid
Solubility	Glycerin is miscible with water, alcohol and methanol. One part of glycerin dissolve in 11 part of ethyl acetate and in about 500parts of ethyl ether. It is in soluble in benzene, chloroform, ether, mineral oil, fixed and

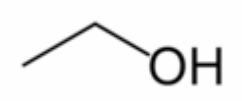
	volatile oils, halogenated hydrocarbons and aromatic hydrocarbons.
Melting point	17.9°C
Hygroscopicity	medium to high
Relative density	1.258-1.263 g/cm3 at °25C
Surface tension	63.4 mN/m at °20C
Viscosity	1490 mPas at °20C, 954 mPas at °25C
Osmolarity	2.6%v/v solution is iso-osmotic with serum

Safety

Glycerin is very large oral dose can expert systemic effects, such as headache, thirst and nausea. Injection of large doses may induce convulsion, paralysis and hemolysis. The oral LD50 in mice is 31.5 g/kg and intravenous LD50 in mice is 7.45 g/kg. Glycerin can be used as solvent for parenteral formulations in concentration up to 50% w/v.

8. Ethanol (Kibbe, 2000)

Chemical structure



Nonproprietary names	BP: Ethanol (96%), USP: Alcohol
Synonyms	Ethyl alcohol; ethyl hydroxide; grain alcohol; methyl carbinol.
Chemical Name	Ethanol
Empirical Formula	C_2H_6O
Molecular Weight	46.07
Structural Formula	C_2H_5OH

General properties

Appearance.	Alcohol is a clear, colorless, mobile and volatile liquid with a slight, characteristic odor and burning taste.
Boiling point	78.15°C
Flammability	readily flammable, burning with a blue, smokeless flame.
Flash point	14°C (closed cup)
Solubility	miscible with chloroform, ether, glycerin and water (with rise of temperature and contraction of volume).
Specific gravity	0.8119-0.8139 at 20°C

Safety

Ethanol and aqueous ethanol solutions are widely used in a variety of pharmaceutical formulations and cosmetics Ethanol is also consumed in alcoholic beverages.

Ethanol is a central nervous system depressant and ingestion of low to moderate quantities can lead to symptoms of intoxication including muscle incoordination, visual impairment, slurred speech, etc. Ingestion of higher concentrations may cause depression of medullary action, lethargy, amnesia, hypothermia, hypoglycemia, stupor, coma, respiratory depression and cardiovascular collapse. The lethal human bloodalcohol concentration is generally estimated to be 400-500 mg/400 mL.

LD_{50} (guinea pig, IP): 3.41 g/kg[5]

LD_{50} (guinea pig, IV): 2.3 g/kg

LD_{50} (guinea pig, oral): 5.56 g/kg

LD_{50} (rabbit, IP): 0.96 g/kg

LD_{50} (rabbit, IV): 2.37 g/kg

LD_{50} (rabbit, oral): 6.3 g/kg

LD_{50} (rat, IP): 3.75 g/kg

LD_{50} (rat, IV): 1.44 g/kg

LD_{50} (rat, oral): 7.06 g/kg

LD_{50} (hamster, IP): 5.07 g/kg

LD_{50} (mouse pig, IP): 0.93 g/kg

LD_{50} (mouse, IV): 1.97 g/kg

LD_{50} (mouse, oral): 7.5 g/kg

LD_{50} (mouse, SC): 8.29 g/kg

APPENDIX B

Data sheet of formulations for pseudo-ternary phase diagrams.

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water.

sample NO.	%OIL	Weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
1	100	1.5	0	0	0	0		
2	95	1.425	0	0	5	0.075		
3	95	1.425	5	0.075	0	0		
4	90	1.35	0	0	10	0.15		
5	90	1.35	5	0.075	5	0.075		
6	90	1.35	10	0.15	0	0		
7	85	1.275	0	0	15	0.225		
8	85	1.275	5	0.075	10	0.15		
9	85	1.275	7.5	0.1125	7.5	0.1125		
10	85	1.275	10	0.15	5	0.075		
11	85	1.275	15	0.225	0	0		
12	80	1.2	0	0	20	0.3		
13	80	1.2	5	0.075	15	0.225		
14	80	1.2	10	0.15	10	0.15		
15	80	1.2	15	0.225	5	0.075		
16	80	1.2	20	0.3	0	0		
17	75	1.125	0	0	25	0.375		
18	75	1.125	5	0.075	20	0.3		
19	75	1.125	10	0.15	15	0.225		
20	75	1.125	12.5	0.1875	12.5	0.1875		
21	75	1.125	15	0.225	10	0.15		
22	75	1.125	20	0.3	5	0.075		
23	75	1.125	25	0.375	0	0		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water.

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
24	70	1.05	0	0	30	0.45		
25	70	1.05	5	0.075	25	0.375		
26	70	1.05	10	0.15	20	0.3		
27	70	1.05	15	0.225	15	0.225		
28	70	1.05	20	0.3	10	0.15		
29	70	1.05	25	0.375	5	0.075		
30	70	1.05	30	0.45	0	0		
31	65	0.975	0	0	35	0.525		
32	65	0.975	5	0.075	30	0.45		
33	65	0.975	10	0.15	25	0.375		
34	65	0.975	15	0.225	20	0.3		
35	65	0.975	17.5	0.2625	17.5	0.2625		
36	65	0.975	20	0.3	15	0.225		
37	65	0.975	25	0.375	10	0.15		
38	65	0.975	30	0.45	5	0.075		
39	65	0.975	35	0.525	0	0		
40	60	0.9	0	0	40	0.6		
41	60	0.9	5	0.075	35	0.525		
42	60	0.9	10	0.15	30	0.45		
43	60	0.9	15	0.225	25	0.375		
44	60	0.9	20	0.3	20	0.3		
45	60	0.9	25	0.375	15	0.225		
46	60	0.9	30	0.45	10	0.15		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water.

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
47	60	0.9	35	0.525	5	0.075		
48	60	0.9	40	0.6	0	0		
49	55	0.825	0	0	45	0.675		
50	55	0.825	5	0.075	40	0.6		
51	55	0.825	10	0.15	35	0.525		
52	55	0.825	15	0.225	30	0.45		
53	55	0.825	20	0.3	25	0.375		
54	55	0.825	22.5	0.3375	22.5	0.3375		
55	55	0.825	25	0.375	20	0.3		
56	55	0.825	30	0.45	15	0.225		
57	55	0.825	35	0.525	10	0.15		
58	55	0.825	40	0.6	5	0.075		
59	55	0.825	45	0.675	0	0		
60	50	0.75	0	0	50	0.75		
61	50	0.75	5	0.075	45	0.675		
62	50	0.75	10	0.15	40	0.6		
63	50	0.75	15	0.225	35	0.525		
64	50	0.75	20	0.3	30	0.45		
65	50	0.75	25	0.375	25	0.375		
66	50	0.75	30	0.45	20	0.3		
67	50	0.75	35	0.525	15	0.225		
68	50	0.75	40	0.6	10	0.15		
69	50	0.75	45	0.675	5	0.075		
70	50	0.75	50	0.75	0	0		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
71	45	0.675	0	0	55	0.825		
72	45	0.675	5	0.075	50	0.75		
73	45	0.675	10	0.15	45	0.675		
74	45	0.675	15	0.225	40	0.6		
75	45	0.675	20	0.3	35	0.525		
76	45	0.675	25	0.375	30	0.45		
77	45	0.675	27.5	0.4125	27.5	0.4125		
78	45	0.675	30	0.45	25	0.375		
79	45	0.675	35	0.525	20	0.3		
80	45	0.675	40	0.6	15	0.225		
81	45	0.675	45	0.675	10	0.15		
82	45	0.675	50	0.75	5	0.075		
83	45	0.675	55	0.825	0	0		
84	40	0.6	0	0	60	0.9		
85	40	0.6	5	0.075	55	0.825		
86	40	0.6	10	0.15	50	0.75		
87	40	0.6	15	0.225	45	0.675		
88	40	0.6	20	0.3	40	0.6		
89	40	0.6	25	0.375	35	0.525		
90	40	0.6	30	0.45	30	0.45		
91	40	0.6	35	0.525	25	0.375		
92	40	0.6	40	0.6	20	0.3		
93	40	0.6	45	0.675	15	0.225		
94	40	0.6	50	0.75	10	0.15		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
95	40	0.6	55	0.825	5	0.075		
96	40	0.6	60	0.9	0	0		
97	35	0.525	0	0	65	0.975		
98	35	0.525	5	0.075	60	0.9		
99	35	0.525	10	0.15	55	0.825		
100	35	0.525	15	0.225	50	0.75		
101	35	0.525	20	0.3	45	0.675		
102	35	0.525	25	0.375	40	0.6		
103	35	0.525	30	0.45	35	0.525		
104	35	0.525	32.5	0.4875	32.5	0.4875		
105	35	0.525	35	0.525	30	0.45		
106	35	0.525	40	0.6	25	0.375		
107	35	0.525	45	0.675	20	0.3		
108	35	0.525	50	0.75	15	0.225		
109	35	0.525	55	0.825	10	0.15		
110	35	0.525	60	0.9	5	0.075		
111	35	0.525	65	0.975	0	0		
112	30	0.45	0	0	70	1.05		
113	30	0.45	5	0.075	65	0.975		
114	30	0.45	10	0.15	60	0.9		
115	30	0.45	15	0.225	55	0.825		
116	30	0.45	20	0.3	50	0.75		
117	30	0.45	25	0.375	45	0.675		
118	30	0.45	30	0.45	40	0.6		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
119	30	0.45	35	0.525	35	0.525		
120	30	0.45	40	0.6	30	0.45		
121	30	0.45	45	0.675	25	0.375		
122	30	0.45	50	0.75	20	0.3		
123	30	0.45	55	0.825	15	0.225		
124	30	0.45	60	0.9	10	0.15		
125	30	0.45	65	0.975	5	0.075		
126	30	0.45	70	1.05	0	0		
127	25	0.375	0	0	75	1.125		
128	25	0.375	5	0.075	70	1.05		
129	25	0.375	10	0.15	65	0.975		
130	25	0.375	15	0.225	60	0.9		
131	25	0.375	20	0.3	55	0.825		
132	25	0.375	25	0.375	50	0.75		
133	25	0.375	30	0.45	45	0.675		
134	25	0.375	35	0.525	40	0.6		
135	25	0.375	37.5	0.5625	37.5	0.5625		
136	25	0.375	40	0.6	35	0.525		
137	25	0.375	45	0.675	30	0.45		
138	25	0.375	50	0.75	25	0.375		
139	25	0.375	55	0.825	20	0.3		
140	25	0.375	60	0.9	15	0.225		
141	25	0.375	65	0.975	10	0.15		
142	25	0.375	70	1.05	5	0.075		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
143	25	0.375	75	1.125	0	0		
144	20	0.3	0	0	80	1.2		
145	20	0.3	5	0.075	75	1.125		
146	20	0.3	10	0.15	70	1.05		
147	20	0.3	15	0.225	65	0.975		
148	20	0.3	20	0.3	60	0.9		
149	20	0.3	25	0.375	55	0.825		
150	20	0.3	30	0.45	50	0.75		
151	20	0.3	35	0.525	45	0.675		
152	20	0.3	40	0.6	40	0.6		
153	20	0.3	45	0.675	35	0.525		
154	20	0.3	50	0.75	30	0.45		
155	20	0.3	55	0.825	25	0.375		
156	20	0.3	60	0.9	20	0.3		
157	20	0.3	65	0.975	15	0.225		
158	20	0.3	70	1.05	10	0.15		
159	20	0.3	75	1.125	5	0.075		
160	20	0.3	80	1.2	0	0		
161	15	0.225	0	0	85	1.275		
162	15	0.225	5	0.075	80	1.2		
163	15	0.225	10	0.15	75	1.125		
164	15	0.225	15	0.225	70	1.05		
165	15	0.225	20	0.3	65	0.975		
166	15	0.225	25	0.375	60	0.9		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
167	15	0.225	30	0.45	55	0.825		
168	15	0.225	35	0.525	50	0.75		
169	15	0.225	40	0.6	45	0.675		
170	15	0.225	42.5	0.6375	42.5	0.6375		
171	15	0.225	45	0.675	40	0.6		
172	15	0.225	50	0.75	35	0.525		
173	15	0.225	55	0.825	30	0.45		
174	15	0.225	60	0.9	25	0.375		
175	15	0.225	65	0.975	20	0.3		
176	15	0.225	70	1.05	15	0.225		
177	15	0.225	75	1.125	10	0.15		
178	15	0.225	80	1.2	5	0.075		
179	15	0.225	85	1.275	0	0		
180	10	0.15	0	0	90	1.35		
181	10	0.15	5	0.075	85	1.275		
182	10	0.15	10	0.15	80	1.2		
183	10	0.15	15	0.225	75	1.125		
184	10	0.15	20	0.3	70	1.05		
185	10	0.15	25	0.375	65	0.975		
186	10	0.15	30	0.45	60	0.9		
187	10	0.15	35	0.525	55	0.825		
188	10	0.15	40	0.6	50	0.75		
189	10	0.15	45	0.675	45	0.675		
190	10	0.15	50	0.75	40	0.6		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
191	10	0.15	55	0.825	35	0.525		
192	10	0.15	60	0.9	30	0.45		
193	10	0.15	65	0.975	25	0.375		
194	10	0.15	70	1.05	20	0.3		
195	10	0.15	75	1.125	15	0.225		
196	10	0.15	80	1.2	10	0.15		
197	10	0.15	85	1.275	5	0.075		
198	10	0.15	90	1.35	0	0		
199	5	0.075	0	0	95	1.425		
200	5	0.075	5	0.075	90	1.35		
201	5	0.075	10	0.15	85	1.275		
202	5	0.075	15	0.225	80	1.2		
203	5	0.075	20	0.3	75	1.125		
204	5	0.075	25	0.375	70	1.05		
205	5	0.075	30	0.45	65	0.975		
206	5	0.075	35	0.525	60	0.9		
207	5	0.075	40	0.6	55	0.825		
208	5	0.075	45	0.675	50	0.75		
209	5	0.075	47.5	0.7125	47.5	0.7125		
210	5	0.075	50	0.75	45	0.675		
211	5	0.075	55	0.825	40	0.6		
212	5	0.075	60	0.9	35	0.525		
213	5	0.075	65	0.975	30	0.45		
214	5	0.075	70	1.05	25	0.375		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
215	5	0.075	75	1.125	20	0.3		
216	5	0.075	80	1.2	15	0.225		
217	5	0.075	85	1.275	10	0.15		
218	5	0.075	90	1.35	5	0.075		
219	5	0.075	95	1.425	0	0		
220	0	0	0	0	100	1.5		
221	0	0	5	0.075	95	1.425		
222	0	0	10	0.15	90	1.35		
223	0	0	15	0.225	85	1.275		
224	0	0	20	0.3	80	1.2		
225	0	0	25	0.375	75	1.125		
226	0	0	30	0.45	70	1.05		
227	0	0	35	0.525	65	0.975		
228	0	0	40	0.6	60	0.9		
229	0	0	45	0.675	55	0.825		
230	0	0	50	0.75	50	0.75		
231	0	0	55	0.825	45	0.675		
232	0	0	60	0.9	40	0.6		
233	0	0	65	0.975	35	0.525		
234	0	0	70	1.05	30	0.45		
235	0	0	75	1.125	25	0.375		
236	0	0	80	1.2	20	0.3		
237	0	0	85	1.275	15	0.225		
238	0	0	90	1.35	10	0.15		

Table B1. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and water. (continue)

sample NO.	%OIL	weight(g)	%emulsifier	weight(g)	water	weight(g)	appearances	Birefringent
239	0	0	95	1.425	5	0.075		
240	0	0	100	1.5	0	0		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants**.**

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
1	100	1.35	0	0	0	0	0.15		
2	95	1.2825	0	0	5	0.0675	0.15		
3	95	1.2825	5	0.0675	0	0	0.15		
4	90	1.215	0	0	10	0.135	0.15		
5	90	1.215	5	0.0675	5	0.0675	0.15		
6	90	1.215	10	0.135	0	0	0.15		
7	85	1.1475	0	0	15	0.2025	0.15		
8	85	1.1475	5	0.0675	10	0.135	0.15		
9	85	1.1475	7.5	0.10125	7.5	0.10125	0.15		
10	85	1.1475	10	0.135	5	0.0675	0.15		
11	85	1.1475	15	0.2025	0	0	0.15		
12	80	1.08	0	0	20	0.27	0.15		
13	80	1.08	5	0.0675	15	0.2025	0.15		
14	80	1.08	10	0.135	10	0.135	0.15		
15	80	1.08	15	0.2025	5	0.0675	0.15		
16	80	1.08	20	0.27	0	0	0.15		
17	75	1.0125	0	0	25	0.3375	0.15		
18	75	1.0125	5	0.0675	20	0.27	0.15		
19	75	1.0125	10	0.135	15	0.2025	0.15		
20	75	1.0125	12.5	0.16875	12.5	0.16875	0.15		
21	75	1.0125	15	0.2025	10	0.135	0.15		
22	75	1.0125	20	0.27	5	0.0675	0.15		
23	75	1.0125	25	0.3375	0	0	0.15		
24	70	0.945	0	0	30	0.405	0.15		
25	70	0.945	5	0.0675	25	0.3375	0.15		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants.(continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
26	70	0.945	10	0.135	20	0.27	0.15		
27	70	0.945	15	0.2025	15	0.2025	0.15		
28	70	0.945	20	0.27	10	0.135	0.15		
29	70	0.945	25	0.3375	5	0.0675	0.15		
30	70	0.945	30	0.405	0	0	0.15		
31	65	0.8775	0	0	35	0.4725	0.15		
32	65	0.8775	5	0.0675	30	0.405	0.15		
33	65	0.8775	10	0.135	25	0.3375	0.15		
34	65	0.8775	15	0.2025	20	0.27	0.15		
35	65	0.8775	17.5	0.23625	17.5	0.23625	0.15		
36	65	0.8775	20	0.27	15	0.2025	0.15		
37	65	0.8775	25	0.3375	10	0.135	0.15		
38	65	0.8775	30	0.405	5	0.0675	0.15		
39	65	0.8775	35	0.4725	0	0	0.15		
40	60	0.81	0	0	40	0.54	0.15		
41	60	0.81	5	0.0675	35	0.4725	0.15		
42	60	0.81	10	0.135	30	0.405	0.15		
43	60	0.81	15	0.2025	25	0.3375	0.15		
44	60	0.81	20	0.27	20	0.27	0.15		
45	60	0.81	25	0.3375	15	0.2025	0.15		
46	60	0.81	30	0.405	10	0.135	0.15		
47	60	0.81	35	0.4725	5	0.0675	0.15		
48	60	0.81	40	0.54	0	0	0.15		
49	55	0.7425	0	0	45	0.6075	0.15		
50	55	0.7425	5	0.0675	40	0.54	0.15		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
51	55	0.7425	10	0.135	35	0.4725	0.15		
52	55	0.7425	15	0.2025	30	0.405	0.15		
53	55	0.7425	20	0.27	25	0.3375	0.15		
53	55	0.7425	20	0.27	25	0.3375	0.15		
54	55	0.7425	22.5	0.30375	22.5	0.30375	0.075		
55	55	0.7425	25	0.3375	20	0.27	0.075		
56	55	0.7425	30	0.405	15	0.2025	0.075		
57	55	0.7425	35	0.4725	10	0.135	0.075		
58	55	0.7425	40	0.54	5	0.0675	0.075		
59	55	0.7425	45	0.6075	0	0	0.075		
60	50	0.675	0	0	50	0.675	0.075		
61	50	0.675	5	0.0675	45	0.6075	0.075		
62	50	0.675	10	0.135	40	0.54	0.075		
63	50	0.675	15	0.2025	35	0.4725	0.075		
64	50	0.675	20	0.27	30	0.405	0.075		
65	50	0.675	25	0.3375	25	0.3375	0.075		
66	50	0.675	30	0.405	20	0.27	0.075		
67	50	0.675	35	0.4725	15	0.2025	0.075		
68	50	0.675	40	0.54	10	0.135	0.075		
69	50	0.675	45	0.6075	5	0.0675	0.075		
70	50	0.675	50	0.675	0	0	0.075		
71	45	0.6075	0	0	55	0.7425	0.075		
72	45	0.6075	5	0.0675	50	0.675	0.075		
73	45	0.6075	10	0.135	45	0.6075	0.075		
74	45	0.6075	15	0.2025	40	0.54	0.075		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
76	45	0.6075	25	0.3375	30	0.405	0.075		
77	45	0.6075	27.5	0.37125	27.5	0.37125	0.075		
78	45	0.6075	30	0.405	25	0.3375	0.075		
79	45	0.6075	35	0.4725	20	0.27	0.075		
80	45	0.6075	40	0.54	15	0.2025	0.075		
81	45	0.6075	45	0.6075	10	0.135	0.075		
82	45	0.6075	50	0.675	5	0.0675	0.075		
83	45	0.6075	55	0.7425	0	0	0.075		
84	40	0.54	0	0	60	0.81	0.075		
85	40	0.54	5	0.0675	55	0.7425	0.075		
86	40	0.54	10	0.135	50	0.675	0.075		
87	40	0.54	15	0.2025	45	0.6075	0.075		
88	40	0.54	20	0.27	40	0.54	0.075		
89	40	0.54	25	0.3375	35	0.4725	0.075		
90	40	0.54	30	0.405	30	0.405	0.075		
91	40	0.54	35	0.4725	25	0.3375	0.075		
92	40	0.54	40	0.54	20	0.27	0.075		
93	40	0.54	45	0.6075	15	0.2025	0.075		
94	40	0.54	50	0.675	10	0.135	0.075		
95	40	0.54	55	0.7425	5	0.0675	0.075		
96	40	0.54	60	0.81	0	0	0.075		
97	35	0.4725	0	0	65	0.8775	0.075		
98	35	0.4725	5	0.0675	60	0.81	0.075		
99	35	0.4725	10	0.135	55	0.7425	0.075		
100	35	0.4725	15	0.2025	50	0.675	0.075		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
101	35	0.4725	20	0.27	45	0.6075	0.075		
102	35	0.4725	25	0.3375	40	0.54	0.075		
103	35	0.4725	30	0.405	35	0.4725	0.075		
104	35	0.4725	32.5	0.43875	32.5	0.43875	0.075		
105	35	0.4725	35	0.4725	30	0.405	0.075		
106	35	0.4725	40	0.54	25	0.3375	0.075		
107	35	0.4725	45	0.6075	20	0.27	0.075		
108	35	0.4725	50	0.675	15	0.2025	0.075		
109	35	0.4725	55	0.7425	10	0.135	0.075		
110	35	0.4725	60	0.81	5	0.0675	0.075		
111	35	0.4725	65	0.8775	0	0	0.075		
112	30	0.405	0	0	70	0.945	0.075		
113	30	0.405	5	0.0675	65	0.8775	0.075		
114	30	0.405	10	0.135	60	0.81	0.075		
115	30	0.405	15	0.2025	55	0.7425	0.075		
116	30	0.405	20	0.27	50	0.675	0.075		
117	30	0.405	25	0.3375	45	0.6075	0.075		
118	30	0.405	30	0.405	40	0.54	0.075		
119	30	0.405	35	0.4725	35	0.4725	0.075		
120	30	0.405	40	0.54	30	0.405	0.075		
121	30	0.405	45	0.6075	25	0.3375	0.075		
122	30	0.405	50	0.675	20	0.27	0.075		
123	30	0.405	55	0.7425	15	0.2025	0.075		
124	30	0.405	60	0.81	10	0.135	0.075		
125	30	0.405	65	0.8775	5	0.0675	0.075		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
126	30	0.405	70	0.945	0	0	0.075		
127	25	0.3375	0	0	75	1.0125	0.075		
128	25	0.3375	5	0.0675	70	0.945	0.075		
129	25	0.3375	10	0.135	65	0.8775	0.075		
130	25	0.3375	15	0.2025	60	0.81	0.075		
131	25	0.3375	20	0.27	55	0.7425	0.075		
132	25	0.3375	25	0.3375	50	0.675	0.075		
133	25	0.3375	30	0.405	45	0.6075	0.075		
134	25	0.3375	35	0.4725	40	0.54	0.075		
135	25	0.3375	37.5	0.50625	37.5	0.50625	0.075		
136	25	0.3375	40	0.54	35	0.4725	0.075		
137	25	0.3375	45	0.6075	30	0.405	0.075		
138	25	0.3375	50	0.675	25	0.3375	0.075		
139	25	0.3375	55	0.7425	20	0.27	0.075		
140	25	0.3375	60	0.81	15	0.2025	0.075		
141	25	0.3375	65	0.8775	10	0.135	0.075		
142	25	0.3375	70	0.945	5	0.0675	0.075		
143	25	0.3375	75	1.0125	0	0	0.075		
144	20	0.27	0	0	80	1.08	0.075		
145	20	0.27	5	0.0675	75	1.0125	0.075		
146	20	0.27	10	0.135	70	0.945	0.075		
147	20	0.27	15	0.2025	65	0.8775	0.075		
148	20	0.27	20	0.27	60	0.81	0.075		
149	20	0.27	25	0.3375	55	0.7425	0.075		
150	20	0.27	30	0.405	50	0.675	0.075		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
151	20	0.27	35	0.4725	45	0.6075	0.075		
152	20	0.27	40	0.54	40	0.54	0.075		
153	20	0.27	45	0.6075	35	0.4725	0.075		
154	20	0.27	50	0.675	30	0.405	0.075		
155	20	0.27	55	0.7425	25	0.3375	0.075		
156	20	0.27	60	0.81	20	0.27	0.075		
157	20	0.27	65	0.8775	15	0.2025	0.075		
158	20	0.27	70	0.945	10	0.135	0.075		
159	20	0.27	75	1.0125	5	0.0675	0.075		
160	20	0.27	80	1.08	0	0	0.075		
161	15	0.2025	0	0	85	1.1475	0.15		
162	15	0.2025	5	0.0675	80	1.08	0.15		
163	15	0.2025	10	0.135	75	1.0125	0.15		
164	15	0.2025	15	0.2025	70	0.945	0.15		
165	15	0.2025	20	0.27	65	0.8775	0.15		
166	15	0.2025	25	0.3375	60	0.81	0.15		
167	15	0.2025	30	0.405	55	0.7425	0.15		
168	15	0.2025	35	0.4725	50	0.675	0.15		
169	15	0.2025	40	0.54	45	0.6075	0.15		
170	15	0.2025	42.5	0.57375	42.5	0.57375	0.15		
171	15	0.2025	45	0.6075	40	0.54	0.15		
172	15	0.2025	50	0.675	35	0.4725	0.15		
173	15	0.2025	55	0.7425	30	0.405	0.15		
174	15	0.2025	60	0.81	25	0.3375	0.15		
175	15	0.2025	65	0.8775	20	0.27	0.15		

Table B2. Formulations for sheet pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
176	15	0.2025	70	0.945	15	0.2025	0.15		
177	15	0.2025	75	1.0125	10	0.135	0.15		
178	15	0.2025	80	1.08	5	0.0675	0.15		
179	15	0.2025	85	1.1475	0	0	0.15		
180	10	0.135	0	0	90	1.215	0.15		
181	10	0.135	5	0.0675	85	1.1475	0.15		
182	10	0.135	10	0.135	80	1.08	0.15		
183	10	0.135	15	0.2025	75	1.0125	0.15		
184	10	0.135	20	0.27	70	0.945	0.15		
185	10	0.135	25	0.3375	65	0.8775	0.15		
186	10	0.135	30	0.405	60	0.81	0.15		
187	10	0.135	35	0.4725	55	0.7425	0.15		
188	10	0.135	40	0.54	50	0.675	0.15		
189	10	0.135	45	0.6075	45	0.6075	0.15		
190	10	0.135	50	0.675	40	0.54	0.15		
191	10	0.135	55	0.7425	35	0.4725	0.15		
192	10	0.135	60	0.81	30	0.405	0.15		
193	10	0.135	65	0.8775	25	0.3375	0.15		
194	10	0.135	70	0.945	20	0.27	0.15		
195	10	0.135	75	1.0125	15	0.2025	0.15		
196	10	0.135	80	1.08	10	0.135	0.15		
197	10	0.135	85	1.1475	5	0.0675	0.15		
198	10	0.135	90	1.215	0	0	0.15		
199	5	0.0675	0	0	95	1.2825	0.15		
200	5	0.0675	5	0.0675	90	1.215	0.15		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
201	5	0.0675	10	0.135	85	1.1475	0.15		
202	5	0.0675	15	0.2025	80	1.08	0.15		
203	5	0.0675	20	0.27	75	1.0125	0.15		
204	5	0.0675	25	0.3375	70	0.945	0.15		
205	5	0.0675	30	0.405	65	0.8775	0.15		
206	5	0.0675	35	0.4725	60	0.81	0.15		
207	5	0.0675	40	0.54	55	0.7425	0.15		
208	5	0.0675	45	0.6075	50	0.675	0.15		
209	5	0.0675	47.5	0.64125	47.5	0.64125	0.15		
210	5	0.0675	50	0.675	45	0.6075	0.15		
211	5	0.0675	55	0.7425	40	0.54	0.15		
212	5	0.0675	60	0.81	35	0.4725	0.15		
213	5	0.0675	65	0.8775	30	0.405	0.15		
214	5	0.0675	70	0.945	25	0.3375	0.15		
215	5	0.0675	75	1.0125	20	0.27	0.15		
216	5	0.0675	80	1.08	15	0.2025	0.15		
217	5	0.0675	85	1.1475	10	0.135	0.15		
218	5	0.0675	90	1.215	5	0.0675	0.15		
219	5	0.0675	95	1.2825	0	0	0.15		
220	0	0	0	0	100	1.35	0.15		
221	0	0	5	0.0675	95	1.2825	0.15		
222	0	0	10	0.135	90	1.215	0.15		
223	0	0	15	0.2025	85	1.1475	0.15		
224	0	0	20	0.27	80	1.08	0.15		
225	0	0	25	0.3375	75	1.0125	0.15		

Table B2. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 10% of co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	10%of cosolvent(g)	appearances	Birefringent
226	0	0	30	0.405	70	0.945	0.15		
227	0	0	35	0.4725	65	0.8775	0.15		
228	0	0	40	0.54	60	0.81	0.15		
229	0	0	45	0.6075	55	0.7425	0.15		
230	0	0	50	0.675	50	0.675	0.15		
231	0	0	55	0.7425	45	0.6075	0.15		
232	0	0	60	0.81	40	0.54	0.15		
233	0	0	65	0.8775	35	0.4725	0.15		
234	0	0	70	0.945	30	0.405	0.15		
235	0	0	75	1.0125	25	0.3375	0.15		
236	0	0	80	1.08	20	0.27	0.15		
237	0	0	85	1.1475	15	0.2025	0.15		
238	0	0	90	1.215	10	0.135	0.15		
239	0	0	95	1.2825	5	0.0675	0.15		
240	0	0	100	1.35	0	0	0.15		

Table B3. Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants.

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
1	100	1.35	0	0	0	0	0.075	0.075		
2	95	1.2825	0	0	5	0.0675	0.075	0.075		
3	95	1.2825	5	0.0675	0	0	0.075	0.075		
4	90	1.215	0	0	10	0.135	0.075	0.075		
5	90	1.215	5	0.0675	5	0.0675	0.075	0.075		
6	90	1.215	10	0.135	0	0	0.075	0.075		
7	85	1.1475	0	0	15	0.2025	0.075	0.075		
8	85	1.1475	5	0.0675	10	0.135	0.075	0.075		
9	85	1.1475	7.5	0.10125	7.5	0.10125	0.075	0.075		
10	85	1.1475	10	0.135	5	0.0675	0.075	0.075		
11	85	1.1475	15	0.2025	0	0	0.075	0.075		
12	80	1.08	0	0	20	0.27	0.075	0.075		
13	80	1.08	5	0.0675	15	0.2025	0.075	0.075		
14	80	1.08	10	0.135	10	0.135	0.075	0.075		
15	80	1.08	15	0.2025	5	0.0675	0.075	0.075		
16	80	1.08	20	0.27	0	0	0.075	0.075		
17	75	1.0125	0	0	25	0.3375	0.075	0.075		
18	75	1.0125	5	0.0675	20	0.27	0.075	0.075		
19	75	1.0125	10	0.135	15	0.2025	0.075	0.075		
20	75	1.0125	12.5	0.16875	12.5	0.16875	0.075	0.075		
21	75	1.0125	15	0.2025	10	0.135	0.075	0.075		
22	75	1.0125	20	0.27	5	0.0675	0.075	0.075		
23	75	1.0125	25	0.3375	0	0	0.075	0.075		
24	70	0.945	0	0	30	0.405	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
25	70	0.945	5	0.0675	25	0.3375	0.075	0.075		
26	70	0.945	10	0.135	20	0.27	0.075	0.075		
27	70	0.945	15	0.2025	15	0.2025	0.075	0.075		
28	70	0.945	20	0.27	10	0.135	0.075	0.075		
29	70	0.945	25	0.3375	5	0.0675	0.075	0.075		
30	70	0.945	30	0.405	0	0	0.075	0.075		
31	65	0.8775	0	0	35	0.4725	0.075	0.075		
32	65	0.8775	5	0.0675	30	0.405	0.075	0.075		
33	65	0.8775	10	0.135	25	0.3375	0.075	0.075		
34	65	0.8775	15	0.2025	20	0.27	0.075	0.075		
35	65	0.8775	17.5	0.23625	17.5	0.23625	0.075	0.075		
36	65	0.8775	20	0.27	15	0.2025	0.075	0.075		
37	65	0.8775	25	0.3375	10	0.135	0.075	0.075		
38	65	0.8775	30	0.405	5	0.0675	0.075	0.075		
39	65	0.8775	35	0.4725	0	0	0.075	0.075		
40	60	0.81	0	0	40	0.54	0.075	0.075		
41	60	0.81	5	0.0675	35	0.4725	0.075	0.075		
42	60	0.81	10	0.135	30	0.405	0.075	0.075		
43	60	0.81	15	0.2025	25	0.3375	0.075	0.075		
44	60	0.81	20	0.27	20	0.27	0.075	0.075		
45	60	0.81	25	0.3375	15	0.2025	0.075	0.075		
46	60	0.81	30	0.405	10	0.135	0.075	0.075		
47	60	0.81	35	0.4725	5	0.0675	0.075	0.075		
48	60	0.81	40	0.54	0	0	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
49	55	0.7425	0	0	45	0.6075	0.075	0.075		
50	55	0.7425	5	0.0675	40	0.54	0.075	0.075		
51	55	0.7425	10	0.135	35	0.4725	0.075	0.075		
52	55	0.7425	15	0.2025	30	0.405	0.075	0.075		
53	55	0.7425	20	0.27	25	0.3375	0.075	0.075		
54	55	0.7425	22.5	0.30375	22.5	0.30375	0.075	0.075		
55	55	0.7425	25	0.3375	20	0.27	0.075	0.075		
56	55	0.7425	30	0.405	15	0.2025	0.075	0.075		
57	55	0.7425	35	0.4725	10	0.135	0.075	0.075		
58	55	0.7425	40	0.54	5	0.0675	0.075	0.075		
59	55	0.7425	45	0.6075	0	0	0.075	0.075		
60	50	0.675	0	0	50	0.675	0.075	0.075		
61	50	0.675	5	0.0675	45	0.6075	0.075	0.075		
62	50	0.675	10	0.135	40	0.54	0.075	0.075		
63	50	0.675	15	0.2025	35	0.4725	0.075	0.075		
64	50	0.675	20	0.27	30	0.405	0.075	0.075		
65	50	0.675	25	0.3375	25	0.3375	0.075	0.075		
66	50	0.675	30	0.405	20	0.27	0.075	0.075		
67	50	0.675	35	0.4725	15	0.2025	0.075	0.075		
68	50	0.675	40	0.54	10	0.135	0.075	0.075		
69	50	0.675	45	0.6075	5	0.0675	0.075	0.075		
70	50	0.675	50	0.675	0	0	0.075	0.075		
71	45	0.6075	0	0	55	0.7425	0.075	0.075		
72	45	0.6075	5	0.0675	50	0.675	0.075	0.075		
73	45	0.6075	10	0.135	45	0.6075	0.075	0.075		
74	45	0.6075	15	0.2025	40	0.54	0.075	0.075		
75	45	0.6075	20	0.27	35	0.4725	0.075	0.075		
76	45	0.6075	25	0.3375	30	0.405	0.075	0.075		
77	45	0.6075	27.5	0.37125	27.5	0.37125	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
78	45	0.6075	30	0.405	25	0.3375	0.075	0.075		
79	45	0.6075	35	0.4725	20	0.27	0.075	0.075		
80	45	0.6075	40	0.54	15	0.2025	0.075	0.075		
81	45	0.6075	45	0.6075	10	0.135	0.075	0.075		
82	45	0.6075	50	0.675	5	0.0675	0.075	0.075		
83	45	0.6075	55	0.7425	0	0	0.075	0.075		
84	40	0.54	0	0	60	0.81	0.075	0.075		
85	40	0.54	5	0.0675	55	0.7425	0.075	0.075		
86	40	0.54	10	0.135	50	0.675	0.075	0.075		
87	40	0.54	15	0.2025	45	0.6075	0.075	0.075		
88	40	0.54	20	0.27	40	0.54	0.075	0.075		
89	40	0.54	25	0.3375	35	0.4725	0.075	0.075		
90	40	0.54	30	0.405	30	0.405	0.075	0.075		
91	40	0.54	35	0.4725	25	0.3375	0.075	0.075		
92	40	0.54	40	0.54	20	0.27	0.075	0.075		
93	40	0.54	45	0.6075	15	0.2025	0.075	0.075		
94	40	0.54	50	0.675	10	0.135	0.075	0.075		
95	40	0.54	55	0.7425	5	0.0675	0.075	0.075		
96	40	0.54	60	0.81	0	0	0.075	0.075		
97	35	0.4725	0	0	65	0.8775	0.075	0.075		
98	35	0.4725	5	0.0675	60	0.81	0.075	0.075		
99	35	0.4725	10	0.135	55	0.7425	0.075	0.075		
100	35	0.4725	15	0.2025	50	0.675	0.075	0.075		
101	35	0.4725	20	0.27	45	0.6075	0.075	0.075		
102	35	0.4725	25	0.3375	40	0.54	0.075	0.075		
103	35	0.4725	30	0.405	35	0.4725	0.075	0.075		
104	35	0.4725	32.5	0.43875	32.5	0.43875	0.075	0.075		
105	35	0.4725	35	0.4725	30	0.405	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
107	35	0.4725	45	0.6075	20	0.27	0.075	0.075		
108	35	0.4725	50	0.675	15	0.2025	0.075	0.075		
109	35	0.4725	55	0.7425	10	0.135	0.075	0.075		
110	35	0.4725	60	0.81	5	0.0675	0.075	0.075		
111	35	0.4725	65	0.8775	0	0	0.075	0.075		
112	30	0.405	0	0	70	0.945	0.075	0.075		
113	30	0.405	5	0.0675	65	0.8775	0.075	0.075		
114	30	0.405	10	0.135	60	0.81	0.075	0.075		
115	30	0.405	15	0.2025	55	0.7425	0.075	0.075		
116	30	0.405	20	0.27	50	0.675	0.075	0.075		
117	30	0.405	25	0.3375	45	0.6075	0.075	0.075		
118	30	0.405	30	0.405	40	0.54	0.075	0.075		
119	30	0.405	35	0.4725	35	0.4725	0.075	0.075		
120	30	0.405	40	0.54	30	0.405	0.075	0.075		
121	30	0.405	45	0.6075	25	0.3375	0.075	0.075		
122	30	0.405	50	0.675	20	0.27	0.075	0.075		
123	30	0.405	55	0.7425	15	0.2025	0.075	0.075		
124	30	0.405	60	0.81	10	0.135	0.075	0.075		
125	30	0.405	65	0.8775	5	0.0675	0.075	0.075		
126	30	0.405	70	0.945	0	0	0.075	0.075		
127	25	0.3375	0	0	75	1.0125	0.075	0.075		
128	25	0.3375	5	0.0675	70	0.945	0.075	0.075		
129	25	0.3375	10	0.135	65	0.8775	0.075	0.075		
130	25	0.3375	15	0.2025	60	0.81	0.075	0.075		
131	25	0.3375	20	0.27	55	0.7425	0.075	0.075		
132	25	0.3375	25	0.3375	50	0.675	0.075	0.075		
133	25	0.3375	30	0.405	45	0.6075	0.075	0.075		
134	25	0.3375	35	0.4725	40	0.54	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
136	25	0.3375	40	0.54	35	0.4725	0.075	0.075		
137	25	0.3375	45	0.6075	30	0.405	0.075	0.075		
138	25	0.3375	50	0.675	25	0.3375	0.075	0.075		
139	25	0.3375	55	0.7425	20	0.27	0.075	0.075		
140	25	0.3375	60	0.81	15	0.2025	0.075	0.075		
141	25	0.3375	65	0.8775	10	0.135	0.075	0.075		
142	25	0.3375	70	0.945	5	0.0675	0.075	0.075		
143	25	0.3375	75	1.0125	0	0	0.075	0.075		
144	20	0.27	0	0	80	1.08	0.075	0.075		
145	20	0.27	5	0.0675	75	1.0125	0.075	0.075		
146	20	0.27	10	0.135	70	0.945	0.075	0.075		
147	20	0.27	15	0.2025	65	0.8775	0.075	0.075		
148	20	0.27	20	0.27	60	0.81	0.075	0.075		
149	20	0.27	25	0.3375	55	0.7425	0.075	0.075		
150	20	0.27	30	0.405	50	0.675	0.075	0.075		
151	20	0.27	35	0.4725	45	0.6075	0.075	0.075		
152	20	0.27	40	0.54	40	0.54	0.075	0.075		
153	20	0.27	45	0.6075	35	0.4725	0.075	0.075		
154	20	0.27	50	0.675	30	0.405	0.075	0.075		
155	20	0.27	55	0.7425	25	0.3375	0.075	0.075		
156	20	0.27	60	0.81	20	0.27	0.075	0.075		
157	20	0.27	65	0.8775	15	0.2025	0.075	0.075		
158	20	0.27	70	0.945	10	0.135	0.075	0.075		
159	20	0.27	75	1.0125	5	0.0675	0.075	0.075		
160	20	0.27	80	1.08	0	0	0.075	0.075		
161	15	0.2025	0	0	85	1.1475	0.075	0.075		
162	15	0.2025	5	0.0675	80	1.08	0.075	0.075		
163	15	0.2025	10	0.135	75	1.0125	0.075	0.075		
164	15	0.2025	15	0.2025	70	0.945	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
165	15	0.2025	20	0.27	65	0.8775	0.075	0.075		
166	15	0.2025	25	0.3375	60	0.81	0.075	0.075		
167	15	0.2025	30	0.405	55	0.7425	0.075	0.075		
168	15	0.2025	35	0.4725	50	0.675	0.075	0.075		
169	15	0.2025	40	0.54	45	0.6075	0.075	0.075		
170	15	0.2025	42.5	0.57375	42.5	0.57375	0.075	0.075		
171	15	0.2025	45	0.6075	40	0.54	0.075	0.075		
172	15	0.2025	50	0.675	35	0.4725	0.075	0.075		
173	15	0.2025	55	0.7425	30	0.405	0.075	0.075		
174	15	0.2025	60	0.81	25	0.3375	0.075	0.075		
175	15	0.2025	65	0.8775	20	0.27	0.075	0.075		
176	15	0.2025	70	0.945	15	0.2025	0.075	0.075		
177	15	0.2025	75	1.0125	10	0.135	0.075	0.075		
178	15	0.2025	80	1.08	5	0.0675	0.075	0.075		
179	15	0.2025	85	1.1475	0	0	0.075	0.075		
180	10	0.135	0	0	90	1.215	0.075	0.075		
181	10	0.135	5	0.0675	85	1.1475	0.075	0.075		
182	10	0.135	10	0.135	80	1.08	0.075	0.075		
183	10	0.135	15	0.2025	75	1.0125	0.075	0.075		
184	10	0.135	20	0.27	70	0.945	0.075	0.075		
185	10	0.135	25	0.3375	65	0.8775	0.075	0.075		
186	10	0.135	30	0.405	60	0.81	0.075	0.075		
187	10	0.135	35	0.4725	55	0.7425	0.075	0.075		
188	10	0.135	40	0.54	50	0.675	0.075	0.075		
189	10	0.135	45	0.6075	45	0.6075	0.075	0.075		
190	10	0.135	50	0.675	40	0.54	0.075	0.075		
191	10	0.135	55	0.7425	35	0.4725	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)	appearances	Birefringent
192	10	0.135	60	0.81	30	0.405	0.075	0.075		
193	10	0.135	65	0.8775	25	0.3375	0.075	0.075		
194	10	0.135	70	0.945	20	0.27	0.075	0.075		
195	10	0.135	75	1.0125	15	0.2025	0.075	0.075		
196	10	0.135	80	1.08	10	0.135	0.075	0.075		
197	10	0.135	85	1.1475	5	0.0675	0.075	0.075		
198	10	0.135	90	1.215	0	0	0.075	0.075		
199	5	0.0675	0	0	95	1.2825	0.075	0.075		
200	5	0.0675	5	0.0675	90	1.215	0.075	0.075		
201	5	0.0675	10	0.135	85	1.1475	0.075	0.075		
202	5	0.0675	15	0.2025	80	1.08	0.075	0.075		
203	5	0.0675	20	0.27	75	1.0125	0.075	0.075		
204	5	0.0675	25	0.3375	70	0.945	0.075	0.075		
205	5	0.0675	30	0.405	65	0.8775	0.075	0.075		
206	5	0.0675	35	0.4725	60	0.81	0.075	0.075		
207	5	0.0675	40	0.54	55	0.7425	0.075	0.075		
208	5	0.0675	45	0.6075	50	0.675	0.075	0.075		
209	5	0.0675	47.5	0.64125	47.5	0.64125	0.075	0.075		
210	5	0.0675	50	0.675	45	0.6075	0.075	0.075		
211	5	0.0675	55	0.7425	40	0.54	0.075	0.075		
212	5	0.0675	60	0.81	35	0.4725	0.075	0.075		
213	5	0.0675	65	0.8775	30	0.405	0.075	0.075		
214	5	0.0675	70	0.945	25	0.3375	0.075	0.075		
215	5	0.0675	75	1.0125	20	0.27	0.075	0.075		
216	5	0.0675	80	1.08	15	0.2025	0.075	0.075		
217	5	0.0675	85	1.1475	10	0.135	0.075	0.075		
218	5	0.0675	90	1.215	5	0.0675	0.075	0.075		
219	5	0.0675	95	1.2825	0	0	0.075	0.075		
220	0	0	0	0	100	1.35	0.075	0.075		
221	0	0	5	0.0675	95	1.2825	0.075	0.075		
222	0	0	10	0.135	90	1.215	0.075	0.075		

Table B3 Formulations sheet for pseudo-ternary phase diagram of system containing oil, surfactants and 5% of each combined co-surfactants. (continue)

sample NO.	%OIL	weight(g)	%emulsifier1	weight(g)	%emulsifier2	weight(g)	5%of cosolvent 1(g)	5%of cosolvent 2(g)o	appearances	Birefringent
223	0	0	15	0.2025	85	1.1475	0.075	0.075		
224	0	0	20	0.27	80	1.08	0.075	0.075		
225	0	0	25	0.3375	75	1.0125	0.075	0.075		
226	0	0	30	0.405	70	0.945	0.075	0.075		
227	0	0	35	0.4725	65	0.8775	0.075	0.075		
228	0	0	40	0.54	60	0.81	0.075	0.075		
229	0	0	45	0.6075	55	0.7425	0.075	0.075		
230	0	0	50	0.675	50	0.675	0.075	0.075		
231	0	0	55	0.7425	45	0.6075	0.075	0.075		
232	0	0	60	0.81	40	0.54	0.075	0.075		
233	0	0	65	0.8775	35	0.4725	0.075	0.075		
234	0	0	70	0.945	30	0.405	0.075	0.075		
235	0	0	75	1.0125	25	0.3375	0.075	0.075		
236	0	0	80	1.08	20	0.27	0.075	0.075		
237	0	0	85	1.1475	15	0.2025	0.075	0.075		
238	0	0	90	1.215	10	0.135	0.075	0.075		
239	0	0	95	1.2825	5	0.0675	0.075	0.075		
240	0	0	100	1.35	0	0	0.075	0.075		

APPENDIX C

Analysis of Cvclosporin A

1. Valiation of HPLC method

1.1 Specificity

Figure B1, Line **A** showed the chromatogram in the presence of non-active ingredients SMEDDs (Blank SMEDDs) diluted in water. Line **B** showed the chromatogram in the presence of non-active ingredients SMEDDs (Blank SMEDDs) diluted in Simulated Gastric Fluid without pepsin solutions. This system interfered basal line, thus it was not used as medium for dissolution study. Line **C** showed the chromatogram in the presence of non-active ingredients SMEDDs-DP (Blank SMEDDs-DP). Line **D** showed the chromatogram in the presence of 0.05 mg/ml cyclosporin A. It indicated that the other ingredients did not interfere with peaks of cyclosporin. Thus, this method having high specificity could be used for analysis of cyclosporin.

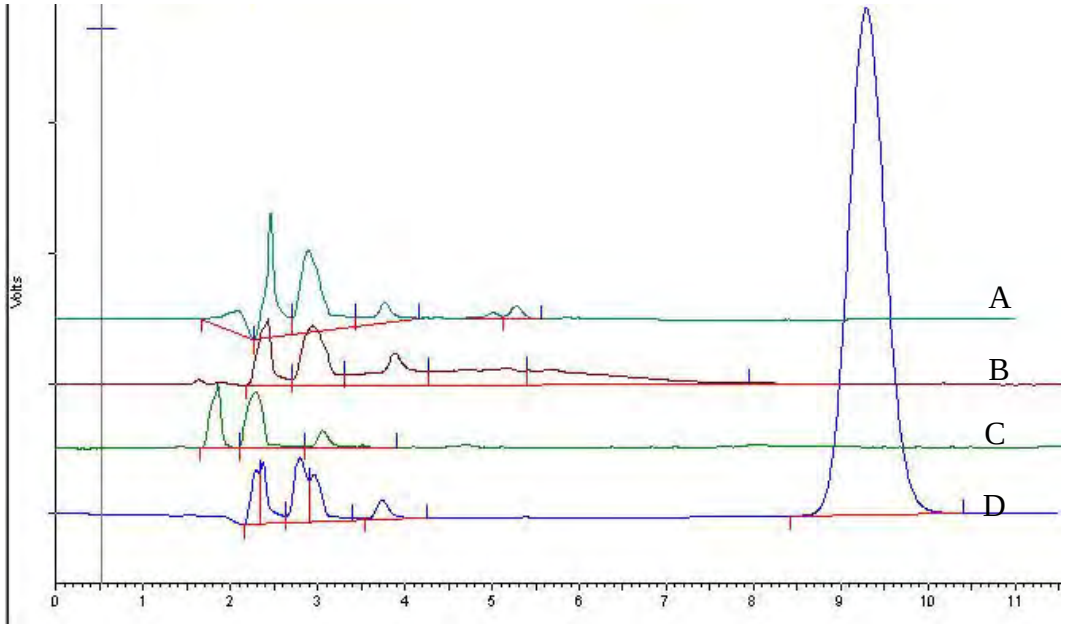


Figure C1. Chromatogram of non-active ingredients in the formulation.

Line A Chromatogram of blank SMEDDs diluted in water

Line B Chromatogram of blank SMEDDs diluted in simulated gastric fluid without pepsin solutions**.**

Line C Chromatogram of blank SMEDDs-DP diluted in water**.**

Line D Chromatogram of 0.05 mg/ml of cyclosporin A**.**

1.2 Accuacy

The accuracy of an analytical method was the closeness of the test results obtained by that method to the true value. It is usually calculated as percentage of recover by the assay of the known added amount of analyze in the sample. The percentages of analytical recoveries of each concentration are shown in Table B1.The mean of percentage of analytical recovered closely to 100%, with a low %CV indicated the high accuracy of this method. Thus, it could be used for analysis of cyclosporin A in all concentrations studied.

Table C1. The percentage recovery of Cyclosporin A

Actual concentration of cyclosporin A base (µg/ml)	%recovery of Cyclosporin A			Mean	SD	%CV
	1	2	3			
0.001	91.45	92.90	92.58	92.31	0.76	0.82
0.005	97.02	96.68	100.74	98.15	2.25	2.29
0.01	101.85	100.19	103.14	101.73	1.48	1.45
0.05	109.65	108.27	108.034	108.65	0.87	0.08
0.1	108.80	106.27	104.67	106.58	2.09	1.95

1.3 Precision

The precision of an analytical method was the degree of agreement among individual test results when the method was applied repeatedly to multiple samplings of a homogeneous sample. The precision of analytical method was usually expressed as the standard deviation or relative standard deviation (coefficient of variation). Table B2 and B3 illustrated the data of within and between run precision, respectively. All coefficients of variation values were small so it indicated that the HPLC method used was precise for quantitative analysis of cyclosporin A concentration in the range studied.

TableC2. Data within run precision of Cyclosporin A

Number	Area under curve		
	1st day	2nd day	3rd day
1	214775	208492	212811
2	211448	211255	215138
3	217347	210646	212575
Average	214523.3	210131	213508
SD	2957.54	1451.71	1416.54
%CV	1.37	0.69	0.66

TableC3. Data between run precision of Cyclosporin A

Number	Area under curve				
	0.001(mg/ml)	0.005(mg/ml)	0.01(mg/ml)	0.05(mg/ml)	0.1(mg/ml)
1	29528	109217.3	214523.3	1097599	2142667
2	29509.33	109902.3	210131	1092882	2164950
3	29066	108607.3	213508	1089041	2135706
Average	29367.78	109242.3	212524	1093174	2147774
SD	261.5138	647.8619	2299.552	4286.799	15276.58
%CV	0.890479	0.59305	1.08202	0.392142	0.711275

1.4 Linearity

The linearity of analytical method was its ability to elicit test results that are directly. Or by a well-defined mathematical transformation, proportional to the concentration of analyze in samples within a given range. Figure B2 showed that the relationship between peak area ratios and cyclosporin A concentrations is linear with a correlation of determination (R^2) value of 0.9999 in cyclosporin A. This result indicated that HPLC method was acceptable for qualitative analysis of cyclosporin A in the range studied.

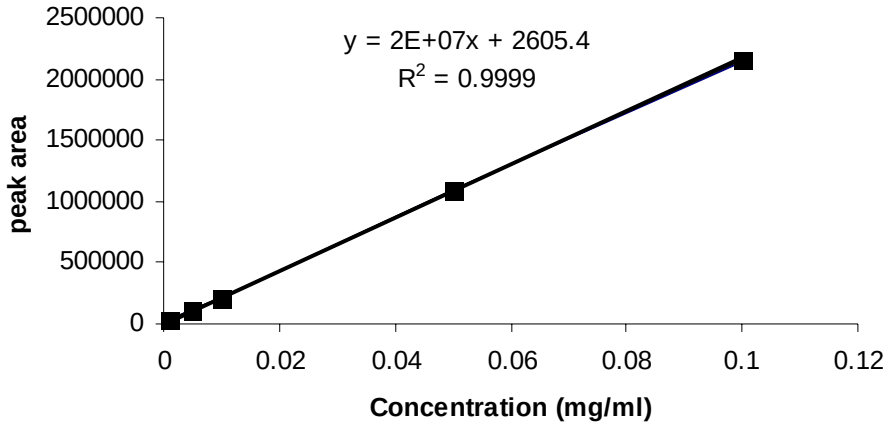


Figure C2. Standard curve of cyclosporin A

APPENDIX D

Results

Table D 1. Solubility of cyclosporine A in oils with various co-solvents.

Sample		Weight(g)	Concentration(w/w)	Average(w/w)	SD
100%Oil	**1**	0.0078	25.78	25.23	1.31
	2	0.0086	23.73		
	3	0.0078	26.18		
100% Cremophor EL	**1**	0.0495	23.31	23.54	0.40
	2	0.0499	23.31		
	3	0.0494	24.00		
100% Tween 80	**1**	0.0483	21.98	22.92	1.17
	2	0.0479	24.23		
	3	0.0464	22.57		
100% Solutol HS 15	**1**	0.0478	27.62	26.29	1.16
	2	0.0476	25.75		
	3	0.0504	25.49		
5% EtOH in oil	**1**	0.0088	53.23	64.49	10.29
	2	0.0087	66.84		
	3	0.0101	73.40		
10% Et in oil	**1**	0.0089	79.56	70.19	20.79
	2	0.0083	84.65		
	3	0.0088	46.37		
20% Et in oil	**1**	0.0081	77.98	81.78	12.87
	2	0.0089	71.23		
	3	0.0088	96.12		
5% Pg in oil	**1**	0.0095	47.43	57.54	12.70
	2	0.0095	53.40		
	3	0.0087	71.80		
10% Pg in oil	**1**	0.0090	78.73	74.22	10.28
	2	0.0087	81.48		
	3	0.0089	62.46		

Table D 1. Solubility of cyclosporine A in oils with various co-solvents (continue).

Sample		Weight(g)	Concentration(w/w)	Average(w/w)	SD
20% Pg in oil	**1**	0.0091	88.72	94.82	5.76
	2	0.0088	27.06		
	3	0.0092	27.03		
20% Pe in oil	**1**	0.0094	29.83	30.40	0.57
	2	0.0090	30.42		
	3	0.0090	30.96		
5% Gy in oil	**1**	0.0092	25.44	25.81	0.42
	2	0.0089	26.26		
	3	0.0090	25.72		
10% Gy in oil	**1**	0.0090	26.95	25.75	1.32
	2	0.0095	24.35		
	3	0.0095	25.95		
20% Gy in oil	**1**	0.0097	29.62	28.22	1.33
	2	0.0083	28.07		
	3	0.0090	26.97		

Table D2 .% Content uniformity SMEDDs25CyA after 7 days at ambient condition.

capsules	total per 1 tab	%Labeled amount
1	25.7930625	103.1723
2	25.870125	103.4805
3	25.0400625	100.1603
4	25.699125	102.7965
5	25.5733125	102.2933
6	25.6133125	102.4533
7	25.85775	103.431
8	25.43325	101.733
average	25.61	102.44
SD	0.274623504	1.098494

Table D3.% Content uniformity SMEDDs25CyA at 40 °C 75RH condition after storage after 2 months.

capsules	total per 1 tab	%Labeled amount
1	25.7584375	103.0338
2	25.756875	103.0275
3	25.4443125	101.7773
average	25.65320833	102.6128
SD	0.180910785	0.723643

Table D4. % Content uniformity SMEDDs25CyA at 40 °C 75RH condition after storage 4 months

capsules	total per 1 tab	%Labeled amount
1	25.6326875	102.5308
2	25.6365625	102.5463
3	25.283625	101.1345
average	25.517625	102.0705
SD	0.202659206	0.810637

Table D5. % Content uniformity SMEDDs100CyA after 7 days at ambient condition.

capsules	total per 1 tab	%Label
1	103.5092	103.5092
2	101.8558	101.8558
3	99.85233	99.85233
4	103.8294	103.8294
5	102.341	102.341
6	105.011	105.011
average	102.7331	102.7331
SD	1.800845	1.800845

Table D6. % Content uniformity SMEDDs100CyA at 40°C 75RH condition after storage after 2 months.

capsules	total per 1 tab	%Label
1	103.8233	103.8233
2	100.2879	100.2879
3	99.31398	99.31398
average	101.1417	101.1417
SD	2.372814	2.372814

Table D7. % Content uniformity SMEDDs100CyA at 40°C 75RH condition after storage after 4 months.

capsules	total per 1 tab	%Label
1	101.8077	101.8077
2	101.6305	101.6305
3	102.8262	102.8262
average	102.0881	102.0881
SD	0.645271	0.645271

Table D8. Cumulative of %dissolution of SMEDDs25CyA at ambient condition after 7 days.

capsules	Time (minutes)						
	5	**10**	**20**	**30**	**40**	**50**	**60**
1	12.0125	28.87513	73.40318	91.00213	94.52778	93.34719	94.21605
2	22.1645	39.66965	81.14613	94.63593	93.53557	97.85739	97.32181
3	22.553	28.98653	57.71214	77.79513	95.47723	99.71598	100.2268
average	18.91	32.51043	70.75381	87.81106	94.51353	96.97352	97.25487
SD	5.976568	6.200309	11.93952	8.862296	0.970908	3.275102	3.005912

Table D9. Cumulative of %dissolution of SMEDDs25CyA at ambient condition after 2 mouths.

capsules	Time (minutes)						
	5	**10**	**20**	**30**	**40**	**50**	**60**
1	27.2765	40.19777	60.79452	77.96924	87.3787	95.83509	94.043
2	5.8955	11.17896	44.27316	67.47669	91.08034	97.60185	98.11309
3	11.7275	16.32578	55.16136	77.58518	91.90925	96.62189	99.89262
average	14.9665	22.5675	53.40968	74.3437	90.12276	96.68627	97.34957
SD	11.05238	15.48361	8.398817	5.95011	2.412299	0.885138	2.99862

Table D10. Cumulative of %dissolution of SMEDDs25CyA at 40°C 75RH condition after 4 mouths.

capsules	Time (minutes)						
	5	**10**	**20**	**30**	**40**	**50**	**60**
1	17.082	37.93782	53.51449	81.11065	87.07448	93.64144	101.324
2	32.9515	44.16502	59.84037	89.5976	97.43349	97.43349	101.6399
3	36.359	60.56859	68.02514	91.43524	97.72673	100.3552	100.9413
average	28.7975	47.55714	60.46	87.38116	94.07823	97.14336	101.3017
SD	10.28798	11.6905	7.275142	5.507604	6.0672	3.366248	0.349791

Table D11. Cumulative of %dissolution of SMEDDs 100CyA at ambient condition after 7 days

capsules	Time (minutes)						
	5	**10**	**20**	**30**	**40**	**50**	**60**
1	0.213055	42.93568	89.20303	98.36766	101.8435	104.5683	106.1924
2	0.312315	60.14444	99.41164	103.4356	105.8207	104.9713	106.2131
3	0.164166	34.49804	90.95301	103.7097	104.9626	102.8491	104.6875
average	0.229845	45.85939	93.18922	101.8377	104.2089	104.1296	105.6976
SD	0.075488	13.07079	5.459342	3.008242	2.092984	1.127073	0.87489

Table D12. Cumulative of %dissolution Dissolution of SMEDDs 100CyA at ambient condition after storage 2 months

capsules	Time (minutes)						
	5	**10**	**20**	**30**	**40**	**50**	**60**
1	0.116845	27.34722	45.59727	91.02473	97.96344	101.7745	103.16
2	0.091308	29.18856	60.81491	92.01536	98.68364	101.5364	103.6186
3	0.133703	36.6802	90.23667	97.98904	102.1921	103.7789	107.9589
average	0.113952	31.07199	65.54962	93.67638	99.61306	102.3633	104.9125
SD	0.021345	4.943343	22.69321	3.767579	2.262352	1.231735	2.648244

Table D13. Cumulative of %dissolution of SMEDDs100CyA at 40°C 75RH condition after storage 4 months

capsules	Time (minutes)					
	10	**20**	**30**	**40**	**50**	**60**
1	67.66213	74.05712	85.6443	91.59764	97.15659	103.4452
2	52.46013	84.44348	87.93192	91.11597	98.00496	102.119
3	73.09713	79.81847	87.09422	93.65307	94.63783	102.7029
average	64.40646	79.43969	86.89015	92.12223	96.59979	102.7557
SD	10.69677	5.203528	1.15738	1.347446	1.751262	0.664693

Table D14. % Content uniformity SMEDDsCyA-DP 7 days at ambient condition.

capsules	total per 1 tab	%Label
1	23.1325625	92.53025
2	23.32325	93.293
3	23.4506875	93.80275
average	23.30216667	93.20867
SD	0.160107024	0.640428

Table D15. % Content uniformity OS25CyA-DP 7 days at ambient condition.

capsules	total per 1 tab	%Label
1	23.0570625	92.22825
2	23.1243125	92.49725
3	23.4258125	93.70325
average	23.20239583	92.80958
SD	0.196384561	0.785538

Table D16. % Content uniformity SMEDDsCyA-DP after heat-cooling condition 5 cycles.

capsules	total per 1 tab	%Label
1	23.30375	93.215
2	23.0560625	92.22425
3	23.1918125	92.76725
average	23.183875	92.7355
SD	0.12403438	0.496138

Table D17. % Content uniformity OSCyA-DP after heat-cooling condition 5 cycles.

capsules	total per 1 tab	%Label
1	23.14375	92.575
2	23.4486875	93.79475
3	23.10975	92.439
average	23.2340625	92.93625
SD	0.186646505	0.746586

Table D18. Cumulative of %dissolution of SMEDDsCyA-DP at ambient conditionafter 7 days.

capsules	Time (minutes)					
	10	**20**	**30**	**40**	**50**	**60**
1	61.3115	80.03312	77.67882	79.49553	82.88729	87.69673
2	35.9135	56.61564	72.3592	80.21158	81.99833	84.9586
3	6.659	52.29759	65.5209	78.03272	77.46417	86.81775
average	34.628	62.98211	71.85297	79.24661	80.78326	86.49102
SD	27.34892	14.9236	6.094746	1.110551	2.908583	1.397999

Table D19. Cumulative of %dissolution of OSCyA-DP at ambient condition after 7 days.

capsules	Time (minutes)					
	10	**20**	**30**	**40**	**50**	**60**
1	0	0.7305	36.22381	3.30947	1.71487	3.72053
2	0	1.413	29.83863	14.34838	7.006735	5.803775
3	0	13.04448	3.470245	13.17601	14.68603	12.60438
average	0	5.062658	23.17756	10.27795	7.802543	7.376228
SD	0	6.920874	17.36307	6.063283	6.522093	4.645983

Table D20. Cumulative of %dissolution of SMEDDsCyA-DP after heat-cooling condition 5cycles.

capsules	Time (minutes)					
	10	**20**	**30**	**40**	**50**	**60**
1	0.014515	35.78502	55.22822	77.18128	82.73989	86.39195
2	0.11933	24.61383	46.41178	82.72537	79.12248	83.74243
3	0.012285	23.01329	62.1218	67.50009	79.12248	83.74243
average	0.04871	27.80404	54.58726	75.80225	80.32828	84.6256
SD	0.061169	6.9579	7.874598	7.70575	2.08851	1.529701

Table D21. Cumulative of %dissolution of OSCyA-DP after heat-cooling condition 5 cycles.

capsules	Time (minutes)					
	10	**20**	**30**	**40**	**50**	**60**
1	0	0	0	0	0	0
2	0	1.218	1.15768	54.01014	32.343	12.6643
3	0	20.989	36.64389	32.343	13.00713	34.2141
average	0	7.402333	12.60052	28.78438	15.11671	15.6261
SD	0	11.78215	20.83021	27.18035	16.27437	17.29828

Table D22. Bulk density, tab density and % compressibility of Avicel

Sample NO	bulk density	tab dendity	%Compessibitily
1	0.333333	0.434783	23.33333
2	0.331126	0.434783	23.84106
3	0.333333	0.436681	23.66667
Average	0.332597	0.435415	23.61369
S.D.	0.001275	0.001096	0.257976

Table D23. Bulk density, tab density and % compressibility of Anhydrous lactose

Sample NO.	bulk density	tab dendity	%Compessibitily
1	0.588235	0.740741	20.58824
2	0.581395	0.735294	20.93023
3	0.591716	0.740741	20.11834
Average	0.587116	0.738925	20.5456
S.D.	0.005251	0.003145	0.40762

Table D24. Bulk density, tab density and % compressibility of Dicalcium phosphate

Sample NO.	bulk density	tab dendity	%Compessibitily
1	0.588235	0.833333	29.41176
2	0.588235	0.833333	29.41176
3	0.588235	0.826446	28.82353
Average	0.588235	0.831038	29.21569
S.D.	0	0.003976	0.339618

Table D25. Bulk density, tab density and % compressibility of Activated charcoal

Sample NO.	bulk density	tab dendity	%Compessibitily
1	0.212766	0.37037	42.55319
2	0.211416	0.37037	42.91755
3	0.211864	0.37037	42.79661
Average	0.212016	0.37037	42.75578
S.D.	0.000687	6.8E-17	0.185577

APPENDIX D

Data in statistical process

TableD1. ANOVA test for study of mean particle size from various dilution ratio

ANOVA

mean particle size (nm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	273.863	3	91.288	63.284	.000
Within Groups	11.540	8	1.443		
Total	285.403	11			

TableD2. Multiple Comparisons test for mean particle size from various dilution ratio

Multiple Comparisons

Mean particle size (nm)

Scheffe

(I) Ratio	(J) Ratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ratio 1:50	Ratio 1:100	9.80000*	.98065	.000	6.3750	13.2250
	Ratio 1:200	12.70000*	.98065	.000	9.2750	16.1250
	Ratio 1:500	5.60000*	.98065	.003	2.1750	9.0250
Ratio 1:100	Ratio 1:50	-9.80000*	.98065	.000	-13.2250	-6.3750
	Ratio 1:200	2.90000	.98065	.101	-.5250	6.3250
	Ratio 1:500	-4.20000*	.98065	.018	-7.6250	-.7750
Ratio 1:200	Ratio 1:50	-12.70000*	.98065	.000	-16.1250	-9.2750
	Ratio 1:100	-2.90000	.98065	.101	-6.3250	.5250
	Ratio 1:500	-7.10000*	.98065	.001	-10.5250	-3.6750
Ratio 1:500	Ratio 1:50	-5.60000*	.98065	.003	-9.0250	-2.1750
	Ratio 1:100	4.20000*	.98065	.018	.7750	7.6250
	Ratio 1:200	7.10000*	.98065	.001	3.6750	10.5250

*. The mean difference is significant at the 0.05 level.

TableD3. ANOVA test for study of mean particle size of SMEDDs before and after loaded cyclosporine A

ANOVA

mean particle size(nm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	316.752	2	158.376	88.950	.000
Within Groups	10.683	6	1.781		
Total	327.435	8			

TableD4. Multiple Comparisons test for mean particle size from various dilution ratio

Multiple Comparisons

mean particle size (nm)

Scheffe

(I) cyclosporin A load	(J) cyclosporin A load	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
no medicine	25 mg loaded	-8.89000*	1.08950	.001	-12.3843	-5.3957
	100 mg loaded	-14.40000*	1.08950	.000	-17.8943	-10.9057
25 mg loaded	no medicine	8.89000*	1.08950	.001	5.3957	12.3843
	100 mg loaded	-5.51000*	1.08950	.007	-9.0043	-2.0157
100 mg loaded	no medicine	14.40000*	1.08950	.000	10.9057	17.8943
	25 mg loaded	5.51000*	1.08950	.007	2.0157	9.0043

*. The mean difference is significant at the 0.05 level.

TableD5. ANOVA test for study of % content of cyclosporin A in SMEDDs 25CyA after storage at ambient condition 7 days and accelerated condition after 2 and 4 months

ANOVA

% CyA Content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.449	2	.224	.217	.809
Within Groups	9.313	9	1.035		
Total	9.762	11			

TableD6. ANOVA test for study of % content of cyclosporin A in SMEDDs 100CyA after storage at ambient condition 7 days and accelerated condition after 2 and 4 months

ANOVA

CyA Content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.095	2	2.547	.810	.475
Within Groups	28.309	9	3.145		
Total	33.403	11			

TableD7. Paired samples T Test for study of % content of cyclosporin A in SMEDDsCyA-DP after storage at ambient condition 7 days and heat-cooling 5 cycles

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	7days - after 5 cycles	.47317	1.00292	.57904	-2.01823	2.96457	.817	2	.500

TableD8. Paired samples T Test for study of % content of cyclosporin A in OSCyA-DP after storage at ambient condition 7 days and heat-cooling 5 cycles

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	7 days - after 5 cycle	-.12667	1.29498	.74766	-3.34357	3.09024	-.169	2	.881

APPENDIX F

Particle mean diameter

TableF1. MeanParticle diameter of $40C_{300}40C_{EL}20T_{80}$+5EtOH 5 Gly in various dilution ratios**.**

Dilution ratio		MeanParticle diameter (nm)	average(nm)	SD
1:50	1	84.7	85.40	0.89
	2	85.1		
	3	86.4		
1:100	1	74.6	75.60	0.95
	2	76.5		
	3	75.7		
1:200	1	72.5	72.70	0.20
	2	72.7		
	3	72.9		
1:500	1	77.9	79.80	2.01
	2	81.9		
	3	79.6		

TableF2. MeanParticle diameter of selected SMEDDs.

Formulation		MeanParticle diameter (nm)	average(nm)	SD
$40C_{300}40C_{EL}20T_{80}$+5EtOH5Gly	1	96.60	97.5	0.79
	2	98.10		
	3	97.80		
$40C_{300}50C_{EL}10T_{80}$+5EtOH5Gly	1	74.60	75.59	0.96
	2	76.50		
	3	75.70		
$35.5C_{300}32.5C_{EL}32.5T_{80}$+5EtOH5PG	1	58.93	58.95	0.02
	2	58.96		
	3	58.97		
$35C_{300}40C_{EL}25T_{80}$+5EtOH5PG	1	40.00	40.00	0
	2	40.00		
	3	40.00		

TableF3. MeanParticle diameter of SMEDDs before and after loaded cyclosporine A.

Formulation		**MeanParticle diameter (nm)**	**average(nm)**	**SD**
SMEDDs (no medicine)	1	60.31	60.30	0.74
	2	59.55		
	3	61.03		
SMEDDs25CyA	1	70.59	69.19	1.36
	2	69.10		
	3	67.87		
SMEDDs100CyA	1	76.59	74.70	1.71
	2	74.25		
	3	73.25		

VITAE

Miss Nichthima Paengnakorn was born on 17 th August 1981, in Chaing mai, Thailand. She graduated the Bachelor of Science in Pharmacy in 2004 from Faculty of Pharmaceutical Science. Chaing mai University. And attending the Master'Degree program in Phamaceutical Science at Chulalongkorn University in 2004.