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DEVELOPMENT OF PARENTERAL PEPTIDE DRUG DELIVERY SYSTEM VIA MICROEMULSION TECHNOLOGY

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พรรณเพ็ญ วัฒนาอาษากิจ : การพัฒนาระบบนำส่งยาเปปไทด์ชนิดฉีดโดยใช้เทคโนโลยีไมโครอิมัลชัน. (DEVELOPMENT OF PARENTERAL PEPTIDE DRUG DELIVERY SYSTEM VIA MICROEMULSION TECHNOLOGY) อ. ที่ปรึกษา : รศ. ดร. กาญจน์พิมล ฤทธิเดช 314 หน้า ISBN 974 -17-0823-8

ไมโครอิมัลชันหลายชนิดเตรียมจากสารที่ทางเภสัชกรรมยอมรับว่าปลอดภัย ในการทดลองสารลดแรงตึงผิวที่ใช้คือฟอสโฟลิปิดและทวีน 80 สารลดแรงตึงผิวร่วมคือโพรพิลีนไกลคอล, โพลีเอธิลีนไกลคอล 400, กลีเซอรอลและกรดคาโปรอิก ในส่วนของน้ำมันไอโซโพลพิลเมอริสเตท, เอทิลโอลีเอท, ไตรกลีเซอไรด์โมเลกุลขนาดกลางและน้ำมันถั่วเหลืองถูกนำมาใช้ ระบบเตรียมจากส่วนประกอบดังกล่าวและนำมาสร้างเป็นเฟสไดอะแกรม เพื่อศึกษาพื้นที่การเกิดไมโครอิมัลชัน ตรวจสอบผลกระทบจากชนิดและอัตราส่วนสารประกอบที่ใช้ซึ่งมีต่อชนิดไมโครอิมัลชัน, ความหนืด, ขนาดอนุภาค ระบบที่ได้นำมาเปรียบเทียบกับระบบที่เตรียมจากสารลดแรงตึงผิวร่วมบิวทานอล รวมทั้งประเมินความเป็นไปได้ในการใช้ระบบไมโครอิมัลชันเพื่อควบคุมการปลดปล่อยตัวยาในการทดลองโดยใช้ฟรีันดิฟฟิวชันเซลและสัตว์ทดลองโดยทำในกระต่าย

พบว่าชนิดและอัตราส่วนของสารลดแรงตึงผิว, สารลดแรงตึงผิวร่วม และชนิดของน้ำมันมีผลต่อพื้นที่การเกิดไมโครอิมัลชัน ทั้งนี้เนื่องจากโครงสร้างโมเลกุลและการจัดเรียงตัวซึ่งมีผลต่อความโค้งของอนุภาค เฟสไดอะแกรมที่เตรียมจากฟอสโฟลิปิดสามารถเกิดพื้นที่ไมโครอิมัลชันในระบบที่ประกอบด้วยสารลดแรงตึงผิวร่วมบิวทานอล โพรฟิลีนไกลคอลและกรดคาโปรอิก เฟสไดอะแกรมที่เตรียมจากทวีน 80 ทั้งหมดสามารถพบพื้นที่ไมโครอิมัลชันยกเว้นในกรณีที่มีการใช้น้ำมันถั่วเหลือง วัฏภาคน้ำมันที่มีขนาดโมเลกุลใหญ่มีผลลดพื้นที่การเกิดไมโครอิมัลชันอย่างชัดเจน นอกจากนี้พบว่าพื้นที่การเกิดไมโครอิมัลชันส่วนใหญ่เพิ่มขึ้นเมื่อเพิ่มอัตราส่วนสารลดแรงตึงผิวต่อสารลดแรงตึงผิวร่วมต่อน้ำมัน ระบบฟอสโฟลิปิดไมโครอิมัลชันเกิดเป็นระบบชนิดน้ำกระจายตัวในน้ำมัน ขณะที่ระบบทวีน 80 ไมโครอิมัลชันที่มีปริมาณวัฏภาคภายในสูงเกิดเป็นระบบชนิดน้ำมันกระจายในน้ำ ขนาดอนุภาคจากกล้องจุลทรรศน์อิเลคตรอนโดยวิธีเนกาทีฟสเตนแสดงถึงขนาดของอนุภาคอยู่ในช่วงของไมโครอิมัลชัน การเพิ่มปริมาณสารลดแรงตึงผิวมีผลเพิ่มความหนืดของระบบ ไมโครอิมัลชันมีความคงตัวดียกเว้นระบบที่เตรียมจากกลีเซอรอลเป็นสารลดแรงตึงผิวร่วม ตัวยาบุสเซอเรอลินอะซีเตทมีค่าการกระจายตัวในระบบออคตานอลและน้ำเท่ากับ 0.01 จากการทดลองการปลดปล่อยตัวยาพบว่าทวีนไมโครอิมัลชันสามารถควบคุมการปลดปล่อยตัวยาได้ในระยะเวลา 1-6 วัน การปลดปล่อยตัวยาจากฟอสโฟลิปิดไมโครอิมัลชันมีเพียงประมาณ 10% ซึ่งปลดปล่อยช้ากว่าเมื่อเปรียบเทียบกับยาในสารละลายบัฟเฟอร์ที่มีการปลดปล่อยยาถึงประมาณ 80% ภายใน 2 วัน การศึกษาในสัตว์ทดลองกระต่ายโดยฉีดยา 3.3 มิลลิกรัมบุสเซอเรอลินอะซีเตทไมโครอิมัลชัน ผลสรุปปฏิกิริยาของยาต่อระดับเทสโตสเตอโรนในกระต่ายยังไม่ชัดเจนจากทั้งระบบฟอสโฟลิปิดและทวีน 80 ในการศึกษานานกว่า 30 วัน

สาขาวิชา .....เภสัชกรรม..... ลายมือชื่อนิสิต .....

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KEY WORD : BUSERELIN ACETATE / PARENTERAL MICROEMULSIONS / PEPTIDE DRUG DELIVERY SYSTEM / PROLONGED DRUG RELEASE / PHASE STUDY / VISCOSITY / TRANSMISSION ELECTRON MICROSCOPY / PARTITION COEFFICIENT / TESTOSTERONE / RABBITS.

PHANPHEN WATTANAARSAKIT : THESIS TITLE (DEVELOPMENT OF PARENTERAL PEPTIDE DRUG DELIVERY SYSTEM VIA MICROEMULSION TECHNOLOGY) THESIS ADVISOR : ASSOC. PROF. GARNPIMOL C. RITTHIDEJ, Ph.D. 314 pp. ISBN 974-17-0823-8

Various microemulsions were prepared using pharmaceutically acceptable components. Phospholipid (PC), tween 80 (TW) were used as surfactants; propylene glycol (PG), polyethylene glycol 400 (PEG), glycerol (GR), and caproic acid (CA) as cosurfactants; isopropyl myristate (IPM), ethyl oleate (EO), medium chain triglyceride (MCT), and soybean oil (SBO) as oils. Partial pseudo-ternary phase diagrams were constructed to evaluate the microemulsion existing area. The effects of ingredients and ratios on microemulsion types, viscosity, and appearance were investigated. The obtained systems were also compared to systems containing butanol (BT) as cosurfactant. The potentials of microemulsions to prolong the release of buserelin acetate were accordingly evaluated both *in vitro* using modified Franz diffusion cell and *in vivo* in rabbits.

Types and ratios of surfactants, cosurfactants, and oils used had a pronounced effect on the existing region of microemulsions, likely due to their molecular structures and geometric packing which consequently affected the curvature of droplets. In PC-based systems, microemulsion regions could be produced only from the systems containing cosurfactants of BT, PG, and CA. All TW-based systems yielded microemulsion regions except when SBO was used as oil. Oil of larger molecule size evidently resulted in smaller microemulsion regions. In addition, microemulsion regions were mostly increased when Em /oil ratio increased. PC-based microemulsions were mostly water-in-oil type while TW-based microemulsions with high percentage of internal phase were oil-in-water type**.** Negative stained transmission electron micrographs (TEM) revealed seemingly that the droplet size of the system was in the range of microemulsion. Increasing the amount of surfactant would increase the viscosity of the system. The obtained microemulsion systems were stable except when GR was used as cosurfactant. Buserelin acetate has partition coefficient (C_0/C_w) in octanol-water system 0.01. Most TW-based systems prolonged the release of drug during 1-6 days of the experiment. The drug released from PC-based systems was much slower and quite low with less than 10% while the amount of drug release from buffer solution was about 80% within two days. *In vivo* study by subcutaneous injection of 3.3 mg/ml buserelin acetate both PC-based and TW-based microemulsions would not clearly conclude the effects of drug on testosterone levels over 30 days.

Field of study …...Pharmaceutics…… Student's signature …….
Academic year …….2001…… Advisor's signature …….

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Key: A=1 mg/ml, B=2.5 mg/ml, C=5 mg/ml, D=10 mg/ml, E=20 mg/ml

LIST OF ABBREVIATIONS

λ	=	wavelength of maximum absorption
%	=	percentage
μl	=	microliter (s)
μm	=	micrometer (s)
μg, mcg	=	microgram (s)
<	=	less than
>	=	more than
Aq	=	aqueous
BSA	=	buserelin acetate
BT	=	n-butanol
CA	=	caproic acid
cp	=	centripoint
CV	=	coefficient of variation
e.g.	=	exampli gratia (for example)
E1	=	surfactant
E2	=	cosurfactant
E1/E2 ,Em	=	surfactant/cosurfactant
Em/O	=	Em/Oil
EO	=	ethyl oleate
et al.	=	et alii (and others)
etc.	=	et cetera (and so on)
GR	=	glycerol
HLB	=	hydrophilic lipophilic balance
HPLC	=	high-performance liquid chromatography
hr	=	hour (s)
i.e.	=	id est (that is)
IPM	=	isopropylmyristate
M	=	molarity

LIST OF ABBREVIATIONS

ME	=	microemulsion formulation
MCT	=	medium chain triglyceride
ME BSA	=	BSA loading microemulsion formulation
mg	=	milligram (s)
min	=	minute (s)
ml	=	milliliter (s)
MW	=	molecular weight
nm	=	nanometer (s)
ng	=	nanogram (s)
No	=	number of sample
^{o} C	=	degree Celcius
o/w	=	oil in water
PC	=	phospholipid ,lecithin
PEG	=	polyoxyethylene 400
PG	=	propylene glycol
pH	=	the negative logarithm of the hydrogen ion concentration
pKa	=	the negative logarithm of the dissociation constant
rpm	=	revolution (s) per minute
RT	=	retention time
SBO	=	soybean oil
SD	=	standard deviation
TEM	=	transmission electron microscopy
TW	=	tween 80
w/o	=	water in oil
w/w	=	weight by weight
μ	=	umhos/cm

CHAPTER I

INTRODUCTION

Buserelin [D-Ser(*t*-Bu)-LHRH(1-9)-nonapeptide-ethylamide] acetate, a highly active agonistic analogue of luteinizing hormone-releasing hormone (LHRH), potently inhibits pituitary gonadotropin secretion and suppresses testicular and ovarian steroidogenesis when administered chronically in therapeutic doses. These inhibitory effects are further used in the treatment of hormone-dependent tumors and endometriosis (Brogden, Buckley, and Ward, 1990; Waxman et al., 1989; De Voogt, Adenauer, and Widdra, 1991; Filicori and Flamigni, 1988; Uekama et al., 1989; Sandow et al., 1987; Furr, 1987; Jacobi et al., 1987).

Since LHRH agonists are peptides they all have poor oral potency and so are most effectively given by the parenteral route. In addition, the short biological half-lives of buserelin requires long-term daily injection or frequent nasal application to maintain therapeutic concentration of the drugs. Daily injection is inconvenient for the patient so alternative means of delivery have been developed. Multiple daily dosing from a nasal spray formulation has advantages over subcutaneous injection but give low and variable absorption of drug. Therefore, attention has been directed toward the development of drug delivery systems with controlled-release features to realize the potential and efficacy of LHRH analogues (Debruyne, Weil, and Dernandez del Moral, 1987; Banga and Chien, 1988).

Several approaches have been proposed including the use of implants (Furr, 1987; Jacobi et al., 1987; Debruyne et al., 1987), prolonged-release injectable oily preparation (Uekama et al., 1989), protein conjugation (Toth et al., 1994), improved nasal delivery by cyclodextrin derivative (Matsubara et al., 1995; Abe, Irie, and Uekama, 1995), improved intestinal absorption by carbomer and chitosan (Lueben et al., 1996), buccal delivery (Hoogstraat et al., 1996), and iontophoretic transdermal (Chen and Chien, 1996).

Microemulsion is a system comprising mixture of water, oil and amphiphilic compounds forming thermodynamically stable, homogeneous, optically isotropic

solutions (Paul and Moulik, 1997; Eccleston, 1992; Lindman and Friberg, 1999; Solan, Pons, and Kunieda, 1997). Three distinct microemulsions, including of oil external, water external, and middle phase, can be used for drug delivery, depending upon the type of drug and the site of action. They have been described in the literature as reservoir systems, allowing slow release of drugs and providing a prolonged effect that avoids high concentrations in the blood. Microemulsions are excellent candidates as potential drug delivery systems because of their improved drug solubilization, long shelf-life, and ease of preparation and administration (Bagwe et al., 2001).

Microemulsions formulated with suitable excipients may also prove to be suitable vehicles for delivery of labile (peptide) and poorly soluble drugs. Water in oil microemulsions may be indicated for the parenteral administration of short-half-life hydrophilic drugs. Specifically, peptides and small proteins could be administered parenterally in water in oil microemulsions, with advantages such as protection of the molecule from the biological environment, prolonged release, and reduction of the drug's side effects (Eccleston, 1992; Gasco, 1997). Although hydrophilic drug could diffuse essentially without obstruction, the release was depend on the volume fraction of the dispersed phase (Shinoda et al., 1991).

The formulation of microemulsions for pharmaceutical use requires a thorough understanding of the properties, uses, and limitations of microemulsions (Bagwe et al., 2001). The limits on the use of microemulsions in the pharmaceutical field arise chiefly from the need for all the components to be acceptable, particularly surfactants and cosurfactants. More studies will have to be done to increase the number of biocompatible components (Gasco, 1997; Radomska and Dobrucki, 2000).

The primary aim of this study is to develop parenteral preparation of LHRH analogue by using buserelin as a model peptide drug and water in oil microemulsions, compared with oil in water systems as a delivery system in order to achieve a safe controlled release formulation.

Thus the study is focused on

1. Developing parenteral microemulsions with pharmaceutically acceptable ingredients.
2. Studying the effect of formulation variables including types and ratios of ingredients on physicochemical properties and stability of the systems.
3. Evaluating the *in vitro buserelin acetate release* drug release from both of water in oil and oil in water microemulsions.
4. Evaluating the *in vivo* testosterone suppression from the water in oil microemulsion delivery systems, compared with oil in water systems, in male rabbits.

CHAPTER II

LITERATURE REVIEWS

PEPTIDE AND PROTEIN DRUGS

Since the advent of genetic engineering has resulted in a proliferation of new biopharmaceuticals, the commercial production of proteins and peptides for pharmaceutical purpose is common. The available therapeutic agents produced by this technology is expanding rapidly including of neuropeptides, interferon, macrophage activation factors, tissue plasminogen activator, and experimental agents that may have potential in cardiovascular disease, inflammation, contraception and so on. However, there are some limitations of its chemical and physical properties, including molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, immunogenicity and denaturation, which make them unsuitable for delivery using the normal absorption routes, especially the oral route (Banga and Chien, 1988; Ganderton, 1991; Borchardt et al., 1989; Pitt, 1990; Zhou and Po, 1991a,b).

Because of their susceptibility to the strong acidic environment and the proteolytic enzymes in the gastrointestinal tract, the oral bioavailability of most peptides and proteins is very low. Also, peptides and proteins are high-molecular-weight substances and thus do not easily cross the intestinal mucosa. For systemic delivery of peptide and protein drugs, parenteral administration is currently required in order to achieve their therapeutic activities. However, most peptide/protein drugs cannot accomplish their full range of therapeutic benefits when administered by parenteral route, as limited by the nature of their extremely short-acting biological functions, the repeated injections are often required (Banga and Chien, 1988; Borchardt et al., 1989; Pitt, 1990; Zhou and Po, 1991a,b).

Thus, research programs have been initiated to search for a viable nonparenteral route for the effective systemic delivery of peptide/protein drugs. Another alternative for minimizing the drawbacks of parenteral administration is to develop long-acting parenteral preparations for peptide/protein drugs as has been done (Eschenbach et al., 1989; Banga and Chien, 1988; Pitt, 1990; Zhou and Po, 1991b).

Possible non-parenteral administration routes for delivery of peptide and protein drugs include the nasal, buccal, rectal, vaginal, transdermal, ocular and oral routes. In the absence of an absorption enhancer, these routes are generally much less efficacious than parenteral administration. For example, the oral administration of LHRH required a dose 3000-times higher than that of the parenteral route. The effective doses of LHRH via the other routes, relative to the parenteral route, were 100-times (nasal), 400-times (rectal) and 600-times (vaginal), higher. To date, systemic delivery parenteral administration is believed to be the most efficient route and also the delivery method of choice to achieve therapeutic activity (Zhou and Po, 1991b).

LHRH agonists

Lutenizing hormone-releasing hormone (LHRH), a decapeptide, is a neuro-hormone of hypothalamic origin whose function is to stimulate the synthesis and release of gonadotropins from the gonadotropes in the anterior pituitary (Cook and Sheridan, 2000; Eschenbach et al., 1989; Filicori and Flamigni, 1988; Banga and Chien, 1988).

Since Schally has synthesized LHRH in 1971, a number of analogues of naturally occurring LHRH such as leuprolide, buserelin and goserelin were soon synthesized. Most LHRH analogues are superactive agonists, i.e. they acutely increase gonadotrophin secretion. However, after an initial stimulatory phase lasting 1 to 2 weeks, LHRH agonists cause LHRH receptor down-regulation and suppress pituitary sensitivity. Long term administration of LHRH agonists reduce luteinising hormone and follicle-stimulating hormone secretion and gonadotrophin-dependent gonadal function (Eschenbach et al., 1989; Filicori and Flamigni, 1988).

The clinical applications of LHRH agonists extend from precocious puberty, endometriosis, uterine leiomyomas to hormone-dependent tumours, and to male and female contraceptive, covering a wide dose range (Cook and Sheridan, 2000; Eschenbach et al., 1989; De Voogt et al., 1991; Filicori and Flamigni, 1988).

As LHRH agonists are peptides, they all have poor oral potency and so are most effectively given by the parenteral route. In addition, the short biological half-

lives of LHRH analogues require long-term daily injection or frequent nasal application to maintain therapeutic concentration of the drugs. Daily injection is inconvenient for the patient so alternative means of delivery have been developed. Multiple daily dosing from a nasal spray formulation has advantages over subcutaneous injection but give low and variable absorption of drug. Therefore, attention has been directed toward the development of drug delivery systems with controlled-release features to realize the potential and efficacy of LHRH analogues (Brogden et al., 1990; Waxman et al., 1989; De Voogt et al., 1991; Filicori and Flamigni, 1988; Uekama et al., 1989; Sandow et al., 1987; Furr, 1987; Jacobi et al., 1987; Debruyne et al., 1987; Banga and Chien, 1988; Chen and Chien, 1996).

Several approaches have been proposed to improve the efficacy of various LHRH analogues including injectable gel system and dispersion or encapsulation in PLGA microcapsule (Banga and Chien, 1988). Many studies have been established to develop delivery system of LHRH and its analogues as follows:

- injectable oily preparation of buserelin acetate utilizing β-cyclodextrin which prolonged release for at least 1 month by Uekama et al. (1989);
- two-monthly depot buserelin using lactide-glycolide co-polymer by Waxman et al. (1989);
- intramuscular W/O microemulsion of D-Trp-6 LHRH which can prolong over a few weeks by Gasco et al. (1990);
- once-a-month injectable microspheres of leuprolide acetate by Okada et al. (1991);
- conjugation of LHRH and TRH which demonstrated a longer half-life than when present alone by Toth et al. (1994);
- polyanhydride microspheres containing LHRH analogues which prolonged release for at least 90 days *in vitro* by Mylonas et al. (1995);
- improvement of nasal bioavailability of buserelin by cyclodextrin derivatives by Matsubara et al. (1995);
- enhanced nasal delivery of buserelin by oleic acid solubilized and stabilized in hydroxypropyl-β-cyclodextrin by Abe et al. (1995);
- transdermal iontophoretic permeation of LHRH using polyacrylamide hydrogel reservoir devices by Chen and Chien (1996);

- improve the intestinal absorption of buserelin by carbomer and chitosan by Lueben et al. (1996);
- buccal delivery of buserelin with glycodeoxycholate as an absorption enhancer by Hoogstraate et al. (1996);
- LHRH-loaded polyhedral and spherical/tubular niosomes which can sustain drug released for more than 25 hours by Arunothayanun et al. (1999).

Despite the fact that there are many studies trying to invent slow release depot formulations of LHRH agonists, the attempt to develop more appropriate delivery system is continued in order to further optimize and extend the clinical application of these compounds.

Buserelin

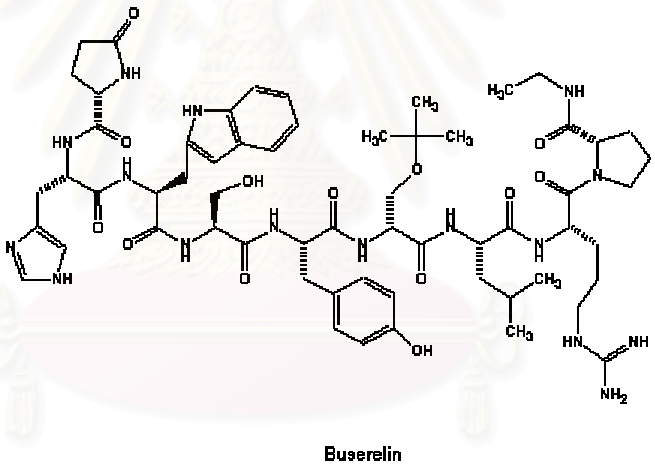


Figure 1: Buserelin structure

Buserelin was produced by scientists at Hoechst AG (West Germany) by substituting at the sixth site in the peptide chain of natural GnRH the amino acid glycine with D-serine-O-tert-butyl ether and by replacing the terminal glycinamide with ethylamide, resulting in a nonapeptide. The molecular structure of buserelin is shown in Figure 1. It is a white powder and the empirical formula is $C_{60}H_{86}N_{16}O_{13}$ $C_2H_4O_2$, with a molecular weight of 1237.3. The chemical name is 6-[O-(1,1-

dimethylethyl)-D-serine]-9-(N-ethyl-L-prolinamide)-10-deglycinamidelutenizing hormone releasing factor, with the sequence of Glp-His-Trp-Ser-Tyr-D-ser(t-Bu)-Leu-Arg-Pro-NHEt. It has LD_{50} of 56mg/kg (mouse, i.v.) ;>1 g/kg (mouse, p.o.), 36 mg/kg (rat, i.v.) ; >400 mg/kg (rat, p.o.). (Kleemann et al., 1999)

It has a serum half-life similar to that of natural GnRH but, after binding to the receptor, is resistant to degradation by arylamidase, resulting in a prolonged and more intense receptor blockade that is, however, completely reversible on cessation of the drug (Eschenbach et al., 1989; De Voogt et al., 1991). The commercial buserelin are available as nasal spray of 10 mg buserelin in 10 gm aqueous solution; 15 mg buserelin in 10 gm aqueous solution; and subcutaneous injection of 6.6 mg buserelin acetate on poly-(D, L-lactide-co-glycolide) 75:25 matrix. Treatment with buserelin is varied depending on the symptoms of patients.

Buserelin is a promising agent in the treatment of a variety of disorders in gynaecology, andrology, paediatrics, and oncology. While a single dose of buserelin stimulates the release of pituitary gonadotrophins, multiple doses produce reversible pituitary desensitization, and this specific blockade of gonadotrophin support to the gonads provides the basis for the drug's efficacy in conditions dependent on sex hormone secretion. Thus, buserelin provides comparable efficacy to orchidectomy or lower incidence of adverse effects. During the early phase of treatment it may be particularly useful in combination with antiandrogens. Buserelin also appears promising in hormone-sensitive premenopausal breast cancer. Extensive studies have proven the value of buserelin in endometriosis, where it produces a transient remission with gradual recurrence of the disease on cessation of treatment. Surgical intervention is necessary in severe disease after buserelin-induced involution of the lesions. In patients with uterine leiomyoma, preliminary data suggests that buserelin may be beneficial in rendering surgery more conservative by reducing fibroid size, although it appears unlikely to preclude surgical intervention.

The use of buserelin to induce a state of reversible hypogonadotrophism before administration of exogenous gonadotrophins is a promising strategy in the treatment of infertility associated with polycystic ovary syndrome and other conditions of infertility with underlying ovarian dysfunction; such a strategy also clearly enhances the efficiency of in vitro fertilization programmes. Initial studies

suggest its potential usefulness as a female contraceptive when administered intermittently in conjunction with a progestrogen. Buserelin represents a first-line treatment of central precocious puberty. In endometriosis the adverse effect profile of buserelin is generally favorable, with hypoestrogenic effects such as hot flushes and vaginal dryness, and decreased libido, predominating. There is no apparent detrimental effect on lipid metabolism. The potential for adverse hypoestrogenic effects on bone mineral content with long term administration remains to be clarified.

De Voogt et al. (1991) have reviewed 28 studies from 1979-1988 of 1522 patients with advanced prostatic cancer, who received buserelin monotherapy. Seventy-five percent of the patients received buserelin nasal spray (3x daily 400 mcg), 20% received subcutaneous injections (2x daily 200 mcg) and 4% received 2 combinations of nasal spray, subcutaneous injections and depot, or depot alone. They concluded that buserelin appears to give results equivalent to orchiectomy in terms of progression-free and overall survival. The adverse reaction profile is also similar and therefore it may be regarded as a standard anti-androgenic therapy for patients with advanced prostatic cancer.

Thus, the LHRH agonist buserelin represents an advance in the treatment of a variety of gynaecological and andrological as well as paediatric and oncological conditions, infertility and other sex-hormone dependent conditions, with a low incidence of adverse treatment effects (Brogden et al., 1990).

Several approaches have been proposed to improve the efficacy of buserelin as following:

Parenteral delivery

injectable oily preparation of buserelin acetate utilizing β-cyclodextrin which prolonged release for at least 1 month by Uekama et al. (1989);

two-monthly depot buserelin using lactide-glycolide co-polymer by Waxman et al. (1989);

Nasal delivery

improvement of nasal bioavailability of buserelin by cyclodextrin derivatives by Matsubara et al. (1995);

enhanced nasal delivery of buserelin by oleic acid solubilized and stabilized in hydroxypropyl-β-cyclodextrin by Abe et al. (1995). The rate and extent of nasal bioavailability of buserelin were remarkably increased by coadministration of oleic acid and hydroxypropyl-β-cyclodextrin, compared with the sole use of the enhancer. This enhancement was ascribable to the lowering of both the enzymatic and physical barriers of the nasal epithelium to the peptide, probably through the facilitated transmucosal penetration of oleic acid solubilized in hydroxypropyl-β-cyclodextrin.

Buccal delivery

Enhance an absorption of buserelin with glycodeoxycholate in pigs by Hoogstraate et al. (1996a,b). Buccal administration of buserelin resulted in rapidly reached steady state plasma levels. The absolute bioavailability of the peptide after buccal delivery for 4 hours could be increased by co-administration of glycodeoxycholate (Hoogstraate et al. 1996b). This study was confirmed by Junginger et al. (1999).

Improve intestinal absorption

improve the intestinal absorption of buserelin by carbomer and chitosan by Lueben et al. (1996).

Kotze et al. (1997) found that the potential use of N-trimethyl chitosan chloride as an absorption enhancer across mucosal surfaces could be an important contribution towards the development of effective delivery systems for hydrophilic drugs such as buserelin.

Bernkop-Schnurch (2000) explained that because of the mucoadhesive properties of chitosan and most of its derivatives, a presystemic metabolism of peptides on the way between the dosage form and the absorption membrane could be strongly reduced. Based on these unique features, the co-

administration of chitosan and its derivatives leads to a strongly improved bioavailability of many perorally given peptide drugs such as buserelin.

Microemulsions with suitable excipients may be suitable vehicles for delivery of peptide drugs. Water in oil microemulsions may be indicated for the parenteral administration of short-half-life hydrophilic drugs. Specifically, peptides and small proteins could be administered parenterally in water in oil microemulsions, with advantages such as protection of the molecule from the biological environment, prolonged release, and reduction of the drug's side effects. In addition, their thermodynamic stability has resulted in easy of preparation, independent of order mixing, and transparency with low viscosity which made it more suitable for this route (Eccleston, 1992; Gasco, 1997).

MICROEMULSIONS

Microemulsions are isotropic and thermodynamically stable multicomponent fluids composed of water, oil, and emulsifier(s). Large amounts of two immicible liquids (i.e., water and oil) can be brought into a single phase, macroscopically homogeneous but microscopically heterogeneous, by addition of an appropriate emulsifier or emulsifier mixture. This unique class of optically clear solutions called microemulsions comprises the colloidal systems that have attracted much scientific and technological interest over the past decade. The characteristic properties of microemulsions include spontaneous formation, optically clear appearance, large interfacial area, low interfacial tension, large solubilization capability, and low viscosity-properties that render these organized solutions unique.

Microemulsions research has been stimulated by the great potential of microemulsions for practical applications primarily because their use for enhanced oil recovery seemed and attractive alternative due to the work of Shah and Hamlin (1971), and over the years a wide spectrum of other applications-ranging from mundane to sophisticated-have emerged and many more are under active development.

Since the increasing demands on the performance of pharmaceutical formulations with respect to, e.g., storage stability, increased dosage levels, greater bioavailability, fewer side effects, controlled release, and biological response (e.g., tissue distribution) constitute the main motivation for drug delivery research. In the last few decades, this research has resulted in the development of e.g., parenteral emulsions, liposomes, liquid crystalline phases, and microemulsions.

Microemulsions are distinctly different from normal emulsions in that the former are thermodynamically stable one-phase systems whereas the latter are kinetically stabilized dispersions. Thus, microemulsions require no work for their formation, and once formed they are " infinitely " stable. Their thermodynamic stability, resulting in ease of preparation and excellent long-term stability, and their ability to solubilize large amount of both polar and non polar drugs, makes these systems an attractive alternative for drug delivery vehicles. Moreover, the dispersed phase could act as a reservoir and lead to a controlled rate delivery system (Kumar and Mittal, 1999; Solans, Pons and Kunieda, 1997; Eccleston, 1992).

Terminology and Definitions

The term "microemulsion" was first introduced by Hoar and Schulman in an article in Nature in 1943, to describe the transparent or translucent systems obtained by titration of an ordinary emulsion having a milky appearance to clarity by addition of a medium chain length alcohols such as pentanol or hexanol. These alcohols were later referred to as cosurfactants or cosolvents. There had been much debate on the use of term "microemulsion". Some prefered the names "swollen micellar solutions" or "solubilized micellar solutions" to describe precisely the systems called microemulsions by Hoar and Schulman.

Some authors defined microemulsions in the wider sense of including both thermodynamically stable systems and kinetically stable emulsion of fine droplet size. The stricter definition of Danielson and Lindman (1981) described microemulsions as optically istropic and thermodynamically stable liquid solutions of oil, water and amphiphile, was probably more widely accepted. It included micellar and reverse micellar solutions, microdroplets of oil and water, and bicontinuous structures, and excluded the various surfactant aggregates and transparent emulsions containing very

small droplets that were long-lived kinetic stability. Friberg (1982), however, pointed out that a wider definition might be more appropriate if o/w microemulsions of high oil content were included.

Microemulsions and micellar solution are, on the other hand, thought to be thermodynamically stable. Their properties are time independent and also the order of mixing. Many investigators have perceived a difference between microemulsions and micelles. In theoretical meaning, microemulsions are the aggregates containing a large amount of solubilisate while micelles is the aggregates of surfactant dispersed in a continuous phase. However, there is no precisely operational method available to distinguish this difference.

Many researchers think that the word “microemulsion” must be kept for those truly single-phase surfactant-oil-water (and often cosurfactant) mixtures in which sizable amounts of both oil and water are cosolubilized in a complex structure that has been described as percolated, bicontinuous, and transient. As will become clear later, from the point of view of applications these structures exhibit extremely interesting properties such as extremely low interfacial tension and high solubilization.

Theories of Microemulsions Formation and Stability

Many approaches have been used to explore the mechanisms of microemulsion formation and stability. Theories of microemulsion formation are classified into three main categories. The interfacial or mixed-film theory has been introduced by Schulman (1959) and Prince (1977). The second theory is the solubilization theory proposed by Shinoda, Friberg and collaborators (1974). The third one is thermodynamic treatment of Ruckenstein (1979), and Overbeek (1987).

Mixed-film theory

In this theory, The spontaneous formation of microemulsion droplets was considered to be due to the formation of a complex film at the oil-water interface by the surfactant and cosurfactant. This caused a reduction in the oil-water interfacial tension to very low values (from close to zero to negative). The interfacial film is considered as a duplex film having different properties on the water side and the oil side, where the interfacial tension is, $\gamma_T = (\gamma_{o/w})_a - \pi$ (1) , where $(\gamma_{o/w})_a$ represents the

oil/water interfacial tension in the present of alcohol (cosurfactant), and π is the spreading pressure of the mixed film. π is attached by the presence of surfactants and penetration of the oil into the hydrocarbon part of the interface. With the expansion of the interface, π increases and γ_T tends to zero favoring dispersion.

The interfacial film must be curved to form small droplets, and the concept of the duplex film was used to explain both the stability of the system and the bending of the interface. A flat duplex film would be under stress because of the different tensions and spreading pressure on either side of it. The reduction of this tension gradient by equalizing the two surface pressures and tensions is the driving force for the film curvature. Both sides of the interface expand spontaneously with penetration of oil and cosurfactant until the two pressures become equal. The side with the higher tension would be concave and would envelope the liquid on that side, making it the internal phase. The pressure gradients, and hence the type of microemulsion, are influenced by the molecular structures of the oil, surfactant, and cosurfactant and the concentrations of each. Since it is generally easier to expand the oil side of an interface (by penetration of the oil or cosurfactant into the hydrocarbon chain area) than the water side, it is easier to form W/O rather than O/W microemulsions. A short-to-medium chain length cosurfactant ensures that the film is flexible enough to readily form around the droplets. The importance of geometric packing to the nature of the microemulsion was also considered by Mitchell and Ninham (1981) and is discussed later under formulation.

The dynamic role of the cosurfactant in microemulsion formation was considered by Gerbacia and Rosano (1973) who observed that the interfacial tension could be temporarily reduced due to the diffusion of cosurfactant through the interface. They proposed that microemulsions be formed by two processes, dispersion and stabilization. The dispersion process involves a transient reduction of interfacial tension to near zero or negative values at which the interface expands to form fine dispersed droplets. Subsequently these absorb more surfactant and cosurfactant until the bulk phase is depleted enough to bring the γ_i positive again, when the second process, that of stabilization, is initiated by the interfacial film of alcohol and surfactant. The stability of the O/W systems was considered to be controlled by the

interfacial charge. If the diffuse double layer at the interface is compressed by high concentrations of counterions, W/O microemulsions are formed.

Solubilization theories

In the solubilization concept introduced by the groups of Gillberg (1970) and Shinoda (1975), microemulsions are considered as swollen micellar systems i.e with oil and water solubilized in normal or reverse micelles stemmed from the studies of three and four component phase diagrams on the one hand and the solubilization studies of water and hydrocarbons by nonionic surfactants on the other.

Thermodynamic theory

Interfacial film theories do not explain why some microemulsions form in the absence of cosurfactant. For microemulsions to form spontaneously, the free energy, ΔG, involved, shown in Equation $\Delta G = \gamma \Delta A$ (2), where ΔA is the inverse in surface area. The equation must be negative.

The theory of Ruckenstein and co-workers (1979) and Overbeek (1985) considered that microemulsion formation is entropically driven and emphasize the calculation of the entropy term. The dispersion of the droplets in the continuous phase increases the entropy of the system and produces a negative free-energy change. This is not very important for large-droplet macroemulsions, but can be very significant when droplets are small as in microemulsions. Ruckenstein and Chi (1975) considered the free energy of formation of microemulsions ΔG_m to consist of three main contributions, as shown in Equation (3).

$$\Delta G_m = \Delta G_1 + \Delta G_2 + \Delta G_3 \qquad (3)$$

where ΔG_1 is the interfacial free energy, including a positive term due to the formation of an uncharged interface and a negative term due to the formation of an electric double layer; ΔG_2 is the free energy of interdroplet interactions, composed of a negative term due to Van der Waals attraction and a positive term due to repulsive double layer interaction; and ΔG_3 is an entropy term for dispersion of droplets into the continuous medium. Later it was shown that accumulation of the surfactant and cosurfactant at the interface results in a reduction of bulk concentration and a decrease

in chemical potential, generating an additional negative free-energy change, the so-called dilution effect. Microemulsions form because the negative free energy changes due to the adsorption of the surfactant and cosurfactant on the generated interface plus the entropy of the dispersion of the droplets in the continuous phase overcome the positive product of the small interfacial tension and the large interfacial area. This thermodynamic treatment was used to explain the role of the cosurfactant and salt in microemulsions formed with ionic surfactants. The cosurfactant produces an additional dilution effect and decreases interfacial tension further. The addition of salt to systems containing ionic surfactants causes similar effects, because it shields the electric field produced by the adsorbed ionic surfactant, facilitating the adsorption of a larger amount of surfactant.

Overbeek (1985) developed a thermodynamic theory that was slightly different from that of Ruckenstein and Chi (1975), in that it included curvature contributions to the interfacial free energy arising from the bending and flexibility of interfaces, although the final conclusions were similar.

The role of the cosurfactant cannot be entirely reconciled to its effect on packing. A highly flexible film is required to form small droplets. The bending of an interface requires work against both interfacial tension and the bending stress of the interface. The bending stress, which is particularly important for very low interfacial tensions and highly curved interfaces, is represented by K, the rigidity (i.e., elastic) constant. The interplay between bending and thermal energies plays an important role in these systems, because thermal fluctuations produce large undulations in surfactant layers when their elastic energy is comparable to the thermal energy. This interplay is expressed in terms of persistence length, which represents the average length of the straight part of the film. The persistence length increases exponentially with K, in such a way that a small reduction of K would drastically decrease the persistence length of the film toward a very curved phase. A large value of K represents a rigid interface for which large energy is required to bend the interface, and a lamellar birefringence phase often forms in the vicinity of the microemulsion region of the phase diagram. The rigidity constant is lowered by a cosurfactant and can cause a transition from lamellar phases to isotropic microemulsions phases. A small value of

K represents a fluid interface for which little energy is necessary for bending, and the interface can become extremely wrinkled to give bicontinuous structures.

Microemulsion Structures

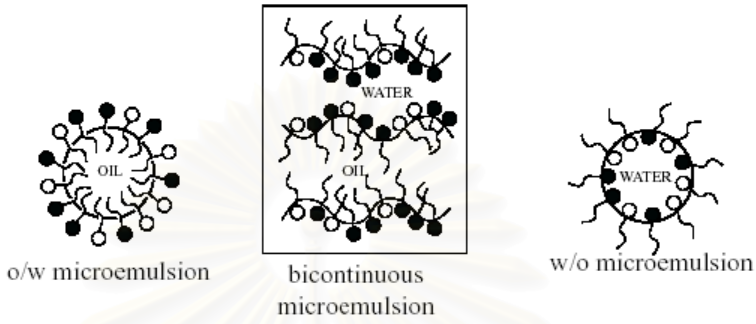


Figure 2: Some possible microemulsion structures

The simplest representation of the structure of microemulsions is the droplet model in which microemulsion droplets (discretes) are surrounded by an interfacial film consisting of both surfactant and cosurfactant molecules, as illustrated in Figure 2. The orientation of the amphiphiles at the interface will, of course, differ in o/w and w/o microemulsions. As shown, the hydrophobic portions of these molecules will reside in the dispersed oil droplets of o/w systems, with the hydrophilic groups protruding in the continuous phase, while the opposite situation will be true of w/o microemulsions.

Whether the systems form o/w or w/o microemulsions is determined to a large extent by the nature of the surfactant. An indication of the type of system that is likely to be favored can be gained by applying an approach proposed by Mitchell and Ninham, which considers the geometry of the surfactant molecule. If the volume of the surfactant molecule is *V*, its head group surface area *a*, and its length *l*, then when the critical packing parameter *V/al* has values of between 0 and 1, o/w systems are likely to form, but when *V/al* is greater than 1, w/o microemulsions are favored. Values of the parameters *V* and *l* can be readily estimated using, for example, equations proposed by Tanford. It should, however, be noted that the critical packing

parameter is based purely on geometric considerations. Penetration of oil and cosurfactant into the surfactant interface and hydration of the surfactant head groups will also influence the packing of the molecules in the interfacial film around the droplets. In many systems, inversion from w/o to o/w microemulsions can occur as a result of changing the composition or the temperature. In general, o/w microemulsions are favored when small amounts of oil are present and w/o systems form in the presence of small amounts of water. Under such conditions the droplet model is a reasonable representation of the system.

In contrast to the discrete microemulsion structure, the structure of bicontinuous microemulsion is more difficult to visualize and therefore its theoretical treatment is complicated. In bicontinuous microemulsion both the aqueous and oil phases are continuous. This has an extremely large interfacial area because of an extremely low interfacial tension, close to zero. Near-zero interfacial tension imply that the interface are unstable and can form and disappear without an energy increase. Condition for the formation of bicontinuous structures are a ratio of oil and water pseudophase close to one, large amounts of surfactant (enough to cover the interface), and zero natural curvature of the interface.

Formulations

Excipient selection

Although several microemulsion systems have been described in the literature, the challenge for the pharmaceutical formulator is to predict which oils and surfactants to select for particular application, taking into consideration their acceptability due to potential toxicity. The formulation of o/w and w/o microemulsions usually involves a combination of four basic components, namely, oil, water, surfactant, and cosurfactant. Even there are no strict rules for choosing the appropriate microemulsion components, there are a number of general guidelines base on empirical observes. A crucial step lies in the choice of surfactant and cosurfactant for the particular oil. The surfactant chosen must : lower interfacial tension to a very small value to aid dispersion processes during the preparation of the microemulsion ; provide a flexible film that can readily deform round small droplets ; have the

appropriate hydrophilic-lipophilic character to provide the correct curvature at the interfacial region for the desired microemulsion type.

The inclusion of a microemulsion in the following review of formulations should be taken to imply that it is of pharmaceutical interest rather than necessarily of use as a dosage form, since several contain ingredients that would limit their application as drug delivery systems. For convenience, microemulsions have been classified according to the surfactant used in their formulation.

Nonionic Surfactants

An early study by Jayakrishnan (1983) examined the solubilization of hydrocortisone by w/o microemulsions containing a mixture of the nonionic surfactants Brij® 35 (polyoxyethylene 23 lauryl ether) and Arlacel® 186 (glycerolmonooleate-propylene glycol), isopropanol as cosurfactant, water, and *n*-alkane. The influence of the concentration of the oil-soluble surfactant (Arlacel® 186), the chain length of the oil, and the alcohol concentration on the amount of water that could be incorporated in the w/o microemulsion were examined. With fixed quantities of the components, the water solubilization increased with increase of the chain length of the alkane between C_8 and C_{16} and increase of the concentration of Arlacel® 186, reaching a maximum at a 5/1 weight ratio of Arlacel® to Brij® . A formulation containing 10 mL decane, 4 mL isopropanol, 5 g Arlacel® 186, and 1 g of Brij® 35 was capable of incorporating about 8 mL of water.

The phase properties of microemulsions prepared using polysorbate/ sorbitol/ isopropyl myristate/water have been reported in a series of papers by Ktistis (1990) and Attwood (1989). A key factor influencing the phase properties is the surfactant/ cosurfactant ratio. Similar studies using polysorbate 80 and 60 have shown a change in the optimum polysorbate/sorbitol ratio (i.e., that producing the largest microemulsion region) from 1/ 2.5 for polysorbate 80 to 1/ 2 for polysorbate 60 to 1/1.5 for polysorbate 40. Such effects were attributed to differences in the packing of surfactant and cosurfactant at the oil/water interface. Gasco (1988) have used microemulsions of similar composition prepared using polysorbate 60 but with phosphate buffer rather than water, to study the in vitro release of propranolol.

Phospholipid

The possibility of forming microemulsions using phospholipids is obviously very appealing in view of low toxicity of these compounds. The problems involved in formulating microemulsions using lecithin have been reviewed by Shinoda (1991). Lecithin is slightly too lipophilic to form spontaneously the zero mean curvature lipid layers needed for balanced microemulsions, and it is necessary both to adjust the HLB and to destabilize the lamellar liquid-crystalline phases, which have a strong tendency to form in these systems. Alteration of the HLB can be achieved by adding short chain alcohols, which make the polar solvent less hydrophilic. In addition, the incorporation of these weakly amphiphilic cosolvents in the polar parts of the lipid layers increases the area of the lipid polar head to produce the required spontaneous curvature of the lipid layers; it also decreases the stability of the lamellar liquid-crystalline phase. These authors have reported the phase properties of the lecithin/propanol/water/*n*-hexadecane systems.

Aboofazeli and Lawrence (1993), recently reported phase properties for systems comprising water/lecithin/alcohol/isopropyl myristate in which the alcohols were *n*-propanol, isopropanol, *n*-butanol, *sec*-butanol, isobutanol, *ter*-butanol, and *n*-pentanol. Fubini and Gasco (1988) have prepared microemulsions of egg lecithin, isopropyl myristate, butanol, and water with three lecithin/butanol ratios within the range examined by Attwood (1992). A variation of this formulation reported by Gallarate (1988) contained octanoic acid to enhance the solubility of timolol in the oil phase and also to facilitate ion-pair formation. The formulations used were egg lecithin (28.7 wt %), 1-butanol (14.9 wt %), isotonic phosphate buffer pH 7.4 (40.0 wt %). The oil phase (16.4 wt %) consisted of solutions of octanoic acid and isopropyl myristate at several weight ratios (10.6/89.4, 15.9/84.1, 21.1/78.9, and 28.4/71.6). Water-in-oil microemulsions containing egg lecithin (13.81 wt %), water (11.03 wt %), hexanol (8.6 wt %), and ethyl oleate (66.5 wt %) were shown to be suitable reservoirs for doxorubicine and 1-demethoxy-daunorubicine.

Surfactant-cosurfactant mixtures

Most single-chain surfactants do not lower the oil-water interfacial tension sufficiently to form microemulsions, nor are they of the correct molecular structure.

The cosurfactant is added to further lower the interfacial tension between the oil and water phase, fluidize the hydrocarbon region of the interfacial film, and influence film curvature. Although the free energy associated with the formation of microemulsions is negative, it is small, and therefore the order of mixing plays an important role in the time taken to reach equilibrium. For example, the equilibruim is established more slowly if the cosurfactant is injected into the oil phase, as its greater solubility in this phase hinders its diffusion into the aqueous phase. Thus, the most appropriate cosurfactant is generally a small molecule, typically an alcohol of short to medium chain length that can diffuse rapidly between the bulk oil and water phase and the interface.

The chain length of the alcohol and cosurfactant influences curvature, with longer-chain alcohols swelling the tail-group region(negative curvature) more than those of shorter chain length. The microemulsion regions are smaller when alcohols of longer chain length are used. This is because the longer chain alcohols favor the formulation of liquid crystal. On the other hand, the shorter chain alcohols are too soluble in the aqueous phase and they are ineffective as cosurfactant. Cosurfactant of short to medium chain length alcohol also ensures that the interfacial film is flexible enough to deform readily around droplets, as their interaction between the primary surfactant molecules decreases both the polar head group interactions and the hydrocarbon chain interactions. The 1-butanol is good cosurfactant in conjunction with polyglycerol fatty acid ester for the formation of microemulsion. As a given surfactant concentration, the maximal solubilization efficacy of a stable microemulsion system can be achieved by adjusting the interfacial curvature and elasticity to optimal values at which the blending stress and the attractive force of the interface are both minimized. The penetration of short chain alcohols into the interface is a possible way to achieve this purpose.

Microemulsion preparation

Since microemulsions are thermodynamically stable, they can be prepared simply by blending oil, water, surfactant and cosurfactant with mild agitation. No significant energy contribution is required.

The usual method of preparing microemulsion is to dissolve the surfactant in the oil and then add the water to solution of oil and surfactant with gentle shaking. The microemulsion rapidly becomes first translucent and then optically clear after a few seconds. The order of mixing the components is generally considered not to be critical since microemulsions form spontaneously. However, Rosano and co-worker (1988) demonstrated that, although microemulsion is a spontaneous process, the driving forces are small and the time taken for these systems to reach an equilibrium interfacial tension can be long. Large transitory fluctuations in interface can occur during the microemulsion mixing process, as the component arrange themselves in such a way that the resulting interfacial and bulk microstructures lead to an overall minimum in the free energy. The time to establish equilibrium is influenced by the order of mixing. This is established more slowly if the cosurfactant is injected into the oil phase, as its greater solubility in this phase hinders its diffusion into the aqueous phase (Eccleston, 1992; Rosano, 1988).

Methods of Characterization (Solans, Pons and Kunieda, 1997)

Microemulsions have been studied using a great variety of techniques. This suggests that characterization of microemulsions is a rather difficult task. This is due to their complexity, namely the variety of structures and components involved in these systems, as well as the limitations associated with each technique. Therefore, complementary studies using a combination of techniques are usually required to obtain a comprehensive view of the physicochemical properties and structure of microemulsions. Several physical techniques are such as light scattering techniques, electron microscopy, phase diagrams, conductivity, dilutability, and viscosity determinations. Each technique contributes to the understanding of the microstructure of the microemulsion system.

Phase behavior studies, with phase diagram determinations, are essential in the study of surfactant systems. They provide information on the boundaries of the different phases as a function of composition variables and temperature, and, more important, structural organization can be also inferred. In addition, phase behavior studies allow comparison of the efficiency of different surfactants for a given application. It is important to note that simple measurements and equipment are required in this type of study. The boundaries of one-phase regions can be assessed

easily by visual observation of samples of known composition. However, long equilibration times in multiphase regions, can make these determinations long and difficult.

The phase behavior of interest for microemulsion studies involves at least three components; water, surfactant, and oil. Although most of the formulations of practical interest consist of more than three components, study of simple systems with the basic three, four, etc. components from which they are formulated is a prerequisite to understanding the behavior of complex systems. The phase behavior of three-component systems at fixed temperature and pressure is best represented by a ternary diagram and by a triangular prism. Other useful ways of representing the phase behavior are to keep constant the concentration of one component or the ration of two components. As the define the complete phase behavior becomes extraordinary large and the representation of phase behavior is extremely complex. One approach to characterizing these multicomponent systems is by means of pseudoternary diagrams that combine more than one component in the vertices of the ternary diagram. Most of the phase studies concerning microemulsions have been limited to the determination of one-liquid-isotropic phase boundaries. However, information about the number and compositions of the coexisting phases in equilibrium is of the utmost interest in characterizing these systems.

Electrical conductivity has been widely used to determine the nature of the continuous phase and to detect phase inversion phenomena. The distinction between O/W (high-conductivity) and W/O (low-conductivity) emulsions is quite straightforward. Dilutability by the excess of the dispersed phase are also employed to identify the structure of microemulsion. Oil in water microemulsions are dilutable with water, whereas water in oil systems are not undergo a phase inversion into oil in water microemulsions

The viscosity of microemulsions are macroscopically observable parameters that characterize a given system. There are also exist theoretical work that model microemulsion viscosity as a function of phase composition and phase type in order to predict properties of microemulsions under realistic conditions. Viscosity measurements as a function of volume fraction have been used to determine the hydrodynamic radius of droplets, as well as interactions between droplets and

deviations from spherical shape by fitting the results to appropriate models. Some microemulsions show Newtonian behavior, and their viscosity are similar to that of water. Some special situations of W/O microemulsion type that exhibit rheological behavior very different from that of typical microemulsions. An example of such a case is found in the system lecithin-water-oil, the viscosity increase by the factor of up to 10^6.

Several electron microscopic techniques have been attempted for the characterization of microemulsions. Freeze fracture electron microscopy, a well-established method in the biological field, has been successfully applied to microemulsions. Images showing clear evidence of the microstructure have been obtained. Careful control of the temperature of the sample before freezing and ultrarapid cooling followed by fracture and replication of the fracture face yield images of the microstructure of these systems.

Microemulsions as Drug Delivery Systems

Microemulsions represent an alternative to classical formulations as drug delivery systems, such as percutaneous, peroral, ocular, parenteral and may present new possibilities for therapy that have not yet been investigated (Bhargava, 1987). They are potential colloidal carriers for the targeting of drugs to specific areas and offer several advantages, such as enhanced absorption of drugs, modulation of the kinetics of drug release, and their solubilizing capability combined with the possibility of controlling drug release which improve drug efficacy and reduce unwanted toxic side effects. Optimization of microemulsion formulations by choosing surfactants and cosurfactants suitable for specific routes of administration will be a challenge in future years (Attwood, 1994).

Sustained release from microemulsions

The possibility of controlling the drug release rate by the microemulsion structure and composition as well as by drug partitioning makes microemulsions of interest for controlled-release applications, and several studies on this aspect of the pharmaceutical use of microemulsions.

Due to the wide range of structures occurring, microemulsions display a rich behavior regarding the release of solubilized material. Thus, in an O/W microemulsions, hydrophobic drugs, solubilized mainly in the oil droplets, experience hindered diffusions and are therefore released rather slowly (dependent on the oil/water partitioning of the substance). Water-soluble drugs, on the other hand, diffuse essentially without obstruction (depending on the volume fraction of the dispersed phase) and are released fast. The reverse behavior is expected for W/O microemulsions. For balanced microemulsions, relatively fast diffusion and release occur for both water-soluble and oil-soluble drugs due to the bicontinuous nature of the microemulsions "structure" (Attwood, 1994; Shinoda,1991).

Apart from the microemulsion structure, the microemulsion composition is important for the drug release rate. For example, Osborne (1988) studied the *in vitro* transdermal penetration of radiolabeled water from W/O microemulsions formed by water, octanol, and dioctylsodium sulfosuccinate. It was found that the delivery of the polar water portion in this microemulsion system was highly dependent on the microemulsion composition and, as expected, both the water self-diffusion and the transdermal flux increased on increasing the microemulsion water content. Furthermore, Gasco (1988) studied the release of (lipophilic) prednisone from O/W microemulsions formed by lecithin, butanol, and isopropyl myristate and found that the amount of cosurfactant in the microemulsion affected the drug release rate. More precisely, increasing the butanol concentration resulted in a decreased permeability constant for both hydrophilic and hydrophobic membranes. Similar results were obtained by Trotta (1990) on the release of steroid hormones of varying lipophilicity from microemulsions prepared from Aerosol OT, isopropyl myristate, water, and varying amounts of butanol.

Naturally, the microemulsion structure and composition are highly interrelated, and solubilized drugs generally affect both at the same time. Thus, for microemulsions formed by lecithin, oil, water, and short-chain alcohols (Gasco, 1988), there is a gradual transition toward structures more curved toward the oil (i.e., smaller droplets) on increasing the alcohol concentration (Shinoda, 1991). The smaller oil droplets formed in the presence of butanol hinder the prednisone diffusion more efficiently than larger droplets. Similarly, butanol resulted in a decreased

droplet size for the system investigated by Trotta (1990). On the other hand, butanol was also found to cause an effective enhancement of the drug partitioning to the oil phase (Trotta, 1990) , which also contributes to the decrease in the release rate.

Apart from the microemulsion structure and composition, the drug release rate from a microemulsion is expected to depend on the oil/water partitioning of the drug. In the study by Trotta (1990) on the release of steroid hormones of different lipophilicities from O/W microemulsions, a correlation was found between the partition coefficients and the release rate of the hormones.

Considering the dependence of the release rate on the drug partitioning, it is clear that the rate of release from a microemulsion formulation can be altered by changing the oil/water partitioning of the drug. For example, the effects on the release rate of increasing the effective lipophilicity of timolol by ion-pair formation with octanoic acid was studied by Gallarate (1988). These authors found that on increasing the octanoic acid concentration (i.e., increasing the ion-pair formation), the timolol partitioned more to the oil phase, resulting in an increased penetration of a lipophilic membrane. Similarly, Gasco (1988) used ion-pair formation with octanoic acid in O/W microemulsions to increase the lipophilicity of propranolol in order to obtain a disperse phase acting as a reservoir. It was found that octanoic acid increased the propranolol partitioning to the oil, resulting in a decrease in the drug release rate.

Bello (1994) compared the release of pertechnetate from a lecithin-containing W/O microemulsion and an aqueous solution after subcutaneous administration in rabbits and found that pertechnetate carried by W/O microemulsions was released from the site of administration at a slower rate than that administered from an aqueous solution. Along similar lines, Nastruzzi and Gambari (1994) performed an antitumor evaluation of lecithin-based microemulsion gels containing an aromatic tetrabenzamidine for topical administration in comparison to intraperitoneal injections of the corresponding aqueous solution and found the former formulation to be quite effective in inhibiting the proliferation at the cutaneous and subcutaneous levels.

Parenteral microemulsions

O/W microemulsion are used mainly as carriers of lipophilic drugs in order to attain prolonged release and to administer parenterally lipophilic substances that are

not soluble in water. They can be administered intravenously, intramuscularly, or subcutaneously. Various patents concerning the vectorization of fluorocarbons, calcium antagonists, steroids, and other lipophilic drugs have already been reported (Ziegenmeyer, 1980). O/W microemulsions have been injected into rats with the aim of targeting very lipophilic drugs (Garcia-Celma, 1994) into reticuloendothelial system (RES) and tissues (liver and spleen). The results indicated that the higher the partition coefficient of the drug, the better the targeting, an octanol-water partition coefficient above 10^8 is required to deliver the drug effectively to RES tissues.

W/O microemulsions can be used for subcutaneous and intramuscular administration; they may be indicated for parenteral administration of hydrophilic drugs with the aim of obtaining prolonged release. A microemulsion (surfactant; egg lecithin) containing 0.5 mg/mL insulin, was administered subcutaneously in rabbits (Garcia-Celma, 1992). The results demonstrated that some pharmacokinetic parameters were modified compared to those found with a solution; the half-life of insulin was 1.6 h for the solution and reached 4.3 h for the microemulsion; t_{max} values were 0.7 and 1.8 h, respectively.

The behavior of a microemulsion at the injection site was investigated by observing the release of a radiodiagnostic pertechnetate from a W/O microemulsion and an aqueous solution after subcutaneous administration in rabbits, imaging the administration sites with a gamma camera (Fubini, 1989). Disappearance of pertechnetate in aqueous solution from the injection site was about 10 times faster than that of pertechnetate in the microemulsion. The kinetics followed was of the first order, as usual in subcutaneous injection.

W/O microemulsions may be indicated for the parenteral administration of short-half-life hydrophilic drugs. Specifically, peptides and small proteins could be administered parenterally in W/O microemuslions, with advantages such as protection of the molecule from the biological environment, prolonged release, and reduction of the drug's side effects.

Advantages of the use of microemulsions as carriers of drugs

Microemulsions exhibit several properties that are of particular interest in pharmacy(Gasco,1997). Their thermodynamic stability allows self-emulsification of

the system, whose properties are not dependent on the process followed; the temperature range over which the phases do not separate can be rather wide.

Microemulsions act as supersolvents of drugs (including drugs that are relatively insoluble in both aqueous and hydrophobic solvents), probably as a consequence of the presence of the surfactant and the cosurfactant.

The dispersed phase, lipophilic or hydrophilic (oil-in-water (O/W) or water-in-oil (W/O) microemulsion, respectively), can behave as a potential reservoir of lipophilic or hydrophilic drugs, respectively. The drug will be partitioned between dispersed and continuous phases, and when the system comes into contact with a semipermeable membrane, with skin or mucous membrane, the drug can be transported through the barrier. Drug release with pseudo-zero-order kinetics can be obtained, depending on the volume of the dispersed phase, the partition of the drug among interphase and continuous and dispersed phases, and the transport rate of the drug.

The mean diameter of the droplets in microemulsions (considering a droplet microstructure) is below 100 nm. Such a small particle size yields a very large interfacial area, from which the drug can quickly be released into the external phase when in vitro or in vivo absorption takes place, maintaining the concentration in the external phase close to initial levels.

The technology required to prepare microemulsions is simple, because their thermodynamic stability means that no significant energy contribution is required. Microemulsions can be sterilized by filtration, as the mean diameter of the droplets is below 0.22 μm. Microemulsions have low viscosity. They may become unstable at high or low temperatures, but their formation is reversible (when the temperature returns to the stability range).

Autoxidation of lipids in O/W microemulsions is lower than in emulsions or micellar solutions as shown in a study of oxidation rates, using linoleic acid as a model molecule. Lipophilic and hydrophilic drugs can be carried together in the same microemulsion. The use of microemulsions as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

Factors limiting the use of microemulsions in pharmacy

However, the use of microemulsions as drug delivery systems involves limiting factors, such as the high surfactant content in most of the formulations reported in the literature, the potential toxicity of several components of model microemulsions (surfactants, cosurfactants) and possible adverse effects of these materials on the body, and the concomitant solubilization of other ingredients in the formulation with consequent alterations in stability and effectiveness. To make these surfactant systems pharmaceutically and biologically more useful, it is necessary to explore the feasibility of formulating microemulsions using commercially available nontoxic and safe ingredients. Many studies have been performed *in vitro and in vivo* and various components have been examined.

Other relevant factors are such as : Thermodynamic stability must be maintained, at least over the range of temperature between 4° and 40° C; Pressure must be kept reasonably constant during storage; Salinity may have a great effect on the domains in phase diagrams, as well as on the microemulsion structure itself; The amounts of cosurfactant and surfactant required to form microemulsions are usually higher than those required for emulsions (Gasco, 1997)

CHAPTER III

EXPERIMENTAL

Materials

The following materials were purchased from commercial sources and used as received. Deionized water was used throughout the study.

Model drug

Buserelin acetate (lot no L0002A1, American Peptide Company, California, USA)

Oils

- Isopropyl myristate (IPM, lot no 405657/1 14799, Fluka Chemie AG, Buchs, Switzerland)
- Ethyl oleate (EO, lot no 379540/1 14999, Fluka Chemie AG, Buchs, Switzerland)
- Medium chain triglycerides oil (MCT, lot no 9424318A, Mead Johnson & Company, Evansville, Indiana, USA)
- Super refined soybean oil (SBO, lot no 49H0273, Sigma Chemical, St.Louis, MO, USA)

Surfactants

- Soybean lecithin (PC, phospholipon 90, lot no 70060, Ident no 228183, Rhone-Poulenc Rorer, Nattermann Phospholipid GmBH Company, Cologne, Germany)
- Tween 80 (TW, polyoxyethylene (20) sorbitan monooleate, lot no 392141/ 123799, Fluka Chemie AG, Buchs, Switzerland)

Cosurfactants

- n-Butanol (BT, lot no K23035184632, Fluka Chemie AG, Buchs, Switzerland)

- Propylene glycol (PG, lot no 278972 1287, Fluka Chemie AG, Buchs, Switzerland)
- Polyethylene glycol 400 (PEG400, lot no 83-0242, BASF, Ludwigshafen, Germany)
- Glycerol (GR, lot no 118H0280, Fluka Chemie AG, Buchs, Switzerland)
- Caproic acid (CA, lot no 395414/1 22599, Fluka Chemie AG, Buchs, Switzerland)

Analytical substances

- Chlorpropamide (CP, lot no CP346, GPO, Thailand)
- Acetronitrile (HPLC grade, lot no 99120044, Lab-Scan Asia Co, Ltd., Thailand)
- Sodium phosphate monobasic (Ajax Chemicals, Auburn, N.S.W., Australia)
- Potassium phosphate monobasic (Ajax Chemicals, Auburn, N.S.W., Australia)
- Disodium hydrogen phosphate anhydrous (lot no 9I706290B, Carlo Erba, Italy)
- Sodium hydroxide pellets (lot no 7708 MVKK, Mallinckrodt Baker, S.A. Mexico)
- n-Octanol (lot no 398117/1 32699, Fluka Chemie AG, Buchs, Switzerland)

Miscellaneous

- Aquasil C18 column (5 μm, 150X4.6 mm, Thermo Hypersil, PA, USA)
- Aquasil C18 guard column (20×4 mm, Thermo Hypersil, PA, USA)
- Millex-GV (25 mm, 0.22 μm, PVDF, sterilized, lot no RONN46092, Millipore Corporation, Bedford, MA, USA)
- Millex-HV (4mm, 0.45 μm, PVDF, lot no N9NMB121A, Millipore Corporation, Bedford, MA, USA)
- Nylon membrane filter (47mm, 0.45 μm, lot no 329775, Lida, USA)
- Dialysis membrane (Lot no 10B040530, MW cut off 12000 Dalton, Sigma, St.Louis, MO, USA)

- Hypodermic glass syringe (2 ml, S-T Company, Thailand)
- Needle (0.8×40mm, no 21x1 $^{1/2}$, lot no 01G31, Nissho Nipro Corp., Ltd. Thailand)
- Surgical blade (no 11, Apollo, Thailand)
- Betadine solution (lot no KF143, LFT Ltd., Thailand)
- Steriled water for injection (ANB Laboratories Co., Thailand)

Animal

New Zealand White Rabbit (National Laboratory Animal Central, Mahidol University, Thailand)

Equipment

- Analytical balance (Sartorius, 0117-36, Germany)
- Autoclave (Hirayama Mfg. Corp.,Japan)
- Automated chemiluminescent immunoassay system (IMMULITE, DPC®, LA, USA)
- Conductivity meter (portable check-mate90, Ciba-Corning, UK)
- Dual action shaker (model 28L/B/SH/C, Polyscience, USA)
- High performance liquid chromatography instrument equipped with
  - Liquid chromatograph pump (LC-10AD, Shimadzu, Japan)
  - UV-VIS detector (SPD-10A, Shimadzu, Japan)
  - Recorder (C-R6A Chromatopac, Shimadzu, Japan)
- Transmission electron microscope (JEOL, JEM-200CX, 100 k voltage, Japan)
- Laminar airflow (model VS124, Holten Larminar-Air, Denmark)
- Magnetic stirrer (GEM HS-100, Thailand)
- Magnetic stirrer (model Poly15gray, 31516, H&P Labortechnik GmbH, Germany)
- Modified Keshary-Chien diffusion apparatus
- pH meter (model Φ 50 pH, Beckman, USA)
- Polarized light microscope (The KHC Olympus, Japan)
- Top to bottom rotating tube machine (model EWPC 902/T/R/P, Thailand)

- Ultracentrifuge (model L80, Beckman, USA)
- Ultrapure Water equipped with filter system (Balston, Balston Inc., USA), Boost pump, Option 3 water purifier, Maximum ultrapure water(ELGA, USA)
- Viscometer (Cone and Plate model RVTDCP, Brookfield Engineering Laboratories, INC. Massachusetts, USA)
- Vortex mixer (Vortex-genie, model G 560E,USA)

Methods

1. Formulation study design

The partial pseudo-ternary phase diagrams were constructed to examine the formation of microemulsions using four components of oil, surfactants, cosurfactants, and water. A series of sequential studies were done by varying the compositions and ratios of ingredients as shown in Table 1. The study includes

a) varying types of oils, surfactants, and cosurfactants :

oils : isopropyl myristate (IPM), ethyl oleate (EO) , medium chain triglyceride (MCT), soybean oil (SBO)

surfactants : lecithin (PC), tween 80 (TW)

cosurfactants : butanol (BT), propylene glycol (PG), PEG 400 (PEG), glycerol (GR), caproic acid (CA)

b) the ratio of surfactant to cosurfactant (E1/E2,Em) are at 1:1(A), 1:0.5 (B), and 1:0.25(C).

c) the total amount of surfactant/cosurfactant (Em) to oil are 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 and mostly studied between 7/3 to 3/7.

2. Partial pseudo-ternary phase diagrams study/ microemulsion preparation

2.1 Partial pseudo-ternary phase diagrams studies

The ternary plots were constructed by keeping the ratio of E1/E2 constant and varying the remaining components. Em/oil were held in a fixed ratio and

Table 1 Formulation study design

Oil	E1	E2	EM/OIL (1/9-9/1)	7/3			5/5			3/7		
			EM	A (1/1)	B (1/0.5)	C (1/0.25)	A (1/1)	B (1/0.5)	C (1/0.25)	A (1/1)	B (1/0.5)	C (1/0.25)
IPM	PC	BT	111	111a	111b	111c	111a	111b	111c	111a	111b	111c
		PG	112	112a	112b	112c	112a	112b	112c	112a	112b	112c
		PEG	113	113a	113b	113c	113a	113b	113c	113a	113b	113c
		GR	114	114a	114b	114c	114a	114b	114c	114a	114b	114c
		CA	115	115a	115b	115c	115a	115b	115c	115a	115b	115c
	TW	BT	121	121a	121b	121c	121a	121b	121c	121a	121b	121c
		PG	122	122a	122b	122c	122a	122b	122c	122a	122b	122c
		PEG	123	123a	123b	123c	123a	123b	123c	123a	123b	123c
		GR	124	124a	124b	124c	124a	124b	124c	124a	124b	124c
		CA	125	125a	125b	125c	125a	125b	125c	125a	125b	125c
EO	PC	BT	211	211a	211b	211c	211a	211b	211c	211a	211b	211c
		PG	212	212a	212b	212c	212a	212b	212c	212a	212b	212c
		PEG	213	213a	213b	213c	213a	213b	213c	213a	213b	213c
		GR	214	214a	214b	214c	214a	214b	214c	214a	214b	214c
		CA	215	215a	215b	215c	215a	215b	215c	215a	215b	215c
	TW	BT	221	221a	221b	221c	221a	221b	221c	221a	221b	221c
		PG	222	222a	222b	222c	222a	222b	222c	222a	222b	222c
		PEG	223	223a	223b	223c	223a	223b	223c	223a	223b	223c
		GR	224	224a	224b	224c	224a	224b	224c	224a	224b	224c
		CA	225	225a	225b	225c	225a	225b	225c	225a	225b	225c
MCT	PC	BT	311	311a	311b	311c	311a	311b	311c	311a	311b	311c
		PG	312	312a	312b	312c	312a	312b	312c	312a	312b	312c
		PEG	313	313a	313b	313c	313a	313b	313c	313a	313b	313c
		GR	314	314a	314b	314c	314a	314b	314c	314a	314b	314c
		CA	315	315a	315b	315c	315a	315b	315c	315a	315b	315c
	TW	BT	321	321a	321b	321c	321a	321b	321c	321a	321b	321c
		PG	322	322a	322b	322c	322a	322b	322c	322a	322b	322c
		PEG	323	323a	323b	323c	323a	323b	323c	323a	323b	323c
		GR	324	324a	324b	324c	324a	324b	324c	324a	324b	324c
		CA	325	325a	325b	325c	325a	325b	325c	325a	325b	325c
SBO	PC	BT	411	411a	411b	411c	411a	411b	411c	411a	411b	411c
		PG	412	412a	412b	412c	412a	412b	412c	412a	412b	412c
		PEG	413	413a	413b	413c	413a	413b	413c	413a	413b	413c
		GR	414	414a	414b	414c	414a	414b	414c	414a	414b	414c
		CA	415	415a	415b	415c	415a	415b	415c	415a	415b	415c
	TW	BT	421	421a	421b	421c	421a	421b	421c	421a	421b	421c
		PG	422	422a	422b	422c	422a	422b	422c	422a	422b	422c
		PEG	423	423a	423b	423c	423a	423b	423c	423a	423b	423c
		GR	424	424a	424b	424c	424a	424b	424c	424a	424b	424c
		CA	425	425a	425b	425c	425a	425b	425c	425a	425b	425c

titrated against increasing amounts of deionized water, as shown in the phase diagram of Figure 3.

To prepare system 111a which had Em ratio of A (1/1) and Em/oil ratio of 7/3, the total weight preparation was 10 gm. The amount of 3.5 gm surfactant and 3.5 gm cosurfactant were accurately weighed. The mixture was initially mixed with 3 gm of oil by sitrring with a teflon-coated magnetic bar for short time period to accelerate equilibrium (for PC-base formulations the systems were mixed and stayed over night for equilibrium). Then, the mixture was slowly titrated with the deionized water at the 0.5 ml each time using precision burette. At this point, the mixture would compose of water = 4.76%, Em = 66.67%, oil = 28.57%. After mixing, the system was checked for visual clarity and fluidity. The titration was continued to the point where clear mixture became turbid which was the end point of titration. Region in which the formulation formed visually stable and transparent solution was marked on the phase diagram as microemulsion, with one axis representing water, one representing oil, and the third representing mixture of surfactant and cosurfactant at a fixed weight ratio (as shown in Figure 3). To prepare system 111a which had Em/oil ratio of 5/5, the total weight preparation was 10 gm. The amount of 2.5 gm surfactant and 2.5 gm cosurfactant were accurately weighed. The mixture was initially mixed with 5 gm of oil. The process of titration used were the same as aforementioned. Formulation of 111a which had Em/oil ratio of 3/7 was prepared in the same way. The amount of 1.5 gm surfactant and 1.5 gm cosurfactant were accurately weighed and mixed with 7 gm of oil.

The preparation of system 111b that had Em ratio of B (1/0.5) and Em/oil ratio of 7/3 were processed by the same procedure. The total weight preparation was 10 gm. The amount of 4.67 gm surfactant and 2.33 gm cosurfactant were accurately weighed and mixed with 3 gm of oil by the same process previously described. The system 111b which had Em/oil ratio of 5/5, the amount of 3.33 gm surfactant and 1.67 gm cosurfactant were accurately weighed and mixed with 5 gm of oil. Formulation of 111b that had Em/oil ratio of 3/7 was prepared in the same way. The amount of 2.4 gm surfactant and 0.6 gm cosurfactant were accurately weighed and mixed with 7 gm of oil.

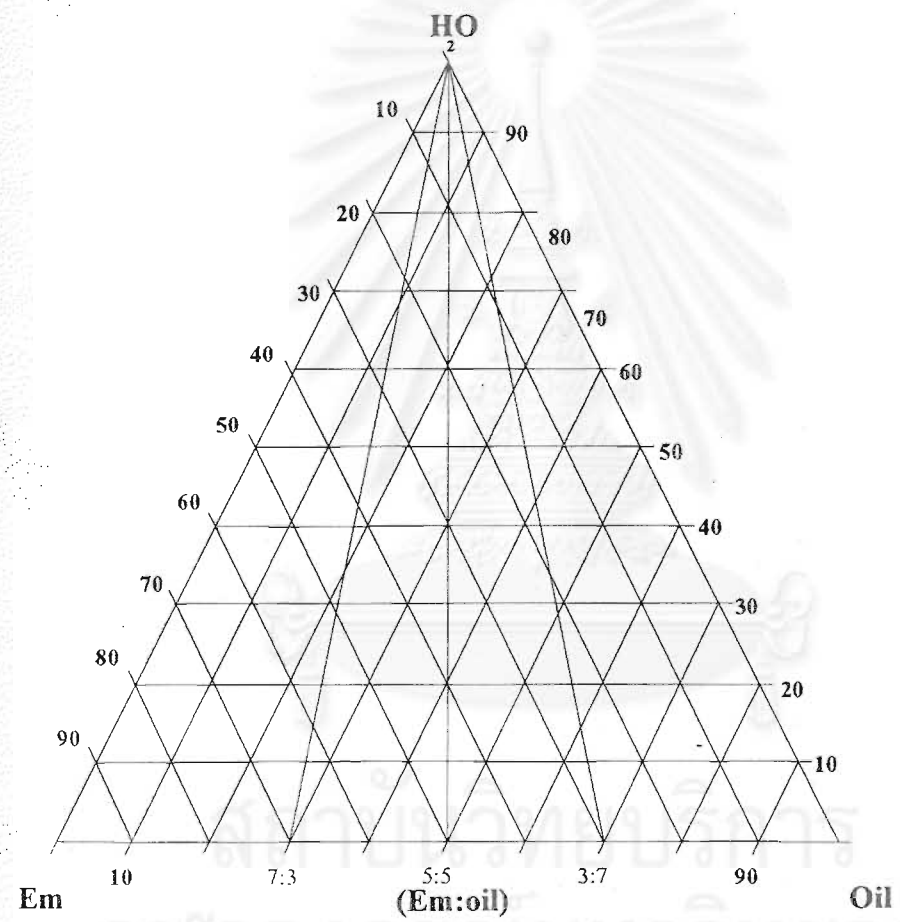


Figure 3 An illustration of a pseudoternary phase diagram construction

Also the system 111c which had Em ratio of C (1/0.25) and Em/oil ratio of 7/3, the weight of surfactant and cosurfactant were 5.60 gm and 1.40 gm, respectively. The mixture was mixed with 3 gm of oil, then following the same process. System 111c which had Em/oil ratio of 5/5 and System 111c which had Em/oil ratio of 3/7, the amount of surfactant and cosurfactant were 4.0 gm and 1.0 gm, 2.4 gm and 0.6 gm, respectively. Then they were mixed with oil of 5 gm and 7 gm and the same processing were done. Other formulations were prepared by the same process and all manipulations were performed at room temperature.

2.2 Preparation of microemulsions

Representative formulations from each system were selected and reprepared for characterization and stability studies. The criterion for selected formulation was the point in which most series were superimposed; the surfactant, cosurfactant, oil, Em ratio, Em/oil ratio, and internal phase can be compared. The composition of the selected microemulsions, formulation ME 1-ME 25, is shown in Table 2, and were prepared by the procedure previously described.

2.3 Preparation of microemulsions containing BSA

Representative formulations from both TW and PC-based systems were selected to evaluate the *in vitro* drug release. Nine formulations from TW and four formulations from PC-based systems, containing high amount of water phase (9-13%), were prepared with buserelin acetate.

To make sure that buserelin acetate was completely dissolved in water phase of the formulation. Approximate amount of 50 mg BSA was added to 1 ml distilled water in screw cap tube and mixed by vortexing. The solution was immersed and shaken in a top to bottom rotator water bath and controlled temperature at 30±1° C, then allowed to equilibrate. At each sampling interval (24, 30, and 48 hours), an aliquot was withdrawn and filtered through 0.45 μm membrane filter (Millex-HV, 4mm, PVDF). Concentrations of BSA in the filtrate were analyzed by HPLC method, after appropriate dilution. The experiment was carried out in triplicate.

Table 2 Composition (% w/w) of the investigated microemulsions

ME	Oil	E1	E2	Em	Em/oil	% w/w Ingredients		
						Oil	Em (E1/E2)	Aq
1	IPM	PC	BT	1/0.5	7/3	28.80	67.20 (44.80 / 22.40)	4.00
2	IPM	TW	BT	1/0.5	7/3	28.80	67.20 (44.80 / 22.40)	4.00
3	IPM	PC	PG	1/0.5	7/3	29.40	68.60 (45.73 / 22.87)	2.00
4	IPM	TW	PG	1/0.5	7/3	28.40	67.20 (44.80 / 22.40)	4.00
5	EO	PC	PG	1/0.5	7/3	29.40	68.60 (45.73 / 22.87)	2.00
6	EO	TW	PG	1/0.5	7/3	28.40	67.20 (44.80 / 22.40)	4.00
7	IPM	TW	CA	1/0.5	7/3	28.40	67.20 (44.80 / 22.40)	4.00
8	IPM	TW	PEG	1/1	7/3	25.95	60.55 (30.28 / 30.28)	13.50
9	IPM	TW	PEG	1/0.5	7/3	27.00	63.00 (42.00 / 21.00)	10.00
10	IPM	TW	PEG	1/0.25	7/3	28.40	63.00 (50.40 / 12.60)	10.00
11	IPM	TW	PEG	1/0.5	7/3	28.57	66.67 (44.45 / 22.22)	4.76
12	IPM	TW	PEG	1/0.25	7/3	28.80	67.20 (53.76 / 13.44)	4.00
13	IPM	TW	GR	1/1	7/3	29.10	67.90 (33.95 / 33.95)	3.00
14	IPM	TW	GR	1/0.5	7/3	29.40	68.60 (45.73 / 22.87)	2.00
15	IPM	TW	GR	1/0.25	7/3	28.80	67.20 (53.76 / 13.44)	4.00
16	EO	PC	PG	1/1	5/5	48.00	48.00 (24.00 / 24.00)	4.00
17	EO	PC	PG	1/0.5	5/5	48.00	48.00 (32.00 / 16.00)	4.00
18	EO	PC	PG	1/0.25	5/5	48.00	48.00 (38.40 / 9.60)	4.00
19	IPM	PC	PG	1/1	5/5	48.00	48.00 (24.00 / 24.00)	4.00
20	MCT	PC	PG	1/1	5/5	49.00	49.00 (24.50 / 24.50)	2.00
21	IPM	PC	PG	1/1	5/5	48.00	48.00 (24.00 / 24.00)	10.00
22	IPM	PC	PG	1/1	5/5	48.00	48.00 (24.00 / 24.00)	15.00
23	EO	PC	PG	1/1	6/4	38.40	57.60 (28.80 / 28.80)	4.00
24	EO	PC	PG	1/1	6/4	36.00	54.00 (27.00 / 27.00)	10.00
25	EO	PC	PG	1/1	6/4	34.00	51.00 (25.50 / 25.50)	15.00

The BSA-containing microemulsion as shown in Table 3 (ME26BSA-ME38BSA) were prepared by the following procedure. To prepare 7 gm of a single preparation, the accurate amount of 23.10 mg buserelin acetate was firstly weighed out and then dissolved in an accurate amount of 0.63 gm distilled water. To ensure complete mixing and solubilization, the drug solution was gently vortexed. The aqueous phase containing BSA was subsequently added to the right amounts (per weight) of the well mixed three components of oil, surfactant, and cosurfactant under predetermined stirring condition.

To sterilize microemulsion preparation (ME BSA 28,32,35,36), the systems were filtered through 0.22 μm Millex-GV, which special for low protein binding and safe for organic systems, by aseptic technique under laminar airflow and all glassware were autoclaved at 121 °C 15 minutes before using.

3. Physicochemical studies

3.1 Physical characterization of microemulsions

3.1.1 Polarized light microscopy

The KHC Olympus (Japan) microscope was employed to examine the nonbirefringence of formulations. A drop of sample was placed between a coverslip and a glass slide and then examined under polarized light.

3.1.2 Dye solubility test

The dye solubility test was performed by adding a drop of water soluble dye (amaranth) solution to the microemulsion. The intense staining of the external phase after the addition of a water soluble dye indicated o/w microemulsion. The addition of a water soluble dye in w/o microemulsion would result in the staining of the droplets.

3.1.3 Dilution test

The dilution test was performed by adding water or oil (IPM) to the microemulsion. If water was easily dispersed in the external phase, the microemulsion

Table 3 Formulations of the buserelin acetate microemulsions for in vitro release

ME	Oil	E1	E2	Em	Em/Oil	% Ingredients		
						Oil	Em (E1/E2)	Aq
26BSA	IPM	TW	PEG	1/0.5	5/5	45.50	45.50 (30.33 / 15.17)	9.00
27BSA	IPM	TW	PEG	1/0.25	5/5	45.50	45.50 (36.40 / 9.10)	9.00
28BSA	IPM	TW	PEG	1/1	7/3	26.10	60.90 (30.45 / 30.45)	13.00
29BSA	IPM	TW	PEG	1/0.25	7/3	27.30	63.70 (50.96 / 12.74)	9.00
30BSA	EO	TW	PEG	1/0.5	7/3	27.30	63.70 (42.47 / 21.23)	9.00
31BSA	EO	TW	PEG	1/0.25	7/3	27.30	63.70 (50.96 / 12.74)	9.00
32BSA	EO	TW	PG	1/0.25	7/3	27.30	63.70 (50.96 / 12.74)	9.00
33BSA	MCT	TW	PG	1/0.25	7/3	27.30	63.70 (50.96 / 12.74)	9.00
34BSA	MCT	TW	PEG	1/0.25	7/3	27.30	63.70 (50.96 / 12.74)	9.00
35BSA	IPM	PC	PG	1/1	5/5	45.50	45.50 (22.75 / 22.75)	9.00
36BSA	EO	PC	PG	1/1	5/5	45.50	45.50 (22.75 / 22.75)	9.00
37BSA	IPM	PC	PG	1/1	6/4	36.40	54.60 (27.30 / 27.30)	9.00
38BSA	EO	PC	PG	1/1	6/4	36.40	54.60 (27.30 / 27.30)	9.00

was o/w type. On the other hand, if oil was dispersible in the external phase the microemulsion was w/o type.

3.1.4 Conductivity measurement

The conductance of microemulsions was determined at room temperature using a portable check-mate90 conductivity meter (Ciba-Corning, UK). Standard solution of 12.88 mS and 1413 μS from Mettler-Toledo were used to calibrate the instrument.

3.1.5 Viscosity measurement

The viscosity of selected systems was monitored by a cone-plated viscometer using cone number-CP 40 (Brookfield Engineering Laboratories, INC. Massachusetts, USA) and was calibrated with liquid with known viscosity. The measurement was conducted at room temperature.

3.1.6 Transmission Electron Microscopy

Samples were viewed using JEM-200CX TEM by negative staining techniques. The specimens were prepared by placing a drop of specimen on a formvar coated 400 mesh copper grid for 15 seconds and wick away excess sample. Then place a drop of 2% phosphotungstic acid on the grid for 1 minute, wick away excess and let the specimen dry completely. Pictures were taken of various fields of interest at various magnifications.

3.1.7 Physical stability of formulation

Shelf-life stability of microemulsions, both as a function of time and storage temperature was routinely evaluated by visual inspection of the samples initially each month for 6 months and viscosity change at 3 months and 6 months. Stable systems were identified as those free of any physical change such as phase separation flocculation and/or precipitation. Stability was monitored at 4, 30, 37, and 50 °C.

3.2 Analytical Assay

3.2.1 HPLC assay for buserelin acetate

The determination of buserelin acetate by reverse phase HPLC assay with UV detection was modified from the method described by Sertl et al (1981). The condition was developed as follows.

Column	: aquasil C18 column, 5 μm, 150X4.6 mm
Guard column	: aquasil C18
Mobile phase	: phosphate buffer pH5.8 : acetonitrile(55:45)
Flow rate	: 1.0 ml/min
Detector wavelength	: 220 nm
Injection volume	: 20 μl
Internal standard	: chlorpropamide
Temperature	: ambient
Retention time	: chlorpropamide 3.81-3.97 minutes
	buserelin acetate 7.39-7.52 minutes

3.2.2 Preparation of mobile phase

2 M NaOH

A 2.0 M sodium hydroxide solution was prepared by accurately weigh 8 gm of sodium hydroxide pellets. Dissolve the pellets and adjust to 100 ml with deionized water.

Phosphate buffer pH 5.8

A 0.05 M sodium dihydrogenphosphate monobasic solution was prepared by accurately weighing 7.8 gm of sodium dihydrogenphosphate monobasic and transferring to 1000 ml volumetric flask. Dissolve the salt and adjust pH to 5.8 with 2 M sodium hydroxide solution then adjust to volume with deionized water.

Mobile phase

Mobile phase composed of 0.05 M phosphate buffer pH 5.8 and acetonitrile in the ratio of 55:45. Freshly prepared mobile phase was produced by mixing 550 ml of phosphate buffer pH 5.8 with 450 ml of acetonitrile (HPLC grade), filtering through 0.45 μm nylon membrane filter, then degasing by sonicator for about 30 minutes.

3.2.3 Preparation of BSA calibration curve

Phosphate buffer pH 7.4 (USP 23, 1995)

A 0.2 M monobasic potassium phosphate solution was prepared by dissolving 27.22 gm of monobasic potassium phosphate in deionized water and diluting to 1000 ml. Place 250 ml of monobasic potussium phosphate solution in a 1000-ml volumetric flask, add 195.50 ml of 2 M sodium hydroxide solution, then adjust water to volume. The buffer solution was then filtered through 0.45 μm membrane filter.

BSA stock solution

A BSA stock solution of 100 μg/ml was prepared by accurately weighing BSA 1.00 mg into 10 ml volumetric flask. The drug was dissolved and adjusted to volume with phosphate buffer pH 7.4.

Internal standard solution

Chlorpropamide stock solution of 15 μg/ml was prepared by dissolving 1.50 mg of chlorpropamide into 100 ml volumetric flask and adjusted with phosphate buffer pH 7.4.

Calibration curve

The standard solution of BSA containing 7.5 μg/ml of CP were prepared by appropriately diluting the BSA stock solution with the same medium and mixing with the internal standard solution, to obtain the final concentrations of 1, 2.5, 5, 10, and 20 μg/ml BSA and 7.5 μg/ml CP. Then these solutions were analyzed by HPLC at 220 nm. The peak area ratio of BSA to CP was used to calculate the BSA concentration and presented in Table 125 and Figures 122-123 of the Appendix D respectively.

Linearity

The linearity of standard curve was evaluated by plotting the standard curve between the peak area ratios of BSA to internal standard versus the

concentrations of BSA. Linear regression analysis was performed. The equation and the coefficient of determination (R^2) were calculated.

Precision

The within run precision was evaluated by analyzing peak area ratios of drug and internal standard of five repetitions of the concentration determined in the same day. The mean, standard deviation of each concentration were determined and shown in Table 126 in Appendix D.

The between run precision was evaluated by analyzing peak area ratios of drug and internal standard of five repetitions of the concentration determined injected on different day. The mean, standard deviation of each concentration were determined and shown in Table 126 in Appendix D.

3.3 Partitioning study

Saturated octanol was prepared by shaking octanol with water at 30±1°C for 24 hours before using. A 5 ml of 50 μg/ml BSA solution was pipetted to a 5 ml saturated octanol in a 25 ml erlenmeyer flask. The mixture was then shaken at 30±1°C in the dual action shaker until the drug was equilibrated between the two phase. At sampling interval of 24, 30, and 48 hours, the mixtures were separated and aqueous phase with BSA was pipetted. The drug was determined by HPLC method and the octanol/water partition coefficient was calculated. The experiment was carried out in triplicate.

4. *In vitro* diffusion study

Formulations

Microemulsion formulations of ME26BSA-ME38BSA, which prepared with surfactant TW and PC, were compared with formulation of BSA in buffer pH 7.4 solution. All of the formulations were freshly prepared.

Diffusion apparatus

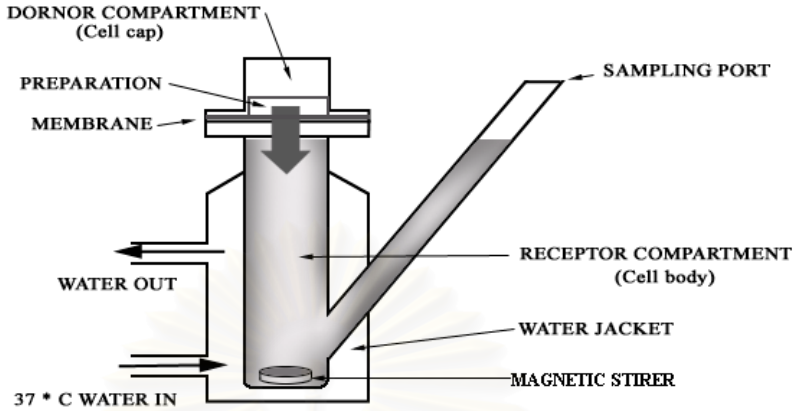


Figure 4 Schematic illustration of modified Keshary-Chien diffusion apparatus

The *in vitro* drug release study of BSA microemulsions was carried out using modified Keshary-Chien diffusion apparatus (Figure 4). The apparatus consists of two glass compartments, 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 phosphate buffer pH 7.4 solution as release medium. Two compartments were separated by dialysis membrane that had a molecular weight cut-off of 12,000-14,000 dalton. Before placing up onto 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 then soaked for 30 minutes in the release medium before using. The cell, with circulating jacket, was allowed to equilibrate and temperature maintained at 37±1 °C before and throughout the experiments.

After equilibration, 1 gm of sample was carefully weighed into the donor part. The two compartments were clamped with the treated membrane in-between. The release medium was carefully filled into the receptor part without air bubble. Then the cell was stirred by magnetic bar at 850 rpm. A 10 ml aliquot of receptor medium was withdrawn at appropriate time intervals and replaced immediately with an equal volume of fresh medium. A portion of the 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.

5. Animal study

Model animal

Young adult male New Zealand white rabbits, weighing about 2-2.5 kg and 2 months old, were acclimatized to the laboratory environment under controlled conditions of light (12 hours dark/12 hours light cycle) and temperature 25±2 °C prior to study. They were allowed free access to tap water and food throughout and were studied when their testosterone value are more than 0.3 ng/ml and their ages were 4 -5 month.

Formulations

BSA microemulsion formulations from TW and PC-based system were selected to be evaluated. Two formulations prepared with surfactant of PC (ME35BSA, ME36BSA) and two of TW (ME28BSA, ME32BSA) were compared with formulation of BSA in buffer solution. All of the formulations were freshly prepared.

Experimental design

At the beginning of the study, all rabbits were collected blood for analyzing of normal testosterone value until their testosterone value are more than 0.3 ng/ml and their ages were 4 -5 month. They were administered subcutaneously with blank ME formulations; 4 group of rabbits (n=5) with each group receiving one of blank formulation. The blood were collected and analyzed for testosterone value for two weeks and left another two weeks for wash out period. Then they were injected with 3.3 mg BSA microemulsion formulations. Four groups (n=4), were injected subcutaneously with each group receiving one of BSA loading microemulsion formulation and were compared with 1 group (n=5) which injected subcutaneously with 3.3 mg BSA in pH 7.4 buffer. The sterilized BSA formulations were slowly injected using 2 ml glass syringe with disposable needle no. $21\times1^{1/2}$ inch

long into the left side (blank formulation) and right side (BSA formulation) of their back neck.

Specimen collection

Blood samples were collected from the lateral ear vein of each rabbit using blade no.11 and 3-5 ml blood were kept and allow to clot in sterile glass tube. Then serum was separated by centrifugation at a speed of 3000 rpm for 20 minutes and stored at -20 °C until subsequent analysis.

Testosterone analysis

Serum testosterone was measured using solid-phase, ligand-labeled, competitive chemiluminescent enzyme immunoassay technique with the IMMULITE Automate Analyzer (Jockenhovel et al., 1996).

The solid phase was coated with a polyclonal rabbit antibody specific for testosterone. The sample and ligand-labeled testosterone were simultaneously introduced into the test unit, and incubated for approximately 30 minutes at 37°C with intermittent agitation. During this time, testosterone in the sample competes with ligand-labeled testosterone for antibody-binding sites on the bead. Unbound material was then removed by a centrifugal wash. An alkaline phosphatase-labeled anti-ligand was introduced, and the test unit was incubated for another 30 minutes cycle. The unbound enzyme conjugate was removed by a centrifugal wash. Substrate was then added, and the test unit was incubated for a further 10 minutes.

The chemiluminescent substrate, a phosphate ester of adamantly dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained of light. The bound complex, as measured by the luminometer, is inversely proportional to the concentration of testosterone in the sample.

CHAPTER IV

RESULTS AND DISCUSSION

Microemulsion formation/partial pseudo-ternary phase diagrams

One of the difficulties in realizing the potential of microemulsions as drug delivery systems through parenteral is the narrow range of acceptable surfactants and cosurfactants and their high concentration usually required. In order to make these systems pharmaceutically and biologically more useful, it is necessary to explore the feasibility of formulating microemulsion using non-toxic, safe substances, commercially available, and pharmaceutically acceptable components. Although there are many types of surfactants, only a few have precedence for use in parenteral products. By far the most popular surfactants used in FDA-approved parenteral products are such as phospholipid and tween 80. Due to naturally occurring non-toxic, metabolized in the same way as fats, most of parenteral lipid formulations used phospholipid as biocompatible surfactant. Many of lecithin-based microemulsions were studied (Attwood et al., 1992; Gallarate et al., 1993; Kovarik et al., 1994; Aboofazali et al., 1995). An interest in using nonionic surfactants is increasing due to their low irritation and high chemical stability. They are compatible with the other classes of surfactants. Microemulsions prepared with nonionic surfactants are increasingly gaining potential in parenteral drug delivery systems (Ho et al., 1996; Constantinides and Scalart, 1997; Radomska and Dobrucki, 2000). Recent reviews showed that tween 80, a nonionic surfactant, can be added into about 60% of all injectable formulations that contain solubilizing, suspending, or emulsifying agents and it is present in about 40 parenteral formulations (Alvarez-Nunez et al., 2000).

In case of cosurfactants, the use of medium and short chain length alcohols as cosurfactants limits the potential use in microemulsions due to their toxic and irritant properties. To date, very little work has examined the use of alternative pharmaceutically acceptable cosurfactants. In this study, ingredients, which have similar structure or properties as alcohol, were selected. Most of them were pharmaceutically acceptable and were present in parenteral products and/or in injectable formulation research, except butanol (Nema, 1997). The latter systems

were prepared in order to compare physical parameters with systems containing non-toxic ingredients.

Therefore, partial pseudo-ternary phase diagrams were constructed by titration a series of surfactant/cosurfactant/oil mixtures with water at ambient temperature following the formulation study design as shown in Table 1. An illustration of a pseudo-ternary phase diagram construction is shown in Figure 3. Such phase diagram had the aqueous phase on the top of the diagram and the fixed weight ratio of surfactant/cosurfactant (Em) and oil were shown on the bottom left and right corner, respectively. Most of pseudo-ternary phase diagram were done at Em to oil ratio of 3/7, 5/5, 7/3. At Em/oil ratio >7/3 and <3/7 are out of the interested area due to the high amount of surfactant used and low probability of existing in microemulsion area, respectively. The shaded areas represented a clear and transparent microemulsion existence range in the systems and were compared. The extension of shade area was found to be dependent on the types and ratios of surfactant to cosurfactant and on the oil used.

Factors affecting microemulsion area on phase behavior

Effect of surfactant

The partial phase diagrams of the PC and TW-base systems are shown in Figures 5-6, respectively. The Figure shows three different Em ratios (surfactant/cosurfactant), A (1:1), B (1:0.5), C (1:0.25), arranged into three columns. Within the column, it represents a set of the systems containing different oils of IPM, EO, MCT, and SBO. Within a set , the overlaid diagrams were the systems containing cosurfactants of BT, PG, PEG, GR, and CA.

It was seen that for PC-base systems in Figure 5, microemulsion regions could be produced only in the systems with cosurfactant of butanol, propylene glycol, and caproic acid. Systems with butanol exhibited comparatively larger area of microemulsion. Small microemulsion areas were from the systems with propylene glycol and caproic acid. In addition, most systems showed no area of microemulsion in the phase diagram. In contrast, most of TW-base systems in Figure 6 with various types and ratios of cosurfactant graphically yielded microemulsion regions. The structure/physicochemical properties of surfactant,

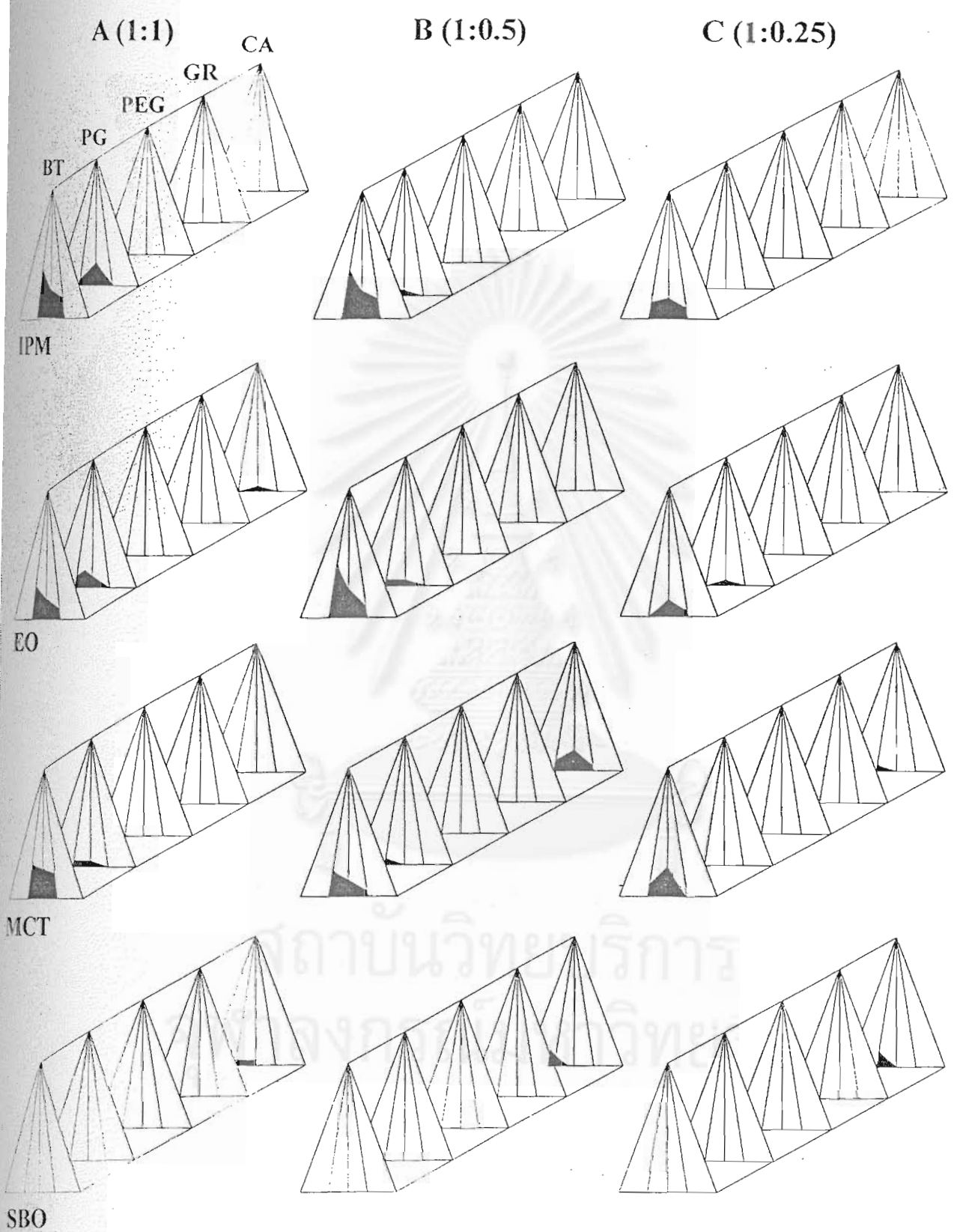


Figure 5 Series of partial pseudoternary phase diagrams for PC-base systems at Em ratio of A (1:1), B(1:0.5), C (1:0.25); Em/oil ratio of 3/7, 5/5, 7/3; four type of oils (IPM, EO, MCT, SBO); and five co-surfactants (BT, PG, PEG, GR, CA)

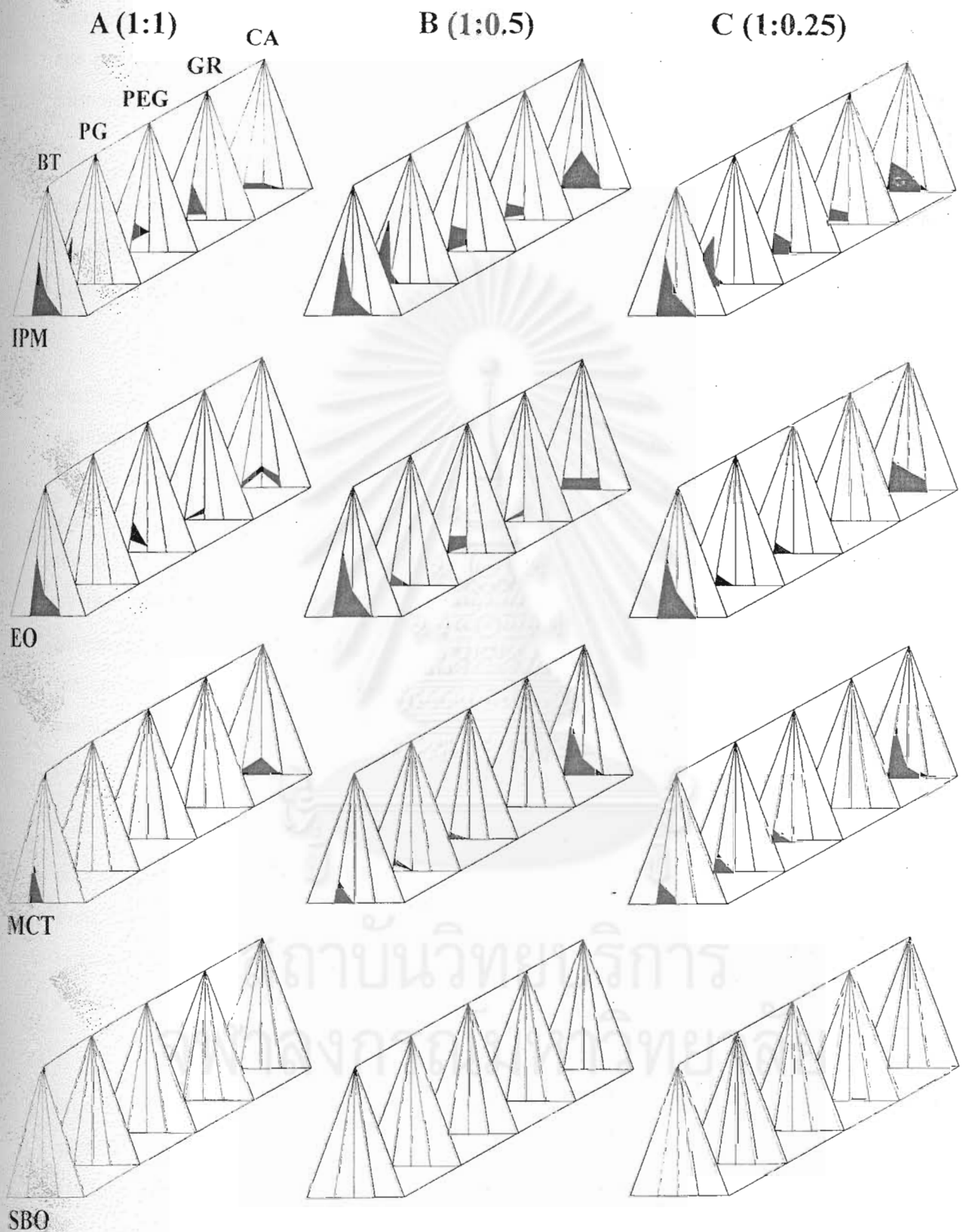


Figure 6 Series of partial pseudoternary phase diagrams for TW-base systems at Em ratio of A (1:1), B(1:0.5), C (1:0.25); Em/oil ratio of 3/7, 5/5, 7/3; four type of oils (IPM, EO, MCT, SBO); and five co-surfactants (BT, PG, PEG, GR, CA)

types of cosurfactant, oils as well as ratio of surfactant to cosurfactant (Em ratios) and Em to oil ratios were seen to have a pronounced effect on the region of existence of the microemulsions.

The explanation for the differences in the partial phase diagrams produced by two types of surfactant, phospholipid and tween80, in this study was the difference in their structures which affected the existed microemulsion area from both systems.

Microemulsion could be formed at a very low interfacial tension. Surfactant was evidently the most important additive in the mixture and its amount determines the total interfacial area. It migrated into the interface to depress interfacial tension to a lower level to form microemulsion. Not only do surfactants lower the interfacial tension, but also their molecular structures affect the curvature of the interface. The nature of the surfactant aggregate was governed by the geometry of its molecule which represented by the critical packing parameter (CPP), the ratio $v/a_o l_e$, where v, a_o and l_e were the hydrocarbon volume, the optimum head group area and the hydrocarbon tail length of an surfactant molecule respectively . This ratio defined the spontaneous curvature of a particular surfactant (Israelachvili et al., 1976).

It could be seen that TW-base system produced more microemulsion area than PC-base system. The microemulsion could be obtained from various types of cosurfactants in the TW surfactant systems than with PC systems. However, systems containing SBO could only form microemulsion with PC but not TW.

Phospholipid has heavily hydrated zwitterionic head group with long hydrocarbon chain. It composes of mainly phosphatidylcholine which is slightly too lipophilic to form microemulsions when used as the sole surfactant. It has high critical packing parameter ratio between hydrocarbon volume, optimum head group area and tail length. For phospholipid, the large hydrophobic core (v) relative to the head group (a_o) gives a large value of $v/a_o\ l_e$ ratio of about 0.8 (Cornell et al., 1986), meaning that it tends to form lamellar phases or bilayers. Tween 80 is an uncharged oxyethylene chain with high HLB of 15. Its HLB value

indicates the extent of preference for surfactant migrating into the interface between water and oil phases to depress the interfacial tension to a lower level to form microemulsion. However, besides the HLB value, geometric packing of surfactants in the interface may be another influencing factor to be considered in term of its effects on the curvature and the fluidity of interface. Its hydrophilic part contains the polyoxyethylene, polyoxypropylene, or polyol derivatives and the hydroxyl group and the hydrophobic part includes saturated or unsaturated fatty acids or fatty alcohols. At the interface, the long and bulky polar group was assumed to act as a shell of oxyetylene chains like the palisade layer (Florence and Attwood, 1998).

From the results, some partial phase diagrams from PC- systems did not exhibit microemulsion region. It meant that the rigid lamellar structure could not be altered by that type and ratio of cosurfactant used, thus microemulsion could not be formed.

Effect of cosurfactant

The effects of cosurfactant types in both PC and TW-based systems on the area of partial phase diagrams are shown in Figures 7-10. Comparison among different cosurfactants in PC-based systems in Figures 7-8 showed that their microemulsion areas were ranks: BT>PG>CA. No microemulsion area was shown in systems of PEG and GR. In TW-base systems in Figures 9-10, their microemulsion areas were ranks: BT>CA>PG>PEG>GR.

In PC-based systems, comparison of the partial phase diagrams among different cosurfactants revealed that the microemulsion region was much larger when butanol was used as the cosurfactant. When propylene glycol was used, smaller microemulsion area was obtained, and the area even decreased in the systems containing caproic acid. This result could be explained by the different hydrophilic head groups and short hydrophobic chain length of the cosufactants. Due to butanol had appropriated structure. Its hydrophilic head group of one hydroxy group and short alkyl chain of four carbon atoms was of sufficient size and length to ensure it resided in the interfacial layer; resulting in altering the rigidity of the interface. Thus, the interfacial layer can be curved enough to form

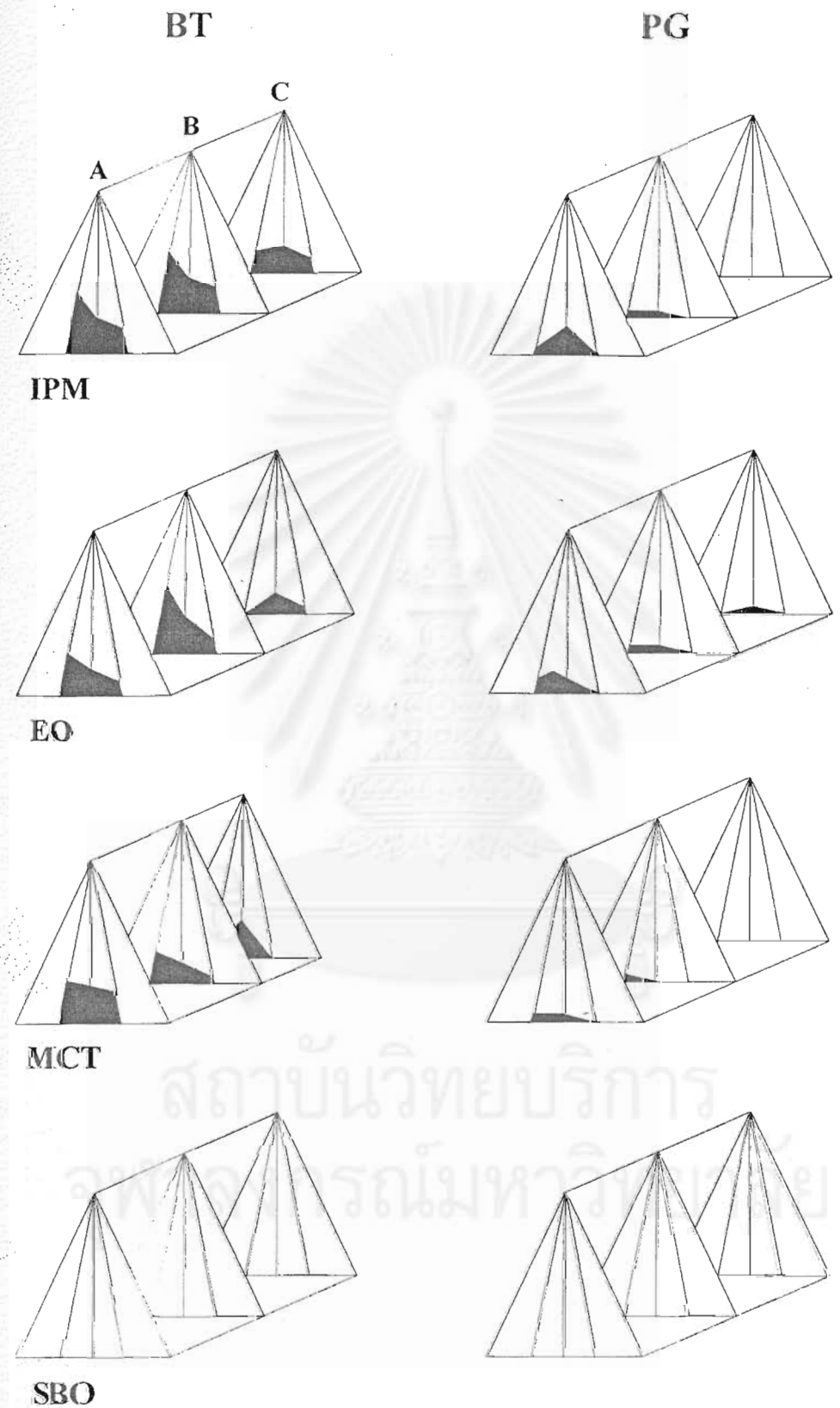


Figure 7 Partial pseudoternary phase diagrams for PC-base systems: comparison of cosurfactants BT and PG with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

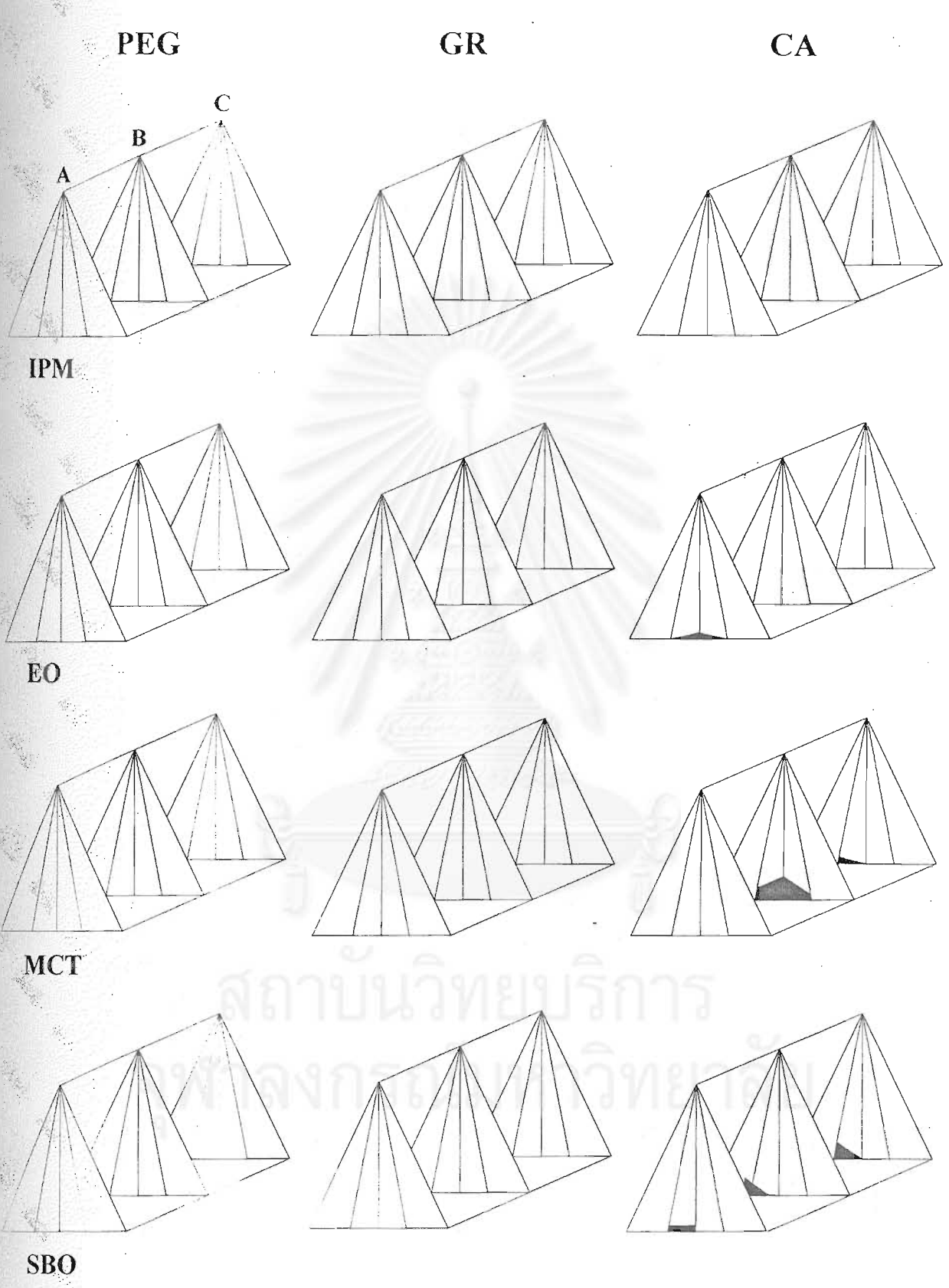


Figure 8 Partial pseudoternary phase diagrams for PC-base systems: comparison of cosurfactants PEG, GR and CA with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

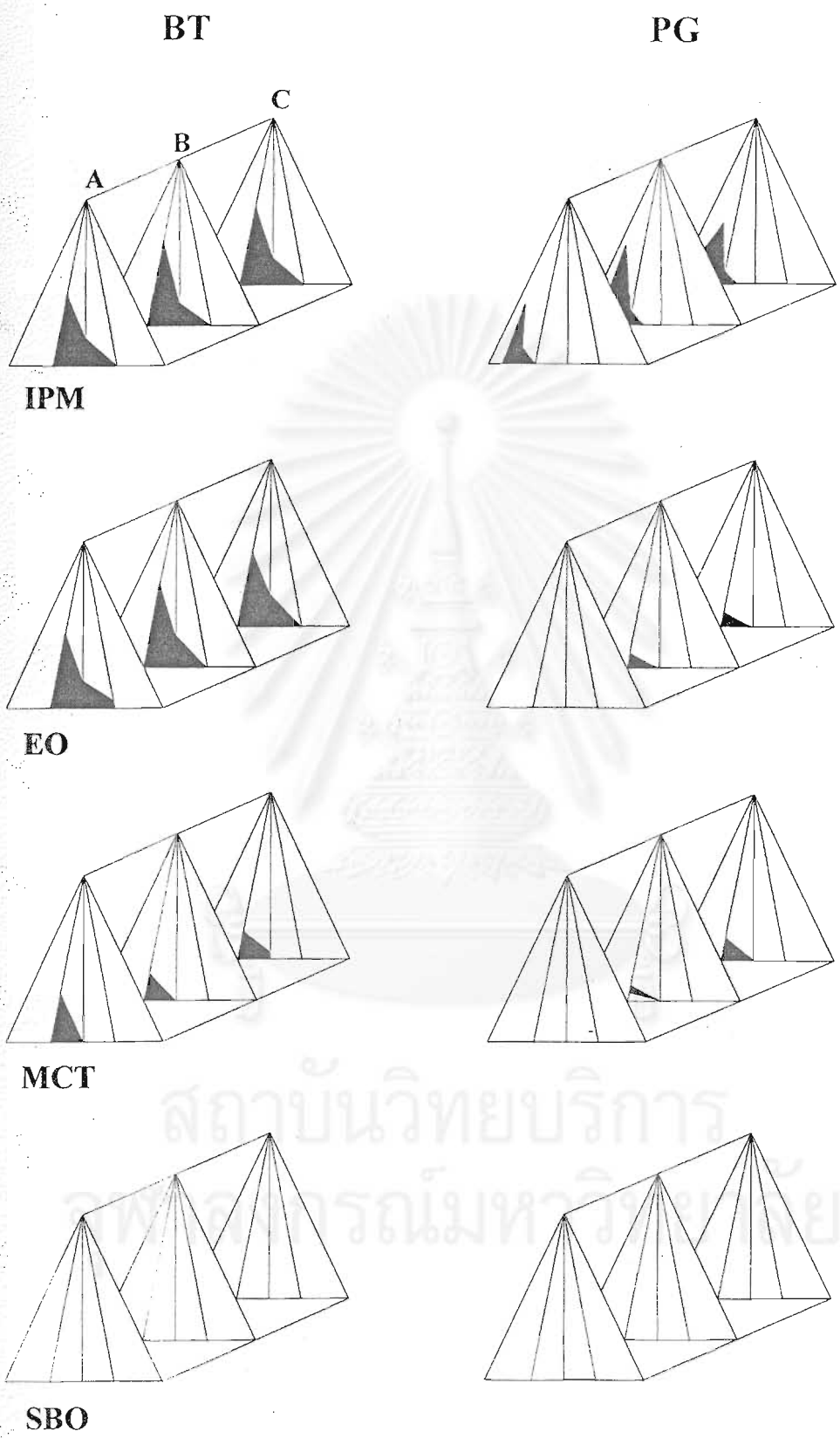


Figure 9 Partial pseudoternary phase diagrams for TW-base systems: comparison of cosurfactants BT and PG with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

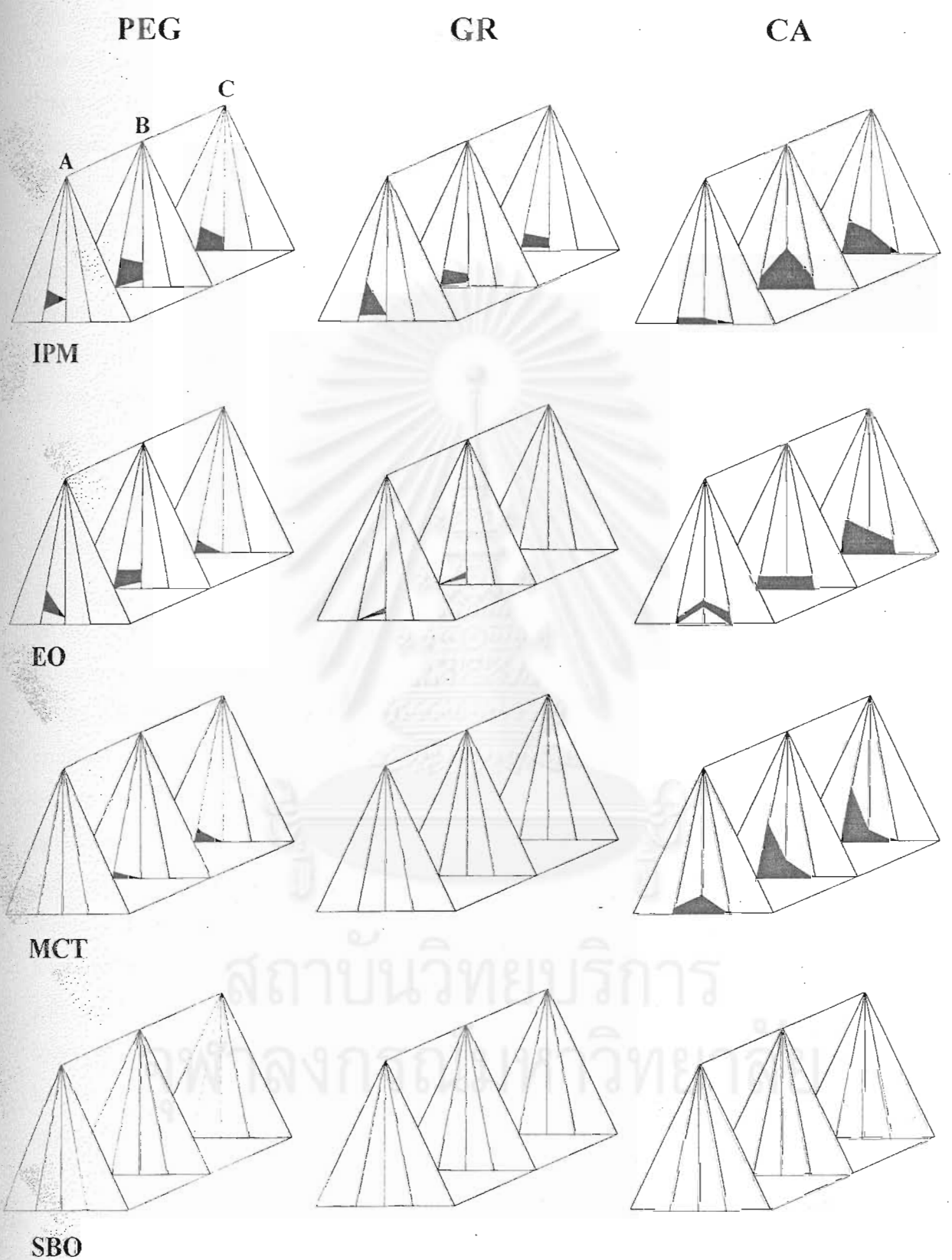


Figure 10 Partial pseudoternary phase diagrams for TW-base systems: comparison of cosurfactants PEG, GR and CA with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

fine droplet and large microemulsion region were obtained. Propylene glycol was relatively more hydrophilic of two hydroxy groups and has shorter alkyl chain length of three carbon atoms than butanol. As expected, the two hydroxy groups need more space to insert at interfacial layer and the short alkyl chain length made it was unsuitable to reside in the interfacial layer; resulted in smaller microemulsion area. Caproic acid had more hydrophobic head group and longer alkyl chain length than butanol. Their structure was inappropriate to stay between surfactant layer compared to butanol. Thus, the smaller area was obtained. For polyethylene glycol and glycerol, their high polarity of more hydroxy groups in hydrophilic part caused them could not penetrate into the interfacial layer. Therefore, no microemulsion area could be observed.

In TW-based systems, the partial phase diagrams were different. Systems with butanol and caproic acid yielded larger microemulsion areas while smaller areas were obtained from the systems of propylene glycol, polyethylene glycol, and glycerol as cosurfactant, respectively. Similarly reasoned, different structure of cosurfactants, could be used to explain this system. Some different results were observed. Caproic acid produced more microemulsion region than propylene glycol, and microemulsion area could be produced with the system containing polyethylene glycol and glycerol. It could be explained that the optimum size/hydrophilicity of head group of butanol and caproic acid, hydroxy and carboxylic acid, were suitable to incorporate into a shell of oxyethylene chains at the interface. Also suitable alkyl chain length which sufficient length to locate in the fatty acid part of tween 80. Comparison among propylene glycol, polyethylene glycol and glycerol, high hydrophilicity and large structure, the cosurfactant should reside only in the hydrophilic region of oxyetylene chains at interfacial layer which had less affect on the curvature and fluidity of the interface.

Figure 11 shows the microemulsion region obtained by using butanol as a co-surfactant with different oils and Em ratios. Large microemulsion regions could be produced in both PC and TW-based systems at all investigated Em ratios and oils except in systems containing of soybean oil. Comparison between microemulsion area from PC and TW-based systems showed that at low

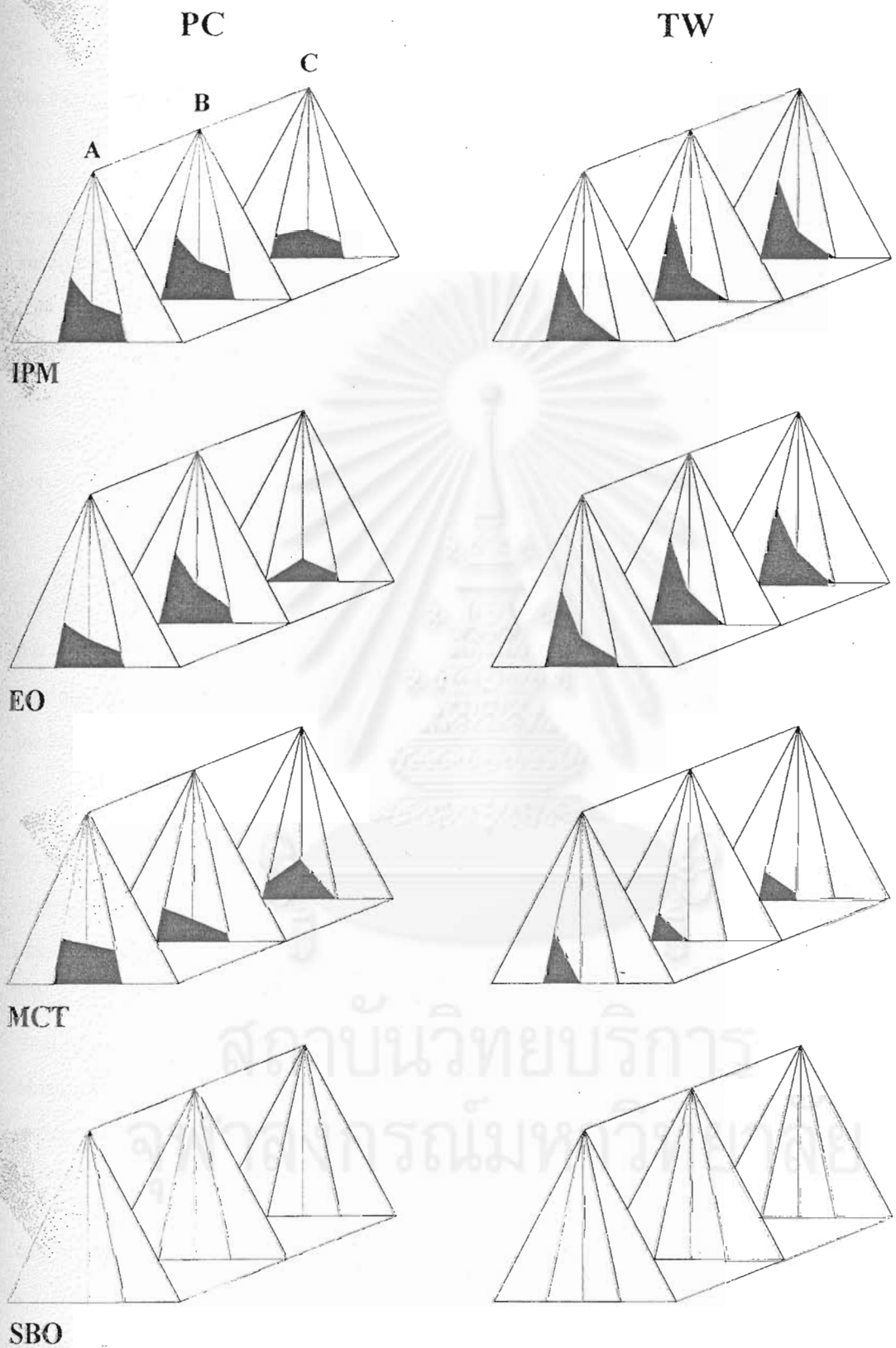


Figure 11 Comparison of partial pseudoternary phase diagrams for systems containing BT as cosurfactants with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

Em/oil ratio (3/7) microemulsion region was not observed for TW-base systems. However, the partial phase diagrams showed some similarities in that increasing the Em/oil ratio (5/5 to 7/3) was due to increase in microemulsion regions.

Figure 12 shows the microemulsion region obtained by using propylene glycol as a cosurfactant with different oils and Em ratios. It could be observed that for PC-based systems, increasing the concentration of surfactant (Em ratio of C; 1/0.25) resulted in smaller microemulsion areas. For TW-based systems, similar results as in systems composed of butanol in Figure 11 were obtained in that at low Em/oil ratio (3/7) microemulsion region could not be observed. The microemulsion regions could be produced at high ratio of Em/oil (> 5/5).

Figure 13 shows the microemulsion region obtained by using polyethylene glycol as a co-surfactant at different oils and Em ratios. Obviously, the difference could be seen between PC and TW-based systems, the microemulsion region could only be produced in TW-base systems. Besides, it mostly occurred in systems of high Em ratio.

Figure 14 shows the microemulsion region obtained by using glycerol as a cosurfactant at different oils and Em ratios. The partial phase diagrams obtained showed some similarities to those obtained when using polyethylene glycol in Figure 13 in that microemulsion area could be produced in TW-based systems. However, the regions of systems using glycerol were much smaller than those of systems using polyethylene glycol. Only systems composed of isopropyl myristate could be clearly seen and while systems composed of ethyl oleate exhibited minute area.

Figure 15 shows the microemulsion region obtained by using caproic acid as a co-surfactant at different oils and Em ratios. For most TW-based systems, it was shown that the microemulsion region was much larger as the concentration of surfactant increased (at higher Em ratio : 1/0.25, at higher Em/oil ratio : 7/3). In addition, the microemulsion area were different with PC-based systems, the microemulsion regions could be produced in the systems composed

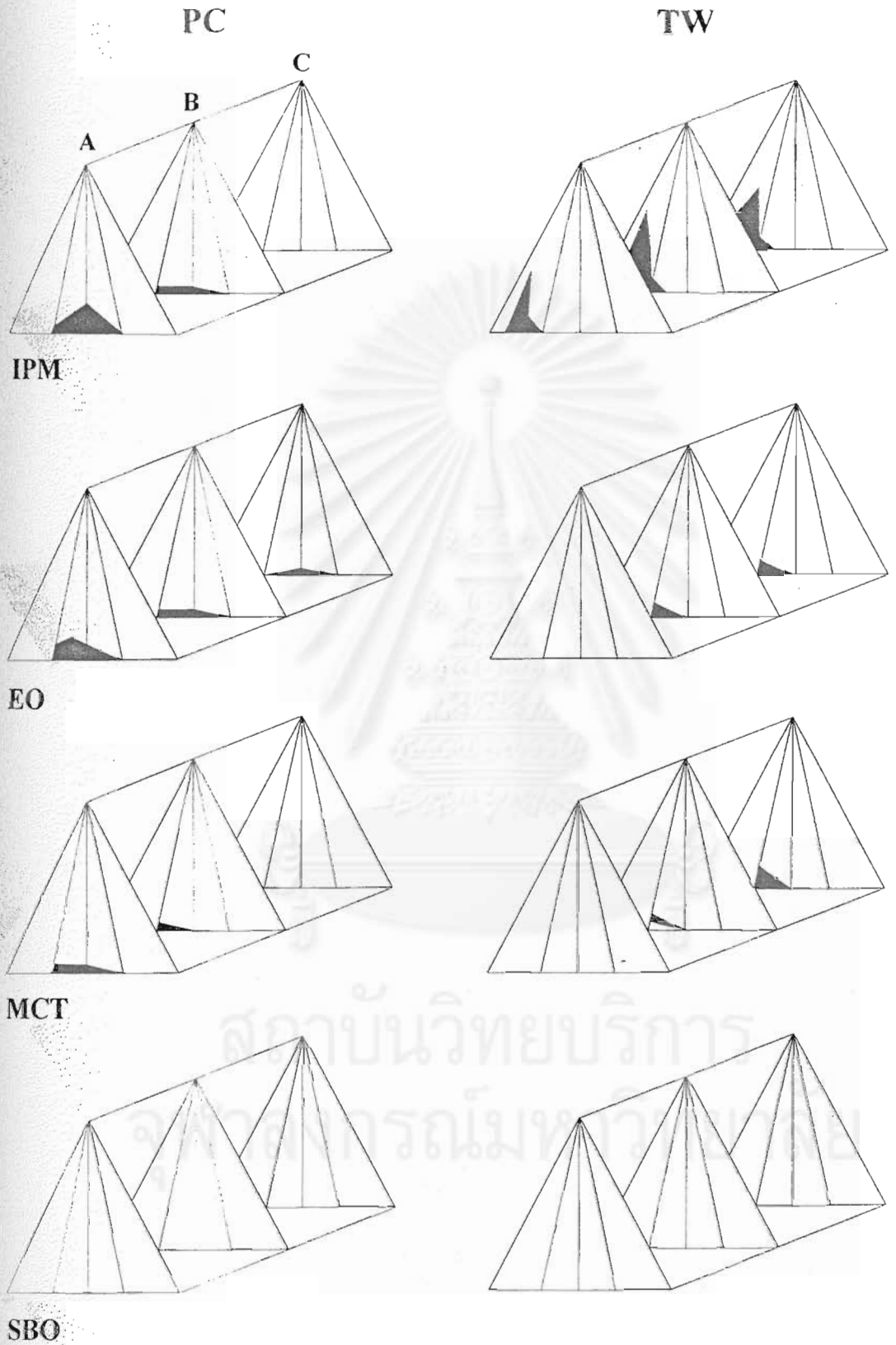


Figure 12 Comparison of partial pseudoternary phase diagrams for systems containing PG as cosurfactants with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

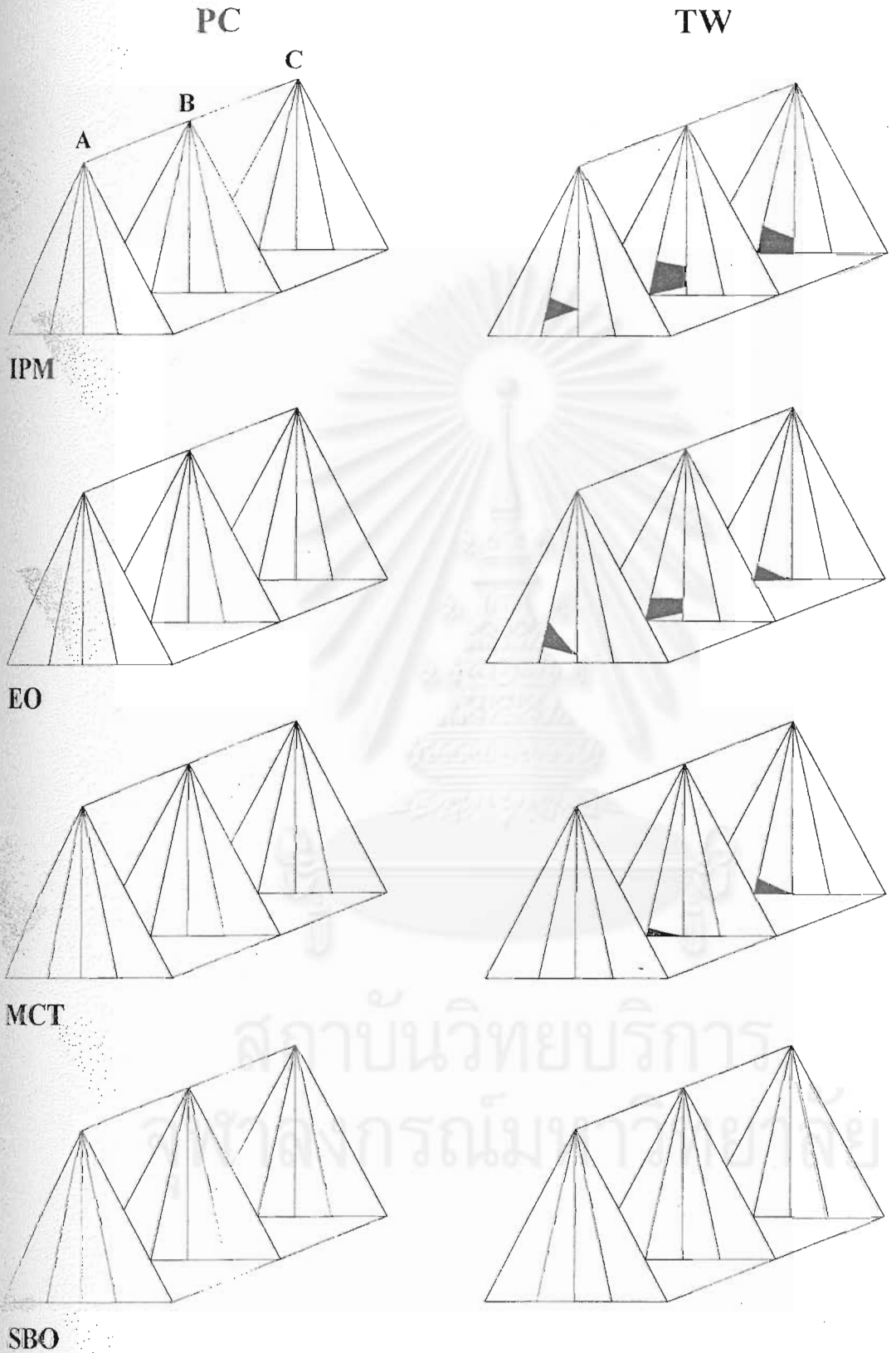


Figure 13 Comparison of partial pseudoternary phase diagrams for systems containing PEG as cosurfactants with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

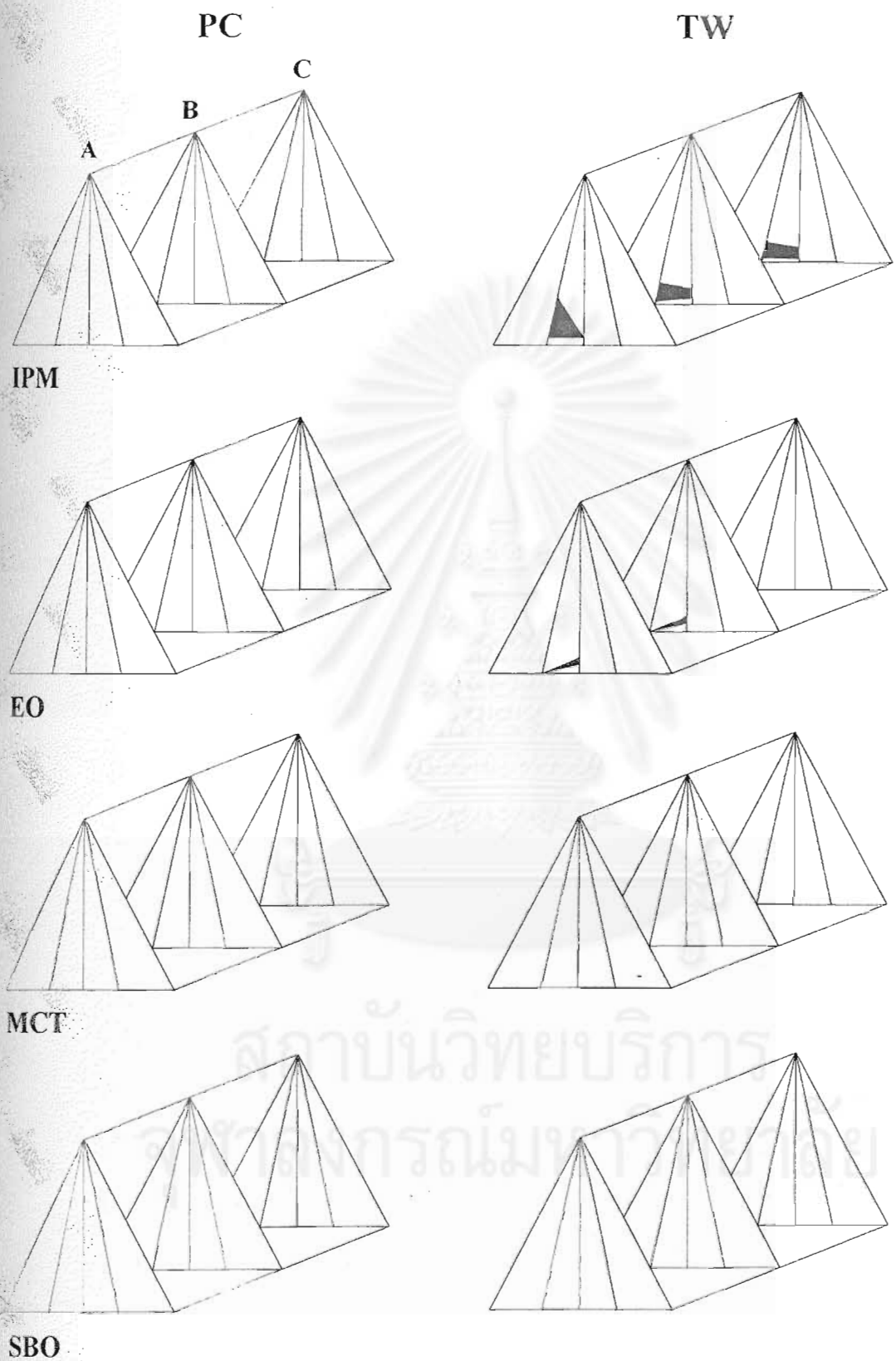


Figure 14 Comparison of partial pseudoternary phase diagrams for systems containing GR as cosurfactants with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

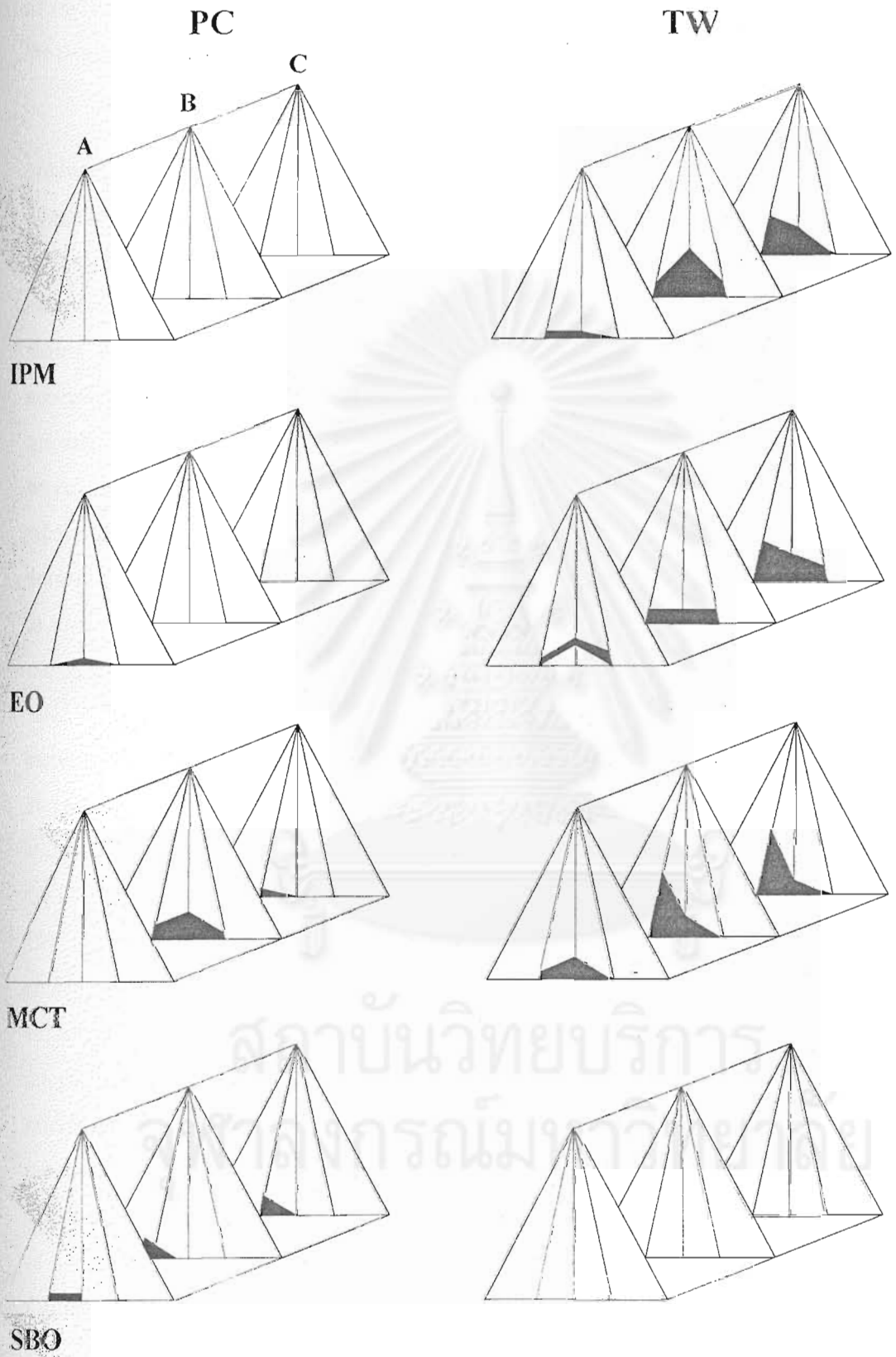


Figure 15 Comparison of partial pseudoternary phase diagrams for systems containing CA as cosurfactants with different oils (IPM, EO, MCT, SBO) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

of soybean oil and medium chain triglyceride oil. Very little area was observed in the system with ethyl oleate oil.

As aforementioned, phospholipid as a single surfactant would not produce microemulsions. This was because the phospholipid molecule was too lipophilic; it had a critical packing parameter of approximately 0.8 favouring the formation of lamellar phases or bilayers. This CPP was further increased in a microemulsion if the oil phase of a microemulsion penetrated into the long alkyl chains of the lecithin molecule. In order to produce a microemulsion, it is necessary to reduce its effective CPP. This could be achieved by the use of suitable cosurfactant. Cosurfactant could alter the effective CPP in one of two ways; either by making the aqueous phase less hydrophilic and/or by incorporation into the interfacial film. As it could distribute between the three domains, namely, the aqueous phase, oil phase and interfacial film, it was possible to gain an idea of the influence of a cosurfactant on the effective CPP by estimating its likely distribution by reference to its aqueous solubility. Water-soluble, hydrophilic cosurfactants would be expected to be distributed primarily between the aqueous phase and in the polar part of the interfacial layer, thereby decreasing the CPP and producing more microemulsions. In contrast, oil-soluble, hydrophobic co-surfactants would be expected to be distributed mainly between the oil phase and in the hydrocarbon parts of interfacial layer, thus increasing the CPP. It could also have a third effect, in that it could reduce the tendency of phospholipid to form highly rigid films, thus allowing the interfacial film sufficient flexibility to take up the different curvatures required to form microemulsions (Aboofazali et al., 1994). Osipow (1963) explained that for microemulsion to be formed, the interfacial film should not be too condensed, otherwise the appropriate curvature required for droplet formation would not be obtained. Indeed, phospholipid was known to form highly rigid film. The addition of a suitable cosurfactant would act to reduce the rigidity of the condensed film formed by phospholipid allowing the formation of microemulsion.

For TW-based systems, cosurfactant intercalated between surfactant molecules. It also acted in the interface, both in the long and bulky nonionic polar group and the long hydrocarbon tail, to reduce the tendency to

form highly rigid films, thus allowing the interfacial film to take up the different curvatures required to form microemulsions.

Comparison among cosurfactants, the results revealed that butanol was the best cosurfactant in both PC and TW-based systems. Butanol, a low molecular weight (74.12) with short chain alcohol ($CH_3CH_2CH_2CH_2OH$), could influence the formation of microemulsions by both interfacial and bulk effects. Their amphiphilic nature, short hydrophobic chain (4 carbon atom) and terminal hydroxyl group, enable them to interact with surfactant monolayers at the interface thereby affecting their packing, which in turn could influence the curvature of the interface and interfacial energy. The amphiphilic nature of low molecular weight cosurfactants also enable them to distribute between the aqueous and oil phase, resulted in altering the chemical composition and hence the relative hydropuilic/lipophilic properties.

Propylene glycol or 1,2-propanediol has three carbon atoms with two hydroxyl groups in the molecule. When used as cosurfactant, it could produce small microemulsion area compared to the systems with butanol. Aboofazeli, et al (1994) had investigated alkanediol (1,2-butanediol, 1,2-pentanediol, and 1,2-hexanediol) as cosurfactants. The results showed that system with 1,2-hexanediol exhibited large clear region covering oil/surfactant axis. It appeared that increasing the chain length of the hydrocarbon moiety resulted in an increase in the extent of clear region by decreasing the stability of the lamellar structure. Their results showed that the long-chain alkanediols were mainly positioned between the phospholipid molecules while those with a short chain were located primarily in the aqueous regions between the phospholipid layers. Propylene glycol is a water soluble, short chain alkanediol and relatively more polar than butanol. Thus it could locate primarily in the aqueous regions and somehow short alkyl chain incorporate between the phospholipid layers, thereby had small effect on decreasing the stability of lamellar structure which resulted in smaller microemulsion area. The similar reason could be explained in TW-based system. Propylene glycol could locate in the bulky hydrophilic region and the relative too short alkyl chain of 3 carbon atoms was insufficient length to reside in

the interfacial layer. Thus it had small effect to alter the flexibility to take up the curvature required to form microemulsion.

Polyethylene glycol containing polyhydroxy group yielded no microemulsion area in the PC-based system. Due to its relatively large structure, it was expected that large molecule could not distribute between phospholipid molecules to reduce the high CPP or flex the curvature of the film. Their high hydrophilicity also would indicate that the long chain structure was primarily located in the aqueous regions between the head group of phospholipid layer. While the small microemulsion area were observed in TW-based systems. The results might be explained that the high hydrophilicity of polyhydroxy group can locate in the hydrophilic region of tween 80 at interfacial layer that affected on the curvature and fluidity of the interface.

Glycerol, which has three hydroxy groups in the molecule, yielded no microemulsion area in the PC-based system. This was similar to the results obtained from the system containing polyethylene glycol. It could be similarly explained that due to its relatively large structure and water solubility, it would be expected that large molecule could not distribute between phospholipid molecules to flex the curvature of the film to form fine droplet. Their high hydrophilicity also would indicate that the long chain structure was primarily located in the aqueous regions between the head group of phospholipid layer. While the small microemulsion area was observed in TW-based systems. The results might be explained that the high hydrophilicity of polyhydroxy group can locate in the hydrophilic region of tween 80 at interfacial layer that affected on the curvature and fluidity of the interface.

Caproic acid, a carboxylic acid head group with six carbon atoms, yielded small microemulsion region in PC-based system but larger region in TW-based system. As the carboxylic acid head group was relatively hydrophobic (Wormuth and Kaler, 1987), it was not surprising that caproic acid gave only a narrow microemulsion region in the PC-based system. Their hydrophobicity implied that cosurfactant would be expected to be distributed mainly between the oil phase and in the hydrocarbon parts of interfacial layer, thus less effect on altering the interfacial curvature to form microemulsion. While the large

microemulsion areas were observed in TW-based systems. The results might be explained that carboxylic head group would be expected to reside in hydrophilic part of tween 80 at interfacial layer and the short alkyl chain with sufficient length to locate in the fatty acid part of tween 80. Then adjusted the curvature and fluidity of the interface to form microemulsion.

Therefore, addition of a cosurfactant resulted in changes in phase behavior of the systems. It altered the packing parameter either by making the aqueous phase less hydrophilic and/or by incorporating it into the interfacial film, and act to increase the fluidity of the surfactant film thus giving the interfacial film sufficient flexibility to take up the different curvatures required to form microemulsion. The concentration of surfactant could sometimes be reduced by the addition of a cosurfactant. Physicochemical properties of the cosurfactant such as the number of carbon atom in backbone, aqueous solubility affected its ability to promote the formation of microemulsion. However, any relationship among co-surfactant use might be influenced by the oil and surfactant combination used in the pseudo-ternary system.

Effect of oil

The effects of oil types in both PC and TW systems are shown in Figures 16-19. In PC-based systems (Figures 16-17), isopropyl myristate and ethyl oleate oils behaved in a similar manner in that they produced systems which gave large microemulsion area in the systems of butanol, small area in the systems of propylene glycol. In addition, small area could be seen in the system of ethyl oleate containing caproic acid. For medium chain triglyceride oil, some similarities were observed.

Butanol still could produce large microemulsion regions and very small area could be seen in systems of propylene glycol. The difference was that systems with caproic acid could produce some microemulsion regions. Distinguished from other oils, the partial phase diagram with soybean oil presented the microemulsion region only with the systems composed of caproic acid.

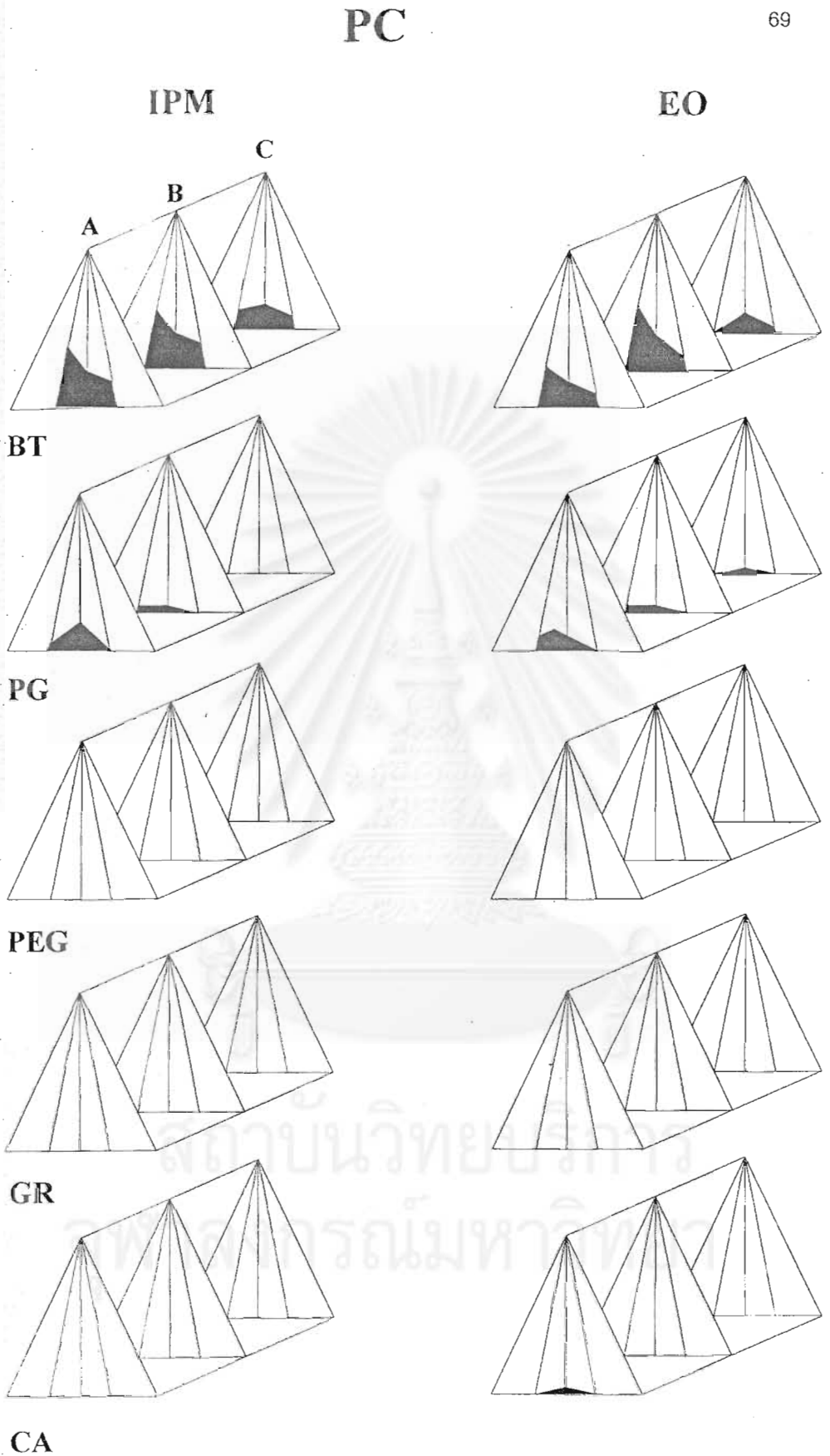


Figure 16 Partial pseudoternary phase diagrams for PC-base systems: comparison of systems containing IPM and EO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

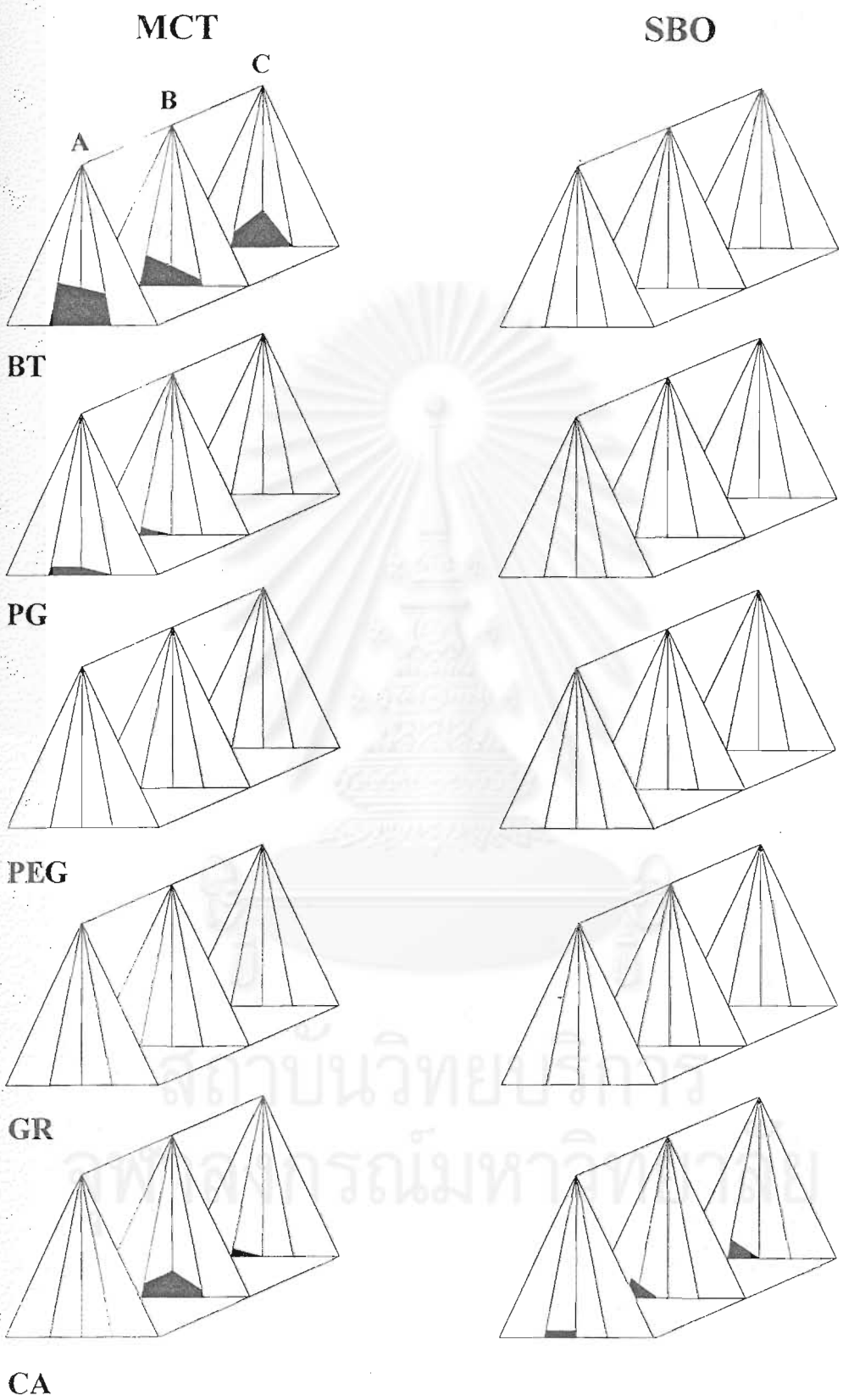


Figure 17 Partial pseudoternary phase diagrams for PC-base systems: comparison of systems containing MCT and SBO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

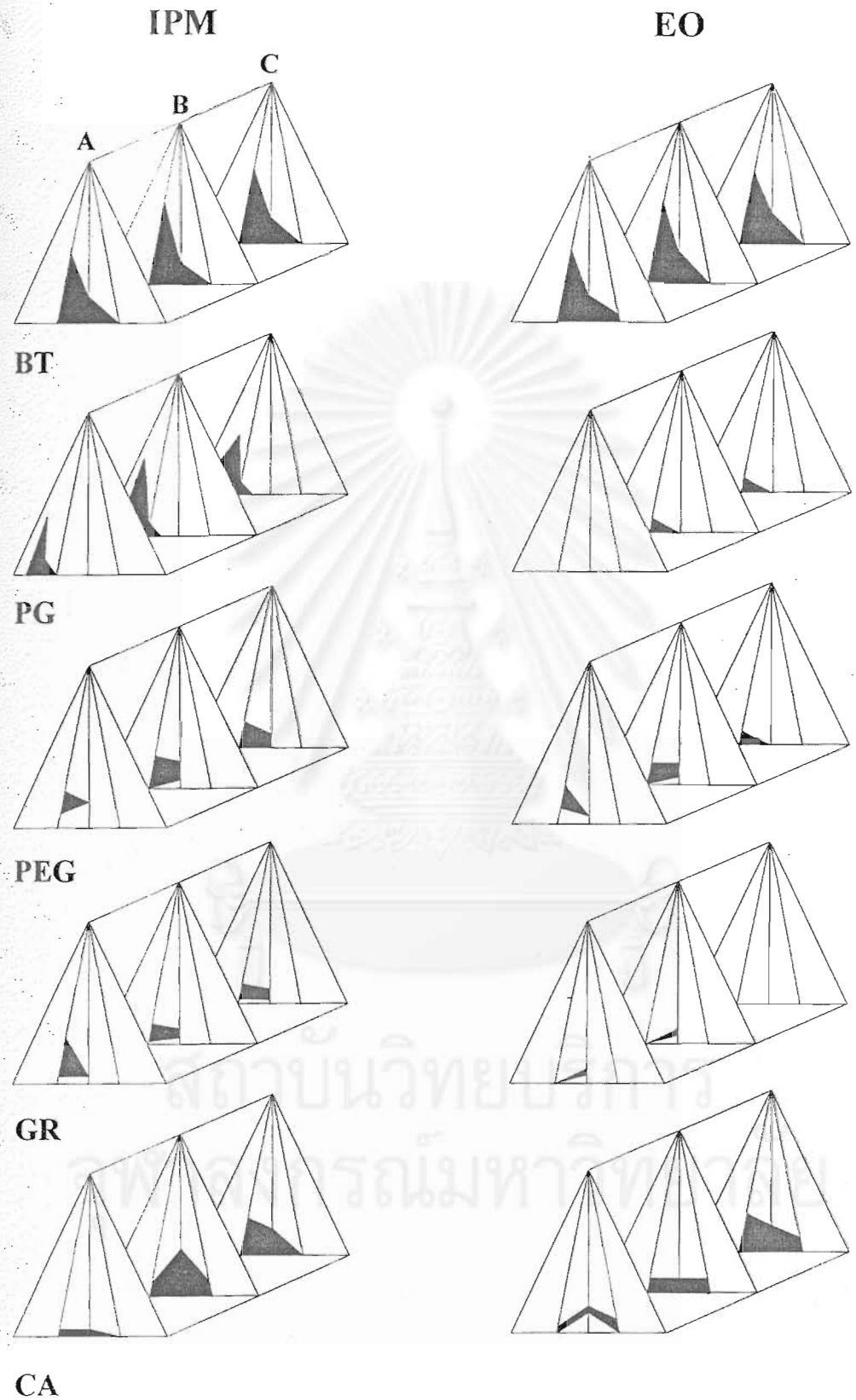


Figure 18 Partial pseudoternary phase diagrams for TW-base systems: comparison of systems containing IPM and EO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

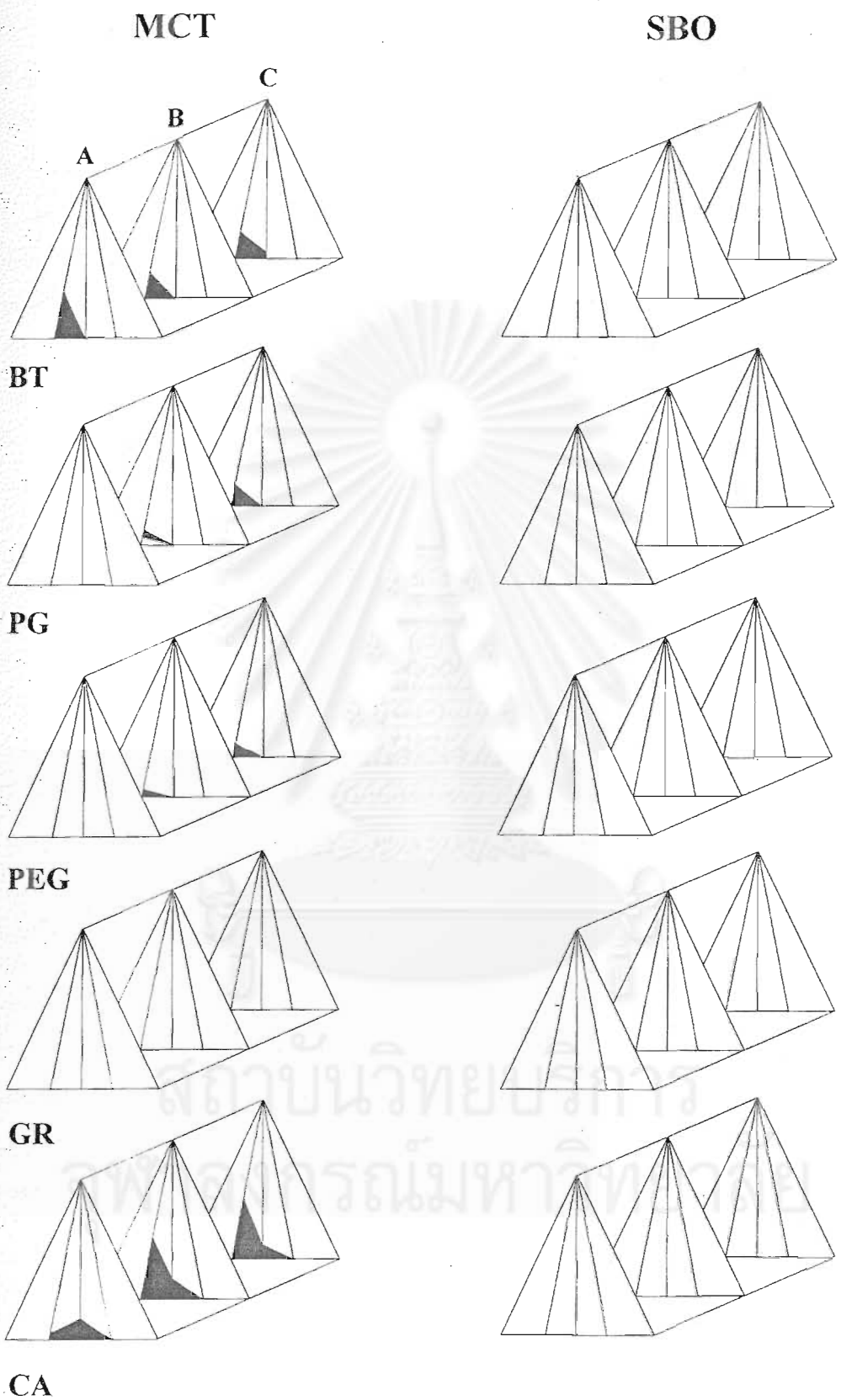


Figure 19 Partial pseudoternary phase diagrams for TW-base systems: comparison of systems containing MCT and SBO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

In TW-based systems shown in Figures 18-19, the microemulsion regions would clearly be seen in most of the systems with isopropyl myristate, ethyl oleate, and medium chain triglyceride oil. No area was observed with the systems of soybean oil. Isopropyl myristate and ethyl oleate oil behaved in a similar manner. They produced large microemulsion areas in the systems of butanol and caproic acid, small areas in the systems of propylene glycol. For medium chain triglyceride oil, the difference in the region was that large microemulsion area was from the systems of caproic acid followed by the systems of butanol, and very small area from the systems with propylene glycol and polyethylene glycol.

Figures 20-23 compared the microemulsion regions between PC and TW-based systems for the systems containing different oils of isopropyl myristate, ethyl oleate, medium chain triglyceride, and soybean oil, respectively. For systems containing isopropyl myristate oil in Figure 20, all partial phase diagrams of TW-based systems yielded microemulsion areas. Large microemulsion regions were obtained in the systems containing butanol and the systems of caproic acid. Other systems yielded smaller areas. For PC-based systems, large microemulsion regions were obtained in the systems containing butanol and smaller areas were in the systems containing propylene glycol. While other systems yielded no microemulsion area.

For systems containing ethyl oleate oil in Figure 21, the phase diagrams obtained showing some similarities to those obtained from the isopropyl myristate system. The difference was the smaller area obtained from the systems containing glycerol and PEG in TW-based system and the small area could be produced in the system containing caproic acid in PC-based system.

For systems containing medium chain triglyceride oil in Figure 22, the phase diagrams obtained showing some similarities to those obtained from the ethyl oleate system. The difference was the smaller areas obtained from the systems containing polyethylene glycol and no microemulsion area in the system containing glycerol in TW-based system. The small area could be produced in the system containing caproic acid of the EM ratio of in PC-based system.

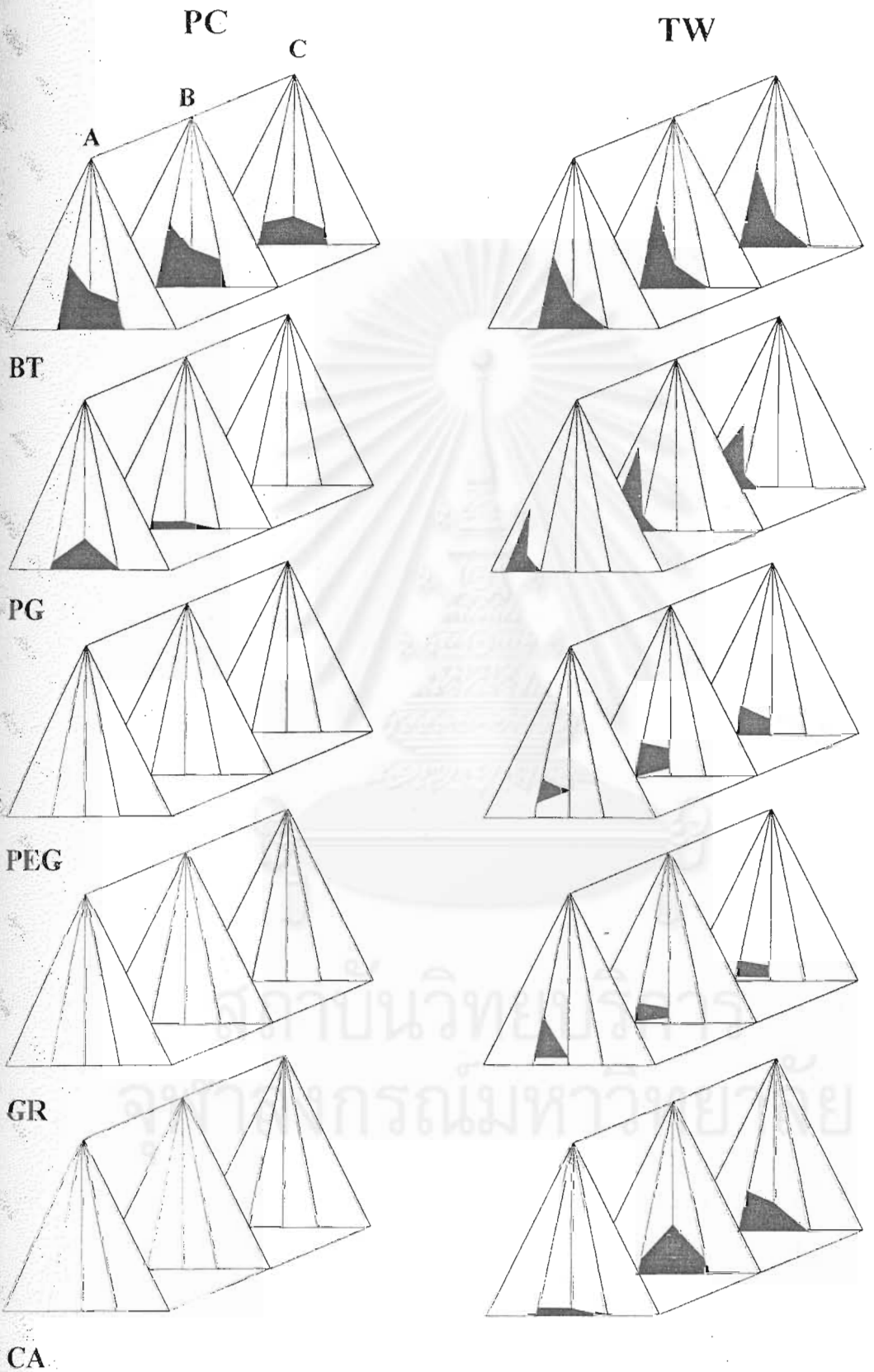


Figure 20 Comparison of partial pseudoternary phase diagrams for systems containing IPM as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

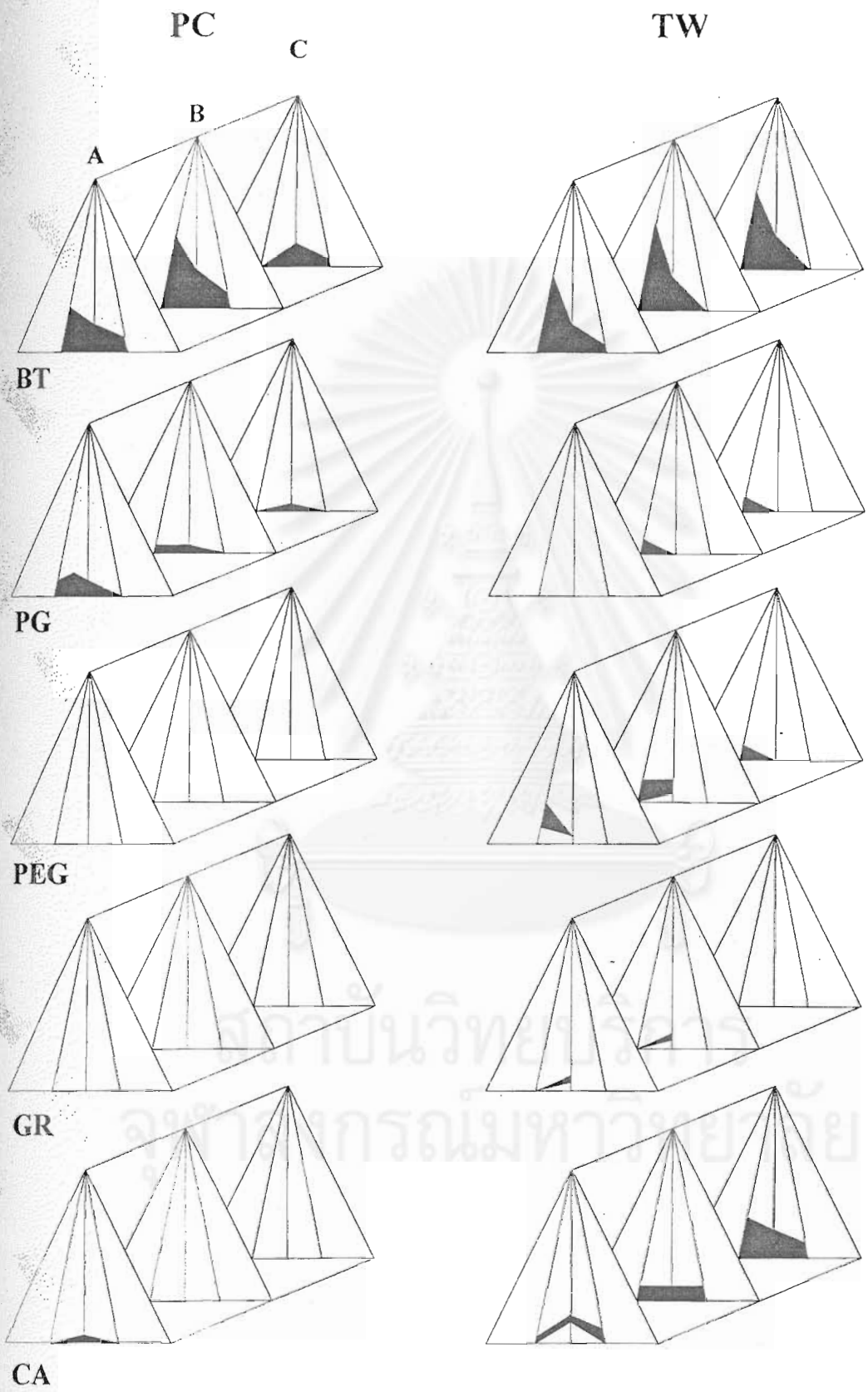


Figure 21 Comparison of partial pseudoternary phase diagrams for systems containing EO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

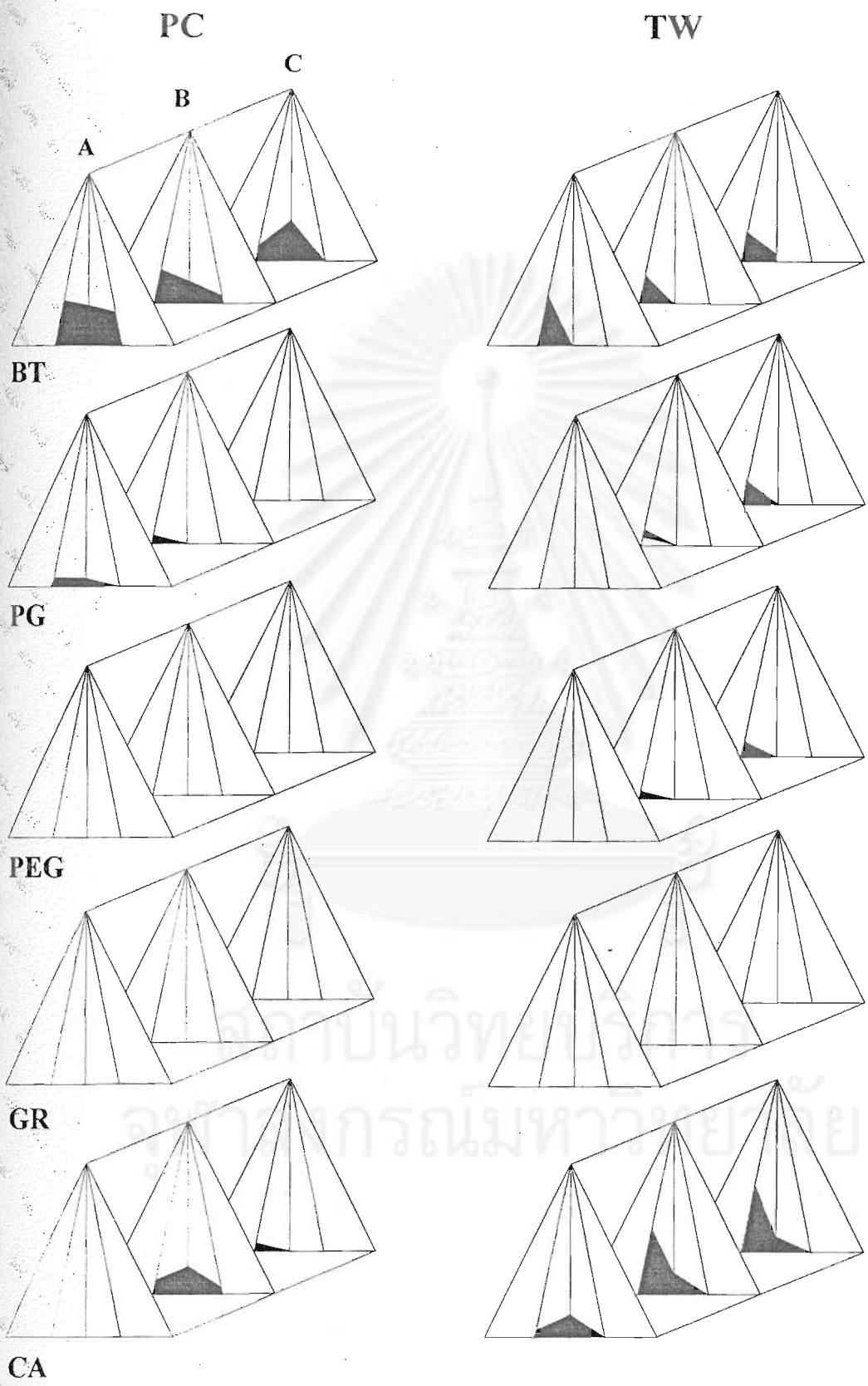


Figure 22 Comparison of partial pseudoternary phase diagrams for systems containing MCT as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

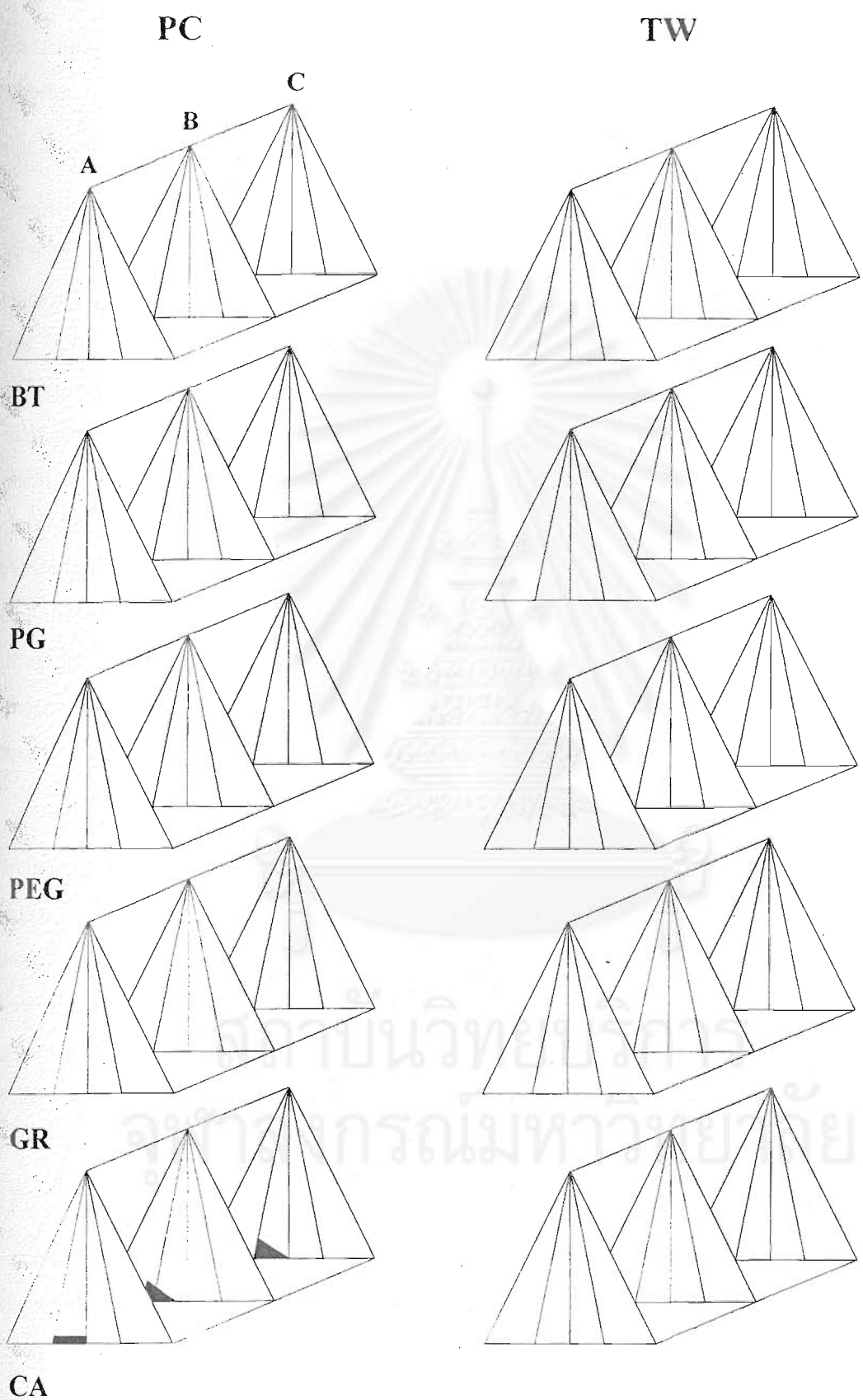


Figure 23 Comparison of partial pseudoternary phase diagrams for systems containing SBO as oil with different cosurfactants (BT, PG, PEG, GR, CA) and Em ratios (A=1:1, B=1:0.5, C=1:0.25)

For systems containing soybean oil in Figure 23, only the system containing caproic acid could produce the microemulsion region in PC-based systems.

It was also well known that the nature of the oil could influence the production of microemulsions. In this study, the phase behavior was influenced by the oil used, in that the oils could be divided by its structure into two groups, namely the ethyl esters (isopropyl myristate and ethyl oleate) and the triglyceride oils (medium chain triglyceride and soybean oil). IPM and EO were slightly different in their structures, isopropyl and ethyl esters of myristic acid and oleic acid, respectively. Comparison between SBO and MCT was that SBO contained mainly long chain fatty acid while MCT contained primarily of short or medium chain fatty acid. Their size were ranked : SBO>MCT>IPM=EO. Aboofazali (1995) studied the effects of oils on phase diagram including isopropyl myristate, medium chain triglyceride, ethyl oleate, and soybean oil and reported the ranking of oil polarity. Therefore, the differences between the two classes of oils used are their sizes and polarity, in that the ethyl esters had smaller size and more polarity than the triglyceride oils.

The results from the partial phase diagrams showed that isopropyl myristate and ethyl oleate can produce larger microemulsion areas than medium chain triglyceride and soybean oils. Due to those of IPM and EO could penetrate more into curved surfactant films than those of MCT and SBO. Thus, microemulsion formation tends to be favored when small oils are present. Similar results were obtained with the systems containing ethyl oleate. Soybean oil, have being the largest size and being the most nonpolar of the oils used, showed no area of the microemulsion in most of the partial phase diagrams.

For the medium chain triglyceride oil generally produced a fairly narrow microemulsion region. This observation was not unexpected since it was known that relatively large oils like the medium chain triglyceride oil used in the study are too large to penetrate into the curved films of the surfactant and as a consequence tended to promote the formation of lamellar phases thereby reducing the extent of the microemulsion region. It should be the effect from cosurfactant

which can produce the microemulsion region in the system containing caproic acid and butanol.

It could be summarized that although differences in phase behavior were seen when using different oils, these differences were generally small, except when using the larger sized oils. The phase diagrams obtained using ethyl oleate as oil were virtually identical to those when using the closely related oil, isopropyl myristate. It appeared that the size of the oils used in this study was the major factor influencing phase behavior, with the larger microemulsion regions seen when the smaller oils were used.

Effect of Em ratios and Em/oil ratios

Comparison of the microemulsion region at three Em ratios of A (1/1), B (1/0.5), C (1/0.25) and Em/oil ratios of 3/7, 5/5, 7/3 between the PC and TW-based systems are shown in Figures 24-26, respectively. It could be seen that for both PC-based and TW-based systems, most systems yielded slightly decrease in microemulsion area against the increase of surfactant concentration (Em ratio of A to C). The different trend could be seen from the TW-based systems containing caproic acid, in that these systems yielded slightly increase in microemulsion area as the increase of surfactant concentration. It was also shown that in most systems, the microemulsion region was increased as the Em/oil ratio increased.

Figure 24 compares the partial phase diagrams at Em ratio of 1/1 between the PC and TW-based systems. It could be seen that for PC-based systems, microemulsion regions could be produced only in the systems containing co-surfactant of butanol, propylene glycol and the three oils of isopropyl myristate, ethyl oleate, or medium chain triglyceride, and the system containing caproic acid and soybean oil. Comparison within TW-based systems showed that most systems with various types and ratios of co-surfactants yielded microemulsion regions in different patterns.

Figure 25 compares the partial phase diagrams at Em ratio of 1/0.5 between the PC and TW-based systems. Some similar results as from the Em ratio of 1/1 were obtained. The difference was that for PC-based systems,

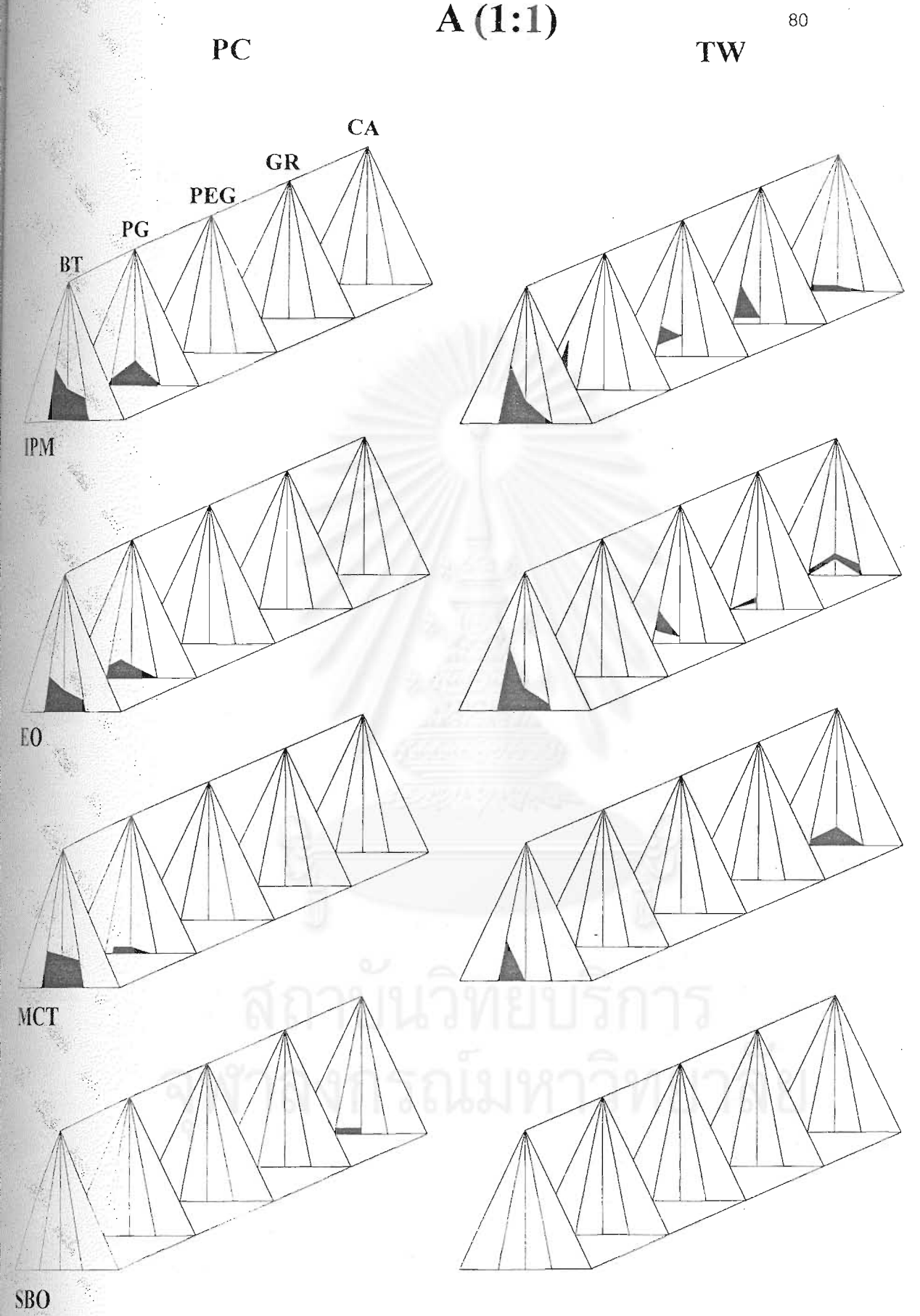


Figure 24 Comparison of partial pseudoternary phase diagrams for systems containing Em ratio of 1:1 with different oil (IPM, EO, MCT, SBO) and cosurfactants (BT, PG, PEG, GR, CA)

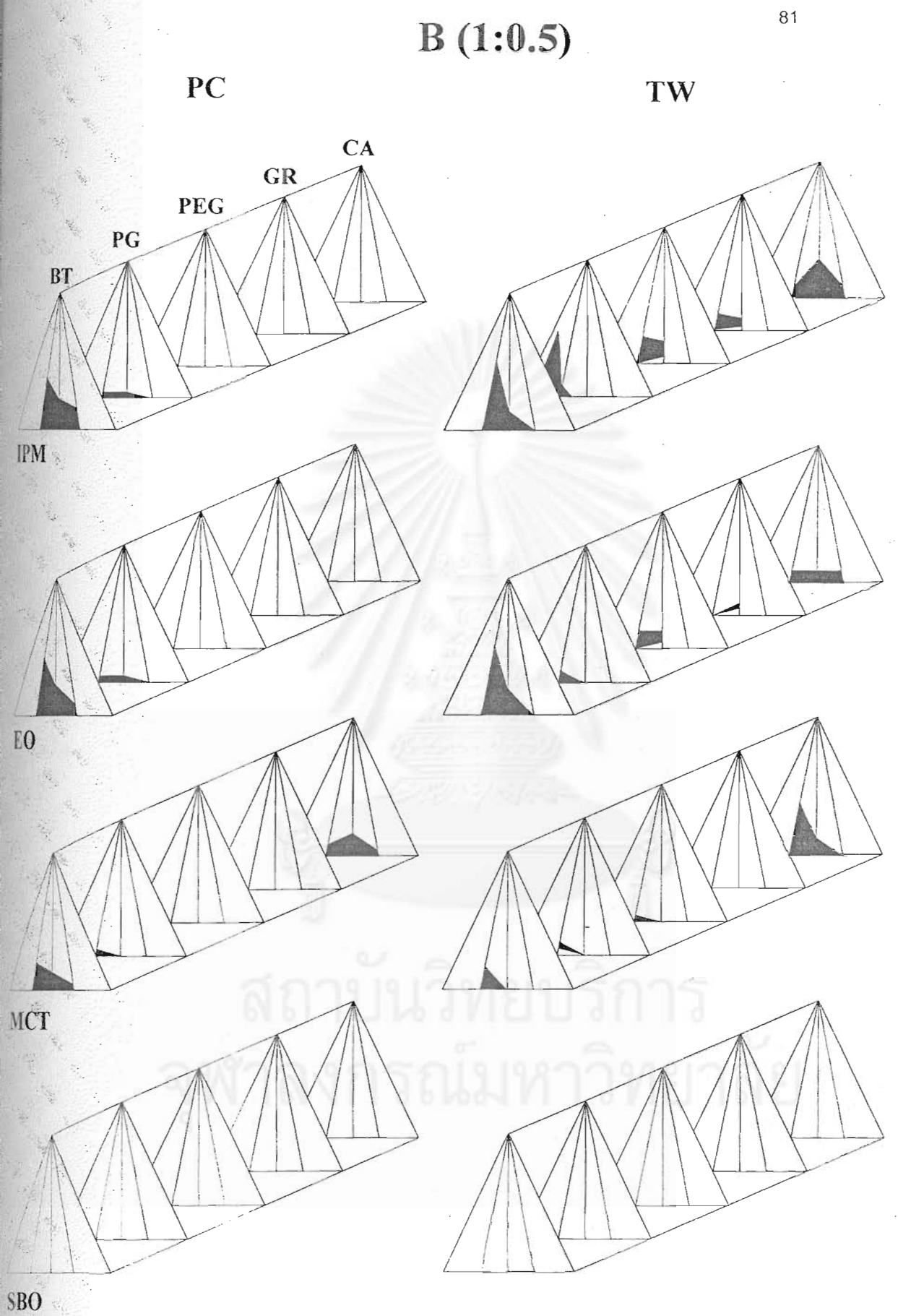


Figure 25 Comparison of partial pseudoternary phase diagrams for systems containing Em ratio of 1:0.5 with different oil (IPM, EO, MCT, SBO) and cosurfactants (BT, PG, PEG, GR, CA)

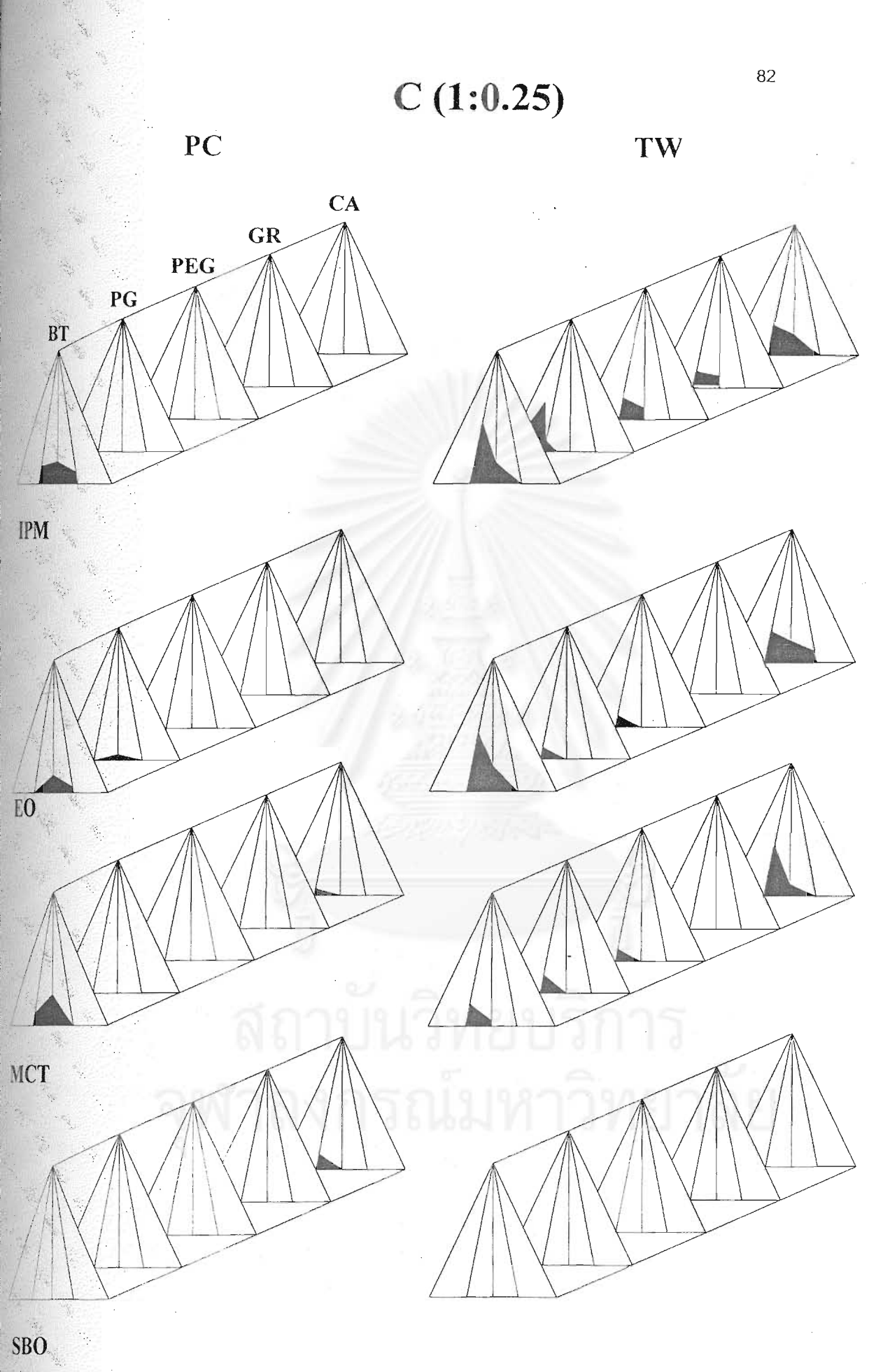


Figure 26 Comparison of partial pseudoternary phase diagrams for systems containing Em ratio of 1:0.25 with different oil (IPM, EO, MCT, SBO) and cosurfactants (BT, PG, PEG, GR, CA)

microemulsion regions were observed from the system containing caproic acid and medium chain triglyceride and no area in the system containing soybean oil. Comparison to TW-based systems, slightly increase of the microemulsion area were obtained.

Figure 26 compares the partial phase diagrams at Em ratio of 1/0.25 between the PC and TW-based systems. Some similar results as from the Em ratio of 1/0.5 and 1/1 were obtained. The microemulsion region was slightly decreased in the PC-based systems but was slightly increased in the TW-based systems.

The results from most systems showed that increasing the Em ratio decreased the microemulsion region. It could be explained that the amount of cosurfactant molecules positioned at the interface would be decreased as the Em ratio increases. Therefore, the rigidity of the interface would increase causing the reduction of the microemulsion area. It was also shown that in most systems, the microemulsion region was increased as the Em/oil ratio increased. This results could be explained that microemulsion possessed a huge interfacial area. To lower those of large interfacial tension, high concentration of surfactant and cosurfactant should be used. The addition of cosurfactant also decrease the concentration of surfactant used.

It could be concluded from the studies that the major factor influencing the phase behavior of microemulsions was the difference in structure/physicochemical properties of surfactant and cosurfactant. Also, the oils, Em ratios, and Em to oil ratios had potentially effect on the region of existence of the microemulsions as well.

Physicochemical characterization

From the partial phase diagrams, the selected microemulsion systems (ME 1-25) which based on the point that most series from phase diagrams were superimposed are presented in Table 2. Criteria used for characterization were the comparison of oil, surfactant, cosurfactant, Em ratio, Em/oil ratio, and internal phase. Their physicochemical characteristics such as types of microemulsion,

droplet sizes, viscosity, and stability were investigated. They were classified as microemulsion according to the transparency with fluidity criterion and being nonbirefringent by polarized light microscopy.

Type of microemulsions

Microemulsion type is another important consideration for pharmaceutical applications. A single method may yield incorrect results. Instead, the type of microemulsion should always be confirmed by mean of other methods. Therefore, dye solubility, dilution, and conductivity test were simultaneously employed to determine the type of microemulsions in this study.

The dye solution test was involved in an addition of a water-soluble dye to the microemulsion. When an amaranth (water soluble dye) was added to each microemulsion, the intense staining of the external phase indicated an o/w microemulsion. In contrast, w/o microemulsion would result in the staining of the droplets of the internal phase. The dilution test, the type was determined by observing the dispersability when diluted with oil or water. If water was easily dispersed in continuous phase, the microemulsion was termed oil-in water (o/w). If oil (IPM) was dispersed in external phase, the microemulsion was termed water- in-oil (w/o) (Ho et al., 1996). In conductivity test, the high conductivity indicated that the continuous phase was water, therefore they were microemulsion of o/w type. In contrast, the conductivity of water in oil was very low which indicated that the continuous phase was less hydrophilic. Thus it was classified to be w/o type.

Table 4 summarizes the results of the tests for microemulsion types determined by three methods. Most PC-based microemulsion systems examined could be diluted with oil or stained at the internal phase with conductivity less than 1 μmhos/cm, indicating that the microemulsions were water-in-oil type. On the other hand, most TW-based systems could be stained at the external phase, diluted with water and having higher conductivity. The well known oil-in-water microemulsions were formed using surfactants within the HLB range of 8 to 18. Due to the high HLB of tween 80, it should be classified to be oil-in-water type. However, some TW-based systems which contained caproic acid (ME7) or

Table 4 Types of selected microemulsions

ME	Stain of dye	Dilution test		Conductivity (μmhos/cm)	Polarized light	Type
	water soluble dye (amaranth)	oil dilution (IPM)	water dilution (water)			
1	IP	NS	S	0.21	NB	w/o
2	EP	NS	NS	2.92	NB	o/w
3	IP	NS	S	0.35	NB	w/o
4	EP	S	S	2.89	NB	o/w
5	IP	NS	S	0.31	NB	w/o
6	EP	S	S	2.77	NB	o/w
7	IP	NS	S	0.11	NB	w/o
8	EP	S	NS	5.63	NB	o/w
9	EP	NS	NS	1.85	NB	o/w
10	EP	NS	NS	2.76	NB	o/w
11	EP	NS	NS	1.14	NB	o/w
12	EP	NS	NS	0.99	NB	o/w*
13	IP	NS	S	0.36	NB	w/o
14	IP	NS	S	0.02	NB	w/o
15	IP	NS	S	0.61	NB	w/o
16	IP	NS	S	0.36	NB	w/o
17	IP	NS	S	0.31	NB	w/o
18	IP	NS	S	0.25	NB	w/o
19	IP	NS	S	0.3	NB	w/o
20	IP	NS	S	0.26	NB	w/o
21	IP	NS	S	0.63	NB	w/o
22	IP	NS	S	0.85	NB	w/o
23	IP	NS	S	0.44	NB	w/o
24	IP	NS	S	0.79	NB	w/o
25	IP	NS	S	1.02	NB	w/o

IP = Internal phase, EP = External phase

S = Separation, NS = No separation

NB = Non birefringence

* = inconclusive

glycerol (ME13-ME15) as cosurfactant displayed different results. Their systems were separated when diluted with water, stained at internal phase, and having low conductivity which implied to be water-in-oil type. This results may be explained by the characteristic of cosurfactant, which distributed and affected in the geometric packing of the surfactant layer at interface, and the low percentage of water. As proposed by Shah et al. (1972), such systems at low water content were of the water-in-oil type with a spherical internal phase. Therefore, these systems should be classified to be water-in-oil type. ME12 cannot be clearly concluded due to the system could be stained at the external phase but having low conductivity.

Droplet Sizes

Droplet size is one of the properties which is usually used to characterize microemulsions. TEM technique is primarily concerned with the examination of bulks specimen. The electron beam passes through the specimen in a TEM analysis. Negative staining technique was used to determine the shapes and dimension of the microstructures at molecular level. The specimen is surrounded by heavy metal atoms that act as an electron barrier. Because the electron beam can pass through the low electron density of the specimen but not through the metallic background, the result is negative staining: a light specimen against a dark background. The image obtained depends primarily on the penetration of the stain into the hydrated regions mostly the hydrophilic regions of the specimen to replace water. As the stain usually dries faster than those of specimen, an increasing viscous cap of stain forms within the hydrophilic regions and a stain envelope surrounds the hydrophobic regions. Thus this technique can be used in both types (w/o and o/w) of microemulsions but cannot separate these types (Hayat and Miller, 1990). Since the transmission electron microscopy (TEM) is the most important technique for the study of microstructures 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 the microemulsion systems.

However, microemulsion cannot be easily imaged with TEM due to some factors. Electron may induce chemical reactions in microemulsion

systems that can change the microstructure. There is insufficient contrast between the microstructures and their surroundings. The overlapping of microstructures in a thicker sample will also make the image difficult to interpret. From this experiment, in some samples the droplets could be clearly seen but some could not. The undetectable results may due to some samples had low internal phase. In addition, some samples had high viscosity which the excess can hardly be drained off thus a netlike or an overlapping pictures were obtained.

During the experiment, the images were viewed at various magnification to see overall of the samples. Then the droplet sizes were analyzed using Scion Image Analyzing software from internet source (http://www.siconcorp.com) to count and calibrate their sizes. The frequency, mean, mode, median, summation, and histogram were obtained using the SPSS version 8 and are shown in Appendix C.

Figures 27-32 compares the effects of ingredients and ratios from some systems of ME1-25. Figure 27 compares the photomicrographs between system containing phospholipid and system containing tween 80. Figure 28 compares the photomicrographs among systems containing different Em ratios and Em/oil ratios. It could be seen that increased Em ratio resulted in increasing aggregates. Figure 29 compares the systems containing different oils. The effect of cosurfactants was compared in Figure 30. The systems containing PEG and GR were compared in Figure 31 and 32, respectively.

The results of droplet sizes could not be compared due to the sample sizes were too small and the obtaining pictures were not clear. However, most systems had droplet size in the range of microemulsion which was under 100 nm.

The microemulsions were also examined for their droplet size by the light scattering technique. Unfortunately, the compositions of these systems showed low intensity of the light scattering which may due to many factors such as the low power of the laser source. So the droplets size could not be detected by this technique.

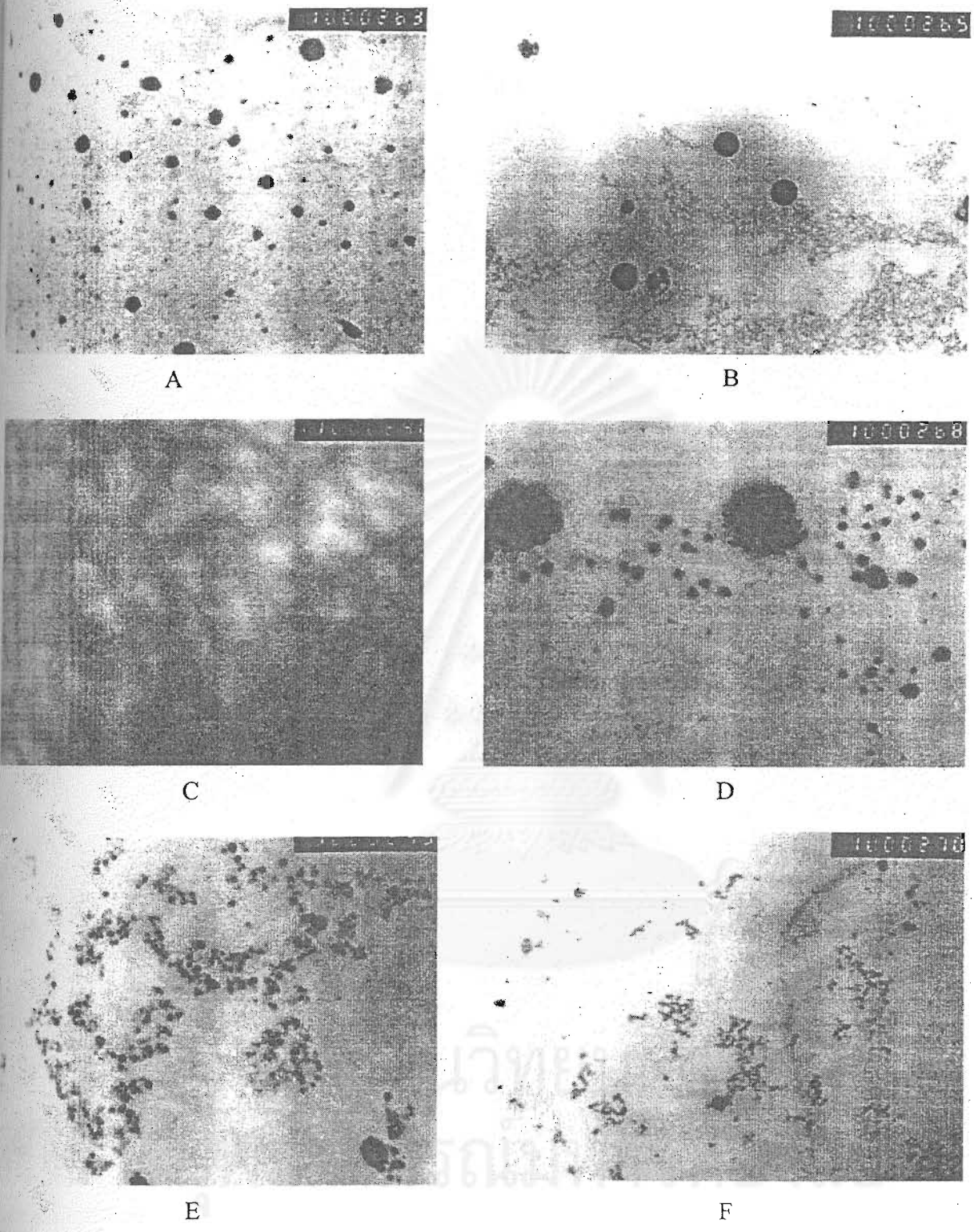


Figure 27 Comparison of TEM photomicrographs between PC and TW-base systems with different oils and cosurfactants

key: A: ME1, IPM-PC-BT, Em=B, Em/oil = 7/3, Aq 4%, x 13200
B: ME2, IPM-TW-BT, Em=B, Em/oil = 7/3, Aq 4%, x 13200
C: ME3, IPM-PC-PG, Em=B, Em/oil = 7/3, Aq 2%, x 13200
D: ME4, IPM-TW-PG, Em=B, Em/oil = 7/3, Aq 4%, x 13200
E: ME5, EO-PC-PG, Em=B, Em/oil =7/3, Aq 2%, x 13200
F: ME6, EO-TW-PG, Em=B, Em/oil =7/3, Aq 4%, x 13200

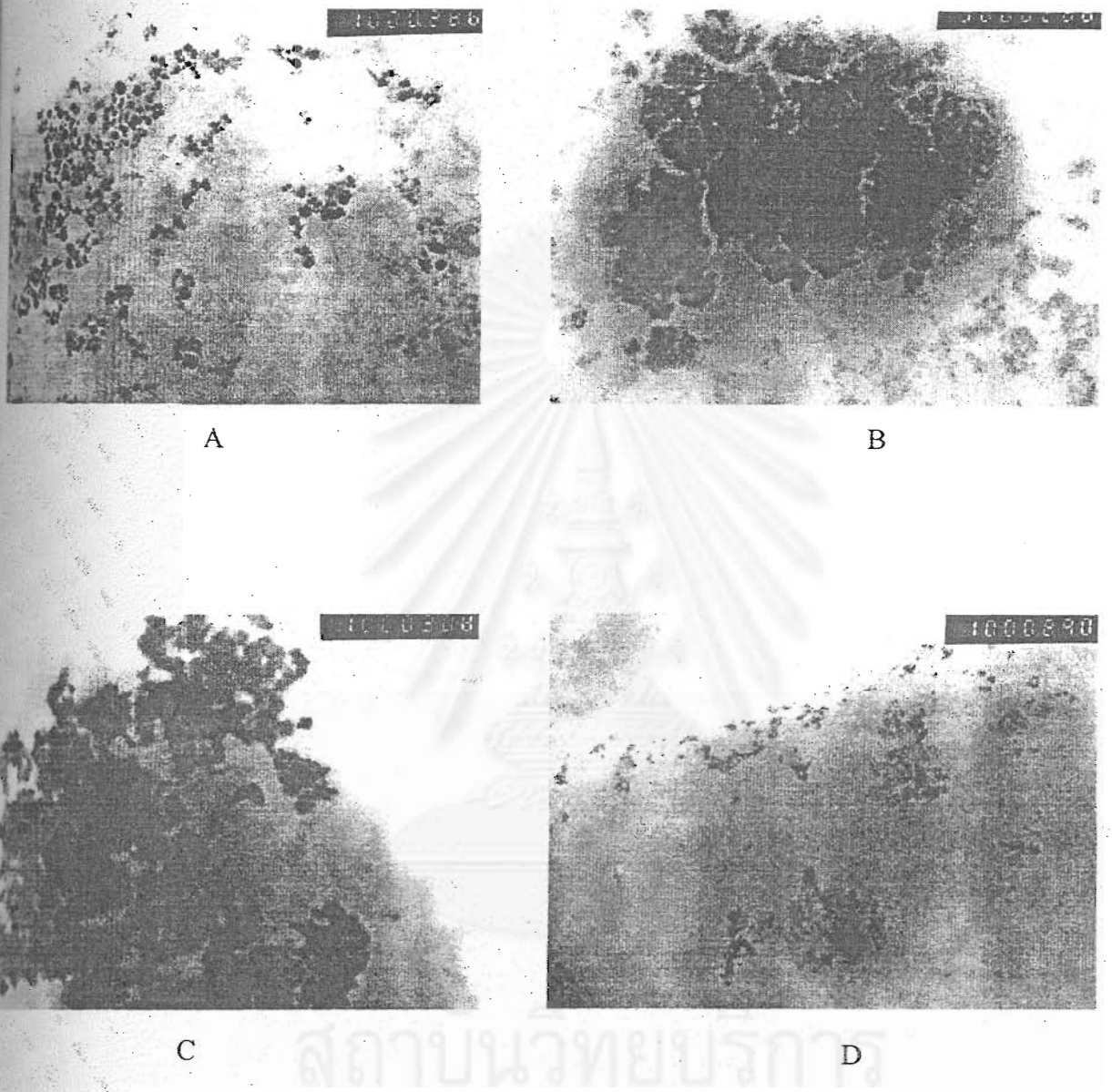
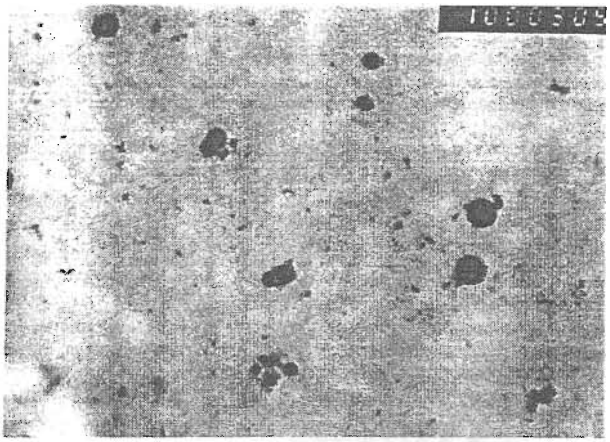
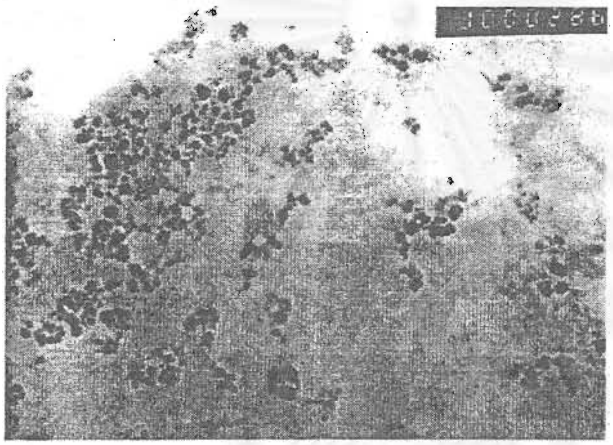


Figure 28 Comparison of TEM photomicrographs of EO-PC-PG systems with different Em ratios and Em/oils ratios

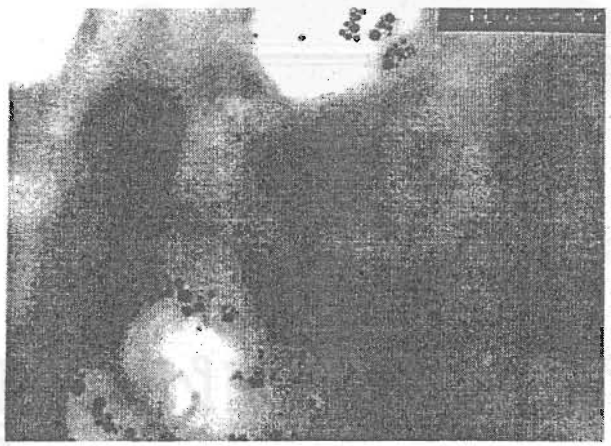
key: A: ME16, EO-PC-PG, Em=A, Em/oil = 5/5, Aq 4%, x 13200
B: ME17, EO-PC-PG, Em=B, Em/oil = 5/5, Aq 4%, x 13200
C: ME18, EO-PC-PG, Em=C, Em/oil = 5/5, Aq 2%, x 13200
D: ME23, EO-PC-PG, Em=A, Em/oil = 6/4, Aq 4%, x 16500



A



B



C

Figure 29 Comparison of TEM photomicrographs for PC-PG systems with different oils

key: A: ME19, IPM-PC-PG, Em=A, Em/oil = 5/5, Aq 4%, x 13200

B: ME16, EO-PC-PG, Em=A, Em/oil = 5/5, Aq 4%, x 13200

C: ME20, MCT-PC-PG, Em=A, Em/oil = 5/5, Aq 2%, x 13200

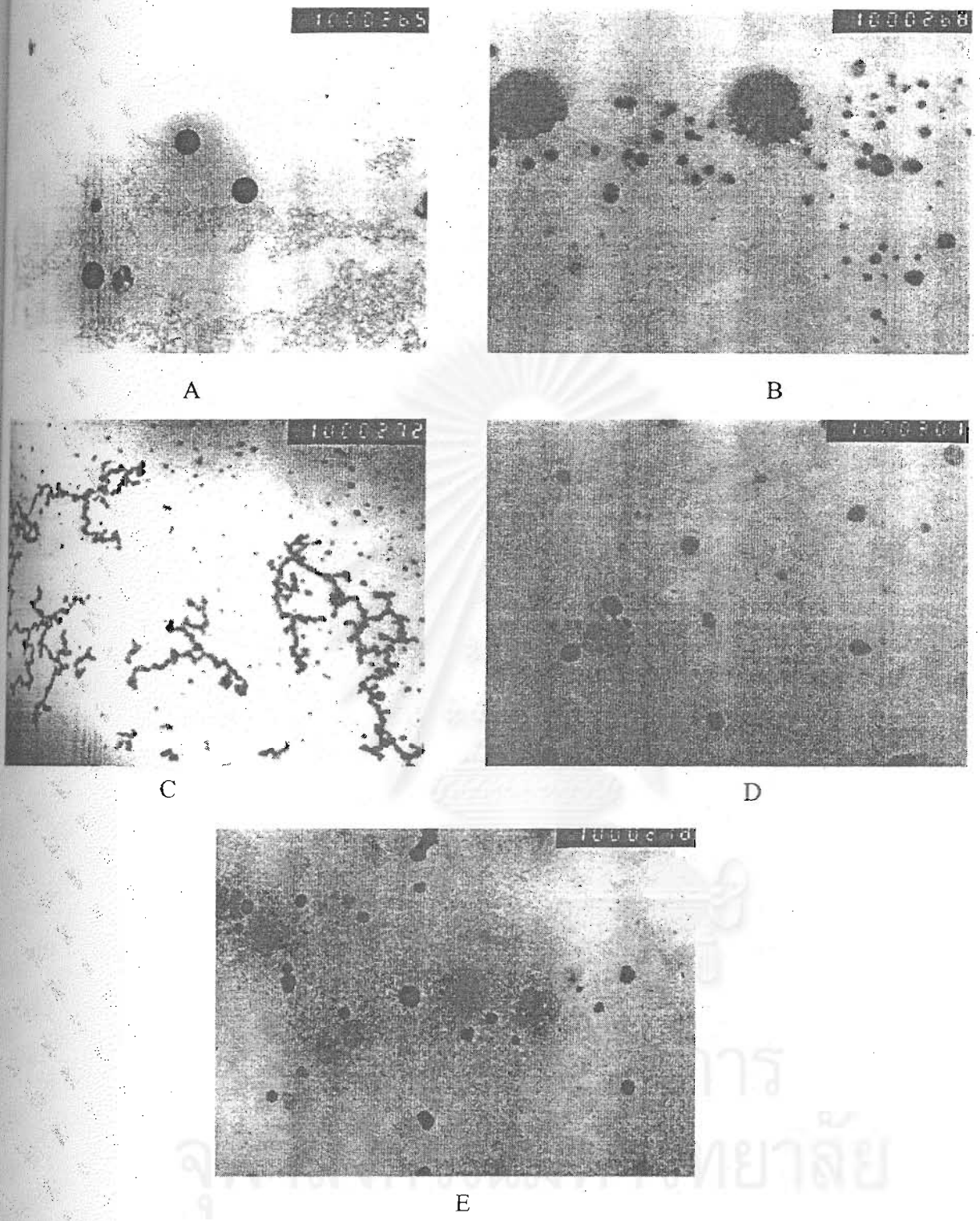


Figure 30 Comparison of TEM photomicrographs for IPM-TW systems with different cosurfactants
key: A: ME2, IPM-TW-BT, Em=B, Em/oil = 7/3, Aq 4%, x 13200
B: ME4, IPM-TW-PG, Em=B, Em/oil = 7/3, Aq 4%, x 13200
C: ME11, IPM-TW-PEG, Em=B, Em/oil = 7/3, Aq 4.76%, x 13200
D: ME14, IPM-TW-GR, Em=B, Em/oil = 7/3, Aq 2%, x 13200
E: ME 7, IPM-TW-CA, Em=B, Em/oil = 7/3, Aq 4%, x 13200

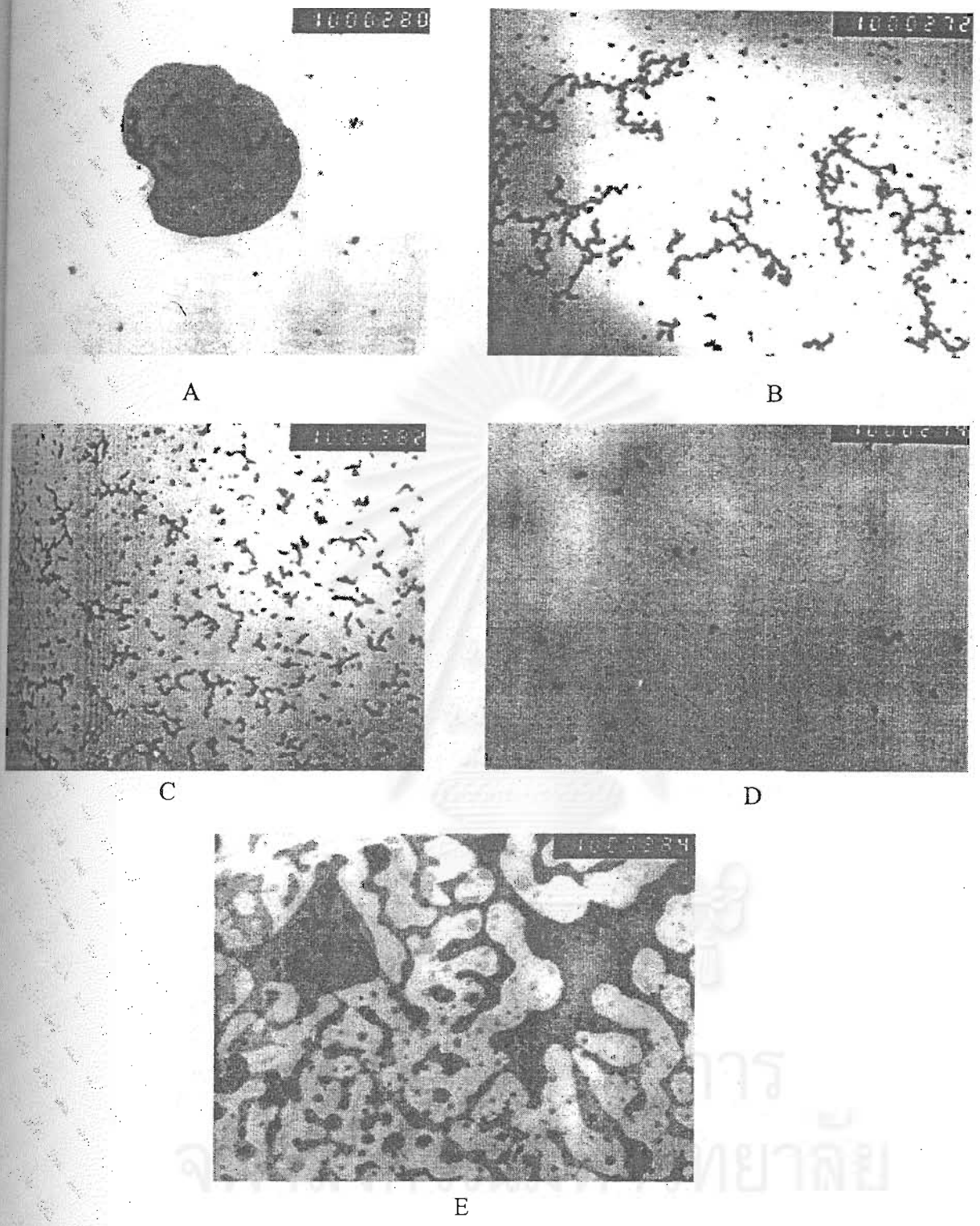
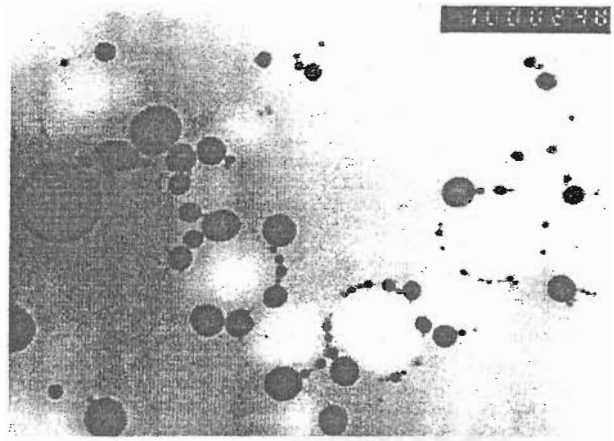
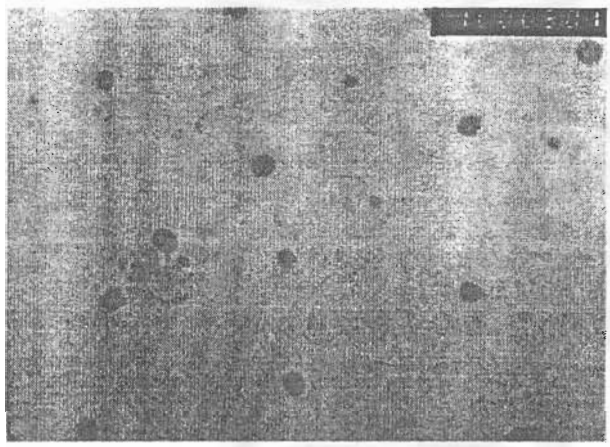


Figure 31 Comparison of TEM photomicrographs for IPM-TW-PEG systems with different Em ratios and percentage of internal phase

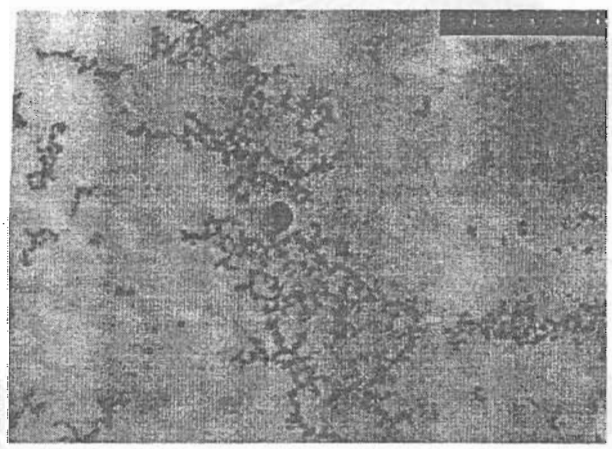
key: A: ME8, IPM-TW-PEG, Em=A, Em/oil = 7/3, Aq13.5%, x 13200
B: ME11, IPM-TW-PEG, Em=B, Em/oil = 7/3, Aq 4.76%, x 13200
C: ME9, IPM-TW-PEG, Em=B, Em/oil = 7/3, Aq10%, x 13200
D: ME12, IPM-TW-PEG, Em=C, Em/oil = 7/3, Aq 4%, x 13200
E: ME10, IPM-TW-PEG, Em=C, Em/oil = 7/3, Aq10%, x 13200



A



B



C

Figure 32 Comparison of TEM photomicrographs for IPM-TW-GR systems with different Em ratios
key: A: ME13, IPM-TW-GR, Em=A, Em/oil = 7/3, Aq 3%, x 13200
B: ME14, IPM-TW-GR, Em=B, Em/oil = 7/3, Aq 2%, x 13200
C: ME15, IPM-TW-GR, Em=C, Em/oil = 7/3, Aq 4%, x 13200

Viscosity

As the viscosity of microemulsion is an important and macroscopically observable parameter, model viscosity as a function of phase composition and phase type was used to characterize a given systems. Viscosity depends largely on the structure, i.e., the type of aggregates that are present, on their interactions, and on the concentration of the system. Because viscosity depends sensitively on the shape and interactions of the droplets dispersed therefore it can be use to monitor microstructure changes in a microemulsion system.

Table 5 summarizes the viscosity of selected microemulsion systems. The viscosity of all systems were between 15.37 to 303.84 cp. ME2 (IPM-TW-BT) had lowest viscosity of 15.37 cp while ME13 had the highest viscosity of 303.84 cp. Figures 33-35 compared the viscosity of the selected microemulsions. As shown in Figure 33A, microemulsion containing phospholipid yielded higher viscosity than the system containing tween 80.

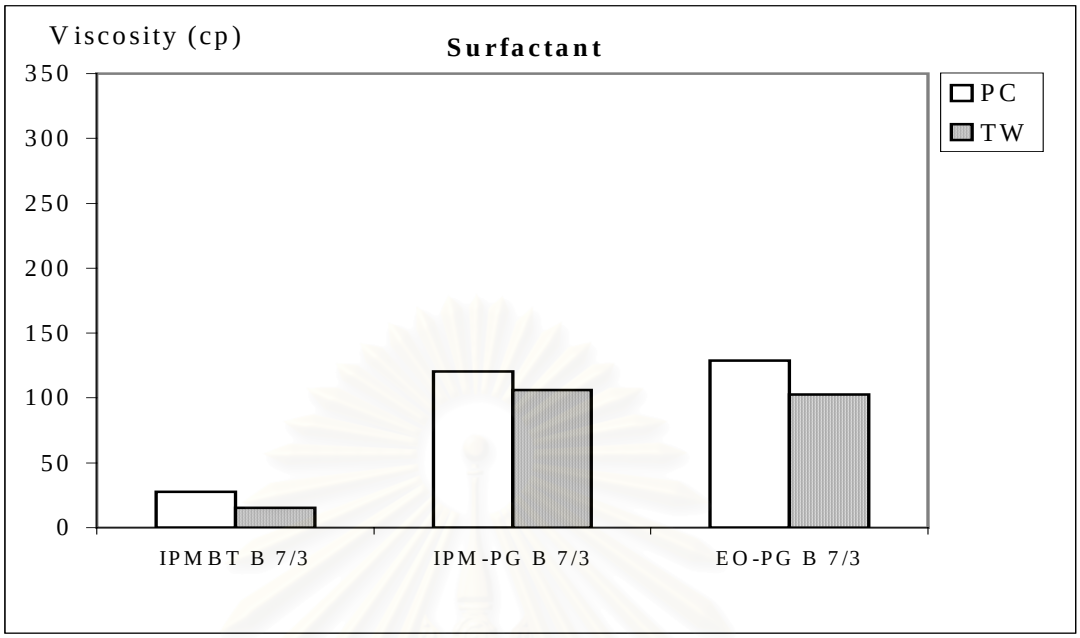
The effect of cosurfactant is shown in Figure 33B. The lowest viscosity was found in the system containing butanol. The system containing caproic acid also yielded low viscosity of 25.11 cp. The viscosity was increased with the systems containing propylene glycol, polyethylene glycol, and glycerol, respectively. ME2 (IPM-TW-BT) had lowest viscosity of 15.37 cp while ME13 had highest viscosity of 303.84 cp.

Comparison among oils showed little effect as shown in Figure 34A. The ester oils, both IPM and EO oil yielded viscosity in the same range and had lower viscosity than the system containing MCT.

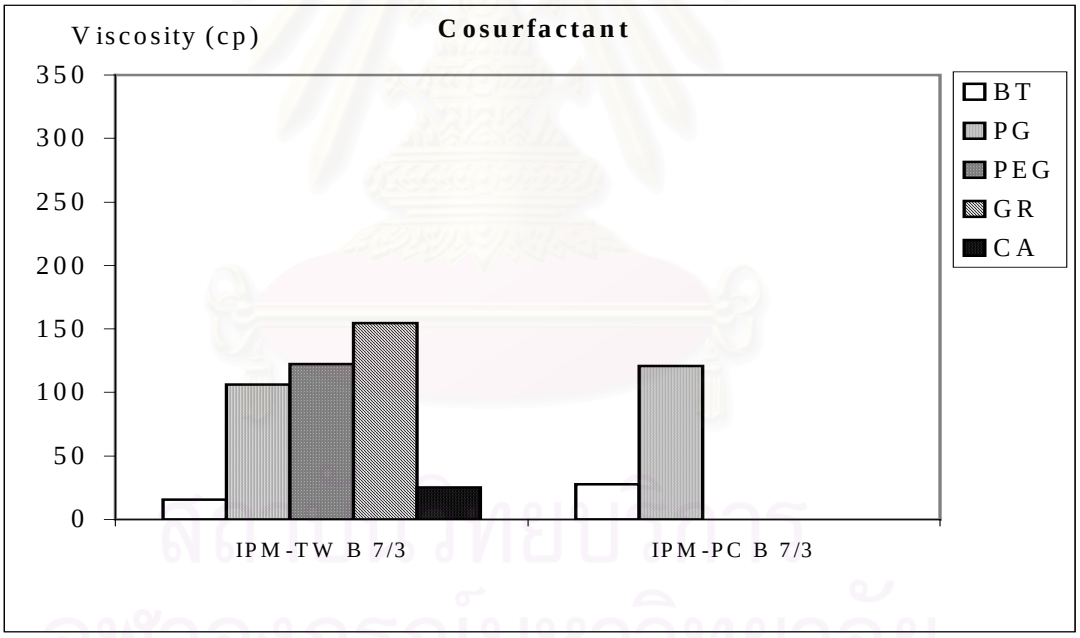
Figure 34B compares E1/E2 ratio. The results showed that Em ratio of C (1/0.25) yielded higher viscosity than B (1/0.5) and A (1/1), respectively. Except the system containing IPM-TW-GR, Em ratio of A yield higher viscosity than Em ratio of B. When the Em ratio increased, it meant that the percentage of surfactant was also increased. As known, the higher amount of surfactants would increase the rigidity of the interface while the higher amount of cosurfactants would increase the flexibility of the film. The effect of Em/oil ratio

Table 5 Viscosity of the investigated microemulsions

ME	Oil	E1	E2	Em	Em/Oil	Aq	Viscosity (cp)	
							Mean	S.D.
1	IPM	PC	BT	1/0.5	7/3	4.00	27.80	0.02
2	IPM	TW	BT	1/0.5	7/3	4.00	15.37	0.01
3	IPM	PC	PG	1/0.5	7/3	2.00	120.64	0.39
4	IPM	TW	PG	1/0.5	7/3	4.00	106.28	0.08
5	EO	PC	PG	1/0.5	7/3	2.00	129.03	0.25
6	EO	TW	PG	1/0.5	7/3	4.00	102.46	0.19
7	IPM	TW	CA	1/0.5	7/3	4.00	25.11	0.07
8	IPM	TW	PEG	1/1	7/3	13.50	111.18	0.33
9	IPM	TW	PEG	1/0.5	7/3	10.00	143.42	0.25
10	IPM	TW	PEG	1/0.25	7/3	10.00	176.42	0.28
11	IPM	TW	PEG	1/0.5	7/3	4.76	122.41	0.05
12	IPM	TW	PEG	1/0.25	7/3	4.00	138.05	0.17
13	IPM	TW	GR	1/1	7/3	3.00	198.00	0.82
14	IPM	TW	GR	1/0.5	7/3	2.00	154.51	0.43
15	IPM	TW	GR	1/0.25	7/3	4.00	303.84	0.90
16	EO	PC	PG	1/1	5/5	4.00	51.35	0.12
17	EO	PC	PG	1/0.5	5/5	4.00	69.68	0.12
18	EO	PC	PG	1/0.25	5/5	4.00	119.36	0.00
19	IPM	PC	PG	1/1	5/5	4.00	50.33	0.03
20	MCT	PC	PG	1/1	5/5	2.00	64.58	0.33
21	IPM	PC	PG	1/1	5/5	10.00	74.58	0.86
22	IPM	PC	PG	1/1	5/5	15.00	89.22	0.94
23	EO	PC	PG	1/1	6/4	4.00	66.49	0.87
24	EO	PC	PG	1/1	6/4	10.00	84.39	0.54
25	EO	PC	PG	1/1	6/4	15.00	121.29	0.90



A

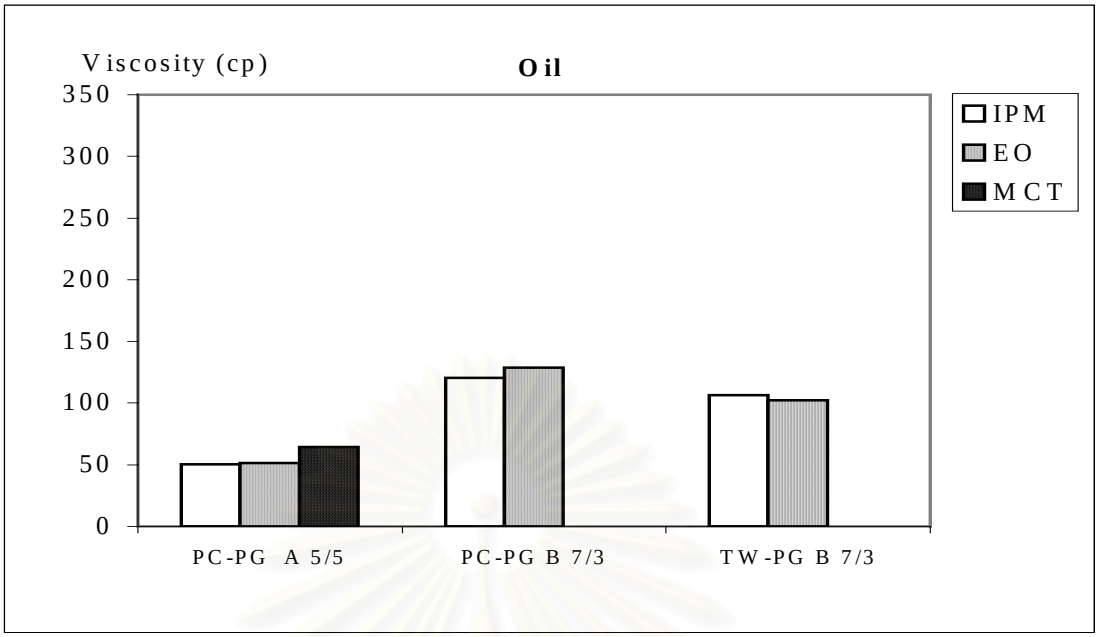


B

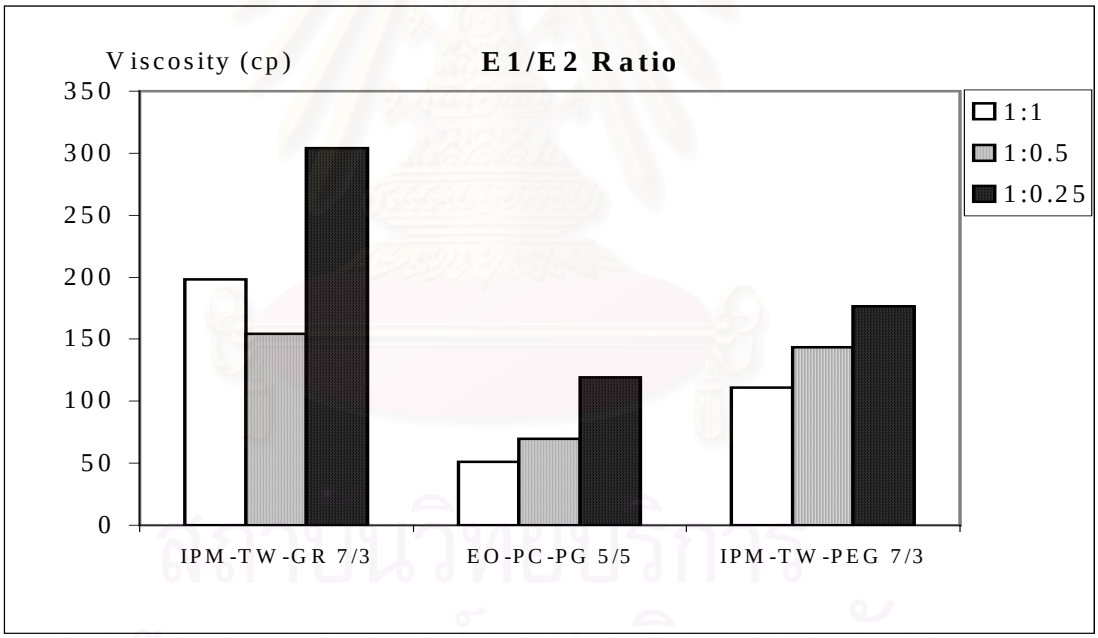
Figure 33 Comparison of viscosity of investigated microemulsions containing different surfactants and cosurfactants

Key: A: for systems containing different surfactants of PC and TW

B: for systems containing different cosurfactants of BT, PG, PEG, GR, and CA



A

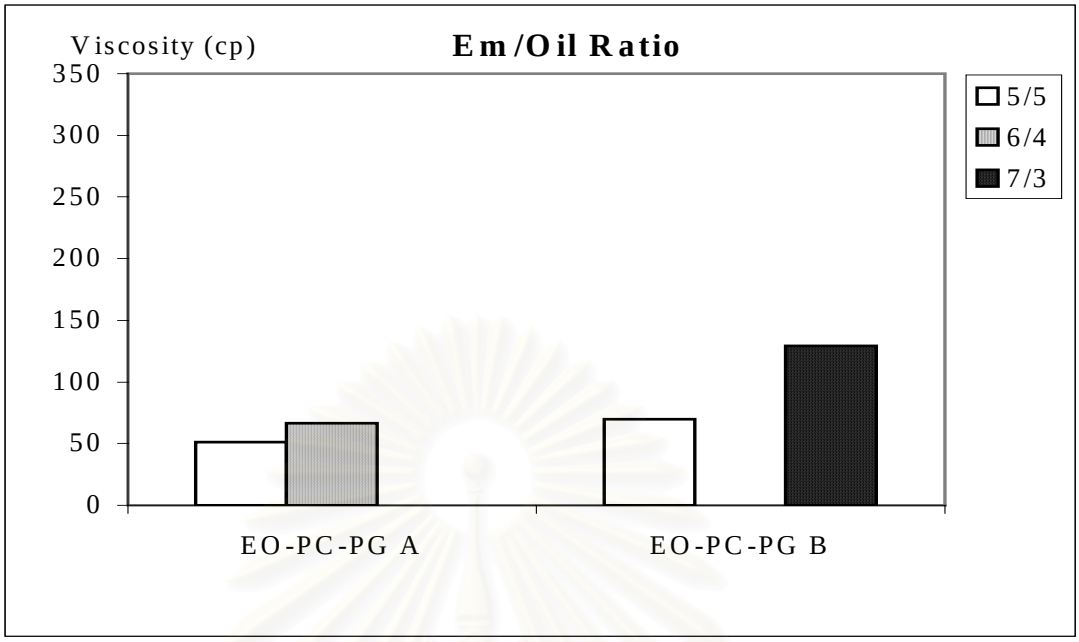


B

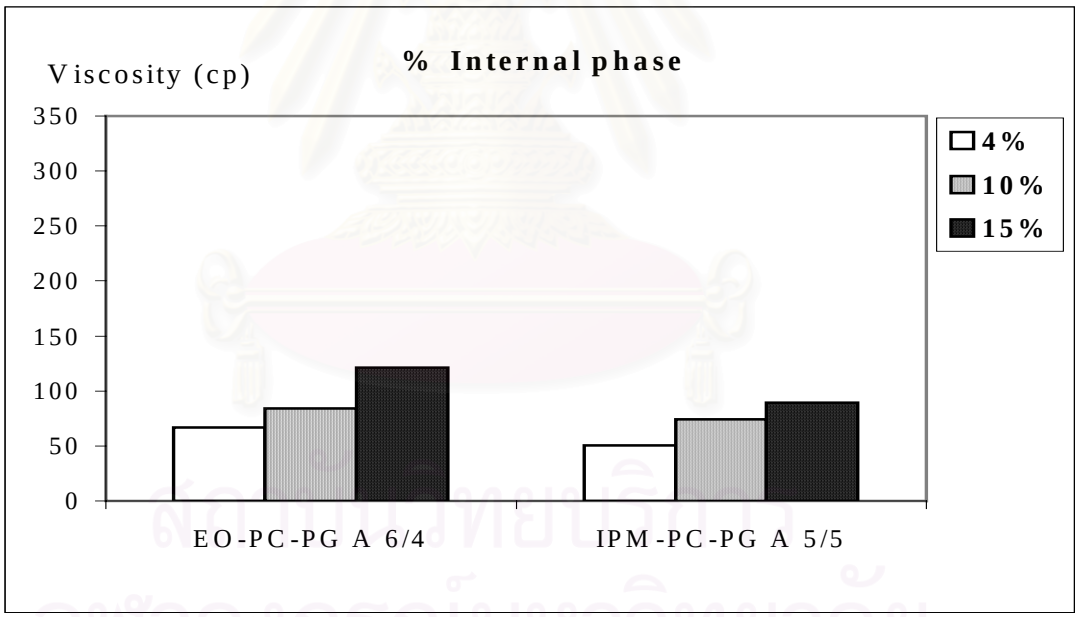
Figure 34 Comparison of viscosity of investigated microemulsions containing different oils and surfactants ratios

Key: A: for systems containing different oils of IPM, EO, and MCT

B: for systems containing different E1/E2 ratios of A(1:1), B(1:0.5), and C(1:0.25)



A



B

Figure 35 Comparison of viscosity of investigated microemulsions containing different surfactant / oil ratio and % internal phase

Key: A: for systems containing different Em/oil ratios of 5/5, 6/4, and 7/3

B: for systems containing different % internal phase of 4%, 10%, and 15%

was explained by increasing the amount of surfactant would increase the viscosity of the system (Figure 35A). Comparison among percentage of the internal phases as shown in Figure 35B revealed that higher of viscosity was obtained with increasing percentage of the internal phases.

Stability

The stability of microemulsions is one of the most important factors to consider. The physicochemical properties of ingredients used may result in instability of these systems. Due to ME21-ME25 could not compare the effect of ingredients, only ME1-ME20 were selected to study their stability. Shelf-life stability of the obtained microemulsions both as a function of time and storage temperatures, monitored at 4°C (refrigerator), 30°, 37°, and 50°C, were evaluated by visual inspection of the samples each month for six months. Investigation of the viscosity change at three and six months, and imaging the droplet sizes by TEM technique were undertaken at six months.

Physical appearance of selected systems are shown in Table 6. By visual inspection criteria, stable systems were identified as those free of any physical changes, such as phase separation, flocculation and/or precipitation. Most preparations stored at various temperatures were single-phase and clear even after 6 months on stability test. This exhibited that these microemulsions were stable during storage period. However, the systems containing glycerol showed unstable as these systems separated during the stability test. The systems of ME3 (IPM-PC-PG), ME8, ME11(IPM-TW-PEG) , ME18 (EO-PC-PG) showed slightly unstable. No changes in color of these systems are observed, the degree of yellowish was due to the ingredients used. The systems containing phospholipid yielded more yellow (+3) color than other systems.

Viscosity measurements are also useful in determining their stability. The viscosity during storage at different temperature of 4°, 30°, 37°, and 50° C were examined for six months, the resulting profile is shown in Table 7 and Figure 36-39. From the results, the viscosity of most microemulsion systems was slightly decreased. When the storage temperature was increased, the viscosity of systems ME13, ME15, and ME18 which contained Em ratio of A and C were

Table 6 Physical appearance of the investigated microemulsions during storage for 6 months at temperature of 4°,30°, 37°, and 50°C

ME	Temperature(C)																							
	30	4						30						37						50					
	month	month						month						month						month					
	0	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	(Y/S)																								
1	3/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
2	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
3	3/-	s1	s1	s1	s1	s1	s1	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
4	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
5	3/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
6	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
7	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
8	1/-	c	c	c	c	c	c	c	c	c	c	c	c	s1	s1	s1	s1	s1	s1	s1	s1	s1	s1	s1	s1
9	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
10	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
11	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	s1	s1	s1	s1	s1	s1	s1	s1	s1	s1
12	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
13	1/-	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3
14	1/-	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3	s3
15	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	s1	s1	s1	s1	s1	s1
16	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
17	3/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
18	3/-	s1	s1	s2	s2	s2	s2	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
19	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
20	2/-	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c

Color : yellow (Y) ; +1, +2, +3
Phase separation : (S) ; s1 = 1-10%, s2 = 11-20%, s3 = 21-30%
Clear = c

Table 7 Viscosity of the investigated microemulsions at initial and after storage for 3 and 6 months at temperature of 4°, 30°, 37° and 50°C

ME	Viscosity (cp)								
	0 months	3 months				6months			
	Initial	4 °C	30°C	37°C	50°C	4°C	30°C	37°C	50°C
1	27.80	24.39	24.62	25.11	25.60	21.64	22.05	22.5	21.42
2	15.37	13.73	13.73	13.84	13.77	14.97	14.34	13.65	13.67
3	120.64	109.71	110.50	109.55	99.82	102.08	100.25	94.78	87.8
4	106.28	93.33	92.87	92.24	89.19	87.28	86.74	87.91	85.37
5	129.03	122.00	130.06	117.04	114.15	106.63	105.05	99.3	95.08
6	102.46	92.68	92.70	92.98	97.96	84.86	85.95	84.97	84.31
7	25.11	21.01	20.80	21.73	21.41	19.93	19.77	19.22	18.67
8	111.18	105.73	107.50	107.99	106.74	100.91	98.29	90.93	93.3
9	143.42	135.41	130.72	133.88	134.53	120.28	117.53	114.15	111.45
10	176.42	167.75	168.73	167.75	163.12	142.08	142.74	142.3	139.85
11	122.41	117.12	119.57	119.87	119.52	105.62	104.56	100.47	97.83
12	138.05	126.66	128.76	125.13	127.61	108.29	109.38	108.4	104.15
13	198.00	155.87	153.36	153.80	116.77	118.54	125.51	111.62	90.94
14	154.51	134.29	124.15	119.30	102.81	108.4	107.26	97.28	80.93
15	303.84	275.50	274.24	273.15	267.60	205.85	221.11	218.98	210.37
16	51.35	48.10	46.63	44.70	43.83	39.32	36.29	34.69	29.62
17	69.68	64.36	62.95	58.85	56.06	49.86	46.98	44.29	35.09
18	119.36	101.59	119.79	104.18	74.94	87.47	98.43	75.46	41.31
19	50.33	45.94	43.76	43.36	40.58	42.42	40.83	37.63	33.47
20	64.58	62.24	63.06	62.35	58.90	60.28	55.86	52.81	49

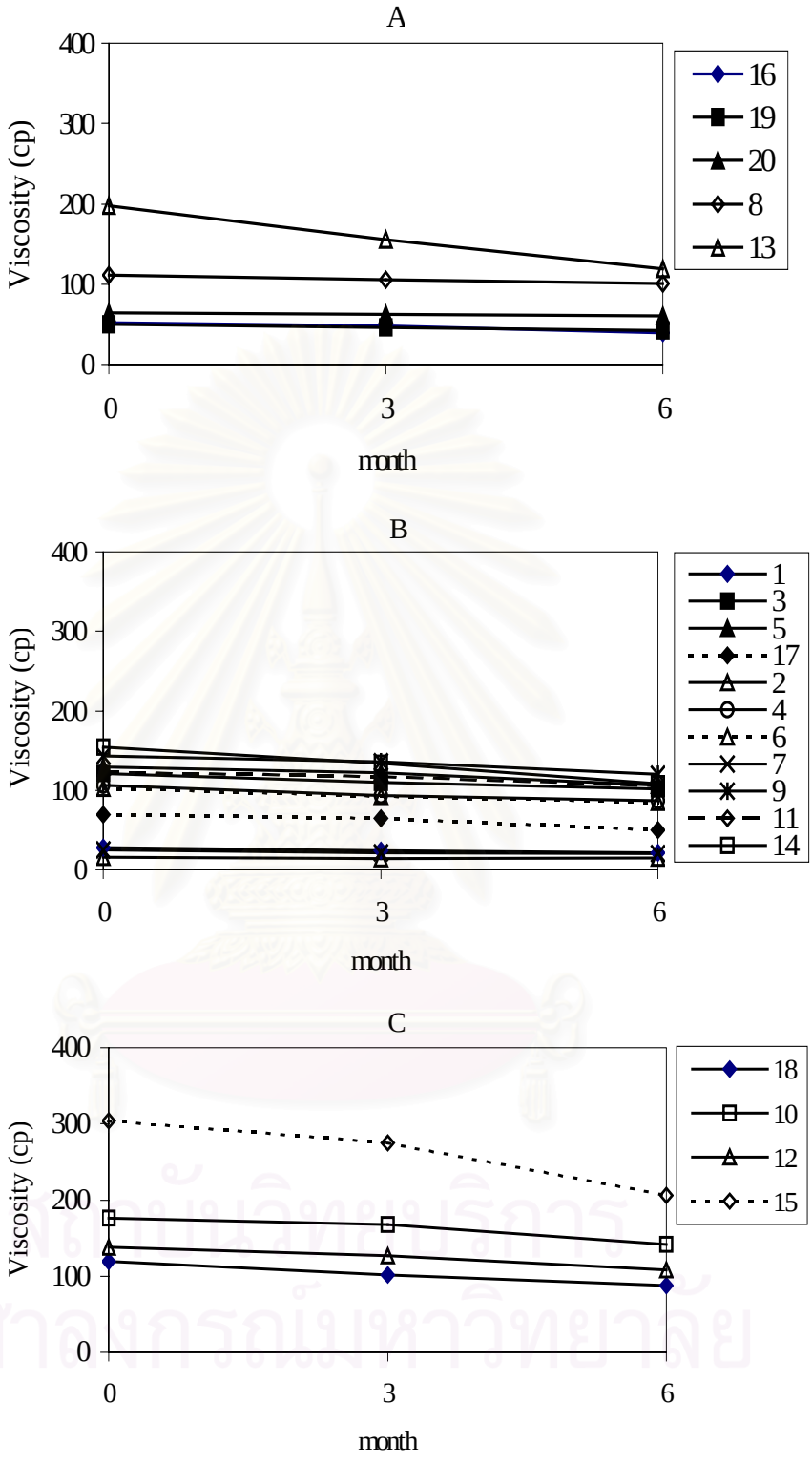


Figure 36 Viscosity profiles of investigated microemulsions at initial and 4 °C in 3 and 6 months

Key: A: for systems containing Em ratio of A (1:1)
B: for systems containing Em ratio of B (1:0.5)
C: for systems containing Em ratio of C (1:0.25)

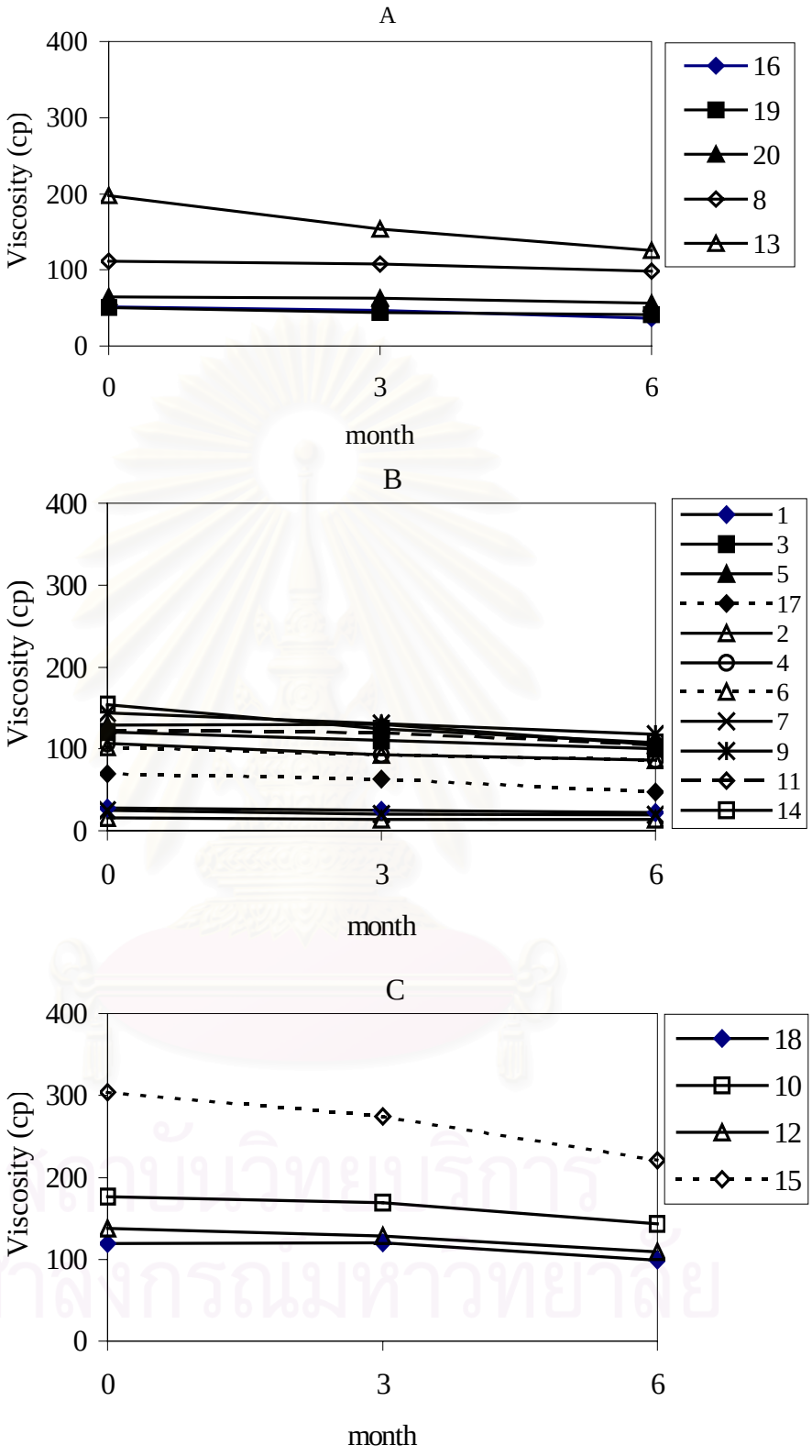


Figure 37 Viscosity profiles of investigated microemulsions at initial and 30 °C in 3 and 6 months
Key: A: for systems containing Em ratio of A (1:1)
B: for systems containing Em ratio of B (1:0.5)
C: for systems containing Em ratio of C (1:0.25)

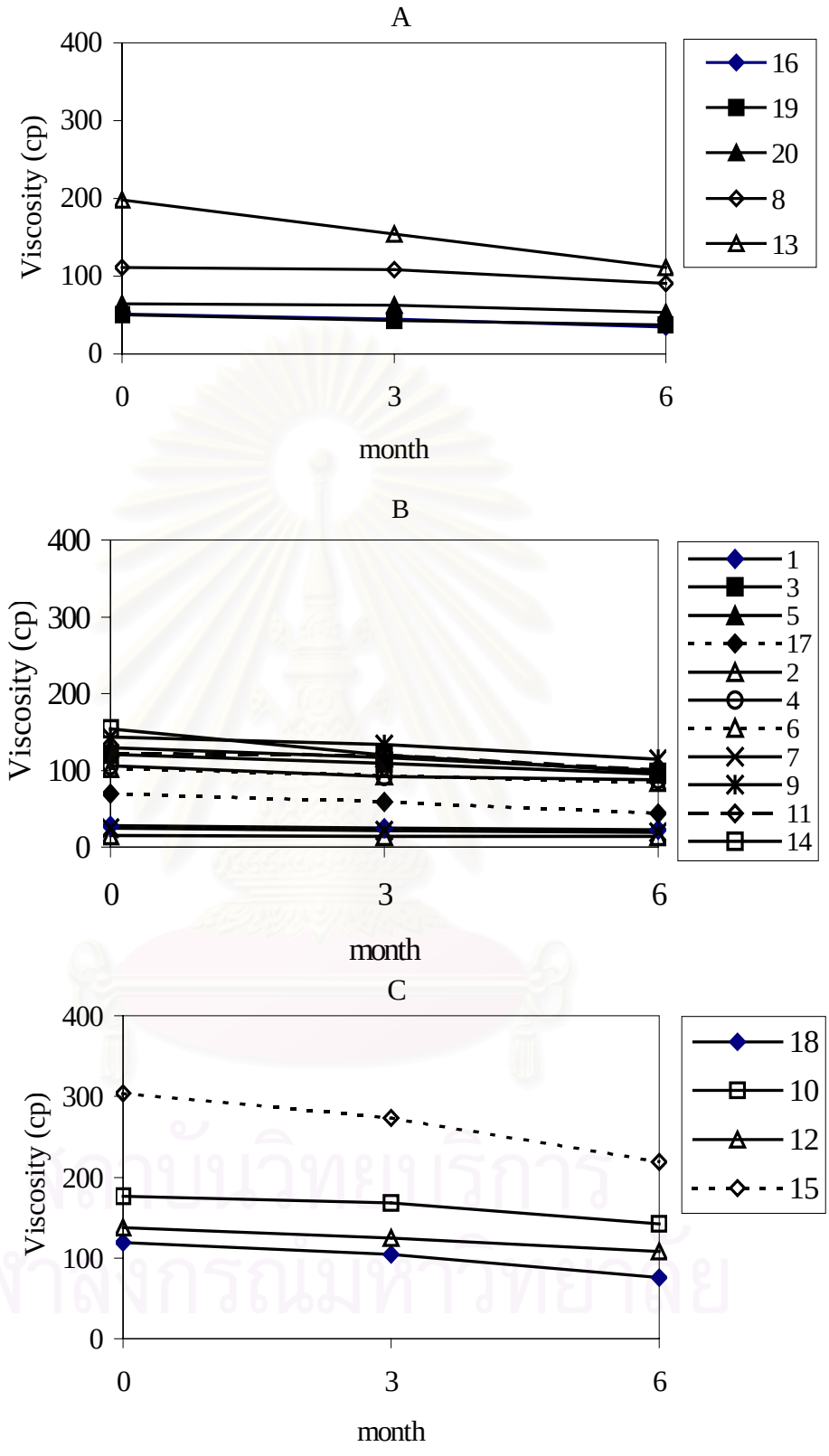


Figure 38 Viscosity profiles of investigated microemulsions at initial and 37 °C in 3 and 6 months
Key: A: for systems containing Em ratio of A (1:1)
B: for systems containing Em ratio of B (1:0.5)
C: for systems containing Em ratio of C (1:0.25)

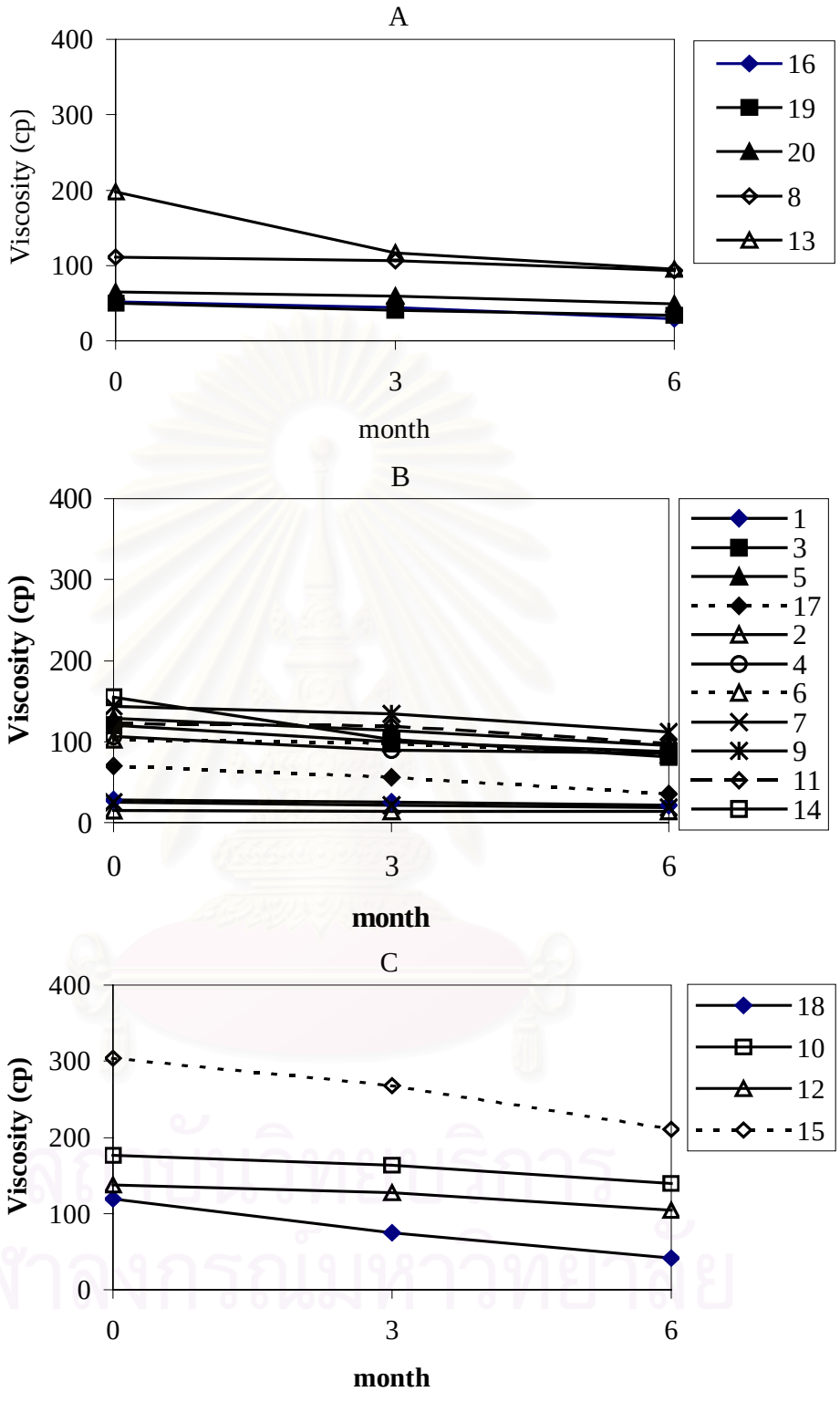


Figure 39 Viscosity profiles of investigated microemulsions at initial and 50 °C in 3 and 6 months

Key: A: for systems containing Em ratio of A (1:1)
B: for systems containing Em ratio of B (1:0.5)
C: for systems containing Em ratio of C (1:0.25)

significantly decreased. While the systems containing Em ratio of B were quite stable after storage at all investigated temperatures.

Droplet size of the investigated microemulsions after storage for six months at temperature of 4°, 30°, 50°C was also examined and the images could be seen in Figures 49-70 in Appendix C. Some systems showed aggregated droplets. As could be seen from the result in table 8, the droplet sizes of most systems were still under 100 nm, except ME7, ME13, ME14.

From the results, microemulsions containing glycerol as cosurfactant were unstable systems. Overall results indicated that most microemulsion systems which contained suitable surfactant, cosurfactant, Em ratio and Em/oil ratio were thermodynamically stable for long periods.

In Vitro Release Study

To study the possibility of using such systems as prolonged release vehicles, buserelin acetate was selected as a model peptide. Buserelin acetate is very hydrophilic and fairly stable in aqueous solution over a wide range of pH and temperature (Powell et al., 1991). Representative formulations from both TW and PC-based systems were selected to evaluate the *in vitro* drug release. Nine formulations from TW and four formulations from PC-based systems that contain high amount of water phase (9-13%), were selected. Their types and viscosity are respectively shown in Table 9-10.

These selected formulations were transparent, fluid, and nonbirefringent. So they were classified as microemulsion. The formulation ME26-ME34 (TW-based systems) could be stained at external phase and have high conductivity. Thus, they were classified as oil-in-water type. In contrast, the ME35-ME38 formulation (PC-based systems) could be diluted with oil or stained at internal phase with conductivity less than 1 μmhos/cm. These qualities could be identified the condition of water-in-oil microemulsions.

From preformulation, buserelin acetate were dissolved in distilled water more than 50 mg/ml. The drug was completely dissolved in water phase of the selected formulations. Table 10 showed the viscosity of the selected

Table 8 Droplet size of the investigated microemulsions at initial and after storage for 6 month at temperature of 4 ,30 ,50 °C

ME	Droplet Size* (nm)			
	0 month	6 months		
	Initial °C	4 °C	30 °C	50 °C
1	77.610(86)	-	18.185(166)	-
2	14.893(54)	-	14.248(173)	16.6441(25)
3	-	-	12.555(15)	-
4	41.2411(70)	-	39.480(211)	20.732(47)
5	34.624(69)	34.243(255)	-	-
6	26.437(66)	-	17.636(172)	-
7	130.589(20)	84.400(160)	13.255(140)	125.828(270)
8	-	15.940(249)	-	23.125(47)
9	35.171(183)	60.912(11)	29.199(42)	29.405(62)
10	38.813(54)	16.351(11)	13.255(42)	53.438(33)
11	43.460(163)	30.747(52)	24.821(132)	10.159(159)
12	-	31.738(110)	-	-
13	120.070(57)	-	-	-
14	178.194(20)	20.029(43)	9.1668(126)	18.336(33)
15	34.016(204)	59.360(47)	73.118(18)	61.903(14)
16	42.999(221)	82.515(39)	-	-
17	33.141(127)	29.899(27)	-	-
18	-	-	-	-
19	47.102(72)	-	-	-
20	46.390(90)	-	-	-

* = Median diameter (sample size, n)

- = Unmeasured

Table 9 Type of microemulsions ME26-ME38

ME No	Stain of dye	Dilution test		Conductivity (μmhos/cm)	Polarized light	Type
	water soluble dye (amaranth)	oil dilution (IPM)	water dilution (water)			
26	EP	S	S	1.91	NB	o/w
27	EP	S	S	2.63	NB	o/w
28	EP	S	S	6.25	NB	o/w
29	EP	S	S	2.41	NB	o/w
30	EP	S	S	2.05	NB	o/w
31	EP	S	S	3.98	NB	o/w
32	EP	S	S	4.27	NB	o/w
33	EP	S	S	3.21	NB	o/w
34	EP	S	S	2.84	NB	o/w
35	IP	NS	S	0.67	NB	w/o
36	IP	NS	S	0.84	NB	w/o
37	IP	NS	S	0.56	NB	w/o
38	IP	NS	S	0.73	NB	w/o

IP = Internal phase, EP = External phase

S = Separation, NS = No separation

NB = Non birefringence

Table 10 Viscosity of the investigated microemulsions before and after BSA loading

No	Viscosity	
	ME	ME BSA
26	148.19	++
27	156.2	++
28	115.1	+
29	180.89	++
30	160.07	++
31	193.97	++
32	167.32	+
33	212.39	++
34	209.88	++
35	78.26	-
36	70.2	-
37	93.47	-
38	97.69	-

Key : - unchanged
+ increased
++ more increased

microemulsions before and after drug loading. The results showed that all of the TW-based systems yielded higher viscosity than the PC-based systems did. Moreover, after buserelin was loaded, the viscosity of TW-based systems significantly increased, but no change be observed in the PC-based systems.

Quantitation of buserelin acetate was determined by comparing peak area ratio with standard curve, prepared daily. The standard curve was linear with the coefficient of determination (r^2) of 0.9999 for concentration from 1 mcg/ml to 20 μg/ml. The within run and between run precision of the standard curves, expressed as the coefficient of variation (%CV), were found to be equal to or lower than 2.02%. The standard curves of the within run and between run precision were shown seen in Appendix D.

Information concerning drug partition in microemulsion is very useful to design optimal microemulsion for controlled drug delivery. However, none of direct method for characterizing partition behaviour of drugs in microemulsion was reported. Thus the partitioning of buserelin acetate in octanol-water system was evaluated and shown in Table 11. The partition coefficient (Co/Cw) of buserelin acetate in the experiment was 0.01.

The study of drug release from a drug-loaded microemulsion was performed by using modified Franz cell apparatus as it was reported to be the most reliable methodology (Grassi et al., 2000). Table 12-13 and Figure 40-42 showed the cumulative amount of buserelin acetate released from TW-based and PC-based microemulsions compared to the drug in buffer solution as a function of time. *In vitro* release of buserelin acetate from different microemulsions were slow and incomplete as compared to the drug release from buffer solution. Most of TW-based systems prolonged the release of drug during 1-6 days of the experiment. The drug released from PC-based systems was much slow and quite low with less than 10%, while the amount of drug release from buffer solution was about 80% in two days of experiment.

The release of drug from microemulsions is governed by two main processes: drug's transafer from the disperse phase to the continuous phase and drug diffusion through the membrane from the continuous phase to the

Table 11 Partition coefficient of buserelin acetate between octanol and distilled water at 30±1°C.

Time (hr)	No	Conc (mcg/ml)	Mean	SD	Cw	Co	Co/Cw
BSA	1	44.0325	45.65974	1.563673	45.6597		
	2	45.7957					
	3	47.1510					
24	1	44.0264	45.21447	1.305374	45.2145	0.4453	0.0098
	2	45.0051					
	3	46.6119					
30	1	45.9036	45.1988	0.815587	45.1988	0.4609	0.0102
	2	45.3874					
	3	44.3054					
48	1	45.2373	45.09164	0.570364	45.0916	0.5681	0.0126
	2	44.4626					
	3	45.5750					

Table 12 In vitro release of buserelin acetate from TW-based microemulsions and buffer solution

	% Drug Release									
Time (day)	PB	26BSA	27BSA	28BSA	29BSA	30BSA	31BSA	32BSA	33BSA	34BSA
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.08	10.26	0.54	1.31	1.09	0.56	0.69	1.31	0.60	0.77	0.79
0.25	28.48	1.49	2.83	4.94	1.48	1.81	3.35	2.41	1.90	2.32
0.42	42.04	3.47	4.83	11.42	2.66	4.29	5.84	5.09	3.31	7.00
1.00	69.62	9.04	7.49	25.81	5.48	8.95	13.06	8.82	11.30	11.46
2.00	82.51	18.32	18.36	38.21	11.90	14.69	21.13	18.16	23.00	18.21
6.00	79.24	27.88	28.34	50.26	15.11	18.68	27.91	32.57	34.84	24.62
10.06	79.58	29.69	31.53	51.10	14.40	18.87	28.67	42.54	35.28	33.97
14.00	80.29	29.89	32.38	52.44	14.46	19.54	28.86	44.47	35.57	31.67
18.00	80.66	30.63	33.09	52.24	14.53	19.47	29.34	46.73	36.03	31.71
21.00	80.77	30.55	32.74	52.13	14.60	19.38	29.08	47.60	36.15	31.85

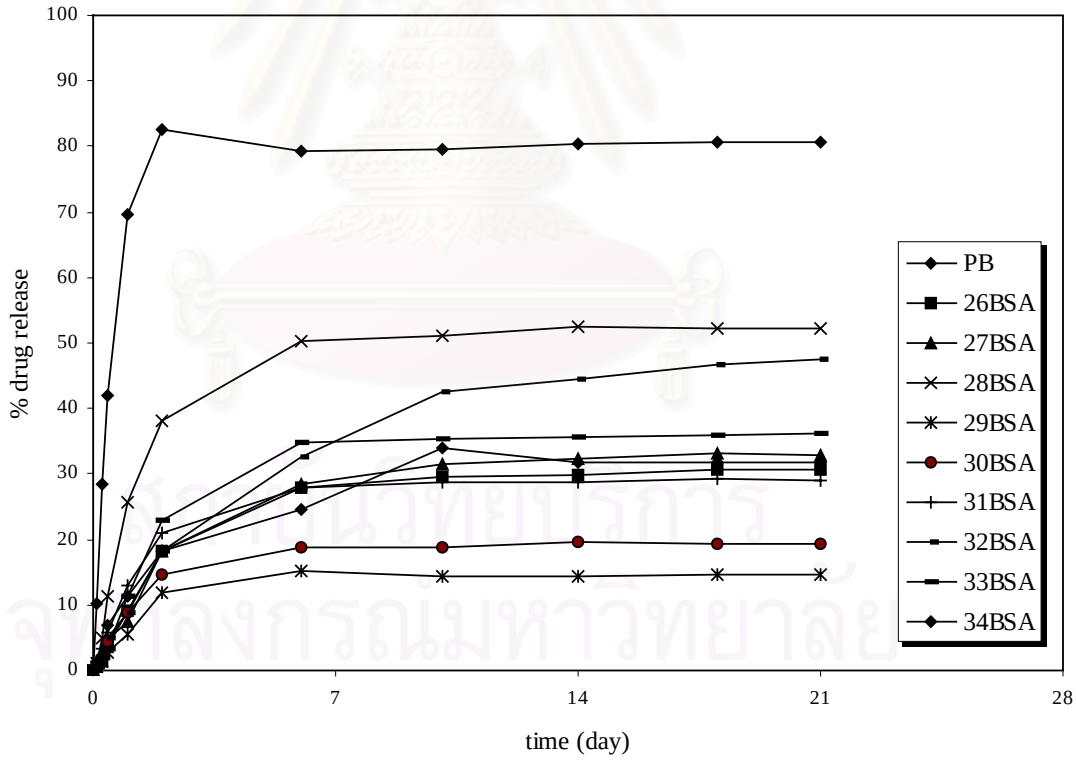


Figure 40 In vitro release of buserelin acetate from various TW-based microemulsions and buffer solution

Table 13 In vitro release of buserelin acetate from PC-based microemulsions and buffer solution

	% Drug Release				
Time (day)	PB	35BSA	36BSA	37BSA	38BSA
0.00	0.00	0.00	0.00	0.00	0.00
0.08	10.26	0.03	0.07	0.01	0.03
0.25	28.48	0.42	0.23	0.11	0.12
0.42	42.04	0.71	0.54	0.45	0.36
1.00	69.62	1.27	0.84	0.80	0.62
2.00	82.51	1.68	1.23	1.12	1.09
6.00	79.24	2.59	2.15	1.95	1.80
10.06	79.58	3.24	4.22	3.03	3.01
14.00	80.29	4.27	8.34	3.44	4.63
18.00	80.66	4.85	10.07	3.55	5.43
21.00	80.77	5.11	10.29	3.63	5.47

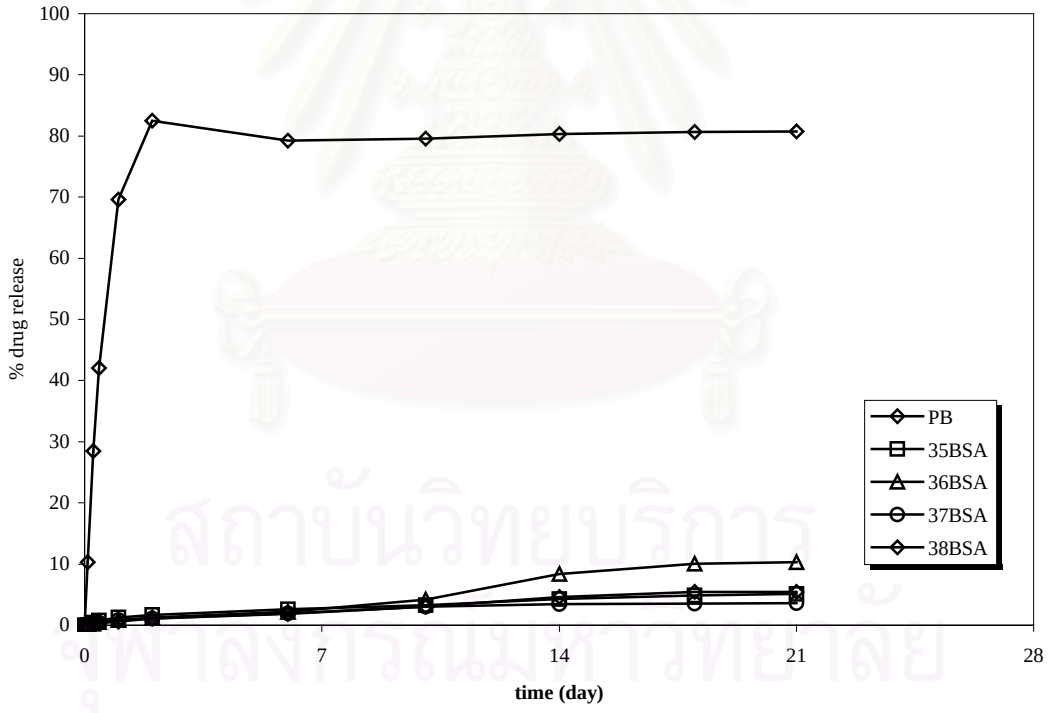


Figure 41 In vitro release of buserelin acetate from various PC-based microemulsions and buffer solution

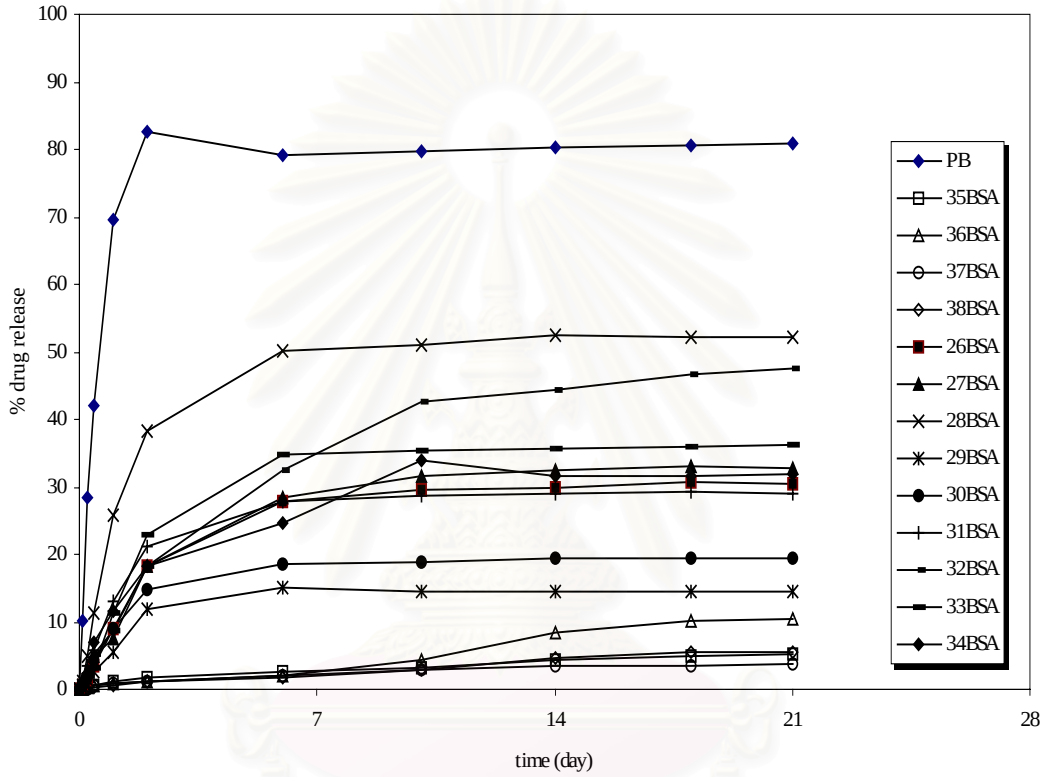


Figure 42 In vitro release of buserelin acetate from TW-base, PC-based microemulsions and buffer solution

sink solution (Trotta, Gasco, and Morel, 1989). It can be explained by the fact that at the beginning of the experiment the donor compartment contained the drug-loaded microemulsion while the receiver was filled by a drug-free aqueous medium. Since the drug-loaded microemulsion was prepared 24 hours before the beginning of the experiment, it can reasonably assume that the initial drug concentration ratio between the microemulsion aqueous and oil phase is that determined by the drug partition equilibrium. At equilibrium, the drug can be distributed among three different phases: the disperse phase, the continuous phase, and the surfactant layer. Buserelin acetate has low value of partition coefficient (Co/Cw = 0.01). It can assume that the drug was likely to stay in water phase, thus buserelin acetate should have high concentration in aqueous phase.

For TW-based systems, oil-in-water type, at the beginning a drug concentration gradient existed between the aqueous of external phase of the donor and the receiver so that the drug molecules leave the microemulsion aqueous phase to reach the receiver fluid crossing the interposed membrane which resulted in the drug concentration increase in the receiver compartment. It should be noted that during the release experiment, the milky emulsion was seen in the donor compartment of TW-based systems. The result was due to the receiver solution penetrated through the donor compartment and then microemulsion was destroyed into an emulsion.

From the release profiles of TW-based systems in Figure 40, the highest percentage of release about 52.13% was obtained from ME28. The drug release was faster than other TW-based formulations. ME28 had high percentage of internal phase (13%) and low amount of Em ratio (1/1) which resulted in comparative low viscosity of the system (115.1cp). It can assume that drug molecule could easily diffuse from continuous phase through the membrane to sink solution. Thus faster and higher drug release was obtained. Comparison to ME28, ME32 also had high percentage of drug release about 47.60% but the release was much slower. It had higher Em ratio of 1/0.25 resulted in higher viscosity (167.32cp) of the system. Drug molecule could not easily diffuse though surfactant layer and viscous medium. In contrast, the lower percentage of drug release was from ME29-ME30. This result could be explained that ME29 had

high Em ratio (1/0.25) and high viscosity of 180.89 cp. Drug might be trapped in surfactant layer. Thus the drug release was only 14.60%. ME30 had lower Em ratio than ME29. It showed slightly higher percentage drug release of 19.38%. For systems of ME 26,27,31,33, and 34, the release of drug obtained were among 29.08-36.15% and the viscosity were 148.19-209.88 cp. It can be seen from the results that various types and ratios of ingredients had affected characteristic of the systems which made the systems difference in viscosity and then on the drug release pattern.

On the other hand, because the PC-based systems (Figure 41) were water-in-oil type, the drug in disperse phase was slowly partitioned through the oil of external phase and then to the sink solution. Unexpectedly, all of the selected PC-base systems formed gel on the membrane resulting in obstruction of the drug through the membrane. Therefore the drug release profile from PC-based systems obtained was quite low. This result may be explained that water diffuse into the phospholipid film then altered the curvature of droplet resulted in rigid bilayer forming and then system became gel-like structure. After several days of experiment, the amount of drug release was slightly increased due to the receiver solution could pass through gel layer resulting in altering the drug partition equilibrium. Then the release profile were obtained. All PC-based systems exhibit similar pattern of low percentage of drug release in during first ten days. Then slightly different was observed, the 36BSA yielded more percentage of drug release. Due to the low percentage of surfactant, degree of gel forming would be lower than system of high amount of surfactant.

In addition, after drug loading, most TW-based systems had significant higher viscosity than unloaded systems. As known, microemulsions system are easily affected by surrounding molecules. In this case, buserelin incorporation had much effect on TW-based system. For PC-based system, the observed viscosity after buserelin acetate loading was unchanged.

When comparing with buffer solution systems, the amount of drug release was about 80% in two days of experiment. The uncontrollable release of drug from solution was expected since no ingredients were existed to prolong its diffusion. Drug in the donor compartment easily diffused through membrane from

the concentration gradient which existed between the donor and the receiver. From the sampling volume of 10 ml, the receiver was maintained sink condition resulted in faster release of the drug profile. The release profile was about 80% might be because buserelin acetate adsorbs glass diffusion cell or drug crystallization might be occurred.

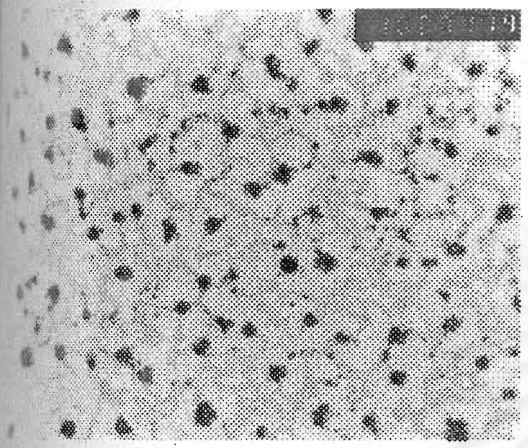
It can be seen from the results that various types and ratios of ingredients had affected characteristics of the systems which made the systems different in viscosity and then on the drug release pattern.

In Vivo Study

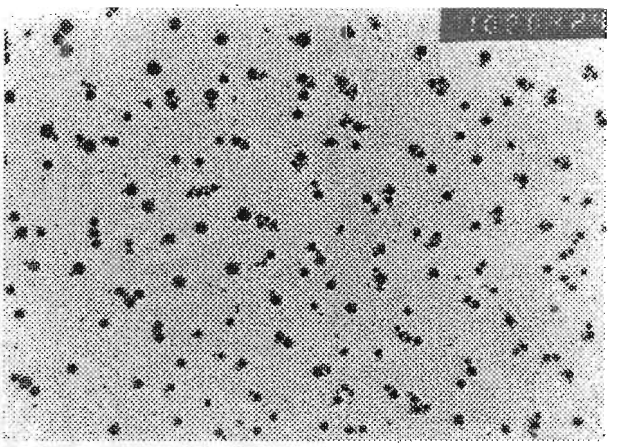
To ascertain whether microemulsions acts as a prolonged release drug carrier *in vivo*, two systems of TW-based and PC-based microemulsions were considered and compared as bases for drug delivery systems. The *in vitro* drug released study could not completely presented the true release from the carriers. Two formulations from TW-based system, 28BSA and 32BSA, were chosen as they had low viscosity and high percentage of the drug released. And two of PC-based system, 35BSA and 36BSA, were selected as they were prepared with low amount of surfactants (Em/oil=5/5). Their viscosity is shown in Table 10. TEM photomicrographs were presented in Figure 43. The results showed that viscosity of TW-based systems were higher than PC-based system.

In the experiment, the mean of normal testosterone value in rabbits was 2.57 ng/ml (N = 20). Silvan, et al 1990 studied variation in testosterone value in male rabbits and reported physiological value of testosterone plasmatic level is between 0.3-10 ng/ml. Schanbacher and Ewing (1975) reported that the testosterone value in adult male rabbits were 1.16±0.26 ng/ml.

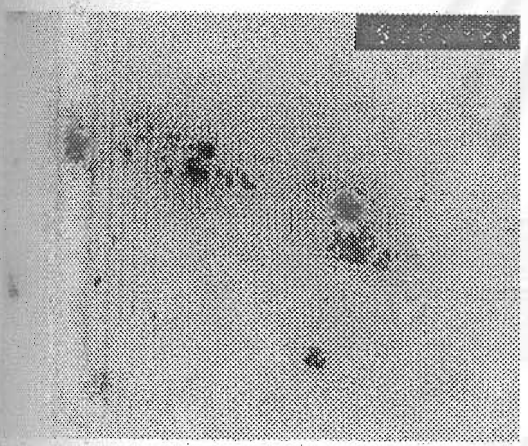
The selected buserelin acetate microemulsions were subcutaneously administered into rabbits at a dose of 3.3 mg/ml. The plot of mean serum testosterone after subcutaneous administration of BSA microemulsions is shown in the following profiles (Figures 44-48). Figure 44 showed mean serum testosterone after subcutaneous of control formulation (3.3 mg/ml BSA in buffer pH 7.4). Figure 45-46 showed mean serum testosterone after subcutaneous in



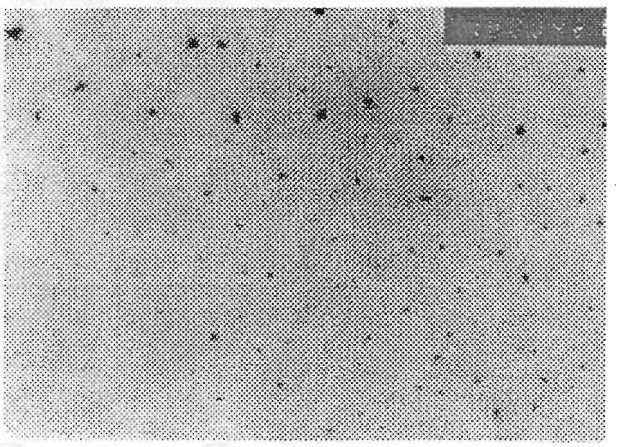
A



B



C



D

Figure 43 TEM micrographs of microemulsion systems containing

Key: A: ME28, IPM-TW-PEG, Em=A, Em/oil = 7/3, Aq 13%, x 16,500

B: ME32, EO-TW-PG, Em=C, Em/oil = 7/3, Aq 9%, x 16,500

C: ME35, IPM-PC-PG, Em=A, Em/oil = 5/5, Aq 9% x 45,000

D: ME35BSA, IPM-PC-PG, Em=A, Em/oil = 5/5, Aq 9% x 16,500

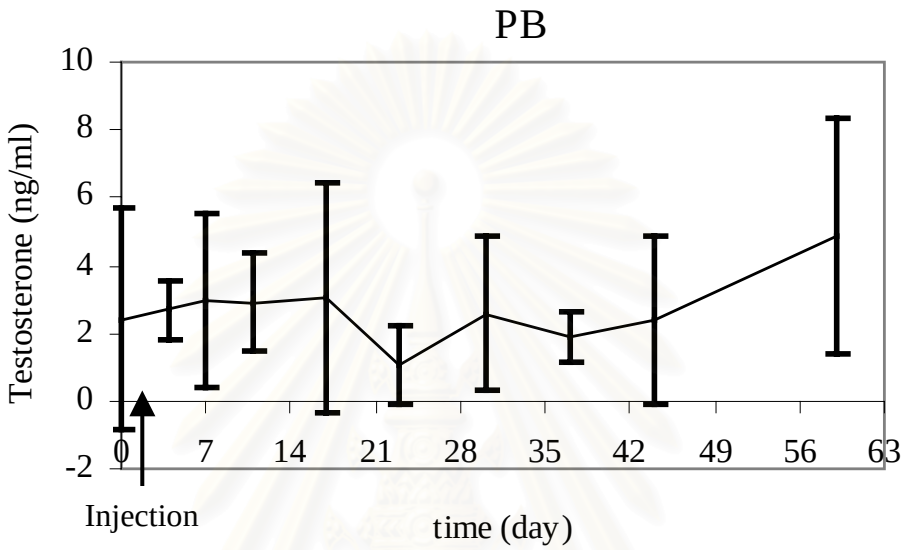
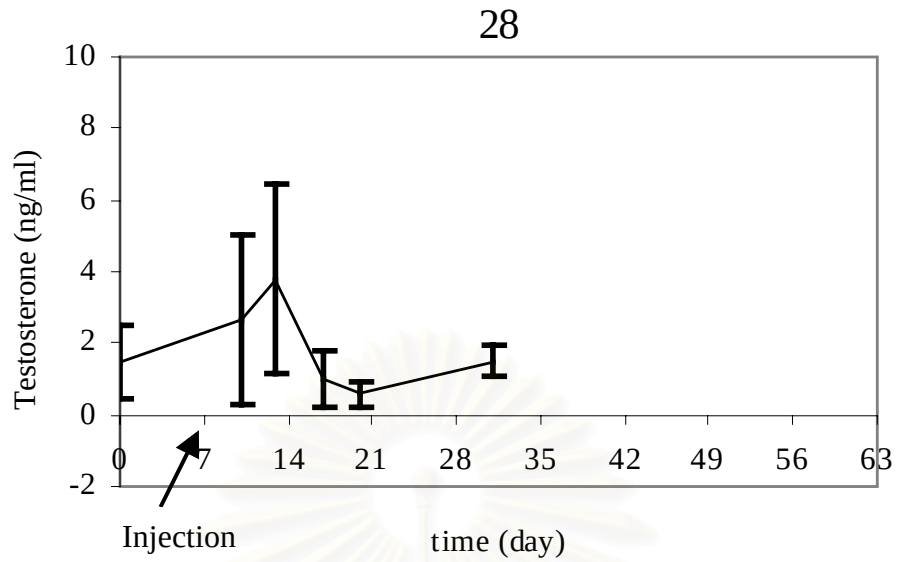
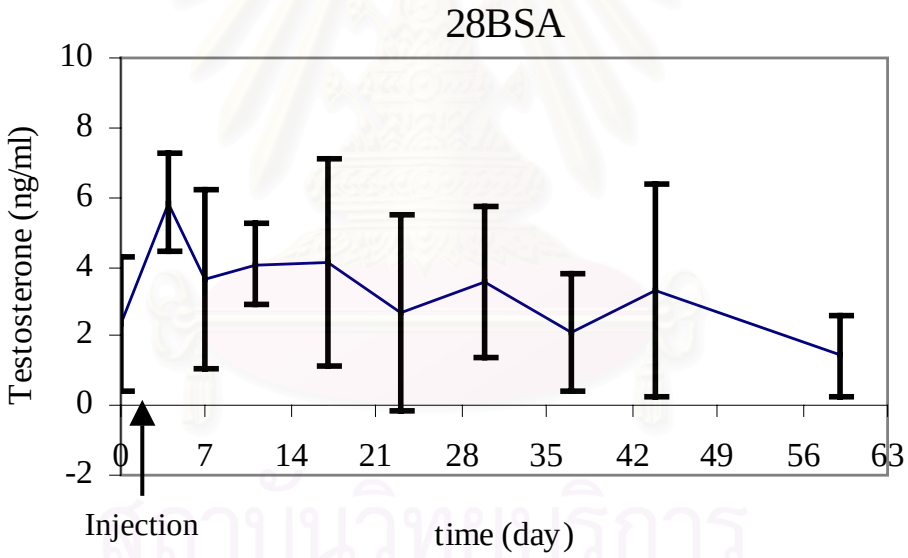


Figure 44 Plot of mean serum testosterone in rabbits after subcutaneous injection of 3.3 mg buserelin acetate buffer solution (injection on day 1)

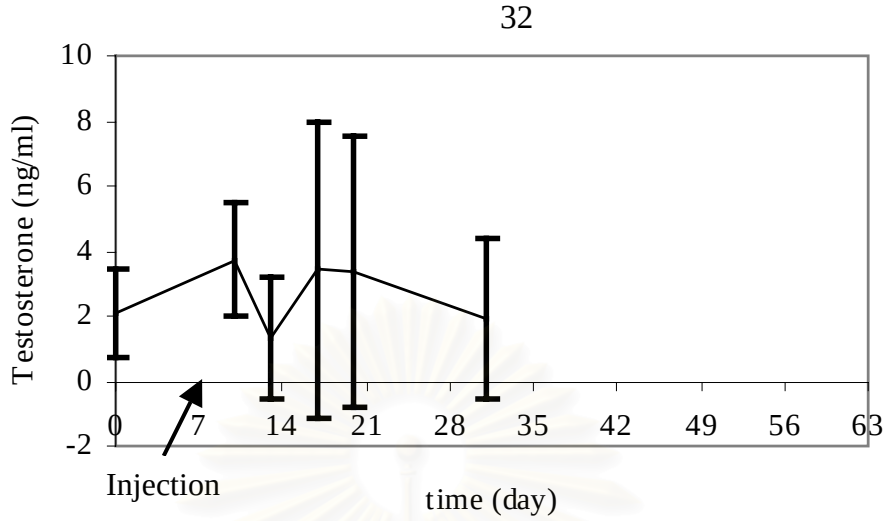


A

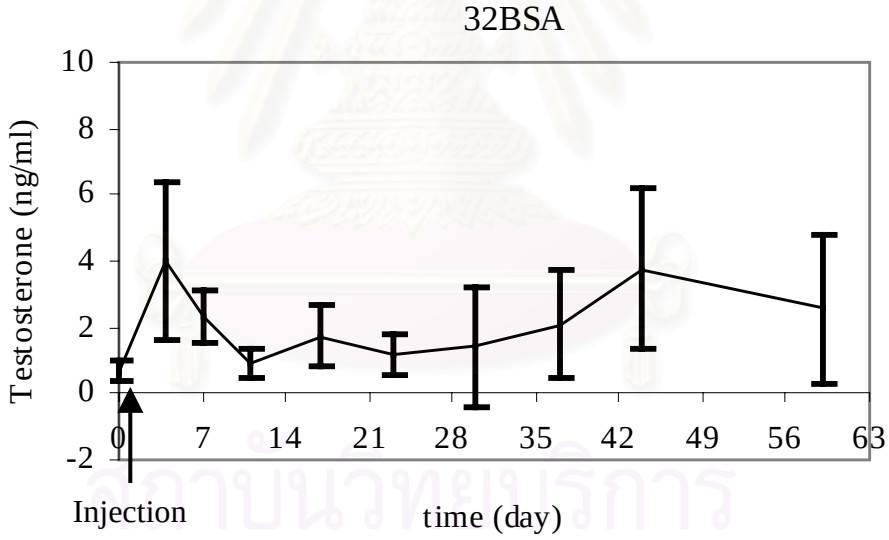


B

Figure 45 Plot of mean serum testosterone in rabbits after subcutaneous injection of blank ME28 (A) and 3.3 mg ME28BSA (B)
Key A: IPM-TW-PEG, Em=A, Em/oil 7/3, Aq 13% (injection on day 7)
B: IPM-TW-PEG, Em=A, Em/oil 7/3, Aq 13% with 3.3 mg BSA, (injection on day 1)

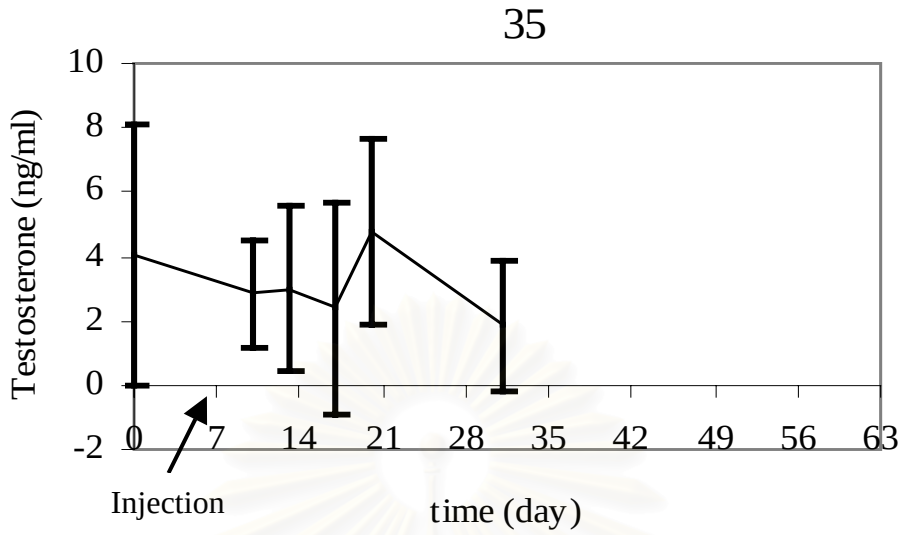


A

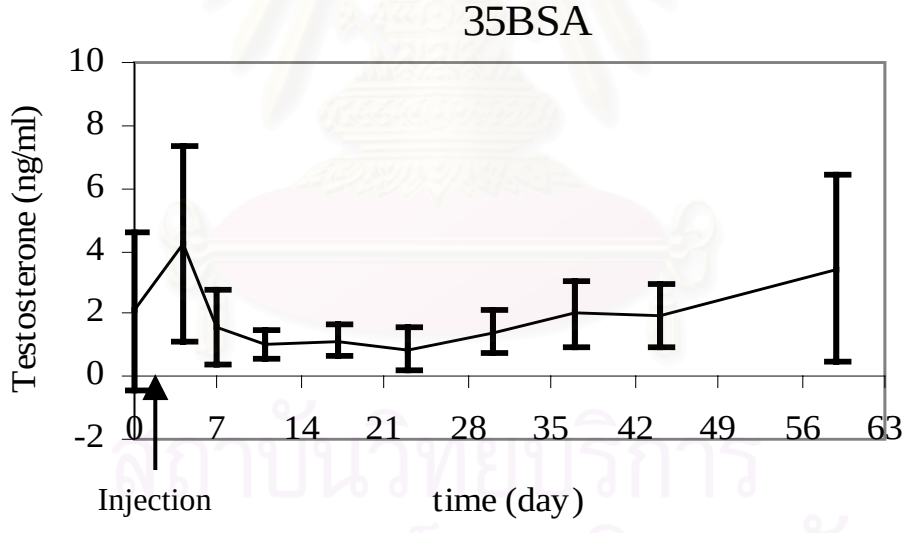


B

Figure 46 Plot of mean serum testosterone in rabbits after subcutaneous injection of blank ME32 (A) and 3.3 mg ME32BSA (B)
Key A: EO-TW-PG,Em=C, Em/Oil 7/3, Aq 9% (injection on day 7)
B: EO-TW-PG,Em=C, Em/Oil 7/3, Aq 9% with 3.3 mg BS (injection on day 1)

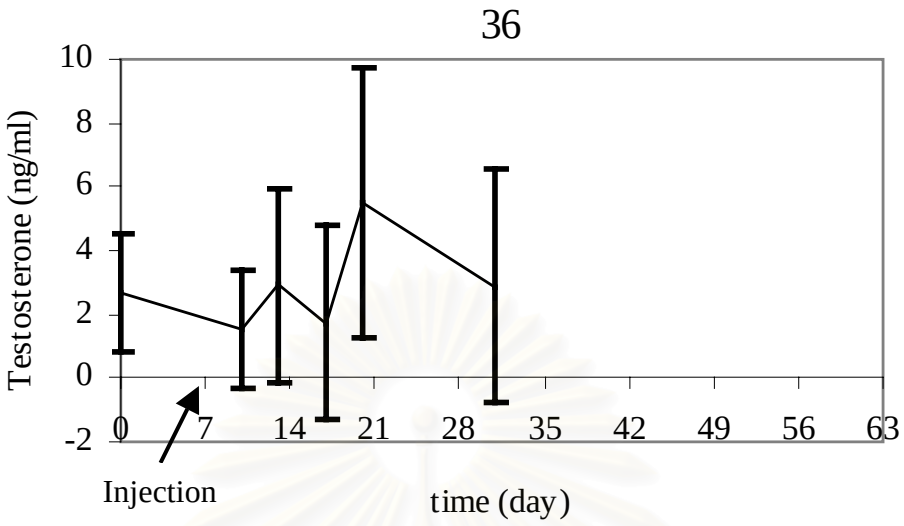


A

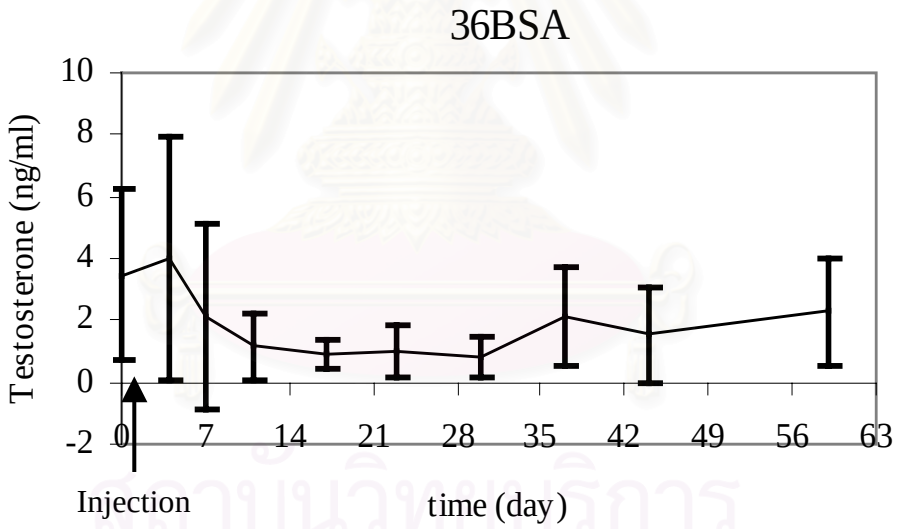


B

Figure 47 Plot of mean serum testosterone in rabbits after subcutaneous injection of blank ME35 (A) and 3.3 mg ME35BSA (B)
Key A: IPM-PC-PG, Em=A, Em/oil 5/5, Aq 9% (injection on day 7)
B: IPM-PC-PG, Em=A, Em/oil 5/5, Aq 9% with 3.3 mg BSA (injection on day 1)



A



B

Figure 48 Plot of mean serum testosterone in rabbits after subcutaneous injection of blank ME36 (A) and 3.3 mg ME36BSA (B)

Key A: EO-PC-PG,Em=A, Em/Oil 5/5, Aq 9% (injection day 7)

B: EO-PC-PG,Em=A, Em/Oil 5/5, Aq 9% with 3.3 mg BSA (injection on day 1)

TW-based microemulsions. There are two parts: 28 and 32 blank formulation (A) and 28 and 32 BSA formulation (B). In Figure 47-48, the plot showed the use of PC-based microemulsions in two formulations: 35 and 36 BSA.

As the mechanism of action of LHRH analogues is acutely increasing gonadotropin secretion. Then, caused GnRH receptor to down-regulate and suppress pituitary sensitivity, resulted in testosterone suppression (Filicori and Flamigni, 1988). From the results, the plot of mean serum testosterone after subcutaneous administration of drug loading microemulsion could not clearly conclude the effect of buserelin acetate due to the small sample size (n=4) which resulted in wide range of variation. However, the variation from PC-based microemulsions was less than TW-based systems and control formulation. In further studies, it might be possible to develop water-in-oil microemulsion (PC-based microemulsion) as alternative long-acting delivery system for parenteral administration of peptide drugs.

CHAPTER V

CONCLUSIONS

The study showed that microemulsions could be prepared using commercially available and pharmaceutically acceptable excipients. In further studies, it might be possible to develop water-in-oil microemulsion (PC-based system) as alternative long-acting delivery system for parenteral administration of peptide drugs. Overall results were as follows:

1. Types and ratios of surfactant, cosurfactant, and oil used had a pronounced effect on the existing region of microemulsions. For PC systems, microemulsion regions could be produced only from the systems containing butanol, propylene glycol, and caproic acid. Most of TW systems yielded microemulsion regions. Systems with butanol produced large area. Oils of larger molecule size evidently resulted in smaller microemulsion regions. In addition, microemulsion region was mostly increased when increasing the Em /oil ratio.

2. Surfactant types had major effected on the formation of a particular structure. Dye solubility, dilution, and conductivity test were simultaneously employed to determine their types. PC-based microemulsions were of water-in-oil type while TW-based microemulsions with high percentage of the internal phase were of oil-in-water type.

3. All ingredients used also have effected on viscosity and appearance. Increasing the amount of surfactant would increase the viscosity of the system.

4. Microemulsion systems were stable except when glycerol was used as cosurfactant.

5. *In vitro* release of buserelin acetate from microemulsions was retarded. Most TW-based systems prolonged the release of drug during 1-6 days of the experiment. The drug released from PC-based systems was much slower and quite low with less than 10%. While the amount of drug release from buffer solution was about 80% in two days. However, in vitro drug released study could not completely presented the true release of drug from the carrier.

6 *In vivo* study in rabbits, subcutaneous injection of 3.3 mg/ml buserelin acetate microemulsion would not clearly conclude the effects of drug on testosterone level over 30 days.

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APPENDICES

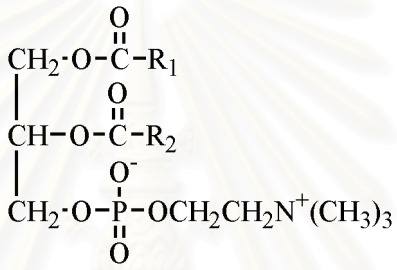
APPENDIX A

Physicochemical properties of substances

1. Lecithin (Kibbe, 2000)

1.1 Name/Compositions

Chemical structure



α-Phosphatidylcholine

where, R1 and R2 are fatty acid which may be different or identical

Scientific names

(3-sn-Phosphatidyl)-choline, soya

1,2-Diacyl-sn-glycero-3-phosphocholine (IUPAC)

Cholinephosphoric acid diglyceride ester

Mean empirical formula: $C_{44}H_{75}O_8PN$

Mean molecular weight: 775 g/mol

Compositions

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist mainly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acid and carbohydrates.

Phospholipon 90/90G compose of

Phosphatidylcholine	93±3 %
Lysophosphatidylcholine	3±3 %

D,l-_-Tocopherol	min.	0.1 %

Typical fatty acid composition:

Palmitic acid	12±2 %
Stearic acid	3±1 %
Oleic acid	10±3 %
Linoleic acid	66±5 %
Linolenic acid	5±2 %

1.2 General properties

Appearance

Lecithin is brown to light yellow, depending on whether it is unbleached or bleached. It has practically no odor and a bland to nut-like taste, similar to soybean oil. In consistency, it may vary from plastic to fluid depending on the free fatty acid content.

Solubility

Lecithin is soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbon, mineral oil and fatty acids such as ethanol, propylene glycol, toluene, hexane, ether, chloroform, petroleum ether. It is practically insoluble in cold vegetable and animal oils, polar solvents and water. However they can disperse in water.

Energy provide	: 9 Cal/g
HLB	: approximately 7
Isoelectric point	: approximately 3.5
pH-value	: 5.5-7.5 (approximately 6.6)

1.3 Safety

Lecithin is a component of cell membranes and is there for consumed as a normal part of diet. Although excessive consumption may be harmful, oral does of up 80 g per day have been used therapeutically in the treatment of tardive dyskinesia. It has been accepted as an additive in parenteral preparations.

1.4 Applications

Pharmaceuticals – parenteral administration:

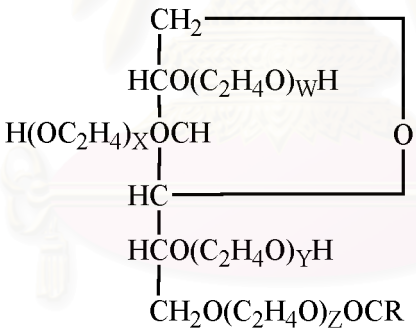
Liposome of Phospholipon 90 prevent damage to the membranes in the liver, produced by drugs or hepatotoxic substance. Hence, these liposomes are particularly suitable carrier systems for drugs.

For years mixed micelles of Phospholipon 90 have been used therapeutically(Essentiale,Lipostabil). This system makes it possible to prepare clear solutions of water-insoluble drugs such as benzodiazepines or vitamins for parenteral administration.

2. Tween 80 (Wade and Weller, 1994)

2.1 Name/Compositions

Chemical structure



W+X+Y+Z = 20

R=Oleic acid

Chemical name

Polyoxyethylene 20 sorbitan monooleate

Molecular formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310 g/mole

2.2 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, ethyl acetate, and methyl alcohol; practically insoluble in liquid paraffin and fixed oils.

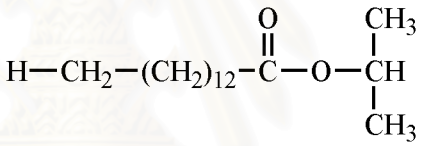
2.3 Safety

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. Isopropyl Myristate (Kibbe, 2000)

3.1 Name/Compositions

Chemical structural



Nonproprietary names;

NF: Isopropyl myristate; BP: Isopropyl myristate;

PhEur: Isopropylis myristas; USP: Isopropyl myristate

Synonyms: Isopropyl ester of myristic acid

Chemical Names and CAS Registry Number

Tetradecanoic acid, 1-methylethyl ester [110-27-0]

Molecular Formular: $C_{17}H_{34}O_2$

Molecular Weight: 270.45

3.2 General properties

Appearance

Isopropyl myristate is a clear, colorless, practically odorless liquid of low viscosity which congeals at about 3 C. It consists of esters of propan-2-ol and saturated high molecular weight fatty acids, principally myristic acid.

Solubility

Soluble in acetone, chloroform, ethanol, ethyl acetate, fats, fatty alcohols, fixed oils, liquid hydrocarbons, toluene, and waxes. Dissolves many waxes, cholesterol, or lanolin. Practically insoluble in glycerin, glycols, and water.

Boiling point: ~140.2 C at 266 Pa (2 mmHg)

Freezing point: = ~3 C

Viscosity (dynamic): 5-7 mPa s (5-7 cP) at ~25 C

Stability and Storage Conditions

Isopropyl myristate is resistant to oxidation and hydrolysis and does not become rancid. It should be stored in a well-closed container in a cool, dry, place and protected from light.

3.3 Safety

Isopropyl myristate is widely used in cosmetics and topical pharmaceutical formulations and is generally regarded as a nontoxic and nonirritant material.

LD_{50} (mouse, oral): 49.7 g/kg

LD_{50} (mouse, SC): 50.2 g/kg

LD_{50} (rat, IP): 79.5 g/kg

3.4 Applications

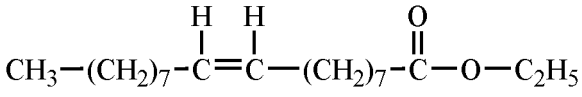
Pharmaceutical Formulation or Technology

Isopropyl myristate is used as a penetration enhancer for transdermal formulations and has been used in conjunction with therapeutic ultrasound and iontophoresis.[2] It has been used in a water-oil-gel prolonged-release emulsion in which isopropyl myristate is the major ingredient of the oil phase.

4. Ethyl Oleate (Kibbe, 2000)

4.1 Name/Compositions

Structural Formula



Nonproprietary Names

NF: Ethyl oleate; BP: Ethyl oleate

PhEur: Ethylis oleas; USP: Ethyl oleate

Synonyms

Ethyl 9-octadecenoate; *Kessco EO*; oleic acid, ethyl ester.

Chemical Name and CAS Registry Number

(Z)-9-Octadecenoic acid, ethyl ester [111-62-6]

Chemical formula: $C_{20}H_{38}O_2$

Molecular Weight: 310.52

4.2 General properties

Appearance

Ethyl oleate occurs as a pale yellow to almost colorless, mobile, oily liquid with a taste resembling that of olive oil and a slight, but not rancid odor.

It consists of the ethyl esters of oleic acid and related high molecular weight fatty acids. A suitable antioxidant may be included.

Boiling point: 205-208 C (some decomposition)

Freezing point: ≈ -32°C

Moisture content: at 20 C and 52% relative humidity, the equilibrium moisture content of ethyl oleate is 0.08%. See also Fig.1.

Solubility

miscible with chloroform, ethanol (95%), ether, fixed oils, liquid paraffin, and most other organic solvents: practically insoluble in water.

Surface tension: 32.3 mN/m (32.3 dynes/cm) at 25 C[3]

Viscosity (dynamic): 3.9 mPa s (3.9 cP) at 25 C[3]

Stability and Storage Conditions

Ethyl oleate should be stored in a cool place in a small, well-filled, air-tight container, protected from light. When a partially filled container is used, the air should be replaced by Nitrogen or another inert gas. Ethyl oleate oxidizes on exposure to air, resulting in an increase in the peroxide value. It remains clear at 5 C, but discolors on standing. Anti-oxidants are frequently used to extend the shelf life of ethyl oleate. Protection from oxidation for over two years has been achieved by storing it in amber glass bottles and the addition of a double or triple mixture of

propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene and citric or ascorbic acid. A concentration of 0.03% w/v of a mixture of propyl gallate (37.5%), butylated hydroxytoluene (37.5%) and butylated hydroanisole (25%) was found to be the best anti-oxidant for ethyl oleate. Ethyl oleate may be sterilized by heating at 150 C for 1 hour.

4.3 Safety

No toxicity data is available in the literature. Generally, Ethyl oleate is considered being a low toxicity, but ingestion should be avoided. Ethyl oleate has been found to cause minimal tissue irritation. No reports of intramuscular irritation during use have been recorded.

Regulatory Status

Included in the FDA Inactive Ingredients Guide (transdermal preparation). Included in parenteral medicines licensed in the UK.

4.4 Applications

Pharmaceutical Formulation or Technology

Ethyl oleate is primarily used as a vehicle in certain parenteral preparations intended for intramuscular administration. It has also been used as a solvent for drugs formulated as biodegradable capsules for subdermal implantation and in the preparation of microemulsions containing cyclosporine.

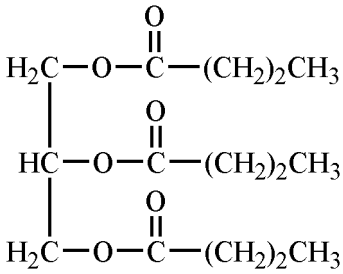
Ethyl oleate is a suitable solvent steroids and other lipophilic drugs. Its properties are similar to those of almond oil and peanut oil. However, it has the advantage that it is less viscous than fixed oils and is more rapidly absorbed by body tissues.

Ethyl oleate has also been evaluated as a vehicle for subcutaneous injection.

5. Medium Chain Triglycerides (Louie and Niemiec, 1986; Wade and Weller, 1994)

5.1 Name/Compositions

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 endosperm of Cocos nucifera L. by hydrolysis, fractionation of the fatty acids were obtained by hydrolysis and then re-esterification to triglycerides. It consists of a mixture of exclusively short or medium chain triglycerides of fatty acid, of which not 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 C_8 and C_{10} saturated fatty acids. Approximate percentages are

Fatty Acid	%
Shorter than C_8	<6
C_8 (caprylic)	67
C_{10} (capric)	23
Longer than C_{10}	<4

5.2 General properties

Appearance

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

Solubility

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 (C_8 and C_{10}): shorter than C_8 (<6%), C_8 or octanoic (67%); C_{10} or decanoic (23%); and larger than C_{10} (<4%).

5.3 Safety

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

6. Soybean oil (Louie and Niemiec, 1986; Wade and Weller, 1994; Kibbe, 2000)

6.1 Name/Compositions

Nonproprietary Names

BP: Soya oil; JP: Soybean oil

PhEur: Sojáe oleum; USP: Soybean oil

Synonyms

Calchem IVO-114; Lipex 107; Lipex 200; soja bean oil; soya bean oil.

Chemical Name and CAS Registry Number

Soybean oil [8001-22-7]

Chemical formula , Molecular Weight

A typical analysis of refined soybean oil indicates the composition of the acids, present as glycerides, to be: linoleic acid 50-57%; linolenicacid 5-10%; oleic acid 17-26%; palmitic acid 9-13, and stearic acid 3-6%. Other acids are present in trace quantities.

6.2 General properties

Description

The USP describes soybean oil as the refined fixed oil obtained from the seeds of the soya plant Glycine soja (Leguminosae). The PhEur describes soybean oil as the refined fixed oil obtained from the seeds of Glycine and Glycine max

(Leguminosae); it may contain a suitable antioxidant. The PhEur also includes a monograph for Soya-bean Oil, Hydrogenated.

Soybean oil is a clear, pale yellow colored, odorless or almost odorless liquid, with a bland taste that solidifies at -10 to -16 C

Density: 0.916-0.922 g/cm^3 at 25 C

Freezing point: -10 to -16 C

Hydroxyl value: 4-8

Interfacial tension: 50mN/m (50 dynes/cm) at 20 C

Refractive index: n_D^{25} = 1.471-1.475

Solubility

Practically insoluble in ethanol (95%) and water; miscible with carbon disulfide, chloroform, ether, and light petroleum.

Surface tension: 25mN/m (25 dynes/cm) at 20 C

Viscosity (dynamic): 172.9 mPa s (172.9 cP) at 0 C

99.7 mPa s (99.7 cP) at 10 C

50.09 mPa s (50.09 cP) at 25 C

28.86 mPa s (28.86 cP) at 40 C

Stability and Storage Conditions

Soybean oil is a stable material if protected from atmospheric oxygen.

The formation of undesirable flavors in soybean oil is accelerated by the presence of 0.01 ppm copper and 0.1 ppm iron which act as catalysts for oxidation; this can be minimized by the addition of chelating agents.

Prolonged storage of soybean oil emulsions, particularly at elevated temperatures, can result in the formation of free fatty acid with a consequent reduction in the pH of the emulsion; degradation is minimized at pH 6-7. However, soybean oil emulsions are stable at room temperature if stored under nitrogen, in a light-resistant glass container. Plastic containers are permeable to oxygen and should not be used for long-term storage since oxidative degradation can occur.

The stability of soybean oil emulsions is considerably influenced by other additives in a formulation.

Soybean oil should be stored in a well-filled, airtight, light-resistant container at a temperature not exceeding 25 C.

6.3 Safety

Soybean oil is widely used intramuscularly as a drug vehicle or as a component of emulsions used in parenteral nutrition regiment; it is also consumed as an edible oil. Generally, soybean oil is regarded as an essentially nontoxic and nonirritant material. However, serious adverse reaction to soybean oil emulsions administered parenterally have been reported. These include cases of hypersensitivity, CNS reactions, and fat embolism. Interference with the anticoagulant effect of warfarin has also been reported.

Anaphylactic reactions have also been reported following the consumption of foods derived from, or containing, soya bean. Recently there has been concern at the concentration of phytoestrogents in some soya-derived products. Administration of soy protein to humans has resulted in significantly decreased serum lipid concentration.

LD_{50} (mouse, IV): 22.1 g/kg; LD_{50} (rat, IV): 16.5 g/kg

Regulatory Status

Included in the FDA Inactive Ingredients Guide (IV injections, oral capsules, and topical preparations). Included in nonparenteral and parenteral medicines licensed in the UK.

6.4 Applications

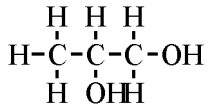
Pharmaceutical Formulation or Technology

In pharmaceutical preparations, soybean oil emulsion are primarily used as a fat source in total parenteral nutrition (TPN) regimens. Although other oils, such as peanut oil, have been used for this purpose, soybean oil is now preferred since it is associated with fewer adverse reactions. Emulsions containing soybean oil have also been used as vehicles for the oral and intravenous administration of drugs; drug substances that have been incorporated into such emulsions include amphotericin, diazepam, retinoids, vitamins, poorly water-soluble steroids, and fluorocarbons. In addition, soybean oil has been includes in formulations of liposomes.

7. Propylene Glycol (Kibbe, 2000)

7.1 Name/Compositions

Structural Formula



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 and CAS Registry Number

1,2-Propanediol [55-57-6]

Chemical Formula: $C_3H_8O_2$

Moleccular Weight: 76.1

7.2 General properties

Density: 1.038 g/cm^3 at 20 C

Osmolarity: a 2.0% v/v aqueous solution is iso-osmotic with serum.

Refractive index: n_D^{20} = 1.4324

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

7.3 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 ethanol, 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 teratogenic or mutagenic. Rats can tolerate a repeated oral daily dose of up to 30 mL/kg in the diet over 6 months, while the dog is unaffected by a repeated oral daily dose of 2 g/kg in the diet for 2 years.

LD_{50} (dog, IV): 25.9 g/kg; LD_{50} (guinea pig, SC): 13-15.5 g/kg

LD_{50} (mouse, IV): 7.6-8.3 g/kg; LD_{50} (mouse, SC): 15.5-19.2 g/kg

LD_{50} (rabbit, IV): 6 g/kg; LD_{50} (rabbit, IM): 5-6.5 g/kg

LD_{50} (rat, IM): 13-20.7 g/kg; LD_{50} (rat, IV): 6.2-12.7 g/kg

LD_{50} (rat, SC): 21.7-29 g/kg

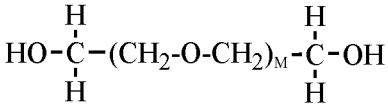
Regulatory Status

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.

8. Polyethylene Glycol 400 (Kibbe, 2000)

8.1 Name/Compositions

Structural Formula



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 and CAS Registry Number

α-Hydro-ω-hydroxy-poly(oxy-1,2-ethanediyl) [25322-68-3]

Chemical Formula: $HOCH_2(CH_2OCH_2)_MCH_2OH$

8.2 General properties

Solubility

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/cm^3 at 25 C for liquid PEGs;

Flash point: 238 C for PEG 400;

Freezing point: 4-8 C for PEG 400;

Refractive index: n_D^{25} = 1.465 for PEG 400;

Moisture content

Liquid polyethylene glycols are very hydroscopic, although hygroscopicity decreases with increasing molecular weight.

8.3 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 quantities of polyethylene glycols can have a laxative effect. 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 may be 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.

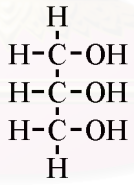
Regulatory Status

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).

9. Glycerine (Louie and Niemiec, 1986; Wade and Weller, 1994; Kibbe, 2000)

9.1 Name/Compositions

Chemical structure



Chemical name

Glycerol, 1,2,3-propanetriol;propane-1,2,3-triol; trihydroxypropane

Molecular formula: $C_3H_8O_3$

Molecular weight: 92.09 g/mole

9.2 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 500 parts of ethyl ether. It is insoluble 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/cm^3 at 25 C

Surface tension : 63.4 mN/m at 20 C

Viscosity : 1490 mPa s at 20 C

954 mPa s at 25 C

Osmolarity : 2.6% v/v solution is iso-osmotic with serum

9.3 Safety

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

10. n-Caproic Acid (Budavari, 1996 : 286)

10.1 Name/Compositions

Chemical structure

$CH_3(CH_2)_4COOH$

Chemical name: Hexanoic acid

Chemical formular: $C_6H_{12}O_2$

Molecilar weight: 116.16

10.2 General properties

Appearance

Oily liquid, goat-like odor

Solubility

Slightly soluble in water (1.082 g/100g), readily soluble in ethanol, ether.

Boiling point: 205 °C

Density: 0.9265

Refractive index: 1.4163

10.3 Safety

LD_{50} orally in rats : 3.0g/kg

11. n-Butanol (Budavari, 1996 : 255)

11.1 Name/Compositions

Chemical structure

$CH_3CH_2CH_2CH_2OH$

Chemical name

Butyl alcohol

Chemical formular: $C_4H_{10}O$

Molecilar weight: 74.12

11.2 General properties

Appearance

Clear, colorless, mobile liquid with a characteristic odor

Solubility

At 25 °C ; 9.1 ml/100 ml water

Boiling point: 117-118°C

Density: 0.810

Refractive index: 1.3993

APPENDIX B

Formulation and viscosity data

Table 14 Composition of all prepared microemulsion formulations

ME	Oil	E1	E2	Em	Em/Oil	% Ingredients				
						Oil	Em	E1	E2	Aq
1	IPM	PC	BT	1/0.5	7/3	28.80	67.20	44.80	22.40	4.00
2	IPM	TW	BT	1/0.5	7/3	28.80	67.20	44.80	22.40	4.00
3	IPM	PC	PG	1/0.5	7/3	29.40	68.60	45.73	22.87	2.00
4	IPM	TW	PG	1/0.5	7/3	28.40	67.20	44.80	22.40	4.00
5	EO	PC	PG	1/0.5	7/3	29.40	68.60	45.73	22.87	2.00
6	EO	TW	PG	1/0.5	7/3	28.40	67.20	44.80	22.40	4.00
7	IPM	TW	CA	1/0.5	7/3	28.40	67.20	44.80	22.40	4.00
8	IPM	TW	PEG	1/1	7/3	25.95	60.55	30.28	30.28	13.50
9	IPM	TW	PEG	1/0.5	7/3	27.00	63.00	42.00	21.00	10.00
10	IPM	TW	PEG	1/0.25	7/3	28.40	63.00	50.40	12.60	10.00
11	IPM	TW	PEG	1/0.5	7/3	28.57	66.67	44.45	22.22	4.76
12	IPM	TW	PEG	1/0.25	7/3	28.80	67.20	53.76	13.44	4.00
13	IPM	TW	GR	1/1	7/3	29.10	67.90	33.95	33.95	3.00
14	IPM	TW	GR	1/0.5	7/3	29.40	68.60	45.73	22.87	2.00
15	IPM	TW	GR	1/0.25	7/3	28.80	67.20	53.76	13.44	4.00
16	EO	PC	PG	1/1	5/5	48.00	48.00	24.00	24.00	4.00
17	EO	PC	PG	1/0.5	5/5	48.00	48.00	32.00	16.00	4.00
18	EO	PC	PG	1/0.25	5/5	48.00	48.00	38.40	9.60	4.00
19	IPM	PC	PG	1/1	5/5	48.00	48.00	24.00	24.00	4.00
20	MCT	PC	PG	1/1	5/5	49.00	49.00	24.50	24.50	2.00
21	IPM	PC	PG	1/1	5/5	48.00	48.00	24.00	24.00	10.00
22	IPM	PC	PG	1/1	5/5	48.00	48.00	24.00	24.00	15.00
23	EO	PC	PG	1/1	6/4	38.40	57.60	28.80	28.80	4.00
24	EO	PC	PG	1/1	6/4	36.00	54.00	27.00	27.00	10.00
25	EO	PC	PG	1/1	6/4	34.00	51.00	25.50	25.50	15.00
26	IPM	TW	PEG	1/0.5	5/5	45.50	45.50	30.33	15.17	9.00
27	IPM	TW	PEG	1/0.25	5/5	45.50	45.50	36.40	9.10	9.00
28	IPM	TW	PEG	1/1	7/3	26.10	60.90	30.45	30.45	13.00
29	IPM	TW	PEG	1/0.25	7/3	27.30	63.70	50.96	12.74	9.00
30	EO	TW	PEG	1/0.5	7/3	27.30	63.70	42.47	21.23	9.00
31	EO	TW	PEG	1/0.25	7/3	27.30	63.70	50.96	12.74	9.00
32	EO	TW	PG	1/0.25	7/3	27.30	63.70	50.96	12.74	9.00
33	MCT	TW	PG	1/0.25	7/3	27.30	63.70	50.96	12.74	9.00
34	MCT	TW	PEG	1/0.25	7/3	27.30	63.70	50.96	12.74	9.00
35	IPM	PC	PG	1/1	5/5	45.50	45.50	22.75	22.75	9.00
36	EO	PC	PG	1/1	5/5	45.50	45.50	22.75	22.75	9.00
37	IPM	PC	PG	1/1	6/4	36.40	54.60	27.30	27.30	9.00
38	EO	PC	PG	1/1	6/4	36.40	54.60	27.30	27.30	9.00

Table 15 Viscosity of the freshly prepared investigated microemulsions at 30°C

ME	N1		N2		N3		Viscosity(cp)	
	Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
1	84.95	27.78	85.05	27.81	85.05	27.81	27.80	0.02
2	47.05	15.39	47.00	15.37	47.00	15.37	15.37	0.01
3	73.60	120.34	73.70	120.50	74.05	121.07	120.64	0.39
4	64.95	106.19	65.05	106.36	65.00	106.28	106.28	0.08
5	78.95	129.08	78.75	128.76	79.05	129.25	129.03	0.25
6	62.60	102.35	62.60	102.35	62.80	102.68	102.46	0.19
7	76.60	25.05	77.00	25.18	76.80	25.11	25.11	0.07
8	68.00	111.18	68.20	111.51	67.80	110.85	111.18	0.33
9	87.75	143.47	87.85	143.63	87.55	143.14	143.42	0.25
10	54.00	176.58	54.00	176.58	53.85	176.09	176.42	0.28
11	74.85	122.38	74.85	122.38	74.90	122.46	122.41	0.05
12	84.35	137.91	84.40	137.99	84.55	138.24	138.05	0.17
13	60.80	198.82	60.55	198.00	60.30	197.18	198.00	0.82
14	47.15	154.18	47.20	154.34	47.40	155.00	154.51	0.43
15	93.20	304.76	92.65	302.97	92.90	303.78	303.84	0.90
16	78.60	51.40	78.65	51.44	78.30	51.21	51.35	0.12
17	42.60	69.65	42.55	69.57	42.70	69.81	69.68	0.12
18	73.00	119.36	73.00	119.36	73.00	119.36	119.36	0.00
19	76.95	50.33	76.90	50.29	77.00	50.36	50.33	0.03
20	39.30	64.26	39.50	64.58	39.70	64.91	64.58	0.33
21	46.15	75.46	45.10	73.74	45.60	74.56	74.58	0.86
22	54.90	89.76	54.90	89.76	53.90	88.13	89.22	0.94
23	40.95	66.95	40.05	65.48	41.00	67.04	66.49	0.87
24	51.40	84.04	52.00	85.02	51.45	84.12	84.39	0.54
25	73.65	120.42	74.15	121.24	74.75	122.22	121.29	0.90
H_2O	1.50	0.49	1.40	0.46	1.80	0.59	0.51	0.07

Table 16 Viscosity of the investigated microemulsions after 3 months storage at 4°, 30°, 37° and 50°C

ME	Temp (°C)	N1		N2		N3		Viscosity(cp)	
		Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
1	4	73.65	24.08	75.65	24.74	74.50	24.36	24.39	0.33
	30	76.60	25.05	74.10	24.23	75.20	24.59	24.62	0.41
	37	76.10	24.88	77.45	25.33	76.80	25.11	25.11	0.22
	50	77.85	25.46	78.80	25.77	78.20	25.57	25.60	0.16
2	4	42.30	13.83	41.50	13.57	42.20	13.80	13.73	0.14
	30	43.00	14.06	41.30	13.51	41.70	13.64	13.73	0.29
	37	41.90	13.70	42.40	13.86	42.70	13.96	13.84	0.13
	50	40.20	13.15	43.15	14.11	42.95	14.04	13.77	0.54
3	4	66.20	108.24	67.20	109.87	67.90	111.02	109.71	1.40
	30	69.05	112.90	67.50	110.36	66.20	108.24	110.50	2.33
	37	67.05	109.63	66.65	108.97	67.30	110.04	109.55	0.54
	50	59.65	97.53	59.85	97.85	63.65	104.07	99.82	3.69
4	4	56.90	93.03	55.50	90.74	58.85	96.22	93.33	2.75
	30	56.10	91.72	57.75	94.42	56.55	92.46	92.87	1.39
	37	55.95	91.48	56.55	92.46	56.75	92.79	92.24	0.68
	50	55.10	90.09	54.45	89.03	54.10	88.45	89.19	0.83
5	4	76.50	125.08	73.55	120.25	73.80	120.66	122.00	2.67
	30	81.50	133.25	78.55	128.43	78.60	128.51	130.06	2.76
	37	70.65	115.51	72.50	118.54	71.60	117.07	117.04	1.51
	50	69.75	114.04	69.60	113.80	70.10	114.61	114.15	0.42
6	4	57.20	93.52	56.00	91.56	56.85	92.95	92.68	1.01
	30	57.20	93.52	55.95	91.48	56.95	93.11	92.70	1.08
	37	57.85	94.58	56.40	92.21	56.35	92.13	92.98	1.39
	50	60.50	98.92	59.35	97.04	59.90	97.94	97.96	0.94
7	4	64.25	21.01	64.10	20.96	64.40	21.06	21.01	0.05
	30	63.60	20.80	63.70	20.83	63.50	20.76	20.80	0.03
	37	66.70	21.81	66.70	21.81	66.00	21.58	21.73	0.13
	50	65.40	21.39	65.50	21.42	65.50	21.42	21.41	0.02
8	4	62.95	102.92	66.60	108.89	64.45	105.38	105.73	3.00
	30	65.95	107.83	65.60	107.26	65.70	107.42	107.50	0.29
	37	65.75	107.50	66.35	108.48	66.05	107.99	107.99	0.49
	50	64.80	105.95	65.75	107.50	65.30	106.77	106.74	0.78
9	4	82.25	134.48	83.40	136.36	82.80	135.38	135.41	0.94
	30	79.90	130.64	80.05	130.88	79.90	130.64	130.72	0.14
	37	82.20	134.40	81.75	133.66	81.70	133.58	133.88	0.45
	50	82.95	135.62	81.65	133.50	82.25	134.48	134.53	1.06
10	4	50.75	165.95	51.65	168.90	51.50	168.41	167.75	1.58
	30	51.65	168.90	51.60	168.73	51.55	168.57	168.73	0.16
	37	51.85	169.55	50.95	166.61	51.10	167.10	167.75	1.58
	50	49.85	163.01	49.90	163.17	49.90	163.17	163.12	0.09

Table 16 Viscosity of the investigated microemulsions after 3 months storage at 4°, 30°, 37° and 50°C (cont.)

ME	Temp (°C)	N1		N2		N3		Viscosity(cp)	
		Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
11	4	71.10	116.25	72.10	117.88	71.70	117.23	117.12	0.82
	30	73.85	120.74	72.40	118.37	73.15	119.60	119.57	1.19
	37	74.45	121.73	72.20	118.05	73.30	119.85	119.87	1.84
	50	72.75	118.95	73.50	120.17	73.05	119.44	119.52	0.62
12	4	78.20	127.86	76.70	125.40	77.50	126.71	126.66	1.23
	30	79.75	130.39	77.75	127.12	78.75	128.76	128.76	1.63
	37	78.60	128.51	74.60	121.97	76.40	124.91	125.13	3.28
	50	77.35	126.47	78.70	128.67	78.10	127.69	127.61	1.11
13	4	48.15	157.45	47.15	154.18	47.70	155.98	155.87	1.64
	30	44.55	145.68	49.25	161.05	46.90	153.36	153.36	7.68
	37	48.50	158.60	45.60	149.11	47.00	153.69	153.80	4.74
	50	69.55	113.71	73.15	119.60	71.55	116.98	116.77	2.95
14	4	80.60	131.78	83.70	136.85	82.10	134.23	134.29	2.53
	30	76.50	125.08	75.35	123.20	75.95	124.18	124.15	0.94
	37	72.95	119.27	72.95	119.27	73.00	119.36	119.30	0.05
	50	63.15	103.25	62.65	102.43	62.85	102.76	102.81	0.41
15	4	83.70	273.70	84.80	277.30	84.25	275.50	275.50	1.80
	30	83.80	274.03	83.90	274.35	83.90	274.35	274.24	0.19
	37	83.95	274.52	83.30	272.39	83.35	272.55	273.15	1.18
	50	81.50	266.51	82.15	268.63	81.85	267.65	267.60	1.06
16	4	74.75	48.89	72.25	47.25	73.65	48.17	48.10	0.82
	30	72.05	47.12	70.45	46.07	71.40	46.70	46.63	0.53
	37	68.05	44.50	68.65	44.90	68.35	44.70	44.70	0.20
	50	68.20	44.60	65.95	43.13	66.90	43.75	43.83	0.74
17	4	39.45	64.50	39.30	64.26	39.35	64.34	64.36	0.12
	30	38.75	63.36	38.25	62.54	38.50	62.95	62.95	0.41
	37	90.65	59.29	89.40	58.47	89.90	58.79	58.85	0.41
	50	85.75	56.08	85.75	56.08	85.65	56.02	56.06	0.04
18	4	62.55	102.27	60.60	99.08	63.25	103.41	101.59	2.25
	30	73.00	119.36	73.65	120.42	73.15	119.60	119.79	0.56
	37	62.30	101.86	64.10	104.80	64.75	105.87	104.18	2.08
	50	44.80	73.25	46.30	75.70	46.40	75.86	74.94	1.47
19	4	70.65	46.21	69.85	45.68	70.25	45.94	45.94	0.26
	30	67.85	44.37	66.05	43.20	66.85	43.72	43.76	0.59
	37	65.95	43.13	67.00	43.82	65.95	43.13	43.36	0.40
	50	62.55	40.91	61.55	40.25	62.05	40.58	40.58	0.33
20	4	37.45	61.23	38.60	63.11	38.15	62.38	62.24	0.95
	30	38.75	63.36	38.40	62.78	38.55	63.03	63.06	0.29
	37	38.40	62.78	37.95	62.05	38.05	62.21	62.35	0.39
	50	89.90	58.79	90.30	59.06	90.00	58.86	58.90	0.14

Table 17 Viscosity of the investigated microemulsions after 6 months storage at 4°, 30°, 37°, and 50°C

ME	Temp (°C)	N1		N2		N3		Viscosity(cp)	
		Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
1	4	63.50	20.76	70.15	22.94	64.90	21.22	21.64	1.15
	30	67.10	21.94	67.50	22.07	67.70	22.14	22.05	0.10
	37	69.70	22.79	68.95	22.55	67.80	22.17	22.50	0.31
	50	65.30	21.35	65.40	21.39	65.80	21.52	21.42	0.09
2	4	46.60	15.24	46.10	15.07	44.60	14.58	14.97	0.34
	30	43.95	14.37	43.90	14.36	43.70	14.29	14.34	0.04
	37	42.10	13.77	41.20	13.47	41.95	13.72	13.65	0.16
	50	42.50	13.90	41.80	13.67	41.10	13.44	13.67	0.23
3	4	62.70	102.51	62.50	102.19	62.10	101.53	102.08	0.50
	30	62.70	102.51	61.30	100.23	59.95	98.02	100.25	2.25
	37	58.60	95.81	57.80	94.50	57.50	94.01	94.78	0.93
	50	54.10	88.45	53.40	87.31	53.60	87.64	87.80	0.59
4	4	53.45	87.39	53.55	87.55	53.15	86.90	87.28	0.34
	30	53.10	86.82	52.90	86.49	53.15	86.90	86.74	0.22
	37	54.35	88.86	53.15	86.90	53.80	87.96	87.91	0.98
	50	52.05	85.10	52.40	85.67	52.20	85.35	85.37	0.29
5	4	63.70	104.15	66.70	109.05	65.25	106.68	106.63	2.45
	30	64.30	105.13	64.10	104.80	64.35	105.21	105.05	0.22
	37	61.20	100.06	60.00	98.10	61.00	99.74	99.30	1.05
	50	58.10	94.99	58.05	94.91	58.30	95.32	95.08	0.22
6	4	51.30	83.88	52.00	85.02	52.40	85.67	84.86	0.91
	30	50.50	82.57	54.85	89.68	52.35	85.59	85.95	3.57
	37	52.00	85.02	51.65	84.45	52.25	85.43	84.97	0.49
	50	52.20	85.35	51.05	83.47	51.45	84.12	84.31	0.95
7	4	60.00	19.62	61.95	20.26	60.85	19.90	19.93	0.32
	30	60.65	19.83	60.25	19.70	60.45	19.77	19.77	0.07
	37	59.15	19.34	58.45	19.11	58.70	19.19	19.22	0.12
	50	57.65	18.85	56.65	18.52	57.00	18.64	18.67	0.17
8	4	63.05	103.09	61.45	100.47	60.65	99.16	100.91	2.00
	30	61.40	100.39	59.90	97.94	59.05	96.55	98.29	1.95
	37	55.20	90.25	56.25	91.97	55.40	90.58	90.93	0.91
	50	56.60	92.54	57.75	94.42	56.85	92.95	93.30	0.99
9	4	75.05	122.71	73.25	119.76	72.40	118.37	120.28	2.21
	30	72.95	119.27	70.85	115.84	71.85	117.47	117.53	1.72
	37	71.35	116.66	69.45	113.55	68.65	112.24	114.15	2.27
	50	67.50	110.36	68.85	112.57	68.15	111.43	111.45	1.10
10	4	44.00	143.88	43.20	141.26	43.15	141.10	142.08	1.56
	30	43.70	142.90	43.55	142.41	43.70	142.90	142.74	0.28
	37	43.40	141.92	43.45	142.08	43.70	142.90	142.30	0.53
	50	42.20	137.99	43.15	141.10	42.95	140.45	139.85	1.64

Table 17 Viscosity of the investigated microemulsions after 6 months storage at 4°, 30°, 37°, and 50°C (cont.)

ME	Temp (°C)	N1		N2		N3		Viscosity(cp)	
		Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
11	4	64.15	104.89	65.15	106.52	64.50	105.46	105.62	0.83
	30	63.30	103.50	64.45	105.38	64.10	104.80	104.56	0.96
	37	62.15	101.62	60.90	99.57	61.30	100.23	100.47	1.04
	50	59.80	97.77	59.85	97.85	59.85	97.85	97.83	0.05
12	4	33.70	110.20	32.65	106.77	33.00	107.91	108.29	1.75
	30	33.80	110.53	32.80	107.26	33.75	110.36	109.38	1.84
	37	33.25	108.73	33.00	107.91	33.20	108.56	108.40	0.43
	50	32.20	105.29	31.60	103.33	31.75	103.82	104.15	1.02
13	4	36.40	119.03	36.15	118.21	36.20	118.37	118.54	0.43
	30	39.75	129.98	37.50	122.63	37.90	123.93	125.51	3.93
	37	34.00	111.18	34.05	111.34	34.35	112.32	111.62	0.62
	50	29.25	95.65	28.90	94.50	28.95	94.67	94.94	0.62
14	4	32.90	107.58	33.50	109.55	33.05	108.07	108.40	1.02
	30	33.20	108.56	32.25	105.46	32.95	107.75	107.26	1.61
	37	30.35	99.24	29.25	95.65	29.65	96.96	97.28	1.82
	50	25.00	81.75	24.35	79.62	24.90	81.42	80.93	1.14
15	4	62.90	205.68	63.05	206.17	62.90	205.68	205.85	0.28
	30	67.85	221.87	67.20	219.74	67.80	221.71	221.11	1.18
	37	68.25	223.18	65.85	215.33	66.80	218.44	218.98	3.95
	50	64.20	209.93	64.60	211.24	64.20	209.93	210.37	0.76
16	4	61.30	40.09	58.95	38.55	60.10	39.31	39.32	0.77
	30	55.70	36.43	55.25	36.13	55.50	36.30	36.29	0.15
	37	53.90	35.25	52.30	34.20	52.95	34.63	34.69	0.53
	50	46.75	30.57	44.00	28.78	45.10	29.50	29.62	0.91
17	4	76.75	50.19	75.65	49.48	76.30	49.90	49.86	0.36
	30	71.10	46.50	72.70	47.55	71.70	46.89	46.98	0.53
	37	67.95	44.44	67.55	44.18	67.65	44.24	44.29	0.14
	50	53.30	34.86	54.10	35.38	53.55	35.02	35.09	0.27
18	4	54.20	88.62	52.75	86.25	53.55	87.55	87.47	1.19
	30	60.45	98.84	59.95	98.02	60.20	98.43	98.43	0.41
	37	46.25	75.62	45.95	75.13	46.25	75.62	75.46	0.28
	50	25.30	41.37	25.25	41.28	25.25	41.28	41.31	0.05
19	4	65.35	42.74	64.45	42.15	64.80	42.38	42.42	0.30
	30	63.15	41.30	61.75	40.38	62.40	40.81	40.83	0.46
	37	57.35	37.51	57.75	37.77	57.50	37.61	37.63	0.13
	50	51.35	33.58	50.95	33.32	51.25	33.52	33.47	0.14
20	4	36.45	59.60	37.40	61.15	36.75	60.09	60.28	0.79
	30	35.50	58.04	32.85	53.71	34.15	55.84	55.86	2.17
	37	31.75	51.91	33.00	53.96	32.15	52.57	52.81	1.04
	50	29.45	48.15	30.40	49.70	30.05	49.13	49.00	0.79

Table 18 Viscosity of the freshly prepared investigated microemulsions at 30°C

ME	N1		N2		N3		Viscosity (cp)	
	Avg.Read	Viscosity	Avg.Read	Viscosity	Avg.Read	Viscosity	Average	S.D.
26	45.00	147.15	45.60	149.11	45.35	148.29	148.19	0.99
27	47.30	154.67	48.15	157.45	47.85	156.47	156.20	1.41
28	71.00	116.09	69.90	114.29	70.30	114.94	115.10	0.91
29	55.55	181.65	55.50	181.49	54.90	179.52	180.89	1.18
30	48.65	159.09	49.30	161.21	48.90	159.90	160.07	1.07
31	59.55	194.73	58.80	192.28	59.60	194.89	193.97	1.47
32	51.50	168.41	51.40	168.08	50.60	165.46	167.32	1.61
33	65.00	212.55	64.60	211.24	65.25	213.37	212.39	1.07
34	64.20	209.93	64.40	210.59	63.95	209.12	209.88	0.74
35	48.35	79.05	47.60	77.83	47.65	77.91	78.26	0.69
36	42.25	69.08	43.30	70.80	43.25	70.71	70.20	0.97
37	56.55	92.46	57.30	93.69	57.65	94.26	93.47	0.92
38	60.50	98.92	59.30	96.96	59.45	97.20	97.69	1.07

APPENDIX C

TEM photomicrographs and droplet size

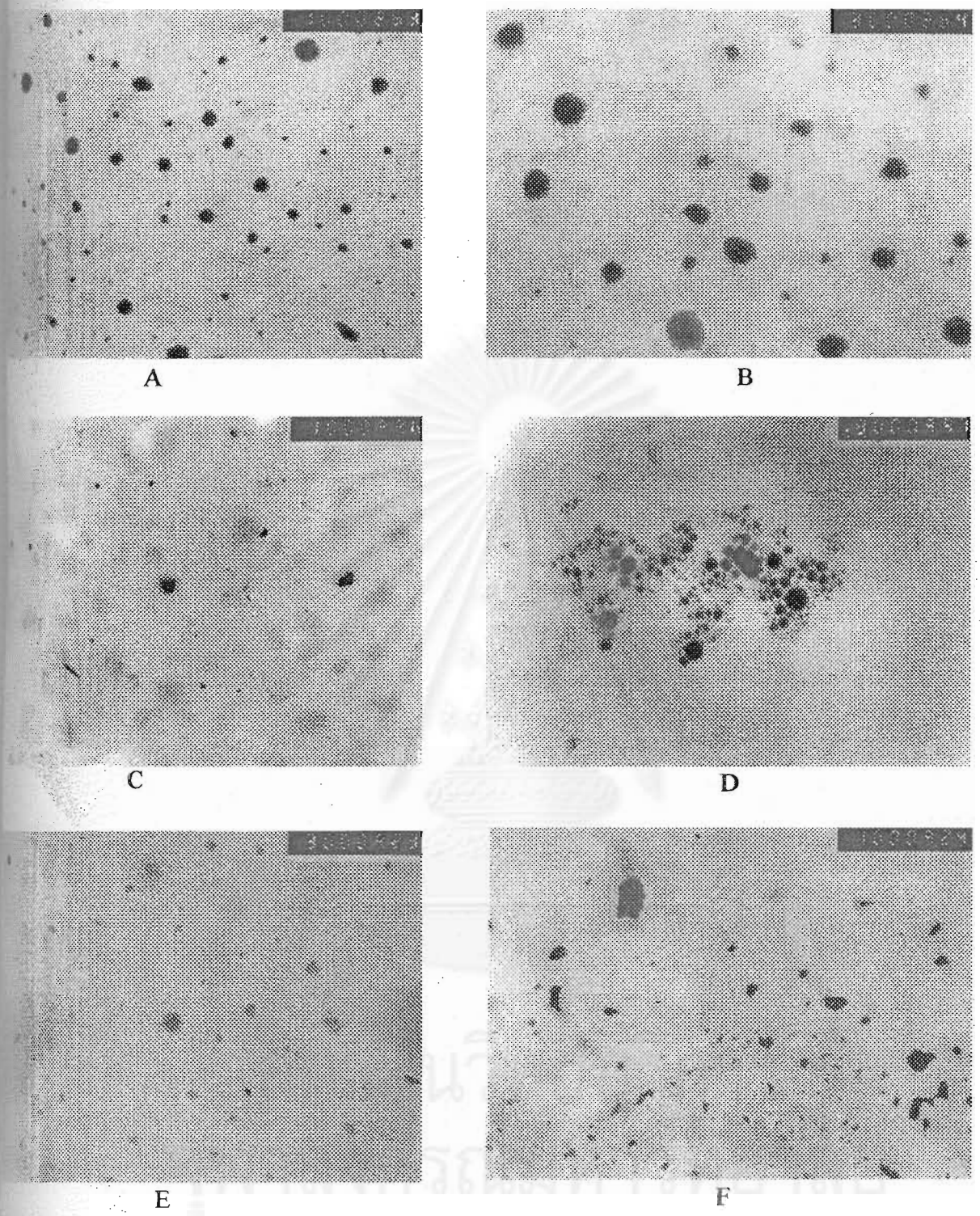


FIGURE 49 TEM photomicrographs of microemulsion system (ME1) containing IPM-PC-BT, Em=B, Em/oil=7/3, Aq 4 %

Key : A initial at 30 °c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

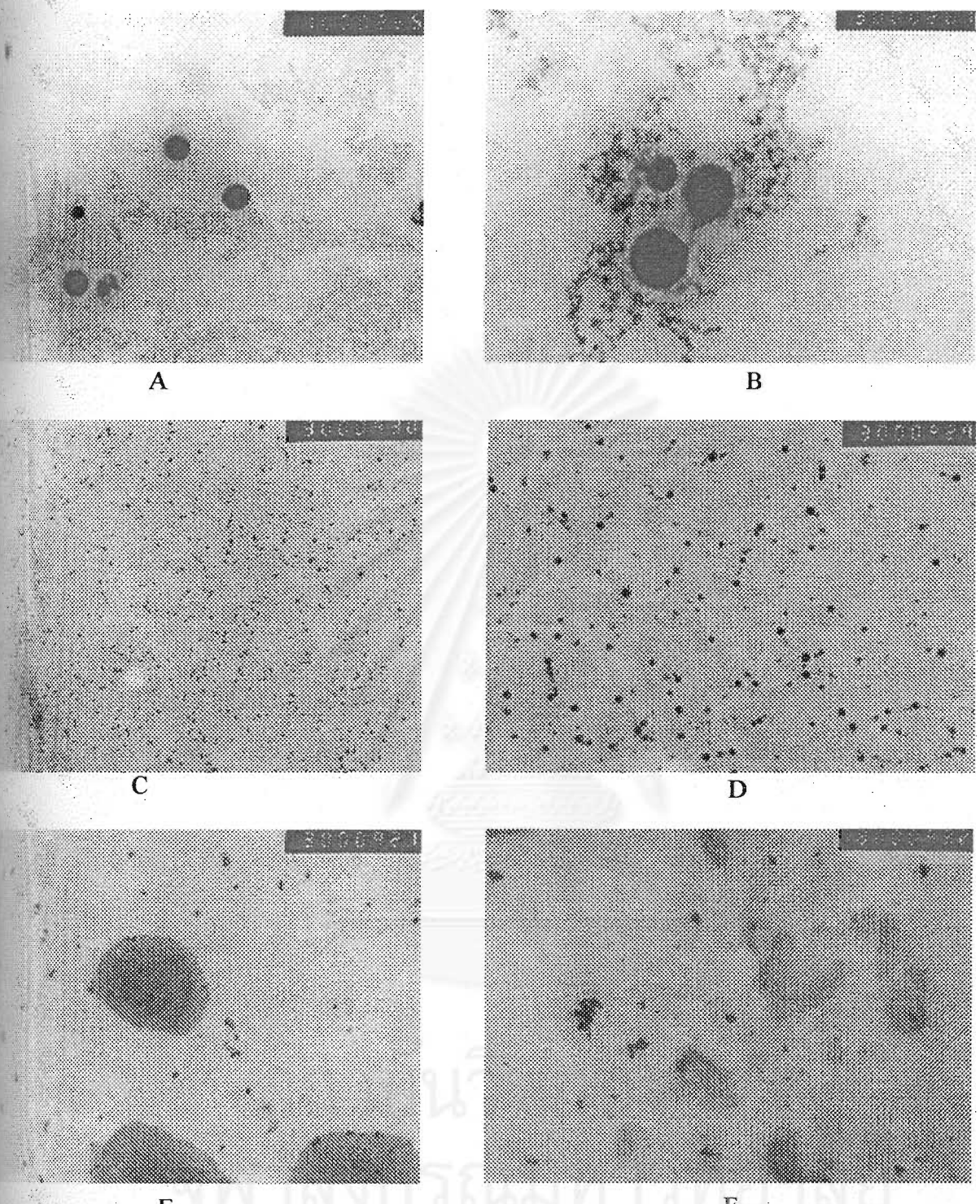


FIGURE 50 TEM photomicrographs of microemulsion system (ME2) containing IPM-TW-BT, Em=B, Em/oil=7/3, Aq 4 %

Key: A initial at 30 °c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30 ° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4 ° c , x 45000 : F 6 months at 50° c , x 45000

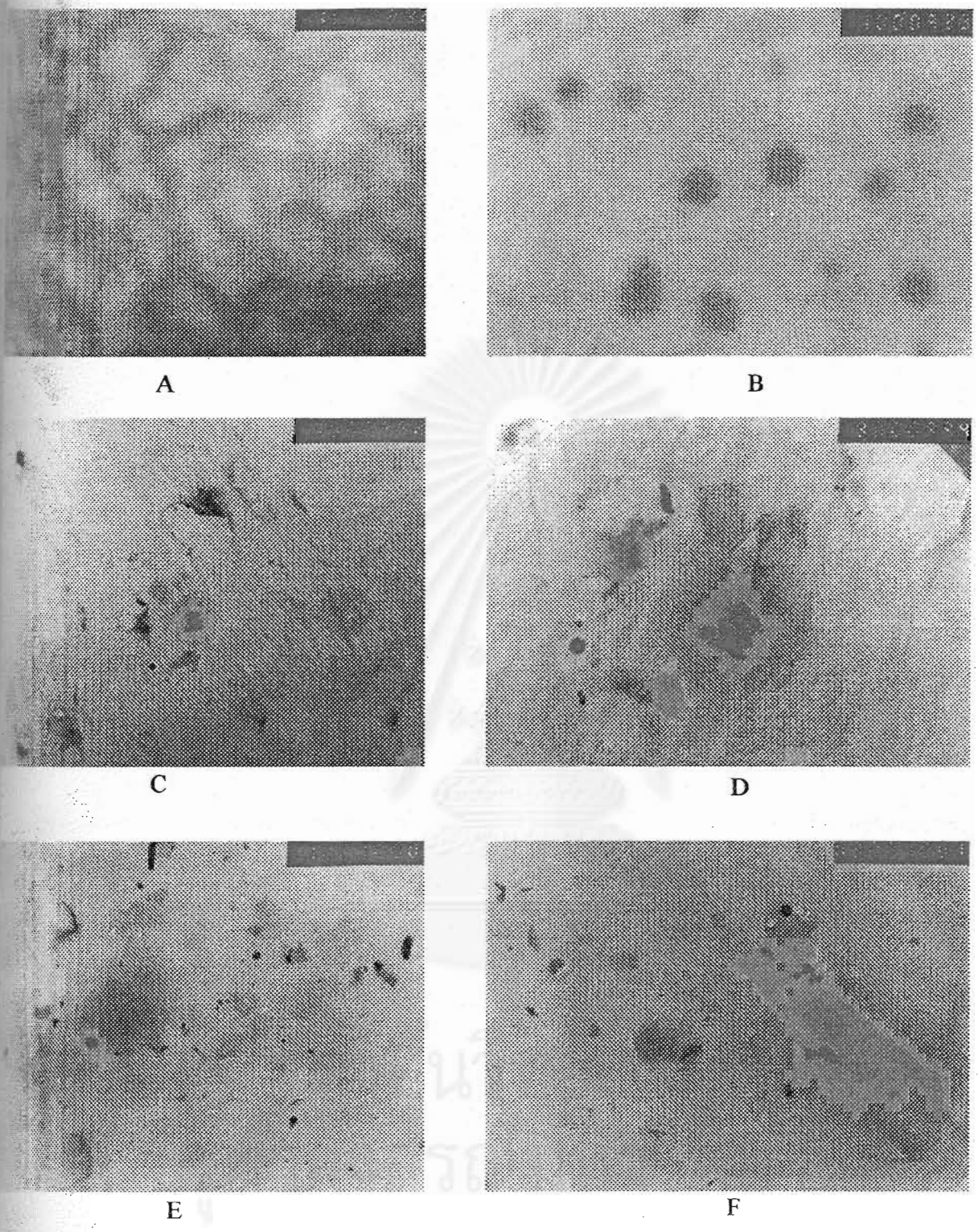


FIGURE 51 TEM photomicrographs of microemulsion system (ME3) containing IPM-PC-PG, Em=B, Em/oil=7/3, Aq 2%

Key : A initial at 30 °c , x 13200 : B 6 months at 30° c , x 16000
C 6 months at 4° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 50° c , x 16500 : F 6 months at 50° c , x 45000

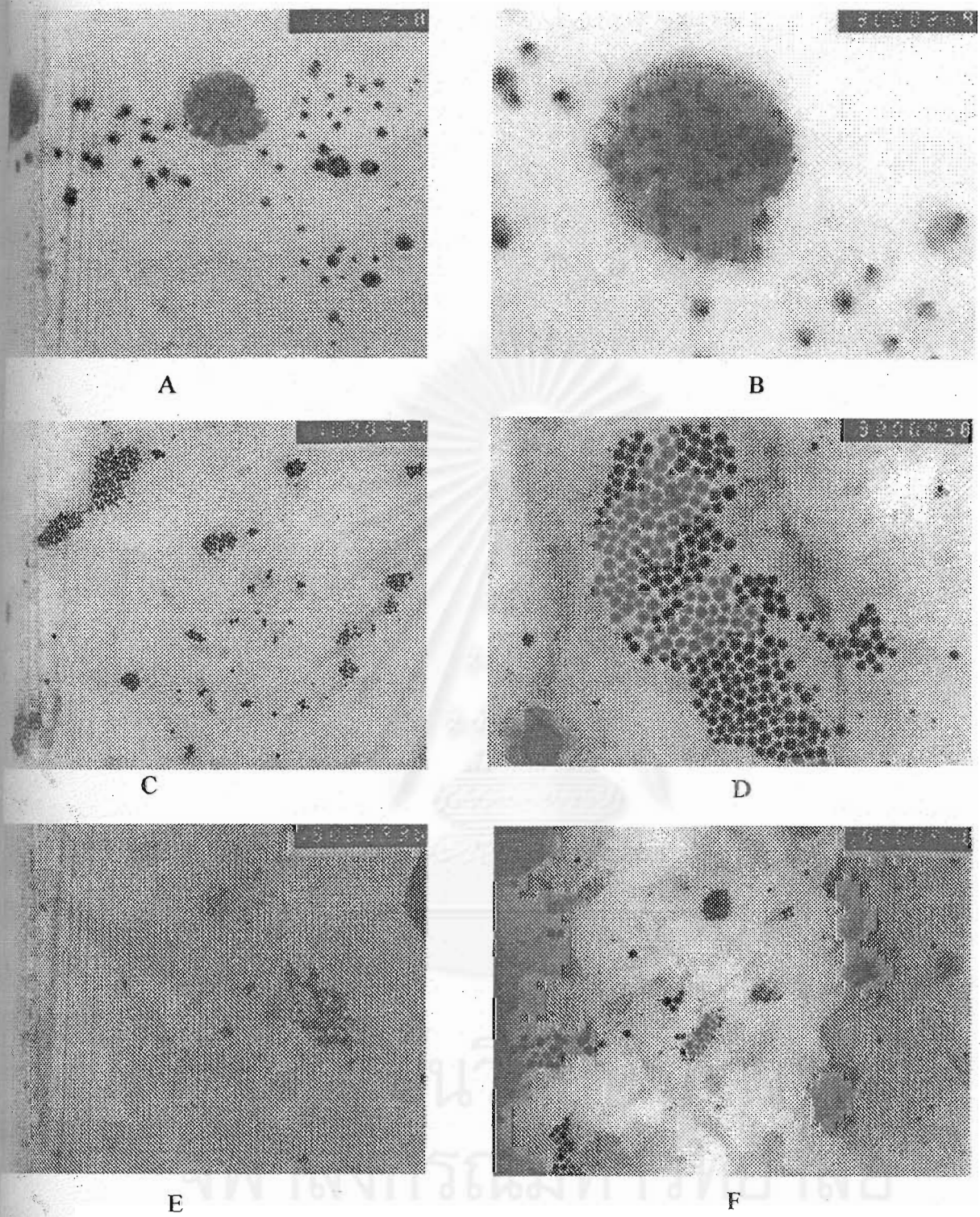


FIGURE 52 TEM photomicrographs of microemulsion system (ME4) containing IPM-TW-PG, Em=B, Em/oil=7/3 ,Aq 4 %

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

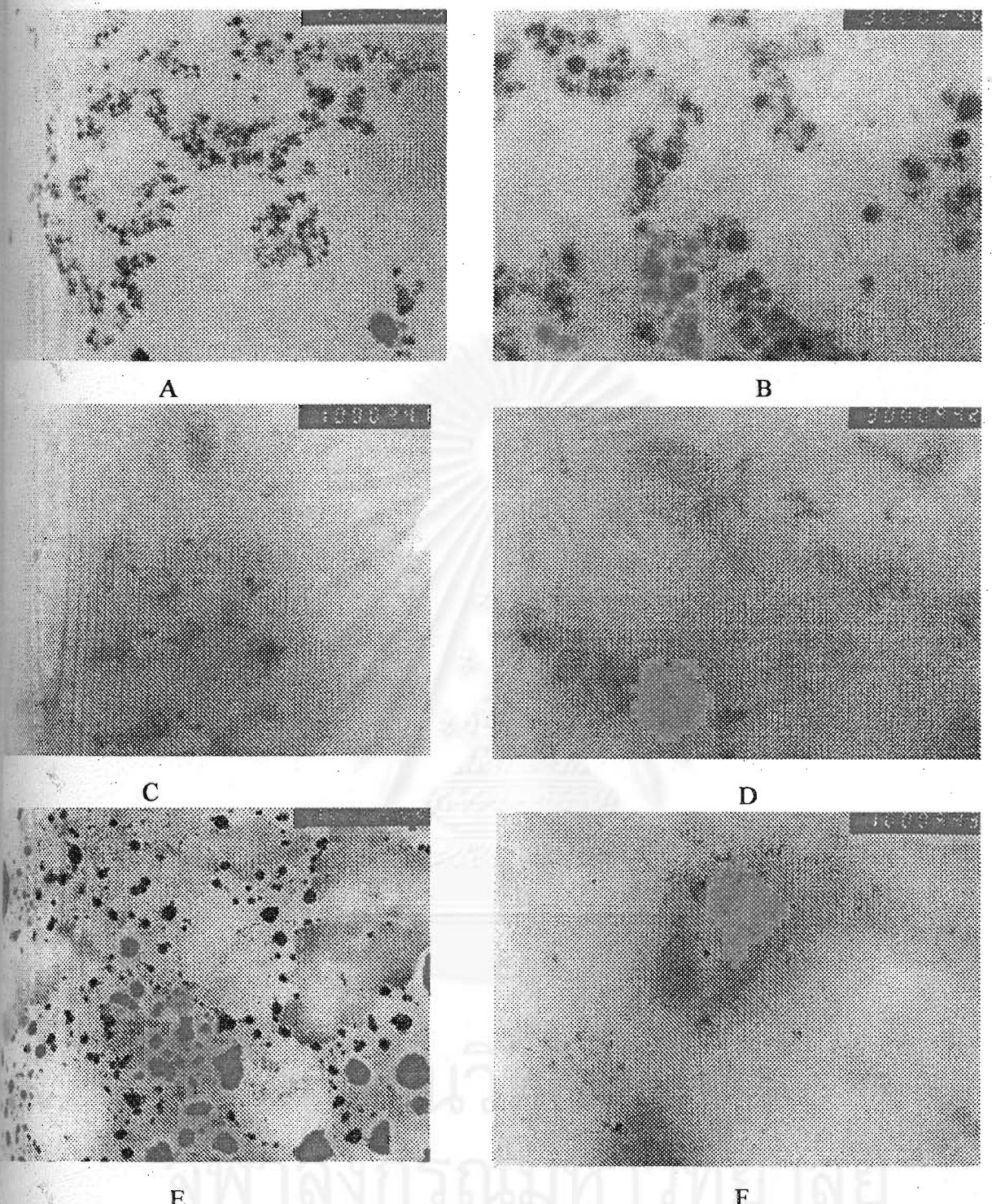


FIGURE 53 TEM photomicrographs of microemulsion system (ME5) containing BO-PC-PG, Em=B, Aq 2%

Key : A initial at 30^0 c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 30^0 c , x 45000
E 6 months at 4^0 c , x 16500 : F 6 months at 50^0 c , x 16500

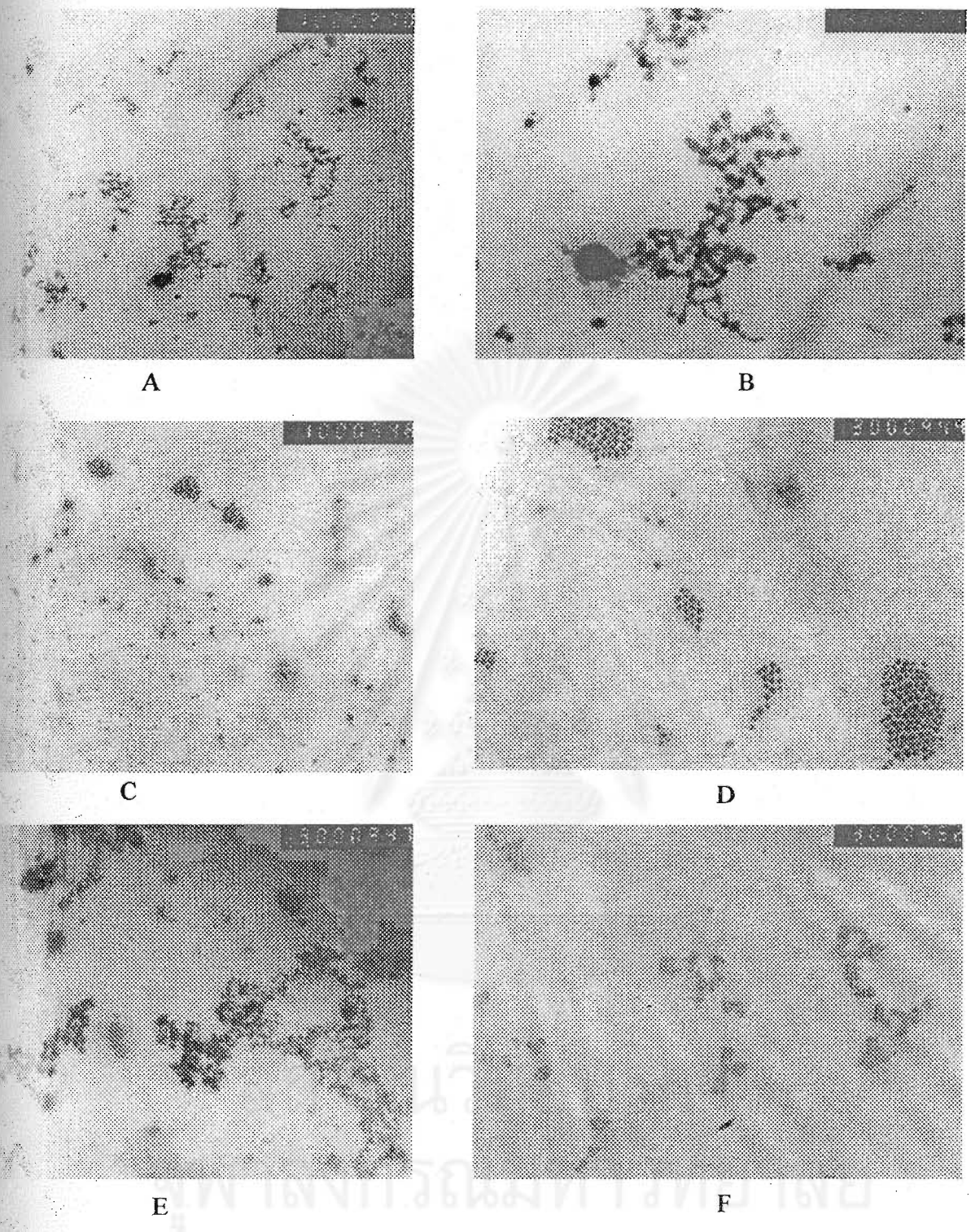


FIGURE 54 TEM photomicrographs of microemulsion system (ME6) containing EO-TW-PG, Em=B, Em/oil=7/3, Aq 4 %

Key : A initia at 30^0 c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 30^0 c , x 45000
E 6 months at 4^0 c , x 45000 : F 6 months at 50^0 c , x 45000

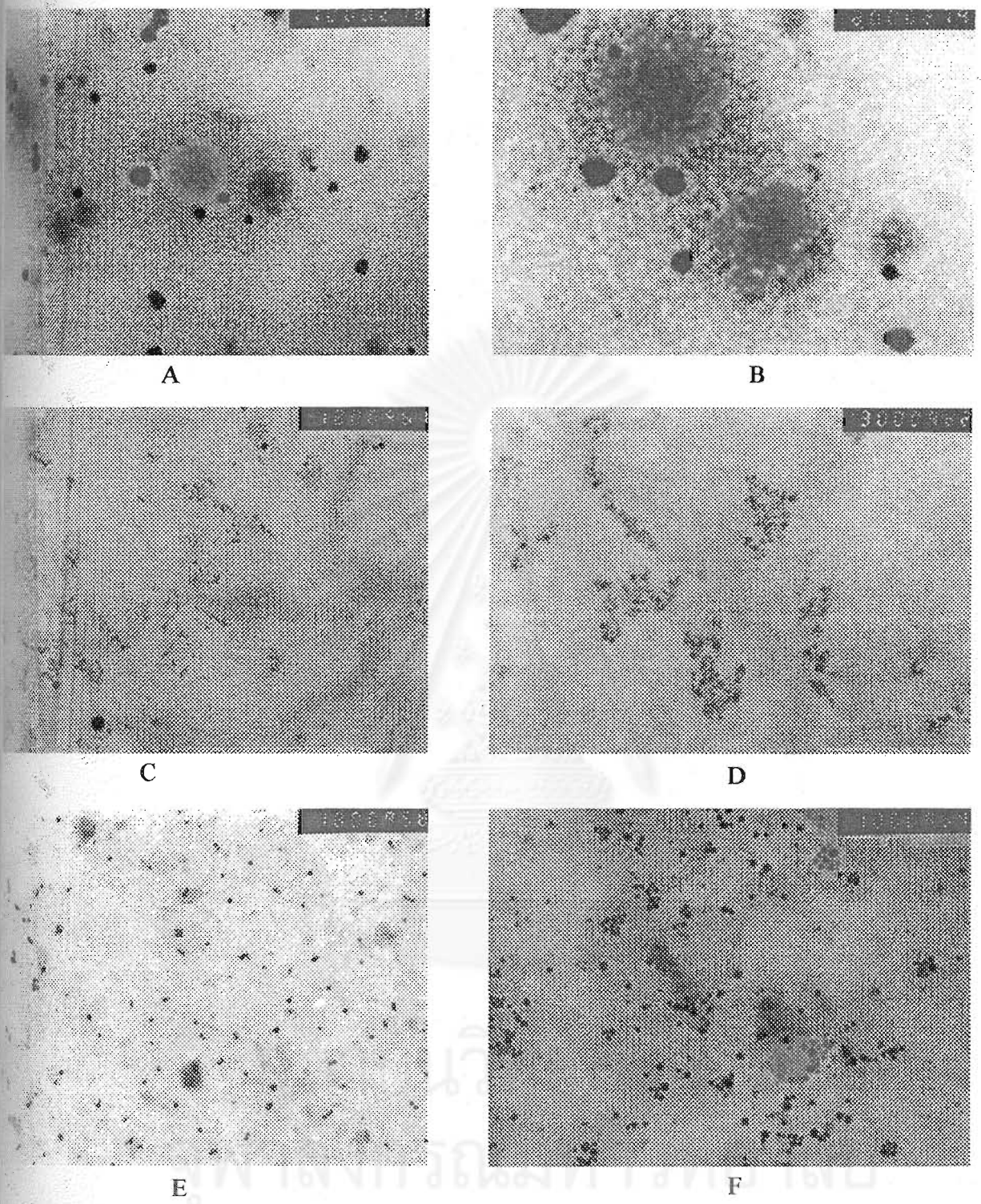


FIGURE 55 TEM photomicrographs of microemulsion system (ME7) containing IPM-TW-CA, Em=B, Em/oil=7/3, Aq 4 %

Key : A initial at 30^{0}c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 30^0 c , x 45000
E 6 months at 4^0 c , x 16500 : F 6 months at 50^0 c , x 16500

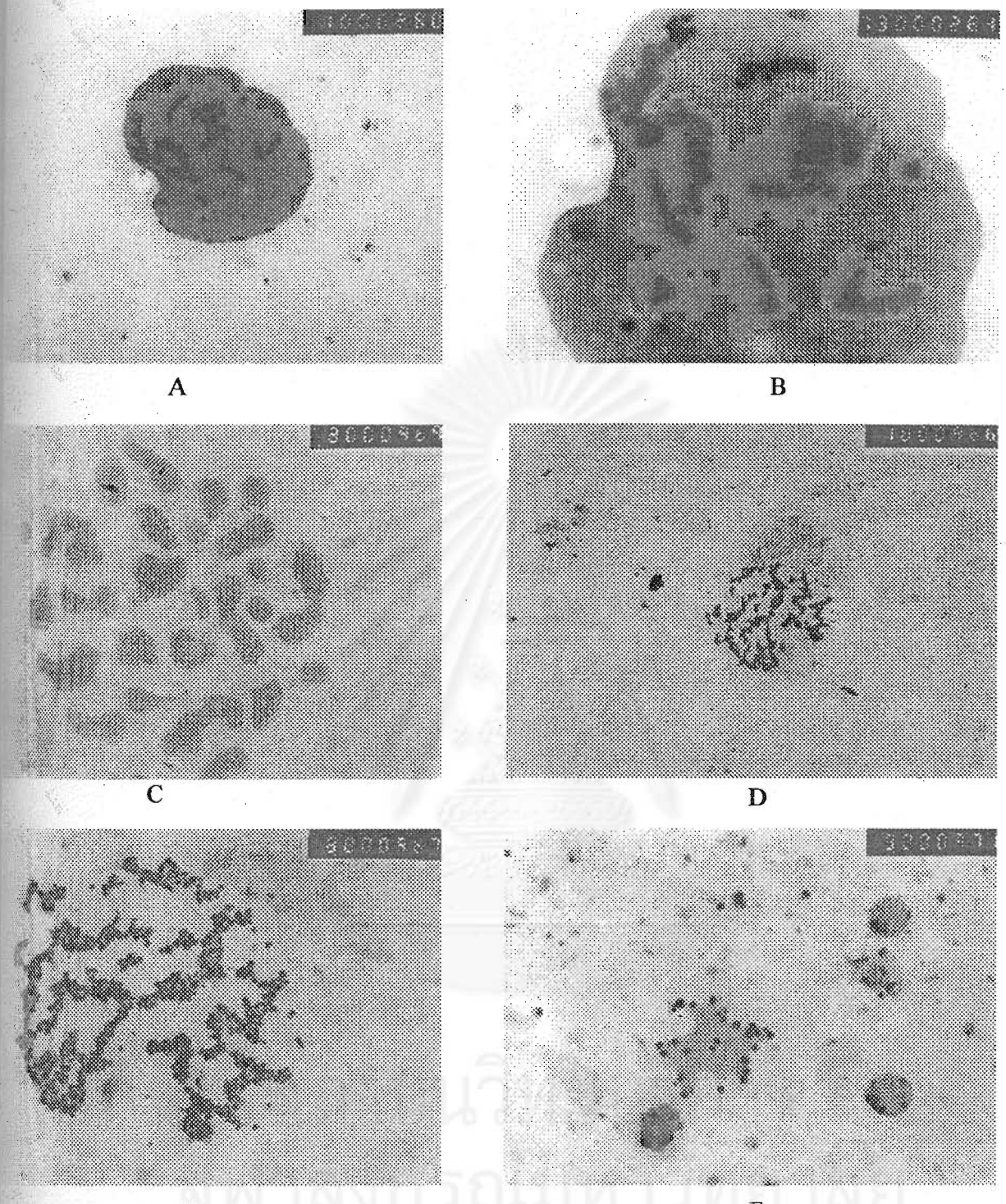
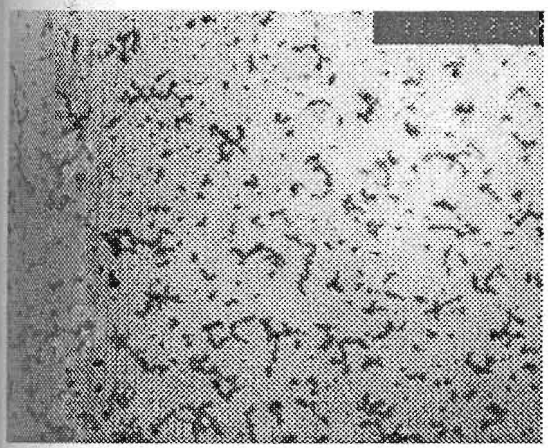
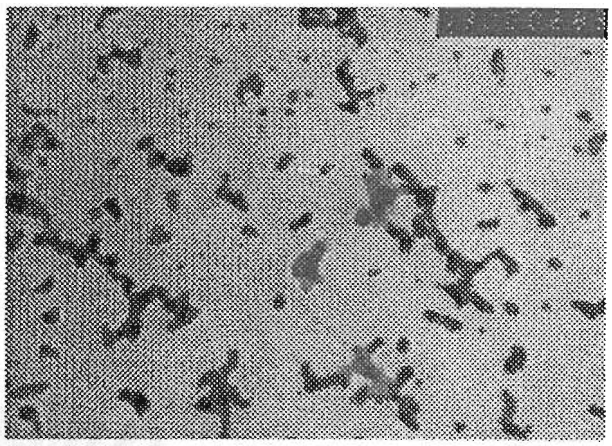


FIGURE 56 TEM photomicrographs of microemulsion system (ME8) containing IPM-TW-PEG, Em=A,Em/oil=7/3,Aq13.5%

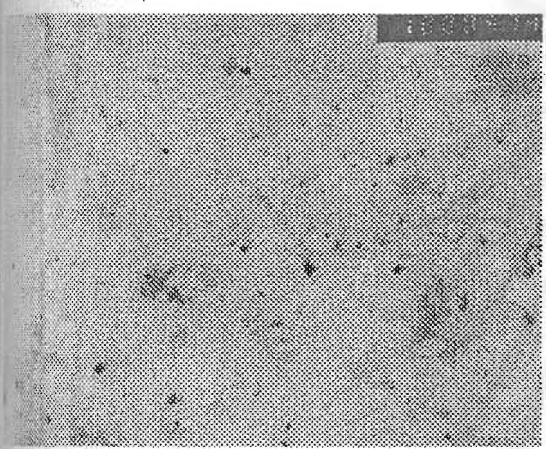
Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 45000 : D 6 months at 4° c , x 16500
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000



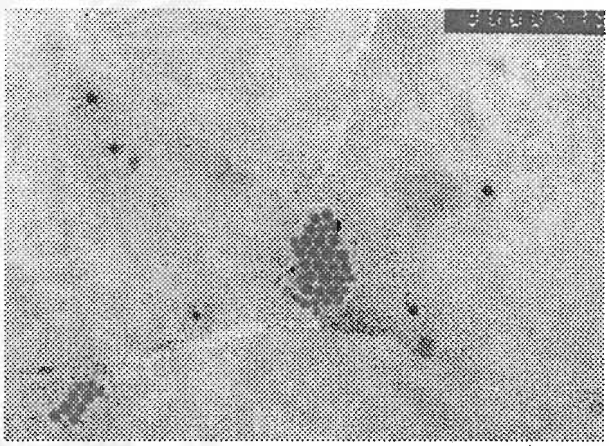
A



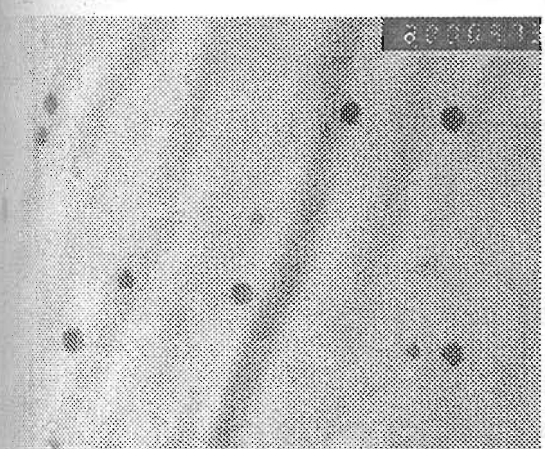
B



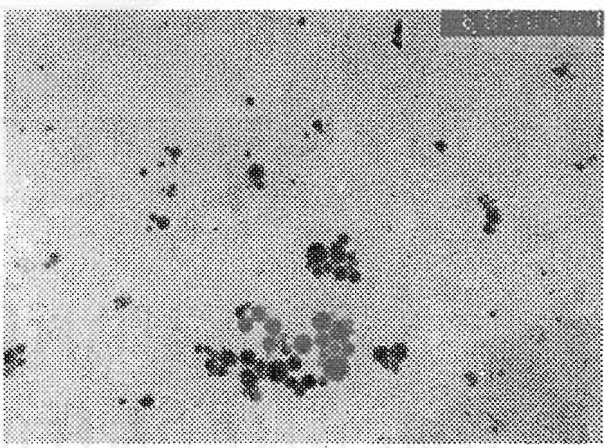
C



D



E



F

FIGURE 57 TEM photomicrographs of microemulsion system (ME9) containing IPM-TW-PEG, Em=B,Em/oil=7/3,Aq10%

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

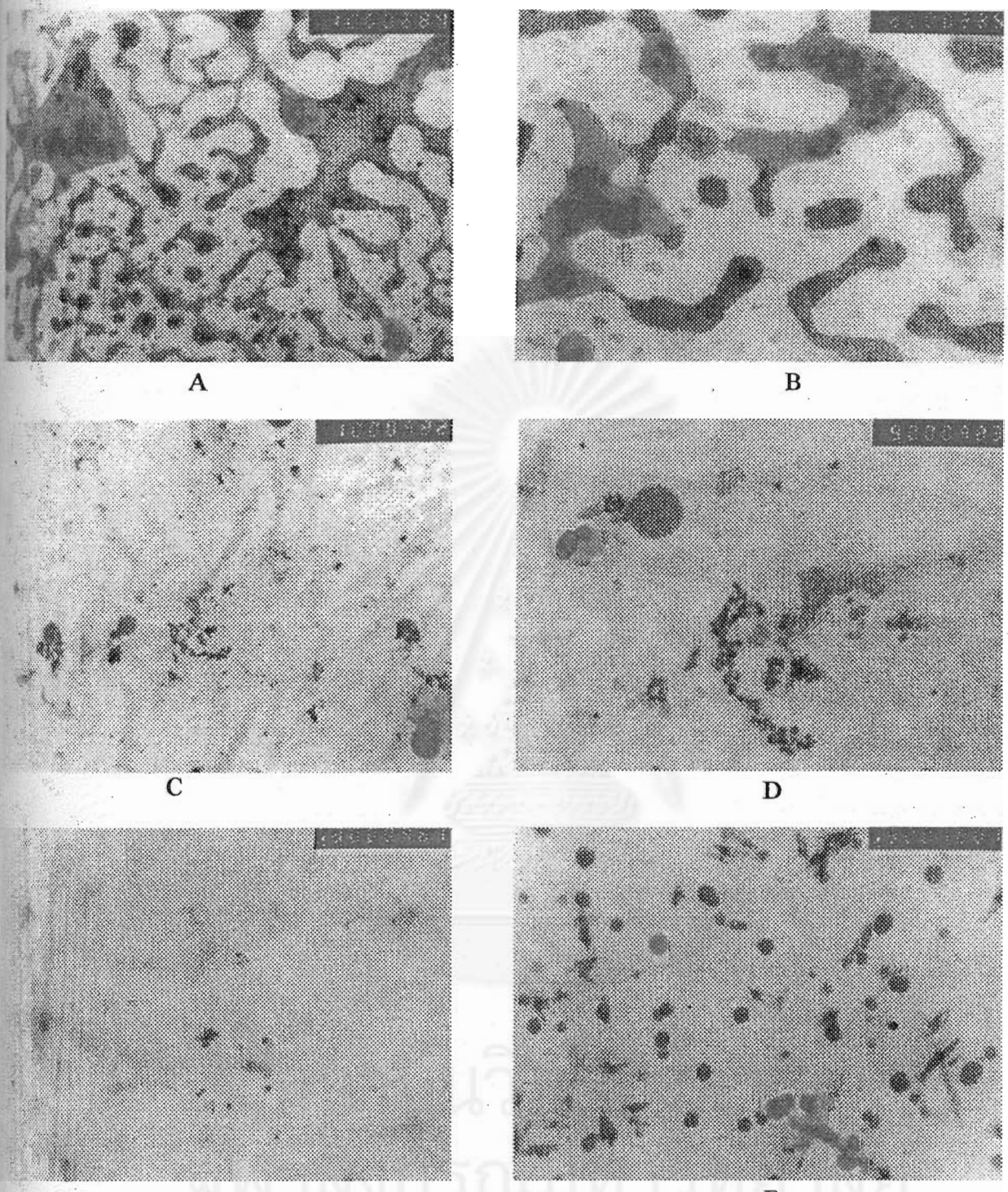


FIGURE 58 TEM photomicrographs of microemulsion system (ME10) containing IPM-TW-PEG, Em=C, Em/oil=7/3, Aq=10%

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

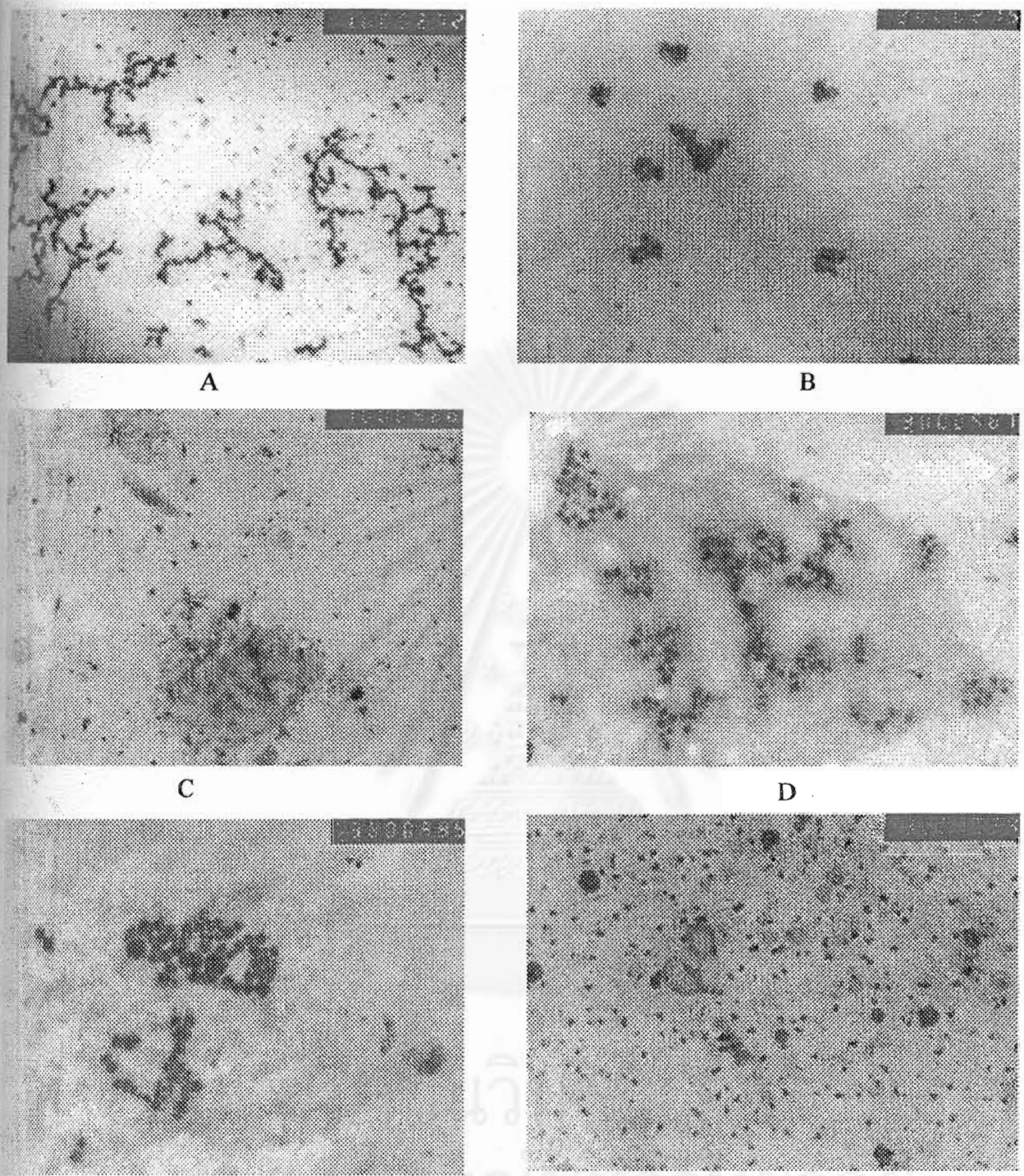


FIGURE 59 TEM photomicrographs of microemulsion system (ME11) containing IPM-TW-PEG, Em=B, Em/oil=7/3, Aq 4.76 %

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

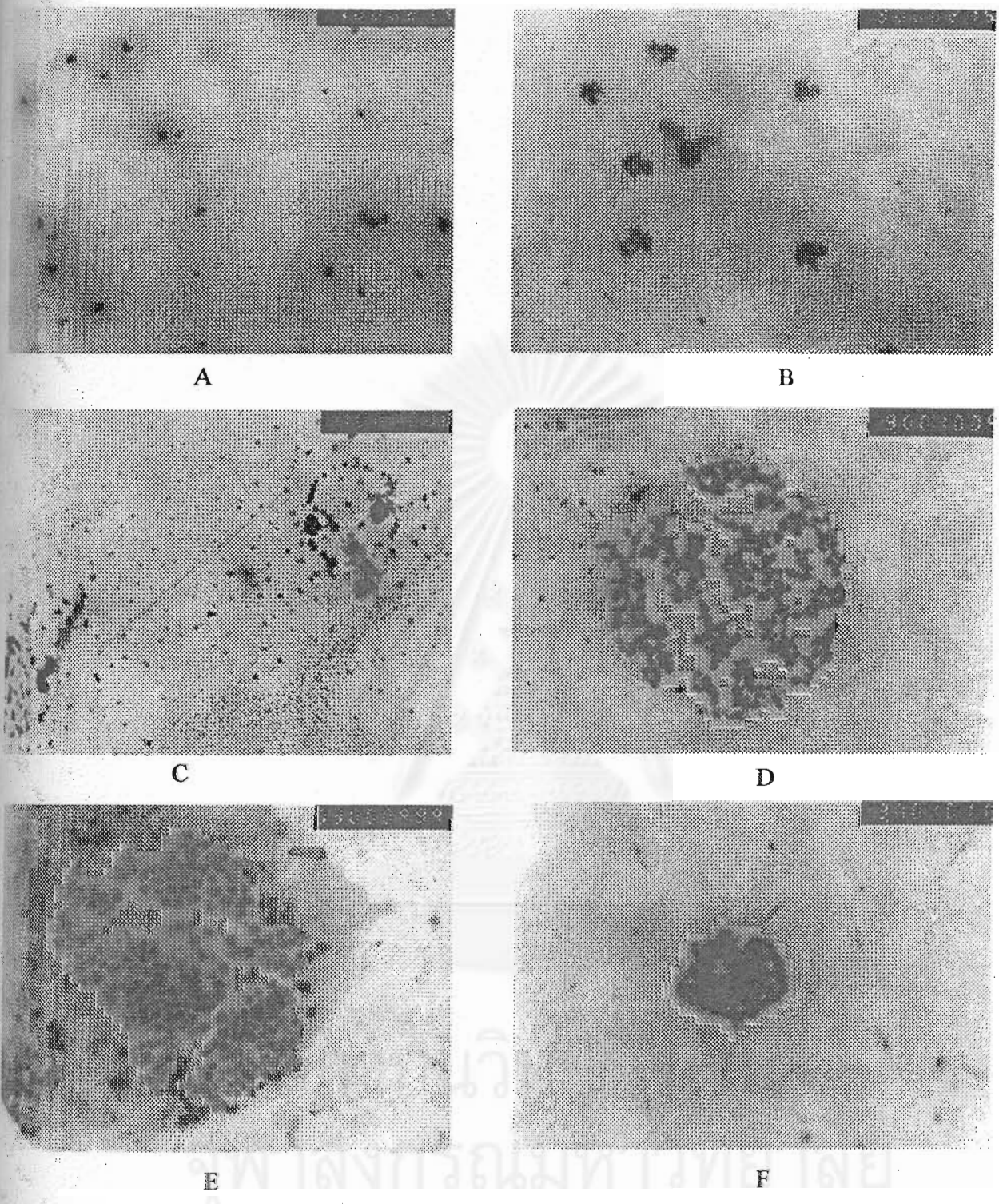
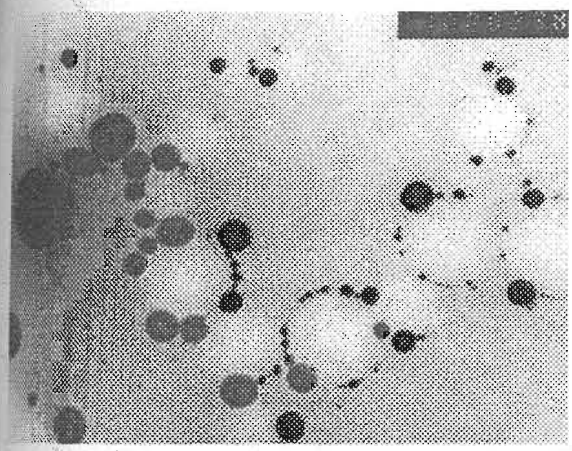
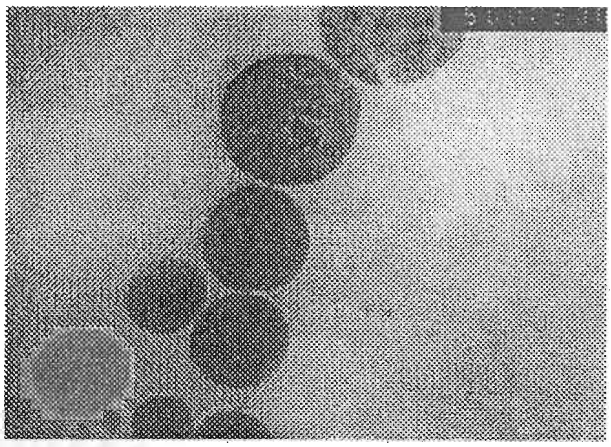


FIGURE 60 TEM photomicrographs of microemulsion system (ME12) containing IPM-TW-PEG, Em=C, Em/oil=7/3, Aq 4 %

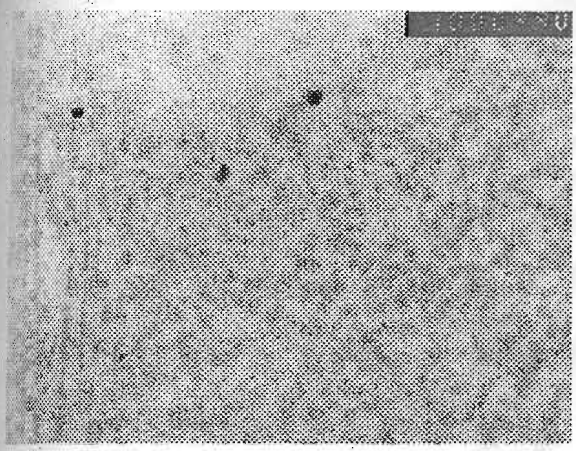
Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000



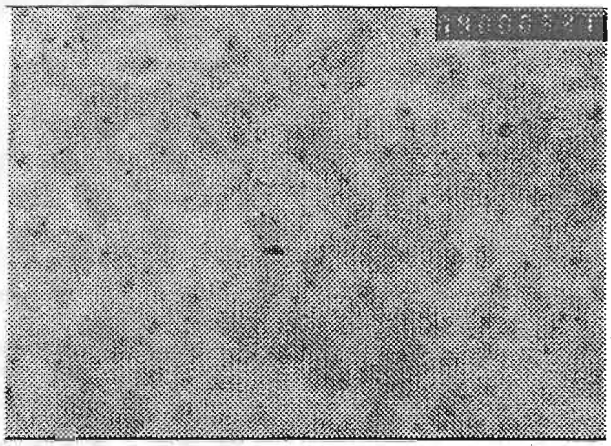
A



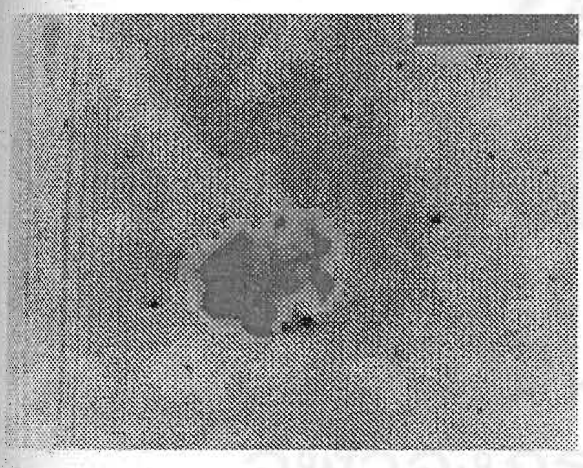
B



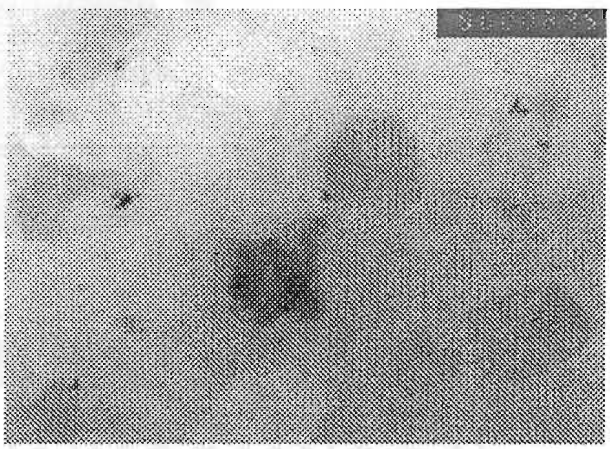
C



D



E



F

FIGURE 61 TEM photomicrographs of microemulsion system (ME13) containing IPM-TW-GR, Em=A, Em/oil=7/3, Aq 3 %

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 66000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000

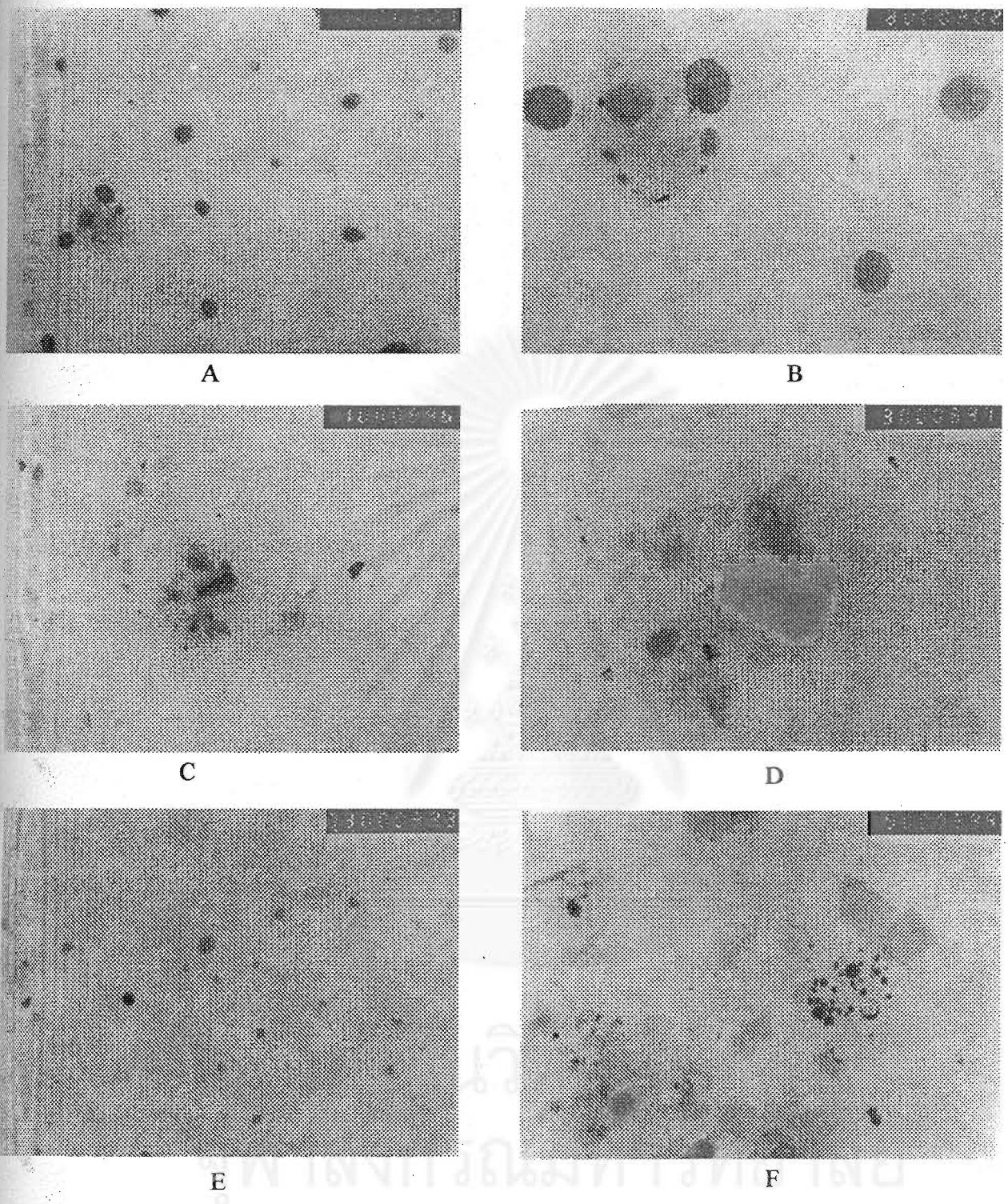
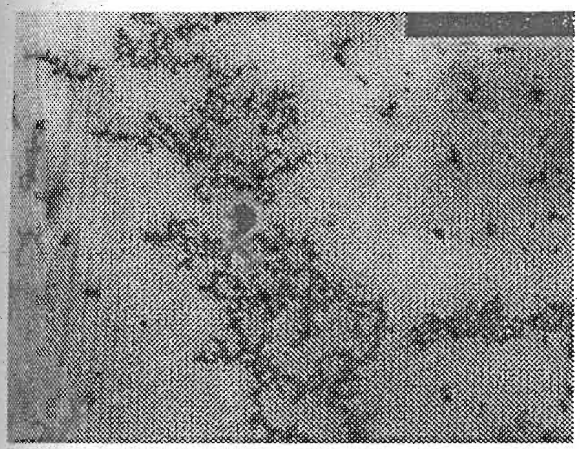
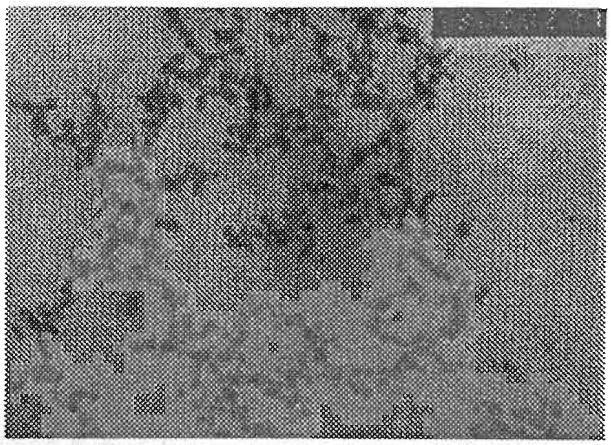


FIGURE 62 TEM photomicrographs of microemulsion system (ME14) containing IPM-TW-GR, Em=B, Em/oil=7/3, Aq 2 %

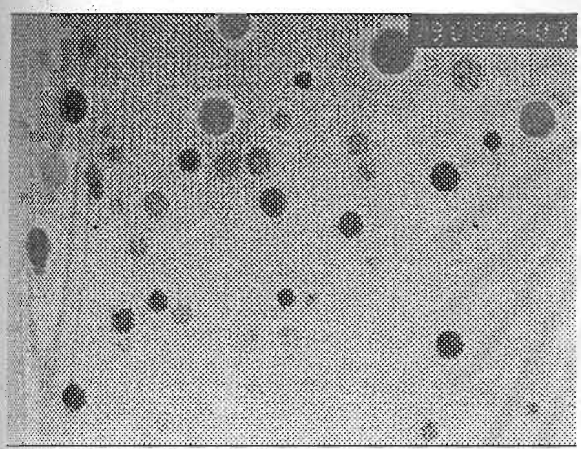
Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 45000 : F 6 months at 50° c , x 45000



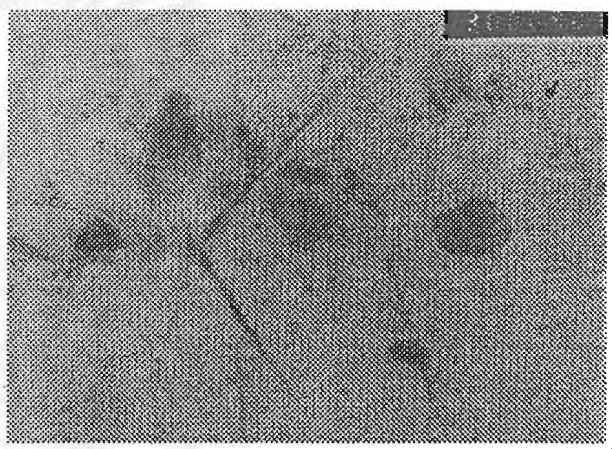
A



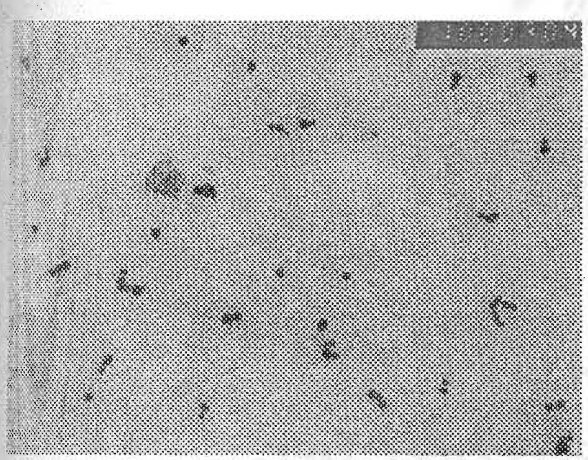
B



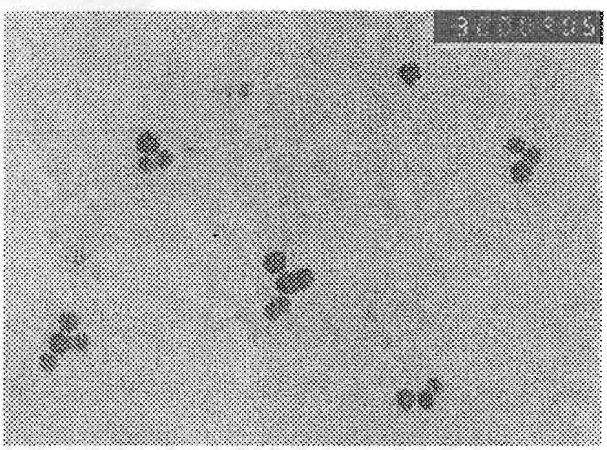
C



D



E



F

FIGURE 63 TEM photomicrographs of microemulsion system (ME15) containing IPM-TW-GR, Em=C, Em/oil=7/3, Aq 4 %

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 45000 : D 6 months at 4° c , x 45000
E 6 months at 50° c , x 16500 : F 6 months at 50° c , x 45000

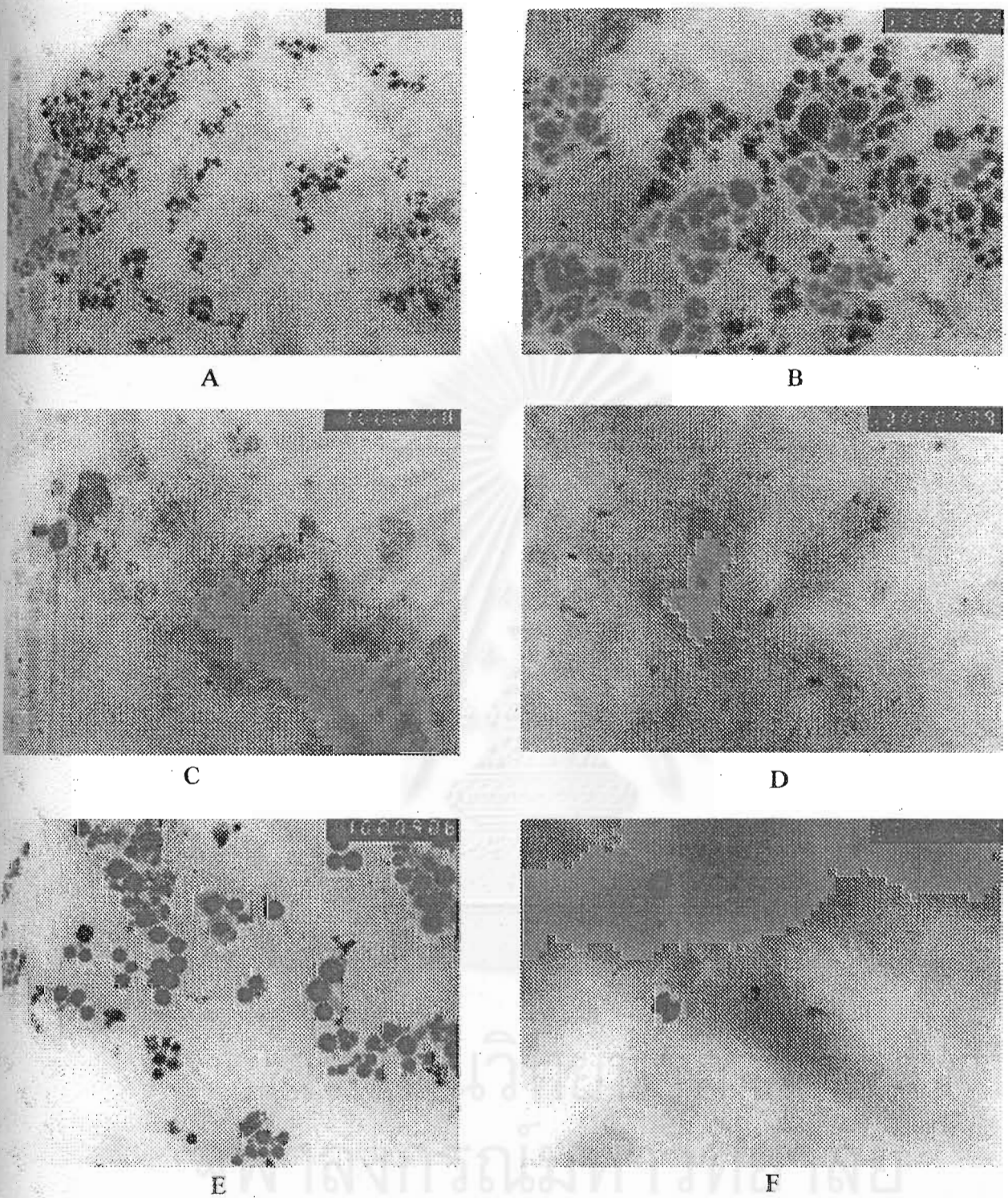


FIGURE 64 TEM photomicrographs of microemulsion system (ME16) containing EO-PC-PG, Em=A, Em/oil=5/5, Aq 4%

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 30° c , x 45000
E 6 months at 4° c , x 16500 : F 6 months at 50° c , x 16500

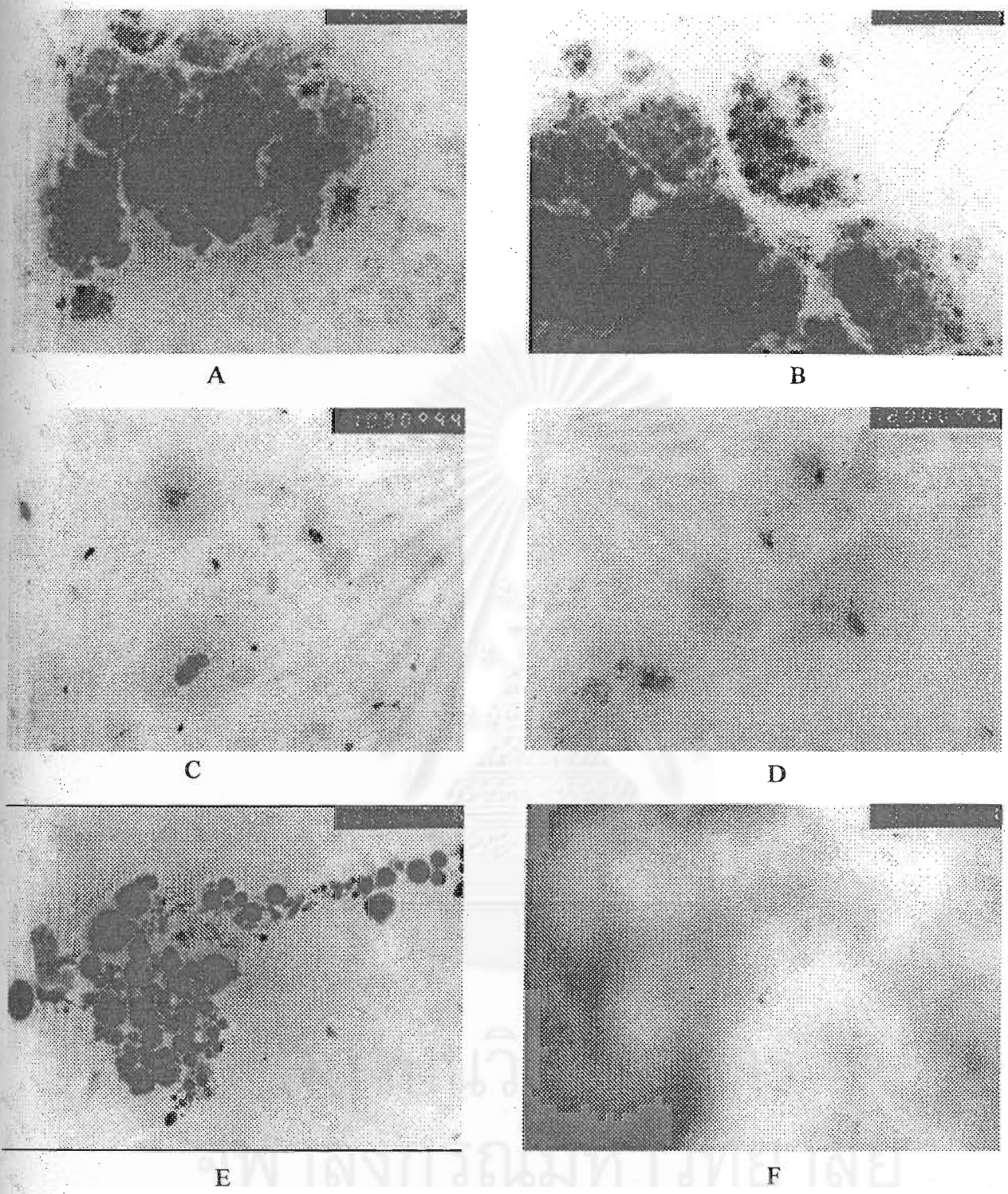
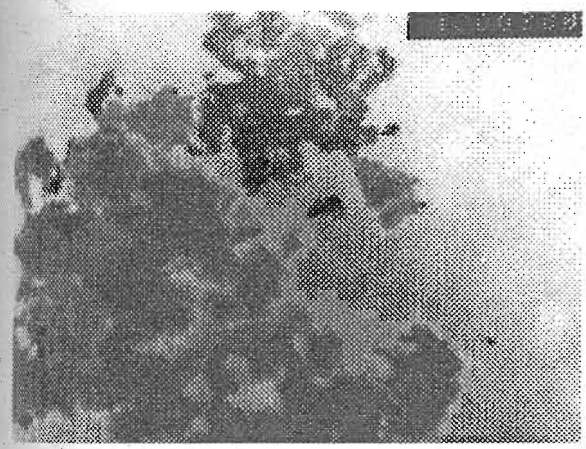
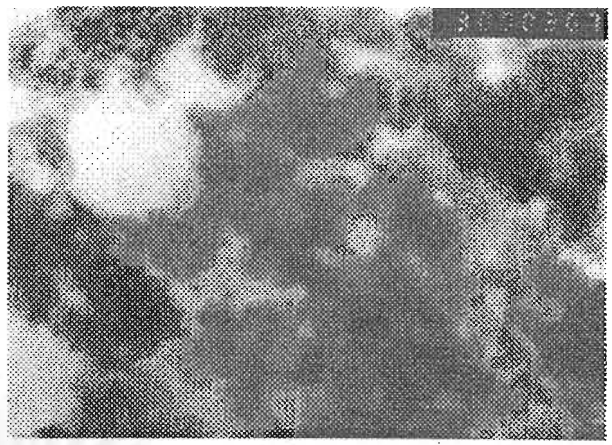


FIGURE 65 TEM photomicrographs of microemulsion system (ME17) containing EO-PC-PG, Em=B, Em/oil=5/5, Aq 4 %

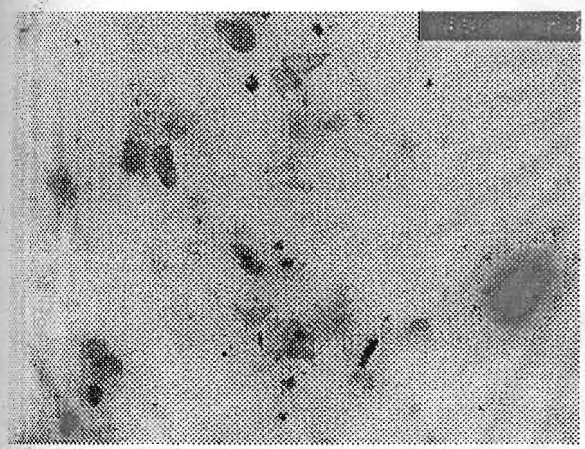
Key : A initial at 30^{0}c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 30^0 c , x 45000
E 6 months at 4^0 c , x 45000 : F 6 months at 50^0 c , x 16500



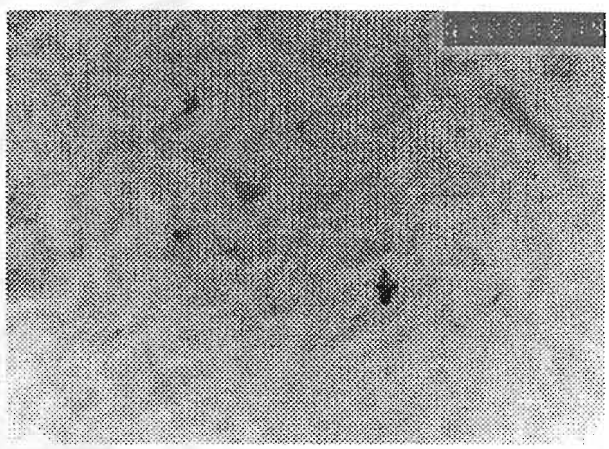
A



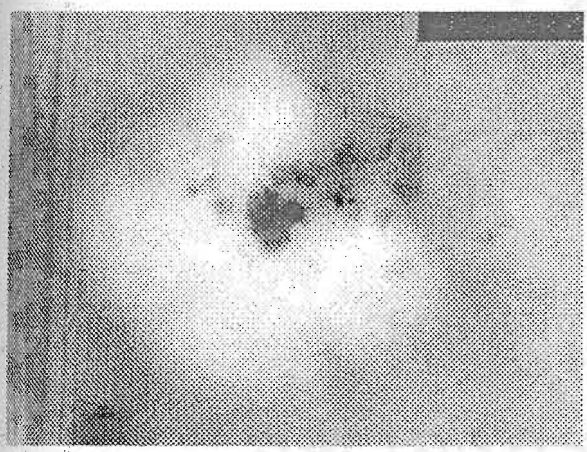
B



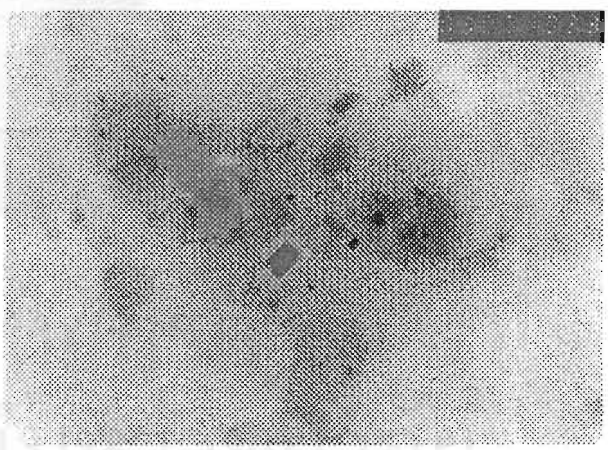
C



D



E



F

FIGURE 66 TEM photomicrographs of microemulsion system (ME18) containing EO-PC-PG, Em=C, Em/oil=5/5, Aq 4 %

Key : A initial at 30° c , x 13200 : B initial at 30° c , x 36000
C 6 months at 30° c , x 16500 : D 6 months at 4° c , x 45000
E 6 months at 50° c , x 16500 : F 6 months at 50° c , x 45000

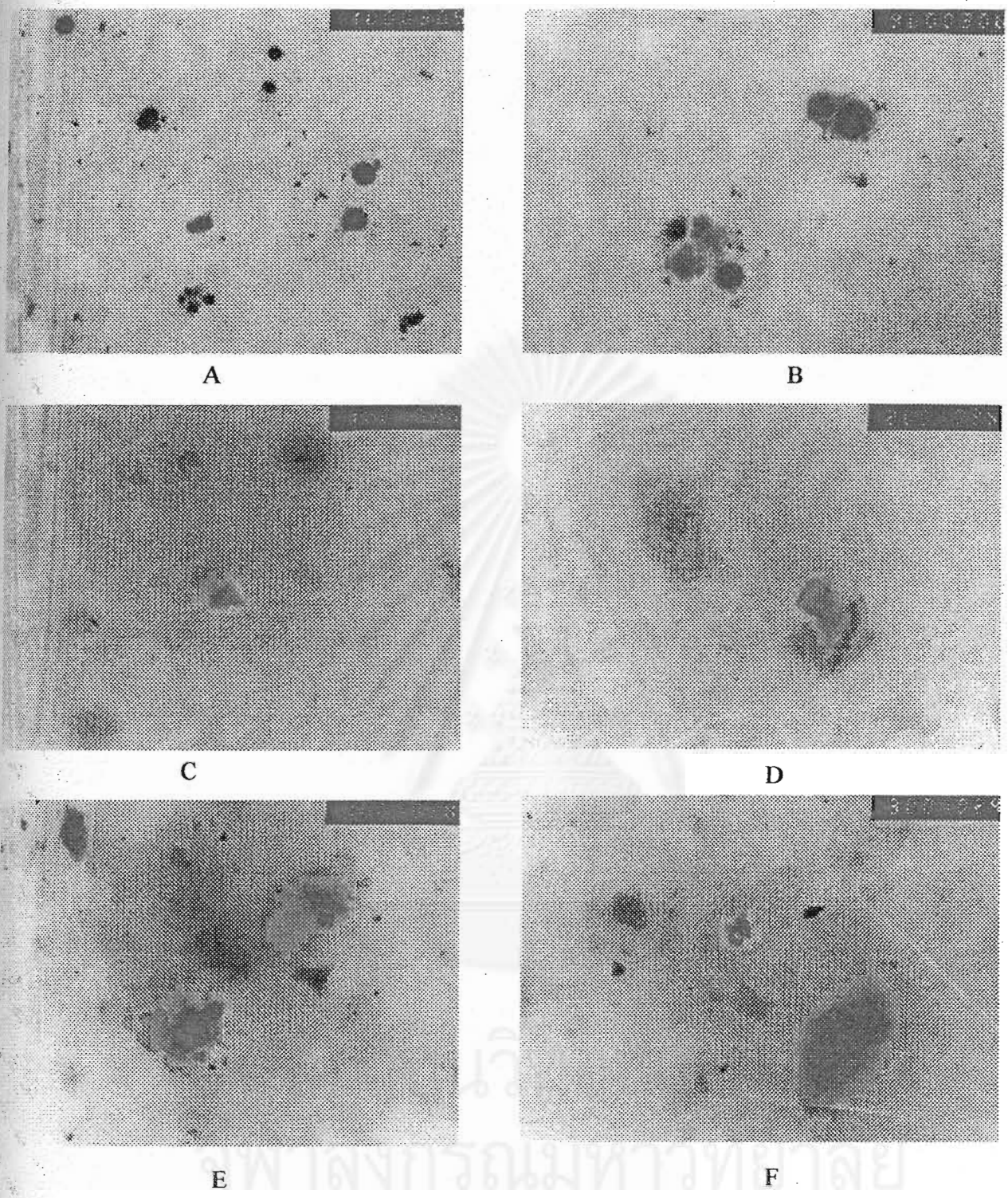


FIGURE 67 TEM photomicrographs of microemulsion system (ME19) containing IPM-PC-PG, Em=A, Em/oil=5/5, Aq 4 %

Key : A initial at 30^0 c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 4^0 c , x 45000
E 6 months at 50^0 c , x 16500 : F 6 months at 50^0 c , x 45000

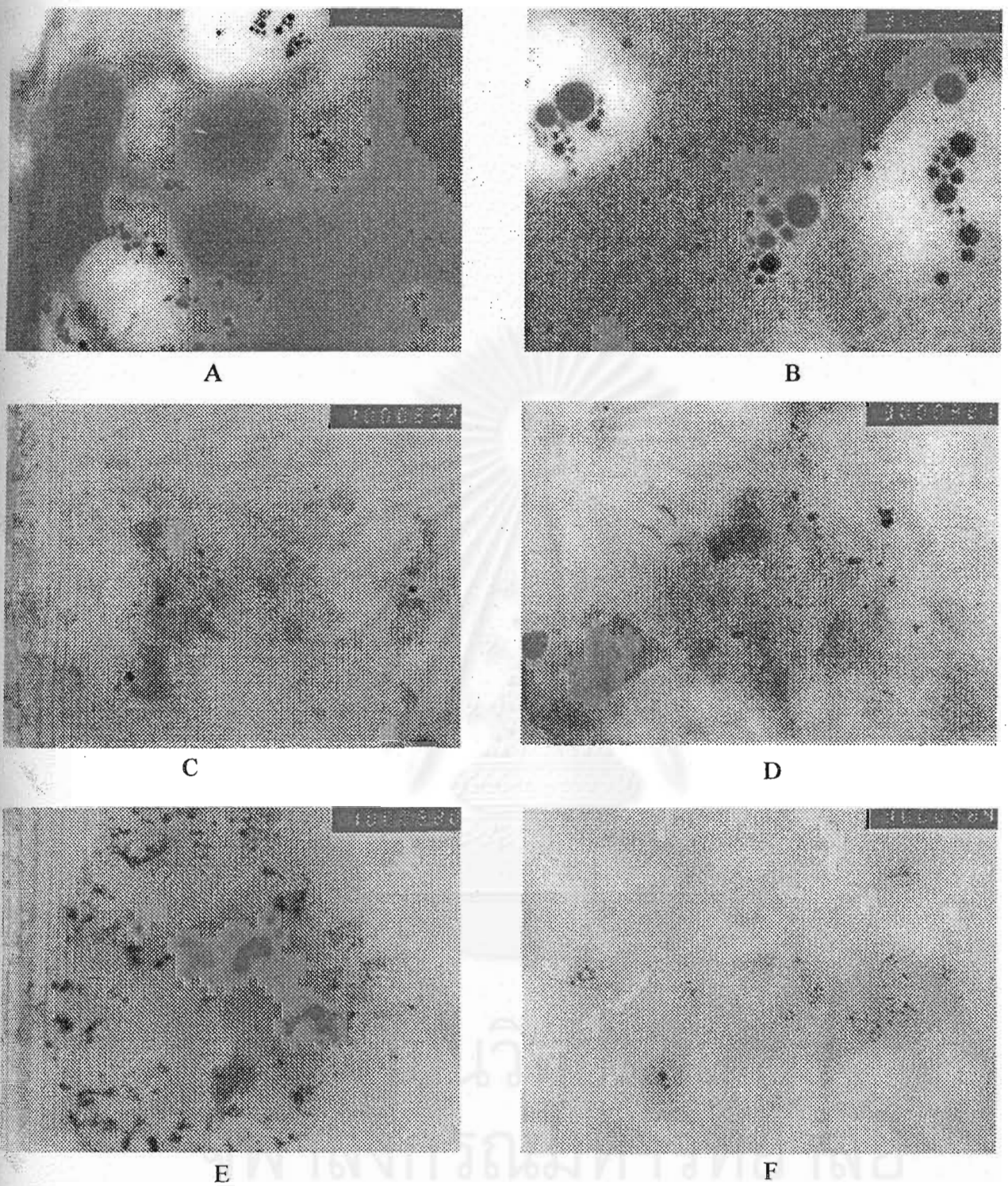


FIGURE 68 TEM photomicrographs of microemulsion system (ME20) containing MCT-PC-PG, Em=A, Em/oil=5/5, Aq 2 %

Key : A initial at 30^{0}c , x 13200 : B initial at 30^0 c , x 36000
C 6 months at 30^0 c , x 16500 : D 6 months at 30^0 c , x 45000
E 6 months at 4^0 c , x 16500 : F 6 months at 50^0 c , x 16500

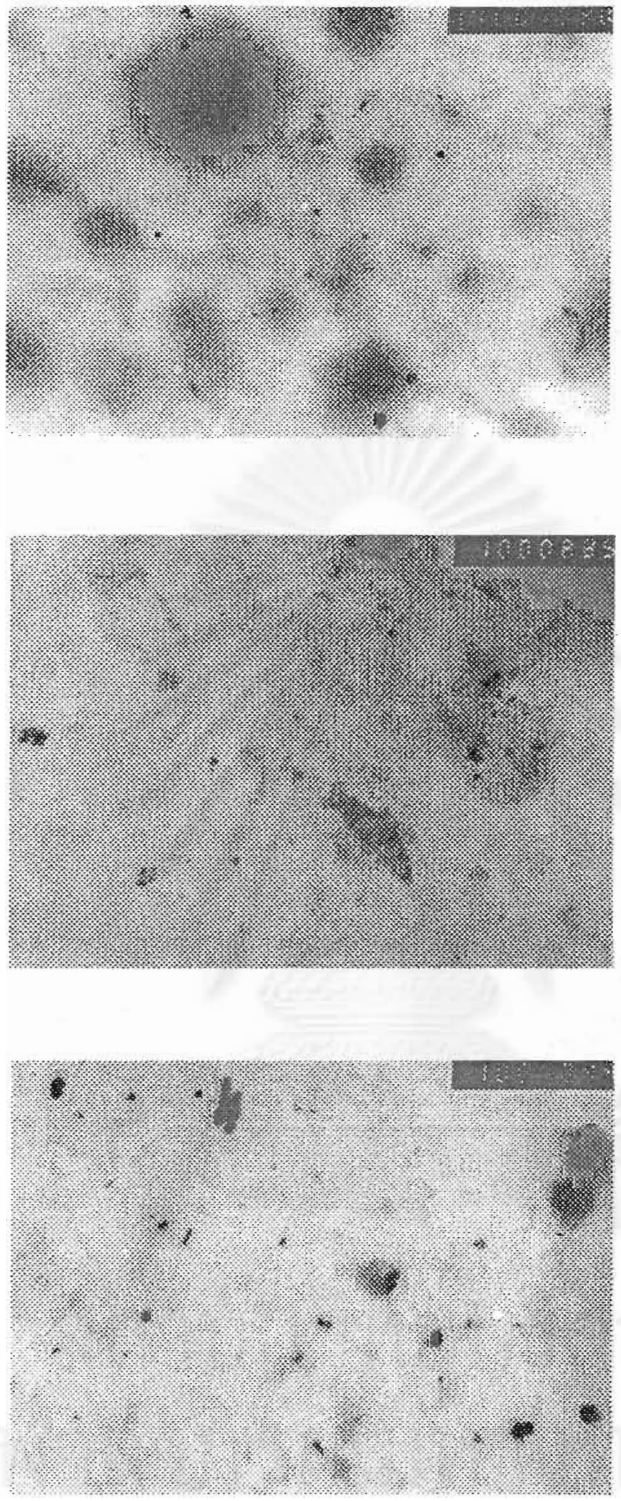
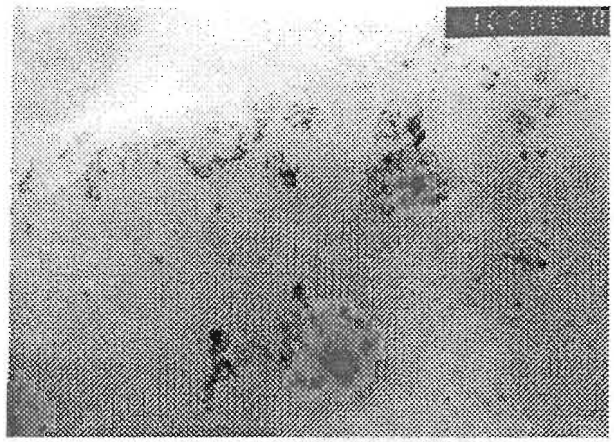
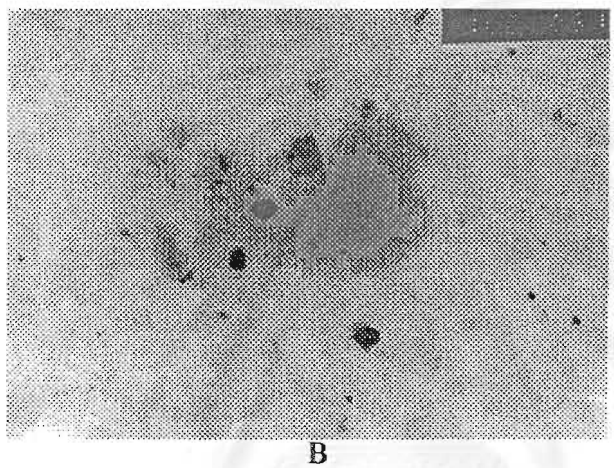


FIGURE 69 TEM photomicrographs of microemulsion system containing IPM-PC-PG (5/5), Em=A, Em/oil=5/5

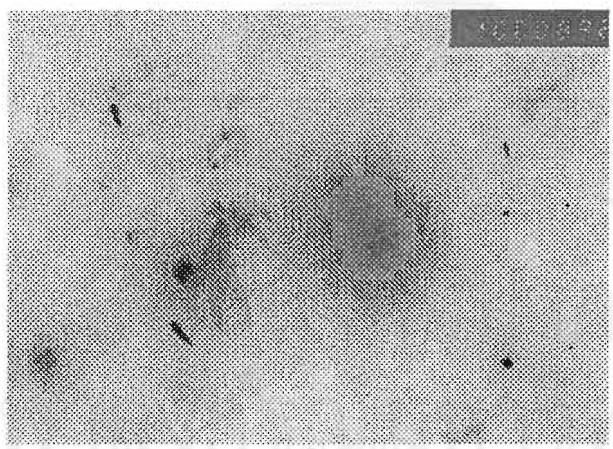
Key : A ME16, 4% aquous phase , at 30°c , x 16500
B ME21, 10% aquous phase , at 30°c , x 16500
C ME22, 15% aquous phase , at 30°c , x 16500



A



B



C

FIGURE 70 TEM photomicrographs of microemulsion system containing EO-PC-PG (6/4), Em=A, Em/oil=6/4

Key : A ME23, 4% aquous phase , at 30°c , x 16500
B ME24, 10% aquous phase , at 30°c , x 16500
C ME25, 15% aquous phase , at 30°c , x 16500

Table 19 Statistical data of microemulsion droplet size of freshly prepared ME1 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	86
	Missing	0
Mean		93.4553
Median		77.6100
Mode		25.87
Std. Deviation		70.8435
Minimum		11.72
Maximum		323.59
Sum		8037.16

a. FORMULAR = ME1-30C-M0 (A)

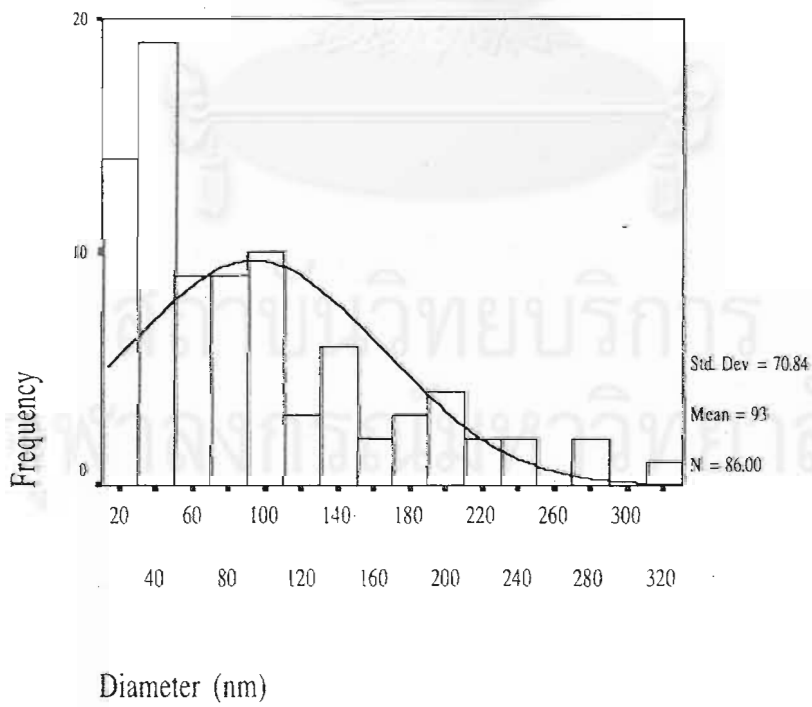


Figure 71 Droplet size histogram of freshly prepared ME1storage at temperature of 30°C

Table 20 Droplet size frequency of freshly prepared ME1 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.72	2	2.3	2.3	2.3
20.01	3	3.5	3.5	5.8
23.44	1	1.2	1.2	7.0
25.87	6	7.0	7.0	14.0
29.30	2	2.3	2.3	16.3
31.73	3	3.5	3.5	19.8
34.16	1	1.2	1.2	20.9
37.59	3	3.5	3.5	24.4
40.02	3	3.5	3.5	27.9
43.45	2	1.2	1.2	29.1
45.88	3	3.5	3.5	32.6
46.89	1	1.2	1.2	33.7
48.31	3	3.5	3.5	37.2
49.32	1	1.2	1.2	38.4
50.74	1	1.2	1.2	39.5
54.17	2	2.3	2.3	41.9
56.60	1	1.2	1.2	43.0
61.03	2	2.3	2.3	45.3
62.46	1	1.2	1.2	46.5
68.32	1	1.2	1.2	47.7
69.32	1	1.2	1.2	48.8
77.61	2	2.3	2.3	51.2
78.62	1	1.2	1.2	52.3
82.47	1	1.2	1.2	53.5
83.47	2	2.3	2.3	55.8
86.91	1	1.2	1.2	57.0
88.33	1	1.2	1.2	58.1
89.33	1	1.2	1.2	59.3
91.76	1	1.2	1.2	60.5
94.19	1	1.2	1.2	61.6
95.19	1	1.2	1.2	62.8
97.62	3	3.5	3.5	66.3
100.05	1	1.2	1.2	67.4
103.48	2	2.3	2.3	69.8
105.91	1	1.2	1.2	70.9
115.20	1	1.2	1.2	72.1
117.63	2	2.3	2.3	74.4
131.78	1	1.2	1.2	75.6
135.21	1	1.2	1.2	76.7
143.50	1	1.2	1.2	77.9
145.93	2	2.3	2.3	80.2
149.36	1	1.2	1.2	81.4
152.80	1	1.2	1.2	82.6
155.23	1	1.2	1.2	83.7
171.80	1	1.2	1.2	84.9
175.65	1	1.2	1.2	86.0
188.38	1	1.2	1.2	87.2
193.23	1	1.2	1.2	88.4
200.10	1	1.2	1.2	89.5
203.53	1	1.2	1.2	90.7
205.96	1	1.2	1.2	91.9
211.82	1	1.2	1.2	93.0
224.96	1	1.2	1.2	94.2
233.25	1	1.2	1.2	95.3
248.41	1	1.2	1.2	96.5
283.57	1	1.2	1.2	97.7
284.99	1	1.2	1.2	98.8
323.59	1	1.2	1.2	100.0
Total	86	100.0	100.0	

Table 21 Statistical data of microemulsion droplet size of ME1 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	166
	Missing	0
Mean		22.1192
Median		18.1850
Mode		14.25
Std. Deviation		17.2607
Minimum		3.39
Maximum		172.02
Sum		3671.79

a. FORMULAR = ME1-30C-M6 (D)

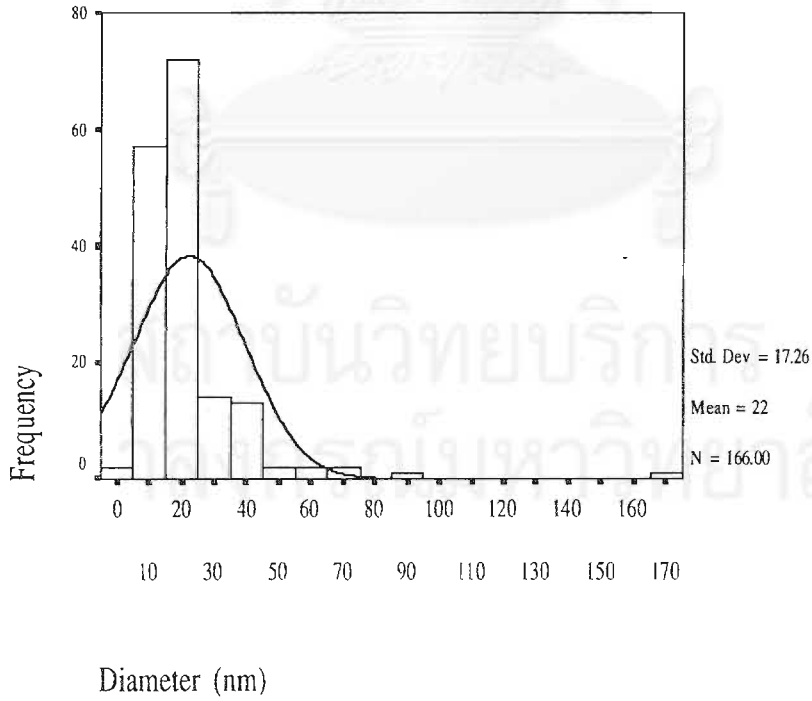


Figure 72 Droplet size histogram of ME1 after 6 months storage at temperature of 30°C

Table 22 Droplet size frequency of ME1 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	2	1.2	1.2	1.2
5.08	1	0.6	0.6	1.8
5.78	2	1.2	1.2	3.0
7.47	3	1.8	1.8	4.8
8.17	1	0.6	0.6	5.4
9.17	2	1.2	1.2	6.6
10.86	8	4.8	4.8	11.4
11.56	8	4.8	4.8	16.3
11.85	1	0.6	0.6	16.9
12.26	1	0.6	0.6	17.5
12.55	6	3.6	3.6	21.1
13.26	6	3.6	3.6	24.7
13.55	1	0.6	0.6	25.3
14.25	9	5.4	5.4	30.7
14.95	8	4.8	4.8	35.5
15.24	1	0.6	0.6	36.1
15.65	3	1.8	1.8	38.0
15.94	5	3.0	3.0	41.0
16.35	1	0.6	0.6	41.6
16.64	7	4.2	4.2	45.8
17.34	1	0.6	0.6	46.4
17.63	5	3.0	3.0	49.4
18.04	1	0.6	0.6	50.0
18.33	6	3.6	3.6	53.6
19.03	3	1.8	1.8	55.4
19.74	2	1.2	1.2	56.6
20.03	3	1.8	1.8	58.4
20.32	1	0.6	0.6	59.0
20.44	1	0.6	0.6	59.6
20.73	6	3.6	3.6	63.3
21.02	2	1.2	1.2	64.5
21.43	1	0.6	0.6	65.1
21.72	2	1.2	1.2	66.3
22.01	1	0.6	0.6	66.9
22.13	1	0.6	0.6	67.5
22.42	5	3.0	3.0	70.5
22.71	1	0.6	0.6	71.1
23.12	3	1.8	1.8	72.9
23.41	3	1.8	1.8	74.7
23.82	1	0.6	0.6	75.3
24.12	6	3.6	3.6	78.9
25.10	1	0.6	0.6	79.5
25.52	1	0.6	0.6	80.1
26.51	1	0.6	0.6	80.7
27.50	2	1.2	1.2	81.9
28.90	1	0.6	0.6	82.5
30.60	3	1.8	1.8	84.3
32.00	1	0.6	0.6	84.9
32.29	1	0.6	0.6	85.5
33.98	1	0.6	0.6	86.1
34.97	2	1.2	1.2	87.3
35.67	1	0.6	0.6	88.0
37.08	1	0.6	0.6	88.6
37.37	4	2.4	2.4	91.0
38.07	1	0.6	0.6	91.6
40.05	1	0.6	0.6	92.2
40.76	1	0.6	0.6	92.8
41.17	1	0.6	0.6	93.4
41.46	2	1.2	1.2	94.6
42.86	1	0.6	0.6	95.2
48.23	1	0.6	0.6	95.8
49.34	1	0.6	0.6	96.4
62.19	1	0.6	0.6	97.0
63.59	1	0.6	0.6	97.6
68.38	1	0.6	0.6	98.2
73.75	1	0.6	0.6	98.8
89.10	1	0.6	0.6	99.4
172.02	1	0.6	0.6	100.0
Total	166	100.0	100.0	100.0

Table 23 Statistical data of microemulsion droplet size of freshly prepared ME2 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	54
	Missing	0
Mean		21.7332
Median		14.8925
Mode		8.47
Std. Diviation		26.6476
Minimum		4.24
Maximum		158.12
Sum		1173.59

a. FORMULAR = ME2-30C-M0 (B)

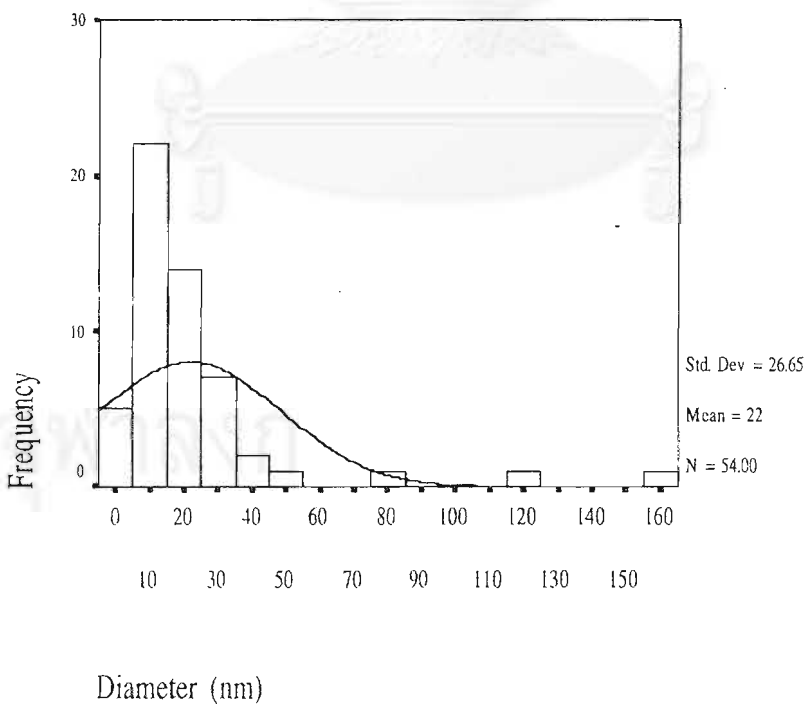


Figure 73 Droplet size histogram of freshly prepared ME2 storage at temperature of 30°C

Table 24 Droplet size frequency of freshly prepared ME2 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
4.24	5	9.3	9.3	9.3
6.35	2	3.7	3.7	13.0
7.23	4	7.4	7.4	20.4
8.47	6	11.1	11.1	31.5
9.35	2	3.7	3.7	35.2
11.46	2	3.7	3.7	38.9
12.34	2	3.7	3.7	42.6
12.70	1	1.9	1.9	44.4
13.58	2	3.7	3.7	48.1
14.46	1	1.9	1.9	50.0
15.33	2	3.7	3.7	53.7
15.69	1	1.9	1.9	55.6
16.57	4	7.4	7.4	63.0
18.32	1	1.9	1.9	64.8
18.69	3	5.6	5.6	70.4
19.57	1	1.9	1.9	72.2
22.04	1	1.9	1.9	74.1
24.68	1	1.9	1.9	75.9
25.92	1	1.9	1.9	77.8
28.03	1	1.9	1.9	79.6
28.39	1	1.9	1.9	81.5
28.91	1	1.9	1.9	83.3
29.79	1	1.9	1.9	85.2
30.15	1	1.9	1.9	87.0
33.14	1	1.9	1.9	88.9
37.37	1	1.9	1.9	90.7
43.72	1	1.9	1.9	92.6
47.96	1	1.9	1.9	94.4
79.50	1	1.9	1.9	96.3
115.12	1	1.9	1.9	98.1
158.12	1	1.9	1.9	100.0
Total	54	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 25 Statistical data of microemulsion droplet size of ME2 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	173
	Missing	0
Mean		16.0829
Median		14.2482
Mode		3.39
Std. Deviation		10.5285
Minimum		3.39
Maximum		59.80
Sum		2782.33

a. FORMULAR = ME2-30C-M6 (D)

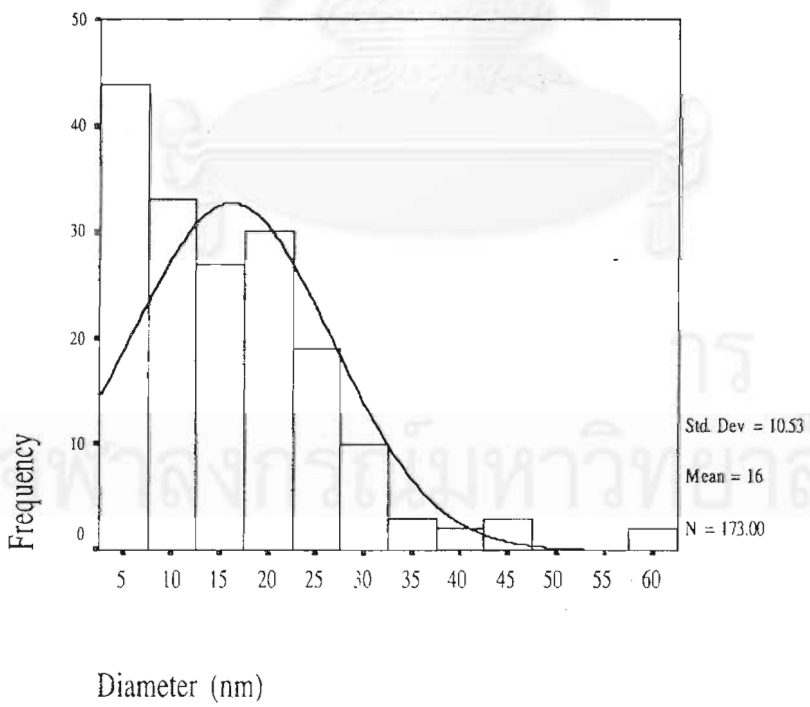


Figure 74 Droplet size histogram of ME2 after 6 months storage at temperature of 30°C

Table 26 Droplet size frequency of ME2 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	19	11.0	11.0	11.0
5.08	5	2.9	2.9	13.9
5.78	9	5.2	5.2	19.1
7.47	11	6.4	6.4	25.4
8.47	2	1.2	1.2	26.6
9.17	2	1.2	1.2	27.7
9.87	4	2.3	2.3	30.1
10.16	6	3.5	3.5	33.5
10.86	8	4.6	4.6	38.2
11.56	7	4.0	4.0	42.2
12.27	4	2.3	2.3	44.5
12.56	2	1.2	1.2	45.7
13.26	4	2.3	2.3	48.0
13.55	2	1.2	1.2	49.1
14.25	10	5.8	5.8	54.9
15.65	2	1.2	1.2	56.1
15.94	2	1.2	1.2	57.2
16.35	4	2.3	2.3	59.5
16.64	1	0.6	0.6	60.1
17.64	4	2.3	2.3	62.4
18.05	1	0.6	0.6	63.0
18.34	5	2.9	2.9	65.9
19.04	2	1.2	1.2	67.1
19.33	1	0.6	0.6	67.6
19.74	1	0.6	0.6	68.2
20.03	1	0.6	0.6	68.8
20.32	1	0.6	0.6	69.4
20.44	1	0.6	0.6	69.9
20.73	3	1.7	1.7	71.7
21.02	2	1.2	1.2	72.8
21.72	3	1.7	1.7	74.6
22.43	5	2.9	2.9	77.5
23.13	1	0.6	0.6	78.0
24.12	5	2.9	2.9	80.9
24.82	2	1.2	1.2	82.1
25.11	2	1.2	1.2	83.2
25.81	2	1.2	1.2	84.4
26.51	6	3.5	3.5	87.9
27.21	1	0.6	0.6	88.4
27.50	2	1.2	1.2	89.6
28.21	2	1.2	1.2	90.8
28.50	1	0.6	0.6	91.3
28.91	2	1.2	1.2	92.5
29.90	1	0.6	0.6	93.1
30.60	1	0.6	0.6	93.6
32.30	1	0.6	0.6	94.2
33.99	2	1.2	1.2	95.4
34.98	1	0.6	0.6	96.0
38.37	1	0.6	0.6	96.5
42.46	1	0.6	0.6	97.1
45.84	1	0.6	0.6	97.7
46.54	1	0.6	0.6	98.3
47.24	1	0.6	0.6	98.8
57.82	1	0.6	0.6	99.4
59.80	1	0.6	0.6	100.0
Total	173	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 27 Statistical data of microemulsion droplet size of ME2 after 6 months storage at temperature of 50°C

Statistics[a]

DIAMETER

N	Valid	25
	Missing	0
Mean		20.1004
Median		16.6441
Mode		3.39
Std. Deviation		16.9315
Minimum		3.39
Maximum		76.44
Sum		502.51

a. FORMULAR = ME2-50C-M6 (F)

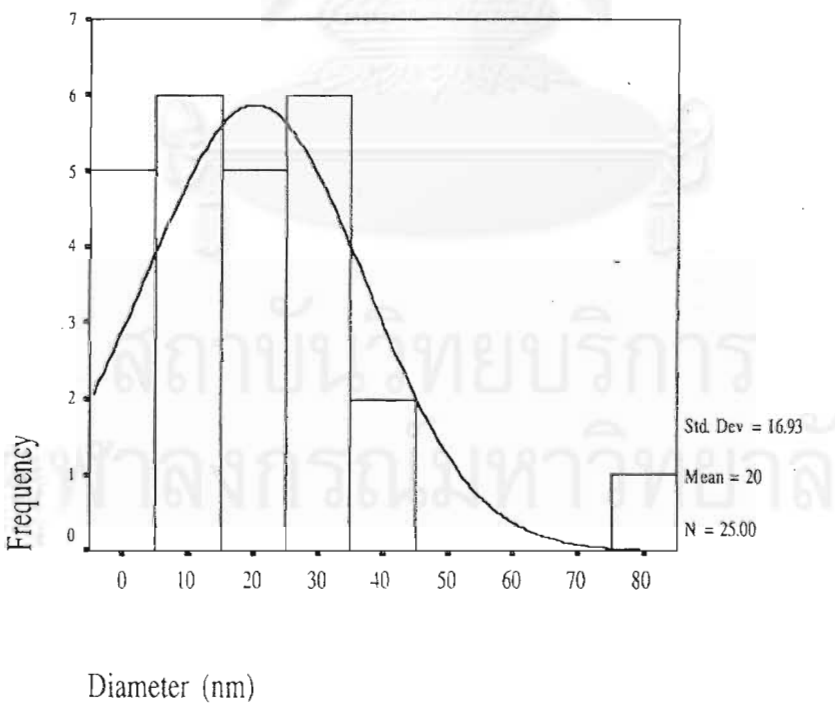


Figure 75 Droplet size histogram of ME2 after 6 months storage at temperature of 50°C

Table 28 Droplet size frequency of ME2 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	5	20.0	20.0	20.0
6.77	1	4.0	4.0	24.0
7.47	2	8.0	8.0	32.0
9.87	3	12.0	12.0	44.0
15.94	1	4.0	4.0	48.0
16.64	1	4.0	4.0	52.0
16.93	1	4.0	4.0	56.0
20.73	1	4.0	4.0	60.0
21.43	1	4.0	4.0	64.0
29.20	1	4.0	4.0	68.0
29.90	1	4.0	4.0	72.0
31.59	1	4.0	4.0	76.0
32.00	1	4.0	4.0	80.0
33.58	1	4.0	4.0	84.0
34.98	1	4.0	4.0	88.0
37.08	1	4.0	4.0	92.0
37.79	1	4.0	4.0	96.0
76.44	1	4.0	4.0	100.0
Total	25	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 29 Statistical data of microemulsion droplet size of ME3 after 6 months storage at temperature of 30°C

Statistics

DIAMETER

N	Valid	15
	Missing	0
Mean		16.3314
Median		12.5555
Mode		3.39
Std. Deviation		12.6360
Minimum		3.39
Maximum		46.54
Sum		244.97

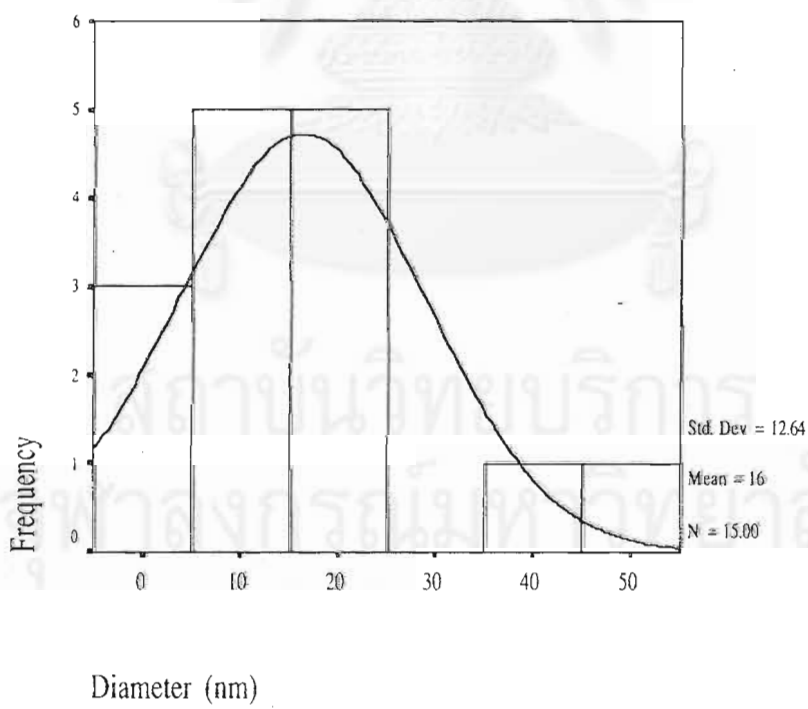


Figure 76 Droplet size histogram of ME3 after 6 months storage at temperature of 30°C

Table 30 Droplet size frequency of ME3 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	20.0	20.0	20.0
5.78	1	6.7	6.7	26.7
10.57	1	6.7	6.7	33.3
11.85	1	6.7	6.7	40.0
12.56	2	13.3	13.3	53.3
15.94	1	6.7	6.7	60.0
16.35	1	6.7	6.7	66.7
19.04	1	6.7	6.7	73.3
20.03	1	6.7	6.7	80.0
23.83	1	6.7	6.7	86.7
39.77	1	6.7	6.7	93.3
46.54	1	6.7	6.7	100.0
Total	15	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 31 Statistical data of microemulsion droplet size of freshly prepared ME4 storage at temperature of 30°C

Statistics[b]

DIAMETER

N	Valid	70
	Missing	0
Mean		56.4132
Median		41.2411
Mode		11.73[a]
Std. Deviation		42.1371
Minimum		11.73
Maximum		203.57
Sum		3948.93

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME4-30C-M0 (A)

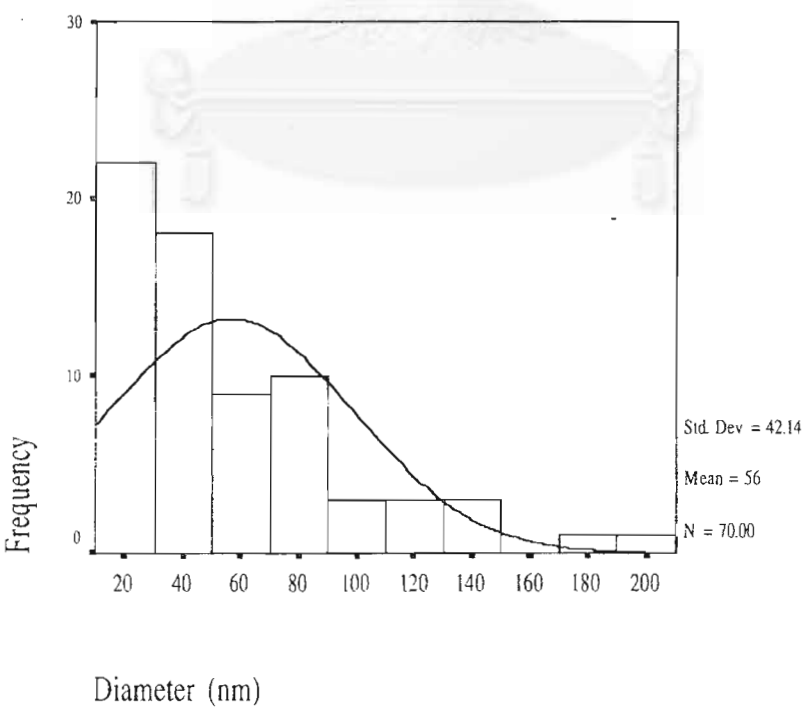


Figure 77 Droplet size histogram of freshly prepared ME4 storage at temperature of 30°C

Table 32 Droplet size frequency of freshly prepared ME4 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	6	8.6	8.6	8.6
17.59	3	4.3	4.3	12.9
20.01	5	7.1	7.1	20.0
23.45	2	2.9	2.9	22.9
25.87	4	5.7	5.7	28.6
28.30	1	1.4	1.4	30.0
29.31	1	1.4	1.4	31.4
31.74	6	8.6	8.6	40.0
34.16	1	1.4	1.4	41.4
35.17	3	4.3	4.3	45.7
37.60	1	1.4	1.4	47.1
40.03	2	2.9	2.9	50.0
42.46	1	1.4	1.4	51.4
46.89	2	2.9	2.9	54.3
49.32	2	2.9	2.9	57.1
55.18	1	1.4	1.4	58.6
61.05	3	4.3	4.3	62.9
62.47	1	1.4	1.4	64.3
63.47	1	1.4	1.4	65.7
66.91	2	2.9	2.9	68.6
69.33	1	1.4	1.4	70.0
75.20	4	5.7	5.7	75.7
77.62	2	2.9	2.9	78.6
80.05	1	1.4	1.4	80.0
81.06	2	2.9	2.9	82.9
88.34	1	1.4	1.4	84.3
92.78	1	1.4	1.4	85.7
97.64	1	1.4	1.4	87.1
106.93	1	1.4	1.4	88.6
112.80	1	1.4	1.4	90.0
128.37	1	1.4	1.4	91.4
129.38	1	1.4	1.4	92.9
131.80	1	1.4	1.4	94.3
142.52	1	1.4	1.4	95.7
149.39	1	1.4	1.4	97.1
185.98	1	1.4	1.4	98.6
203.57	1	1.4	1.4	100.0
Total	70	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 33 Statistical data of microemulsion droplet size of ME4 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	211
	Missing	0
Mean		50.5637
Median		39.4800
Mode		34.69
Std. Deviation		49.2509
Minimum		3.39
Maximum		483.14
Sum		10668.9

a. FORMULAR = ME4-30C-M6 (D)

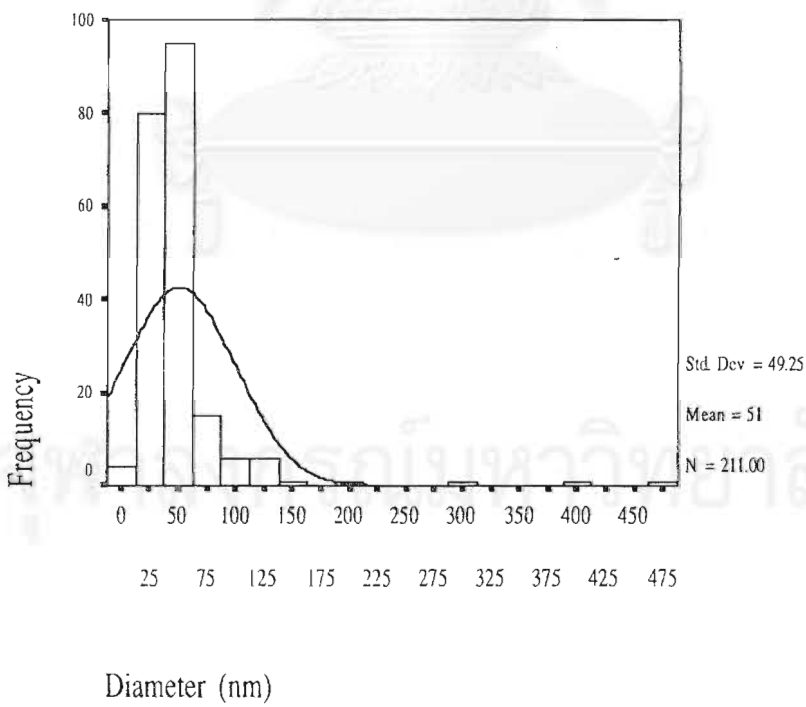


Figure 78 Droplet size histogram of ME4 after 6 months storage at temperature of 30°C

Table 34 Droplet size frequency of ME4 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	2	0.9	0.9	.9
9.87	1	0.5	0.5	1.4
11.85	1	0.5	0.5	1.9
15.65	2	0.9	0.9	2.8
15.94	3	1.4	1.4	4.3
17.64	1	0.5	0.5	4.7
19.04	1	0.5	0.5	5.2
19.33	1	0.5	0.5	5.7
19.74	2	0.9	0.9	6.6
20.44	1	0.5	0.5	7.1
20.73	1	0.5	0.5	7.6
22.72	1	0.5	0.5	8.1
23.42	1	0.5	0.5	8.5
24.12	1	0.5	0.5	9.0
25.52	1	0.5	0.5	9.5
26.51	1	0.5	0.5	10.0
27.50	4	1.9	1.9	11.8
27.92	2	0.9	0.9	12.8
29.90	5	2.4	2.4	15.2
31.30	2	0.9	0.9	16.1
31.59	4	1.9	1.9	18.0
32.30	4	1.9	1.9	19.9
32.59	1	0.5	0.5	20.4
33.29	2	0.9	0.9	21.3
33.70	1	0.5	0.5	21.8
33.99	2	0.9	0.9	22.7
34.69	11	5.2	5.2	28.0
34.98	2	0.9	0.9	28.9
35.39	4	1.9	1.9	30.8
35.68	6	2.8	2.8	33.6
36.38	6	2.8	2.8	36.5
36.67	1	0.5	0.5	37.0
37.08	4	1.9	1.9	38.9
37.37	2	0.9	0.9	39.8
37.79	1	0.5	0.5	40.3
38.08	9	4.3	4.3	44.5
38.78	5	2.4	2.4	46.9
39.07	3	1.4	1.4	48.3
39.48	5	2.4	2.4	50.7
39.77	6	2.8	2.8	53.6
40.47	7	3.3	3.3	56.9
40.76	1	0.5	0.5	57.3
41.17	3	1.4	1.4	58.8
41.46	7	3.3	3.3	62.1
42.17	5	2.4	2.4	64.5
42.87	2	0.9	0.9	65.4
43.16	2	0.9	0.9	66.4
43.86	7	3.3	3.3	69.7
44.56	2	0.9	0.9	70.6
44.85	2	0.9	0.9	71.6
45.26	2	0.9	0.9	72.5
45.55	2	0.9	0.9	73.5
45.84	1	0.5	0.5	73.9
45.96	1	0.5	0.5	74.4
46.25	2	0.9	0.9	75.4
46.54	2	0.9	0.9	76.3
46.95	1	0.5	0.5	76.8
47.24	3	1.4	1.4	78.2
47.95	3	1.4	1.4	79.6
48.65	2	0.9	0.9	80.6
48.94	1	0.5	0.5	81.0
49.35	1	0.5	0.5	81.5
50.34	1	0.5	0.5	82.0
52.04	3	1.4	1.4	83.4
53.73	1	0.5	0.5	83.9
58.11	1	0.5	0.5	84.4
58.52	1	0.5	0.5	84.8
72.07	1	0.5	0.5	85.3
73.76	1	0.5	0.5	85.8
74.75	1	0.5	0.5	86.3
75.45	1	0.5	0.5	86.7
76.15	1	0.5	0.5	87.2
76.85	1	0.5	0.5	87.7
77.15	1	0.5	0.5	88.2
79.13	1	0.5	0.5	88.6
81.94	1	0.5	0.5	89.1
82.93	1	0.5	0.5	89.6
84.04	1	0.5	0.5	90.0
84.33	2	0.9	0.9	91.0
85.32	1	0.5	0.5	91.5
85.61	1	0.5	0.5	91.9
88.71	1	0.5	0.5	92.4
93.50	1	0.5	0.5	92.9
94.20	1	0.5	0.5	93.4
107.34	1	0.5	0.5	93.8
107.46	1	0.5	0.5	94.3
108.45	1	0.5	0.5	94.8
120.01	1	0.5	0.5	95.3
124.39	1	0.5	0.5	95.7
124.80	1	0.5	0.5	96.2
126.79	1	0.5	0.5	96.7
135.37	1	0.5	0.5	97.2
136.95	1	0.5	0.5	97.6
147.93	1	0.5	0.5	98.1
208.14	1	0.5	0.5	98.6
294.16	1	0.5	0.5	99.1
389.64	1	0.5	0.5	99.5
483.14	1	0.5	0.5	100.0
Total	211	100.0	100.0	

Table 35 Statistical data of microemulsion droplet size of ME4 after 6 months storage at temperature of 50°C

Statistics[b]

DIAMETER

N	Valid	47
	Missing	0
Mean		30.6641
Median		20.7327
Mode		3.39[a]
Std. Deviation		35.6888
Minimum		3.39
Maximum		201.24
Sum		1441.21

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME4-50C-M6 (F)

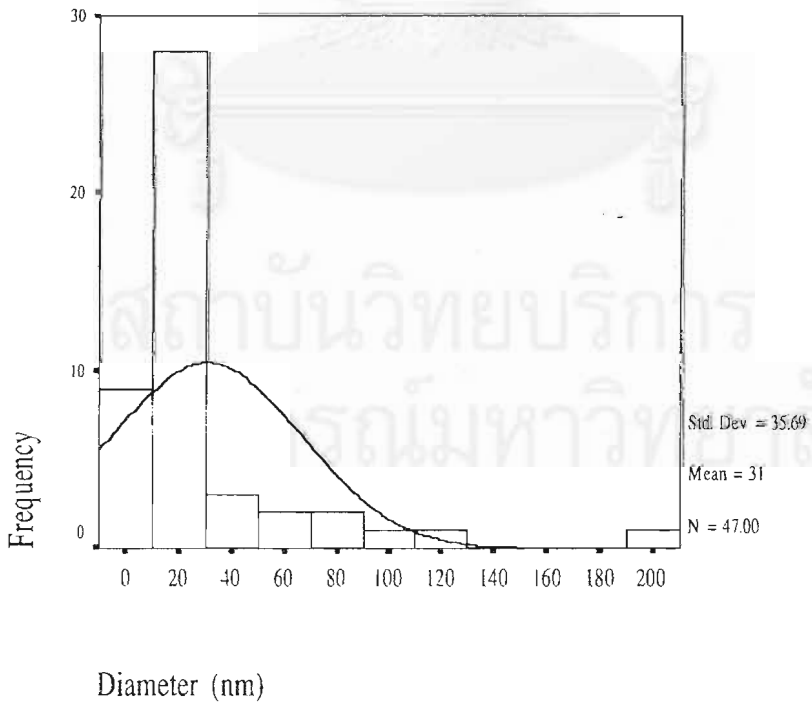


Figure 79 Droplet size histogram of ME4 after 6 months storage at temperature of 50°C

Table 36 Droplet size frequency of ME4 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	6.4	6.4	6.4
5.78	3	6.4	6.4	12.8
6.77	1	2.1	2.1	14.9
7.47	1	2.1	2.1	17.0
9.87	1	2.1	2.1	19.1
10.86	2	4.3	4.3	23.4
13.26	2	4.3	4.3	27.7
14.25	1	2.1	2.1	29.8
15.65	1	2.1	2.1	31.9
15.94	1	2.1	2.1	34.0
16.35	1	2.1	2.1	36.2
16.64	1	2.1	2.1	38.3
17.34	1	2.1	2.1	40.4
18.05	1	2.1	2.1	42.6
19.04	1	2.1	2.1	44.7
20.73	3	6.4	6.4	51.1
21.72	2	4.3	4.3	55.3
22.43	1	2.1	2.1	57.4
22.84	1	2.1	2.1	59.6
23.13	1	2.1	2.1	61.7
24.12	1	2.1	2.1	63.8
24.41	1	2.1	2.1	66.0
26.51	1	2.1	2.1	68.1
28.91	2	4.3	4.3	72.3
29.20	1	2.1	2.1	74.5
29.90	2	4.3	4.3	78.7
30.89	1	2.1	2.1	80.9
36.38	1	2.1	2.1	83.0
46.83	1	2.1	2.1	85.1
51.04	1	2.1	2.1	87.2
58.11	1	2.1	2.1	89.4
80.53	1	2.1	2.1	91.5
89.41	1	2.1	2.1	93.6
93.09	1	2.1	2.1	95.7
124.68	1	2.1	2.1	97.9
201.24	1	2.1	2.1	100.0
Total	47	100.0	100.0	

Table 37 Statistical data of microemulsion droplet size of freshly prepared ME5 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	69
	Missing	0
Mean		43.8061
Median		34.6245
Mode		4.42
Std. Deviation		42.4971
Minimum		4.42
Maximum		244.75
Sum		3022.62

a. FORMULAR = ME5-30C-M0 (B)

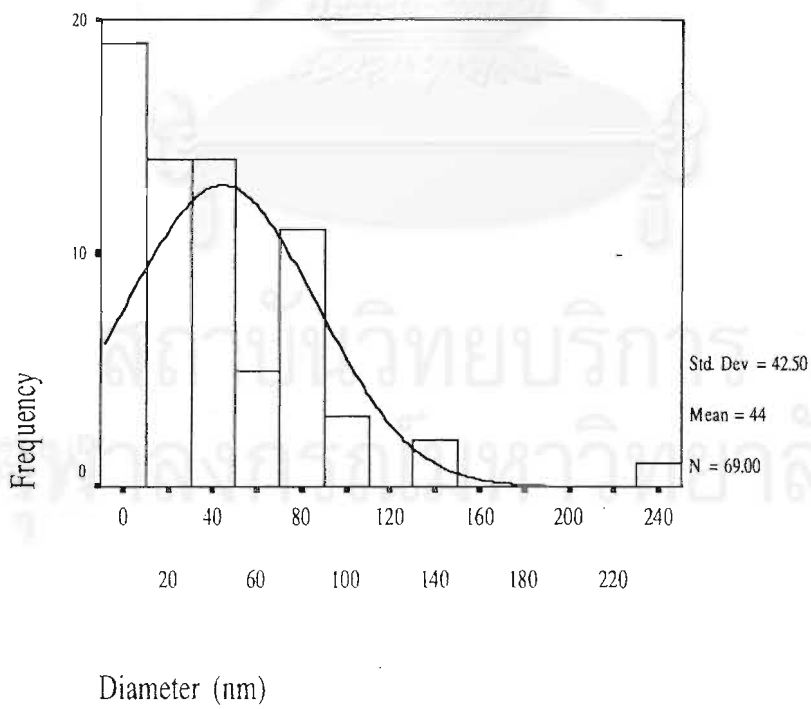


Figure 80 Droplet size histogram of freshly prepared ME5 storage at temperature of 30°C

Table 38 Droplet size frequency of freshly prepared ME5 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
4.42	9	13.0	13.0	13.0
6.63	4	5.8	5.8	18.8
7.55	1	1.4	1.4	20.3
8.85	2	2.9	2.9	23.2
9.76	3	4.3	4.3	27.5
11.97	2	2.9	2.9	30.4
12.89	1	1.4	1.4	31.9
13.27	1	1.4	1.4	33.3
14.18	1	1.4	1.4	34.8
15.48	1	1.4	1.4	36.2
17.69	1	1.4	1.4	37.7
18.61	1	1.4	1.4	39.1
19.15	1	1.4	1.4	40.6
24.86	2	2.9	2.9	43.5
25.78	1	1.4	1.4	44.9
26.16	1	1.4	1.4	46.4
27.99	1	1.4	1.4	47.8
30.20	1	1.4	1.4	49.3
34.62	1	1.4	1.4	50.7
37.75	1	1.4	1.4	52.2
39.96	1	1.4	1.4	53.6
40.88	1	1.4	1.4	55.1
42.56	1	1.4	1.4	56.5
43.47	2	2.9	2.9	59.4
45.30	2	2.9	2.9	62.3
46.60	1	1.4	1.4	63.8
48.05	1	1.4	1.4	65.2
49.73	2	2.9	2.9	68.1
57.28	1	1.4	1.4	69.6
61.70	1	1.4	1.4	71.0
63.00	1	1.4	1.4	72.5
65.21	1	1.4	1.4	73.9
67.95	1	1.4	1.4	75.4
74.59	1	1.4	1.4	76.8
75.50	1	1.4	1.4	78.3
78.10	1	1.4	1.4	79.7
79.55	1	1.4	1.4	81.2
82.14	1	1.4	1.4	82.6
83.06	1	1.4	1.4	84.1
83.97	1	1.4	1.4	85.5
85.65	1	1.4	1.4	87.0
86.18	1	1.4	1.4	88.4
87.48	1	1.4	1.4	89.9
89.69	1	1.4	1.4	91.3
90.23	1	1.4	1.4	92.8
96.86	1	1.4	1.4	94.2
102.96	1	1.4	1.4	95.7
139.04	1	1.4	1.4	97.1
144.38	1	1.4	1.4	98.6
244.75	1	1.4	1.4	100.0
Total	69	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 39 Statistical data of microemulsion droplet size of ME5 after 6 months storage at temperature of 4°C

Statistics[a]

DIAMETER

N	Valid	255
	Missing	0
Mean		57.3244
Median		34.2427
Mode		9.24
Std. Deviation		57.1231
Minimum		9.24
Maximum		309.62
Sum		14617.7

a. FORMULAR = ME5-4C-M6 (E)

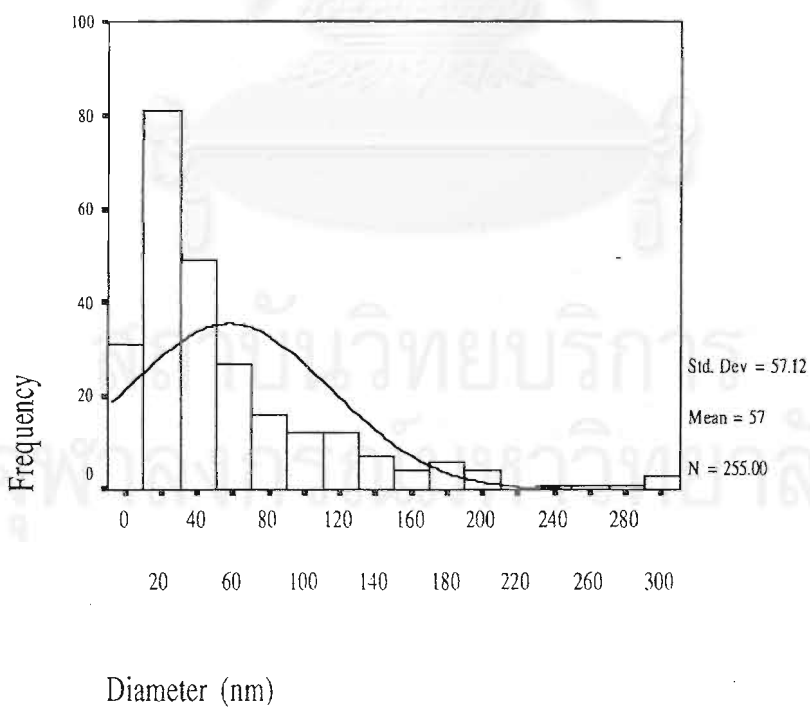


Figure 81 Droplet size histogram of ME5 after 6 months storage at temperature of 4°C

Table 40 Droplet size frequency of ME5 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
9.24	31	12.2	12.2	12.2
13.85	6	2.4	2.4	14.5
15.77	12	4.7	4.7	19.2
18.47	5	2.0	2.0	21.2
20.39	20	7.8	7.8	29.0
23.09	2	0.8	0.8	29.8
25.01	11	4.3	4.3	34.1
26.92	11	4.3	4.3	38.4
27.71	9	3.5	3.5	42.0
29.62	5	2.0	2.0	43.9
31.54	8	3.1	3.1	47.1
32.33	2	0.8	0.8	47.8
33.45	1	0.4	0.4	48.2
34.24	10	3.9	3.9	52.2
36.16	4	1.6	1.6	53.7
36.95	1	0.4	0.4	54.1
38.07	2	0.8	0.8	54.9
38.86	4	1.6	1.6	56.5
40.77	1	0.4	0.4	56.9
42.69	3	1.2	1.2	58.0
43.48	3	1.2	1.2	59.2
44.60	1	0.4	0.4	59.6
45.39	2	0.8	0.8	60.4
46.18	2	0.8	0.8	61.2
47.30	2	0.8	0.8	62.0
48.10	3	1.2	1.2	63.1
50.01	1	0.4	0.4	63.5
51.92	3	1.2	1.2	64.7
52.72	1	0.4	0.4	65.1
54.63	4	1.6	1.6	66.7
56.54	5	2.0	2.0	68.6
59.25	3	1.2	1.2	69.8
61.16	2	0.8	0.8	70.6
62.28	1	0.4	0.4	71.0
63.07	1	0.4	0.4	71.4
63.87	1	0.4	0.4	71.8
64.99	1	0.4	0.4	72.2
67.69	2	0.8	0.8	72.9
69.60	2	0.8	0.8	73.7
70.39	3	1.2	1.2	74.9
75.01	1	0.4	0.4	75.3
78.84	3	1.2	1.2	76.5
81.55	3	1.2	1.2	77.6
83.46	1	0.4	0.4	78.0
86.16	2	0.8	0.8	78.8
86.96	1	0.4	0.4	79.2
88.08	1	0.4	0.4	79.6
89.99	1	0.4	0.4	80.0
90.78	1	0.4	0.4	80.4
91.90	1	0.4	0.4	80.8
94.61	1	0.4	0.4	81.2
99.23	1	0.4	0.4	81.6
100.02	1	0.4	0.4	82.0
101.14	1	0.4	0.4	82.4
102.73	1	0.4	0.4	82.7
103.05	1	0.4	0.4	83.1
103.85	2	0.8	0.8	83.9
105.76	1	0.4	0.4	84.3
108.46	1	0.4	0.4	84.7
111.17	1	0.4	0.4	85.1
113.08	1	0.4	0.4	85.5
114.99	1	0.4	0.4	85.9
115.79	1	0.4	0.4	86.3
116.91	1	0.4	0.4	86.7
117.70	1	0.4	0.4	87.1
119.61	1	0.4	0.4	87.5
120.41	1	0.4	0.4	87.8
124.23	1	0.4	0.4	88.2
126.14	1	0.4	0.4	88.6
128.06	1	0.4	0.4	89.0
129.97	1	0.4	0.4	89.4
131.56	1	0.4	0.4	89.8
133.47	2	0.8	0.8	90.6
135.38	1	0.4	0.4	91.0
140.79	1	0.4	0.4	91.4
143.50	1	0.4	0.4	91.8
148.44	1	0.4	0.4	92.2
151.15	1	0.4	0.4	92.5
155.77	1	0.4	0.4	92.9
156.56	1	0.4	0.4	93.3
166.92	1	0.4	0.4	93.7
170.74	1	0.4	0.4	94.1
176.16	1	0.4	0.4	94.5
181.56	1	0.4	0.4	94.9
185.39	1	0.4	0.4	95.3
186.51	1	0.4	0.4	95.7
187.30	1	0.4	0.4	96.1
194.63	1	0.4	0.4	96.5
201.95	1	0.4	0.4	96.9
203.07	1	0.4	0.4	97.3
208.48	1	0.4	0.4	97.6
236.19	1	0.4	0.4	98.0
250.71	1	0.4	0.4	98.4
271.56	1	0.4	0.4	98.8
293.53	1	0.4	0.4	99.2
297.68	1	0.4	0.4	99.6
309.62	1	0.4	0.4	100.0
Total	255	100.0	100.0	

Table 41 Statistical data of microemulsion droplet size of freshly prepared ME6 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	66
	Missing	0
Mean		31.7979
Median		26.4377
Mode		30.97
Std. Deviation		24.3123
Minimum		3.02
Maximum		140.89
Sum		2098.66

a. FORMULAR = ME6-30C-M0 (B)

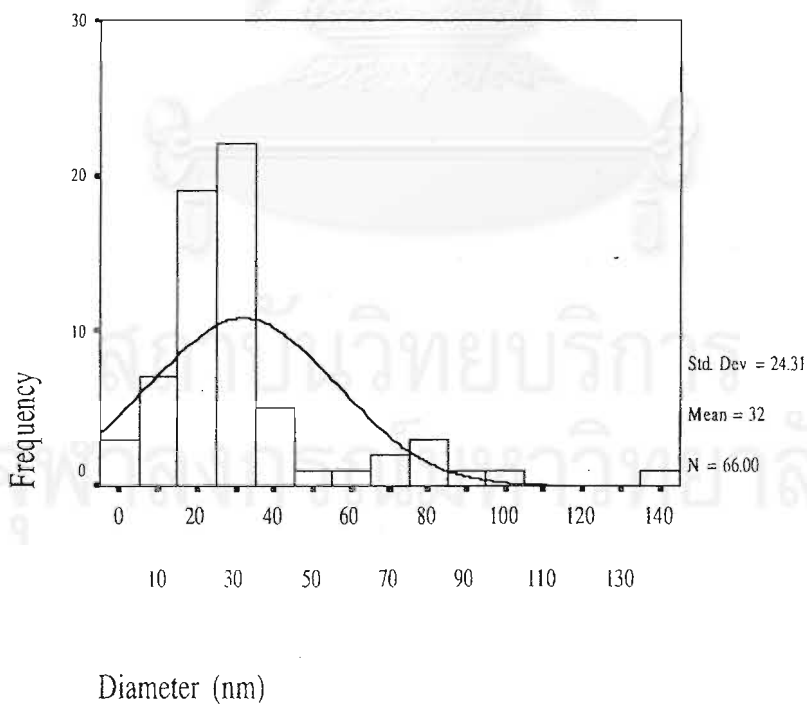


Figure 82 Droplet size histogram of freshly prepared ME6 storage at temperature of 30°C

Table 42 Droplet size frequency of freshly prepared ME6 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.02	3	4.5	4.5	4.5
6.05	1	1.5	1.5	6.1
6.68	2	3.0	3.0	9.1
7.56	1	1.5	1.5	10.6
8.81	1	1.5	1.5	12.1
11.21	2	3.0	3.0	15.2
15.75	1	1.5	1.5	16.7
16.37	1	1.5	1.5	18.2
17.62	1	1.5	1.5	19.7
17.89	1	1.5	1.5	21.2
19.14	1	1.5	1.5	22.7
19.40	1	1.5	1.5	24.2
20.02	2	3.0	3.0	27.3
20.28	1	1.5	1.5	28.8
21.27	1	1.5	1.5	30.3
21.53	2	3.0	3.0	33.3
23.05	2	3.0	3.0	36.4
23.41	1	1.5	1.5	37.9
24.30	2	3.0	3.0	40.9
24.56	1	1.5	1.5	42.4
24.93	1	1.5	1.5	43.9
25.18	1	1.5	1.5	45.5
25.81	2	3.0	3.0	48.5
26.44	2	3.0	3.0	51.5
26.70	1	1.5	1.5	53.0
27.32	1	1.5	1.5	54.5
28.21	2	3.0	3.0	57.6
28.83	1	1.5	1.5	59.1
29.46	1	1.5	1.5	60.6
29.72	2	3.0	3.0	63.6
30.35	1	1.5	1.5	65.2
30.97	5	7.6	7.6	72.7
31.86	1	1.5	1.5	74.2
33.01	1	1.5	1.5	75.8
33.11	1	1.5	1.5	77.3
35.77	1	1.5	1.5	78.8
36.50	1	1.5	1.5	80.3
38.27	2	3.0	3.0	83.3
39.79	1	1.5	1.5	84.8
48.86	1	1.5	1.5	86.4
62.20	1	1.5	1.5	87.9
66.74	1	1.5	1.5	89.4
67.63	1	1.5	1.5	90.9
75.29	1	1.5	1.5	92.4
78.58	1	1.5	1.5	93.9
80.46	1	1.5	1.5	95.5
86.24	1	1.5	1.5	97.0
96.46	1	1.5	1.5	98.5
140.89	1	1.5	1.5	100.0
Total	66	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 43 Statistical data of microemulsion droplet size of ME6 after 6 months storage at temperature of 30°C

Statistics[b]

DIAMETER

N	Valid	172
	Missing	0
Mean		20.1659
Median		17.6368
Mode		14.95[a]
Std. Deviation		11.7078
Minimum		3.39
Maximum		88.01
Sum		3468.54

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME6-30C-M6 (D)

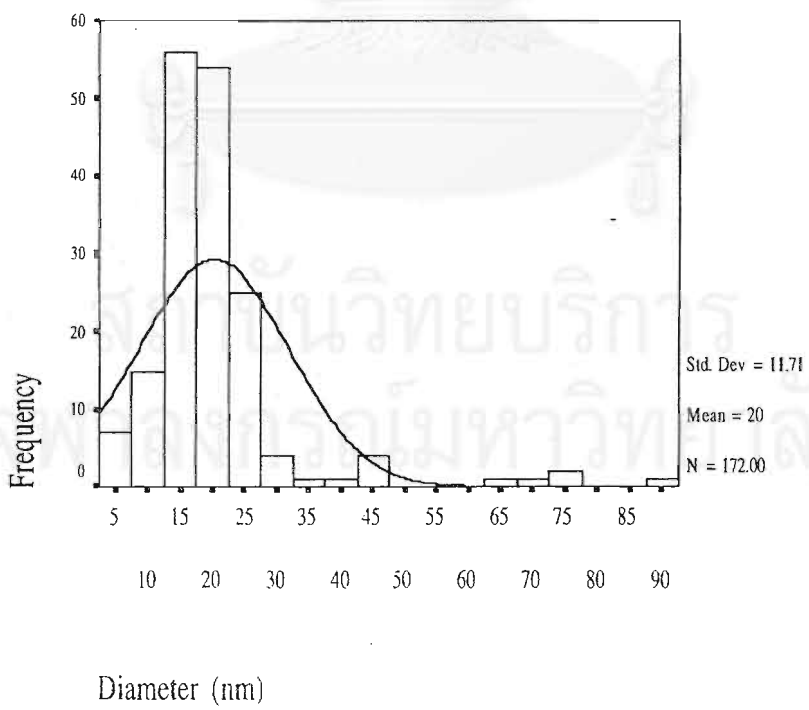


Figure 83 Droplet size histogram of ME6 after 6 months storage at temperature of 30°C

Table 44 Droplet size frequency of ME6 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	1.7	1.7	1.7
5.78	1	0.6	0.6	2.3
6.77	1	0.6	0.6	2.9
7.47	2	1.2	1.2	4.1
8.18	1	0.6	0.6	4.7
9.17	2	1.2	1.2	5.8
9.87	1	0.6	0.6	6.4
10.16	2	1.2	1.2	7.6
10.86	1	0.6	0.6	8.1
11.56	2	1.2	1.2	9.3
11.85	3	1.7	1.7	11.0
12.27	3	1.7	1.7	12.8
12.56	1	0.6	0.6	13.4
13.26	3	1.7	1.7	15.1
13.55	9	5.2	5.2	20.3
13.96	4	2.3	2.3	22.7
14.25	7	4.1	4.1	26.7
14.95	10	5.8	5.8	32.6
15.24	2	1.2	1.2	33.7
15.65	6	3.5	3.5	37.2
15.94	7	4.1	4.1	41.3
16.35	1	0.6	0.6	41.9
16.64	5	2.9	2.9	44.8
17.34	1	0.6	0.6	45.3
17.64	10	5.8	5.8	51.2
18.05	1	0.6	0.6	51.7
18.34	7	4.1	4.1	55.8
19.04	2	1.2	1.2	57.0
19.33	2	1.2	1.2	58.1
20.03	6	3.5	3.5	61.6
20.32	1	0.6	0.6	62.2
20.73	5	2.9	2.9	65.1
21.02	2	1.2	1.2	66.3
21.43	1	0.6	0.6	66.9
21.72	10	5.8	5.8	72.7
22.14	1	0.6	0.6	73.3
22.43	6	3.5	3.5	76.7
22.72	1	0.6	0.6	77.3
23.13	6	3.5	3.5	80.8
23.42	1	0.6	0.6	81.4
24.12	2	1.2	1.2	82.6
24.82	3	1.7	1.7	84.3
25.11	5	2.9	2.9	87.2
25.52	1	0.6	0.6	87.8
25.81	4	2.3	2.3	90.1
26.22	1	0.6	0.6	90.7
27.21	1	0.6	0.6	91.3
27.50	2	1.2	1.2	92.4
28.21	1	0.6	0.6	93.0
30.60	1	0.6	0.6	93.6
33.29	1	0.6	0.6	94.2
42.17	1	0.6	0.6	94.8
43.57	1	0.6	0.6	95.3
45.14	2	1.2	1.2	96.5
45.55	1	0.6	0.6	97.1
64.47	1	0.6	0.6	97.7
69.96	1	0.6	0.6	98.3
74.05	2	1.2	1.2	99.4
88.01	1	0.6	0.6	100.0
Total	172	100.0	100.0	

Table 45 Statistical data of microemulsion droplet size of freshly prepared ME7 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	20
	Missing	0
Mean		110.304
Median		130.590
Mode		11.73
Std. Deviation		78.0071
Minimum		11.73
Maximum		280.18
Sum		2206.08

a. FORMULAR = ME7-30C-M0 (A)

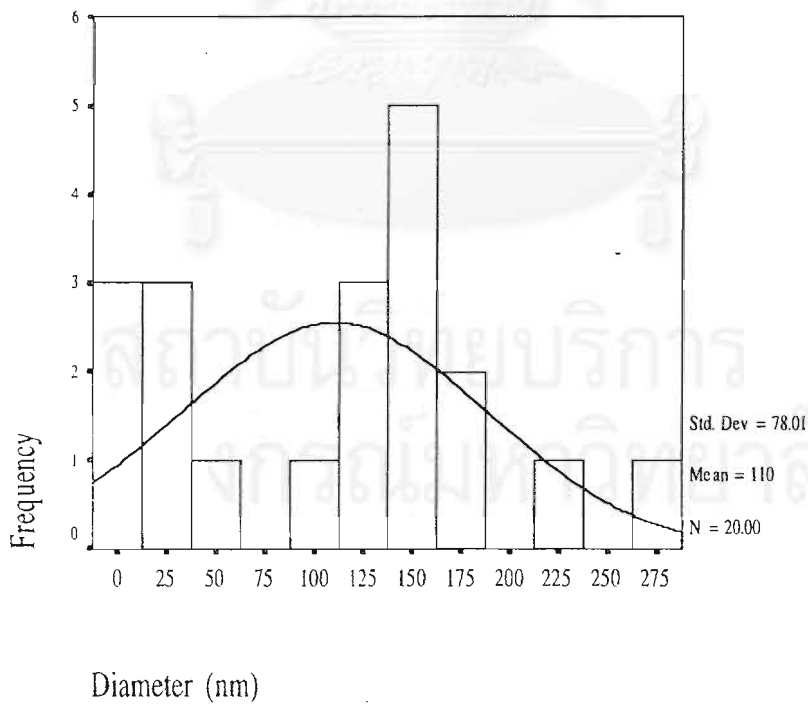


Figure 84 Droplet size histogram of freshly prepared ME7 storage at temperature of 30°C

Table 46 Droplet size frequency of freshly prepared ME7 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	3	15.0	15.0	15.0
17.59	1	5.0	5.0	20.0
20.01	1	5.0	5.0	25.0
29.31	1	5.0	5.0	30.0
37.60	1	5.0	5.0	35.0
97.64	1	5.0	5.0	40.0
112.80	1	5.0	5.0	45.0
129.38	1	5.0	5.0	50.0
131.80	1	5.0	5.0	55.0
140.09	1	5.0	5.0	60.0
148.38	1	5.0	5.0	65.0
149.39	1	5.0	5.0	70.0
154.25	1	5.0	5.0	75.0
157.68	1	5.0	5.0	80.0
168.39	1	5.0	5.0	85.0
174.26	1	5.0	5.0	90.0
222.16	1	5.0	5.0	95.0
280.18	1	5.0	5.0	100.0
Total	20	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 47 Statistical data of microemulsion droplet size of ME7 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	140
	Missing	0
Mean		15.6813
Median		13.2555
Mode		3.39
Std. Deviation		11.8429
Minimum		3.39
Maximum		75.04
Sum		2195.38

a. FORMULAR = ME7-30C-M6 (D)

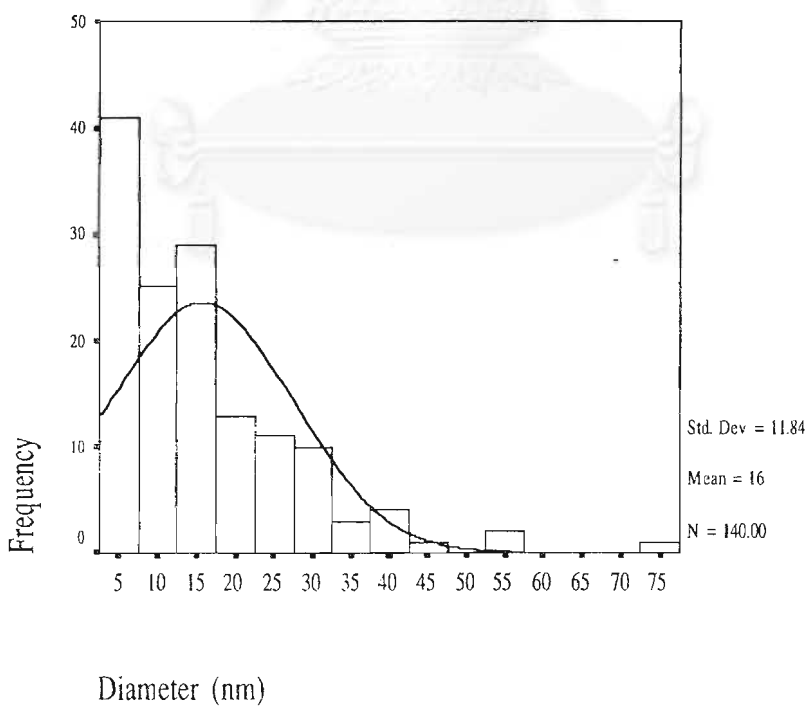


Figure 85 Droplet size histogram of ME7 after 6 months storage at temperature of 30°C

Table 48 Droplet size frequency of ME7 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	20	14.3	14.3	14.3
5.08	5	3.6	3.6	17.9
5.78	8	5.7	5.7	23.6
6.77	3	2.1	2.1	25.7
7.47	5	3.6	3.6	29.3
8.47	2	1.4	1.4	30.7
9.17	5	3.6	3.6	34.3
9.87	3	2.1	2.1	36.4
10.16	4	2.9	2.9	39.3
10.86	4	2.9	2.9	42.1
11.56	4	2.9	2.9	45.0
12.27	3	2.1	2.1	47.1
12.56	3	2.1	2.1	49.3
13.26	2	1.4	1.4	50.7
13.55	3	2.1	2.1	52.9
14.25	8	5.7	5.7	58.6
14.66	1	0.7	0.7	59.3
14.95	6	4.3	4.3	63.6
15.94	2	1.4	1.4	65.0
16.64	3	2.1	2.1	67.1
16.93	1	0.7	0.7	67.9
17.64	1	0.7	0.7	68.6
18.34	1	0.7	0.7	69.3
19.04	1	0.7	0.7	70.0
19.74	2	1.4	1.4	71.4
20.03	2	1.4	1.4	72.9
20.44	2	1.4	1.4	74.3
20.73	3	2.1	2.1	76.4
21.43	1	0.7	0.7	77.1
22.72	1	0.7	0.7	77.9
23.13	2	1.4	1.4	79.3
23.42	1	0.7	0.7	80.0
24.82	2	1.4	1.4	81.4
25.11	1	0.7	0.7	82.1
25.52	1	0.7	0.7	82.9
25.81	1	0.7	0.7	83.6
26.51	1	0.7	0.7	84.3
26.80	1	0.7	0.7	85.0
27.50	1	0.7	0.7	85.7
28.21	1	0.7	0.7	86.4
28.91	1	0.7	0.7	87.1
29.20	2	1.4	1.4	88.6
29.61	1	0.7	0.7	89.3
29.90	1	0.7	0.7	90.0
31.59	3	2.1	2.1	92.1
33.99	2	1.4	1.4	93.6
37.37	1	0.7	0.7	94.3
37.67	1	0.7	0.7	95.0
38.37	1	0.7	0.7	95.7
39.77	1	0.7	0.7	96.4
40.76	1	0.7	0.7	97.1
44.85	1	0.7	0.7	97.9
52.61	1	0.7	0.7	98.6
54.31	1	0.7	0.7	99.3
75.04	1	0.7	0.7	100.0
Total	140	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 49 Statistical data of microemulsion droplet size of ME7 after 6 months storage at temperature of 4°C

Statistics

DIAMETER

N	Valid	160
	Missing	0
Mean		87.4376
Median		84.4009
Mode		17.93[a]
Std. Deviation		41.1968
Minimum		17.93
Maximum		226.30
Sum		13990.0

a. Multiple modes exist. The smallest value is shown

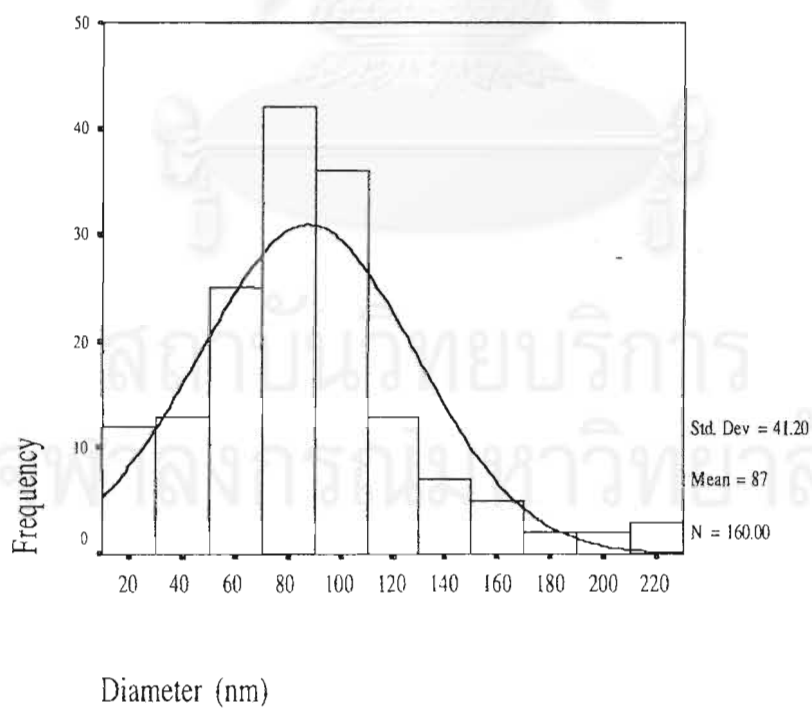


Figure 86 Droplet size histogram of ME7 after 6 months storage at temperature of 4°C

Table 50 Droplet size frequency of ME7 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
17.93	9	5.6	5.6	5.6
26.89	3	1.9	1.9	7.5
30.61	3	1.9	1.9	9.4
39.58	6	3.8	3.8	13.1
44.83	1	0.6	0.6	13.8
48.54	3	1.9	1.9	15.6
52.25	2	1.3	1.3	16.9
53.79	3	1.9	1.9	18.8
57.50	3	1.9	1.9	20.6
61.22	5	3.1	3.1	23.8
62.76	5	3.1	3.1	26.9
64.93	5	3.1	3.1	30.0
66.47	2	1.3	1.3	31.3
70.18	3	1.9	1.9	33.1
71.72	2	1.3	1.3	34.4
73.89	2	1.3	1.3	35.6
75.43	9	5.6	5.6	41.3
79.14	6	3.8	3.8	45.0
82.86	5	3.1	3.1	48.1
84.40	8	5.0	5.0	53.1
88.11	4	2.5	2.5	55.6
89.65	3	1.9	1.9	57.5
90.29	1	0.6	0.6	58.1
91.83	3	1.9	1.9	60.0
93.36	4	2.5	2.5	62.5
95.54	2	1.3	1.3	63.8
97.08	8	5.0	5.0	68.8
100.79	5	3.1	3.1	71.9
102.33	3	1.9	1.9	73.8
104.50	1	0.6	0.6	74.4
107.58	1	0.6	0.6	75.0
109.75	8	5.0	5.0	80.0
111.29	1	0.6	0.6	80.6
113.47	2	1.3	1.3	81.9
115.01	4	2.5	2.5	84.4
118.72	2	1.3	1.3	85.6
122.44	2	1.3	1.3	86.9
123.97	1	0.6	0.6	87.5
127.69	1	0.6	0.6	88.1
131.40	2	1.3	1.3	89.4
136.65	2	1.3	1.3	90.6
140.36	2	1.3	1.3	91.9
141.90	1	0.6	0.6	92.5
150.87	1	0.6	0.6	93.1
162.01	2	1.3	1.3	94.4
165.72	1	0.6	0.6	95.0
168.80	1	0.6	0.6	95.6
186.73	1	0.6	0.6	96.3
188.90	1	0.6	0.6	96.9
200.94	1	0.6	0.6	97.5
203.12	1	0.6	0.6	98.1
215.80	2	1.3	1.3	99.4
226.30	1	0.6	0.6	100.0
Total	160	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 51 Statistical data of microemulsion droplet size of ME7 after 6 months storage at temperature of 50°C

Statistics

DIAMETER

N	Valid	270
	Missing	0
Mean		127.997
Median		125.828
Mode		109.75[a]
Std. Deviation		57.5831
Minimum		17.93
Maximum		306.08
Sum		34559.1

a. Multiple modes exist. The smallest value is shown

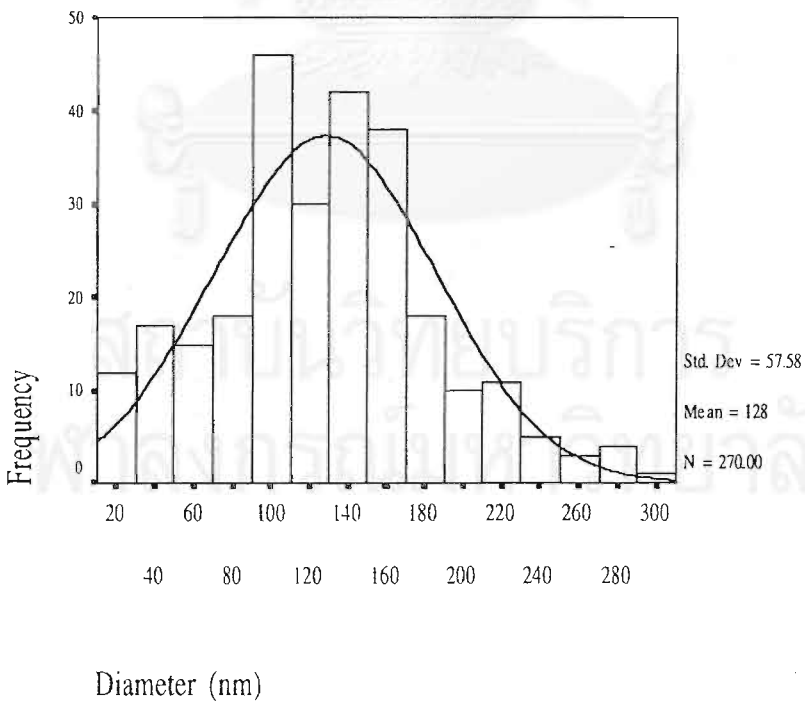


Figure 87 Droplet size histogram of ME7 after 6 months storage at temperature of 50°C

Table 52 Droplet size frequency of ME7 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
17.93	9	3.3	3.3	3.3
26.89	3	1.1	1.1	4.4
35.86	3	1.1	1.1	5.6
39.58	8	3.0	3.0	8.5
44.83	1	0.4	0.4	8.9
48.54	5	1.9	1.9	10.7
52.25	1	0.4	0.4	11.1
53.79	1	0.4	0.4	11.5
57.50	1	0.4	0.4	11.9
61.22	6	2.2	2.2	14.1
64.93	2	0.7	0.7	14.8
66.47	4	1.5	1.5	16.3
70.18	1	0.4	0.4	16.7
71.72	4	1.5	1.5	18.1
73.89	2	0.7	0.7	18.9
75.43	2	0.7	0.7	19.6
79.14	3	1.1	1.1	20.7
82.86	2	0.7	0.7	21.5
84.40	3	1.1	1.1	22.6
88.11	1	0.4	0.4	23.0
91.83	3	1.1	1.1	24.1
93.36	8	3.0	3.0	27.0
95.54	2	0.7	0.7	27.8
97.08	6	2.2	2.2	30.0
100.79	6	2.2	2.2	32.2
102.33	2	0.7	0.7	33.0
104.50	1	0.4	0.4	33.3
106.04	2	0.7	0.7	34.1
107.58	1	0.4	0.4	34.4
108.22	1	0.4	0.4	34.8
109.75	14	5.2	5.2	40.0
111.29	2	0.7	0.7	40.7
113.47	1	0.4	0.4	41.1
115.01	6	2.2	2.2	43.3
117.18	1	0.4	0.4	43.7
118.72	7	2.6	2.6	46.3
120.26	1	0.4	0.4	46.7
122.44	4	1.5	1.5	48.1
123.97	5	1.9	1.9	50.0
127.69	3	1.1	1.1	51.1
131.40	4	1.5	1.5	52.6
132.94	3	1.1	1.1	53.7
135.11	6	2.2	2.2	55.9
136.65	6	2.2	2.2	58.1
140.36	7	2.6	2.6	60.7
144.08	5	1.9	1.9	62.6
145.61	4	1.5	1.5	64.1
147.79	1	0.4	0.4	64.4
149.33	6	2.2	2.2	66.7
153.05	3	1.1	1.1	67.8
154.58	4	1.5	1.5	69.3
158.30	14	5.2	5.2	74.4
160.47	1	0.4	0.4	74.8
162.01	5	1.9	1.9	76.7
165.72	4	1.5	1.5	78.1
167.26	5	1.9	1.9	80.0
169.43	2	0.7	0.7	80.7
170.97	3	1.1	1.1	81.9
174.69	2	0.7	0.7	82.6
176.22	4	1.5	1.5	84.1
179.94	4	1.5	1.5	85.6
183.65	2	0.7	0.7	86.3
185.19	1	0.4	0.4	86.7
188.90	2	0.7	0.7	87.4
192.61	1	0.4	0.4	87.8
196.33	1	0.4	0.4	88.1
197.86	1	0.4	0.4	88.5
201.58	3	1.1	1.1	89.6
205.30	3	1.1	1.1	90.7
209.01	1	0.4	0.4	91.1
210.55	1	0.4	0.4	91.5
212.72	1	0.4	0.4	91.9
214.26	2	0.7	0.7	92.6
215.80	1	0.4	0.4	93.0
217.97	1	0.4	0.4	93.3
219.51	3	1.1	1.1	94.4
226.94	1	0.4	0.4	94.8
228.47	1	0.4	0.4	95.2
230.65	1	0.4	0.4	95.6
239.62	1	0.4	0.4	95.9
241.16	2	0.7	0.7	96.7
248.58	1	0.4	0.4	97.0
253.83	1	0.4	0.4	97.4
255.37	1	0.4	0.4	97.8
266.51	1	0.4	0.4	98.1
270.23	1	0.4	0.4	98.5
275.48	1	0.4	0.4	98.9
284.44	2	0.7	0.7	99.6
306.08	1	0.4	0.4	100.0
Total	270	100.0	100.0	

Table 53 Statistical data of microemulsion droplet size of ME8 after 6 months storage at temperature of 4°C

Statistics[a]

DIAMETER

N	Valid	249
	Missing	0
Mean		20.2701
Median		15.9409
Mode		3.39
Std. Deviation		16.9001
Minimum		3.39
Maximum		127.49
Sum		5047.26

a. FORMULAR = ME8-4C-M6 (E)

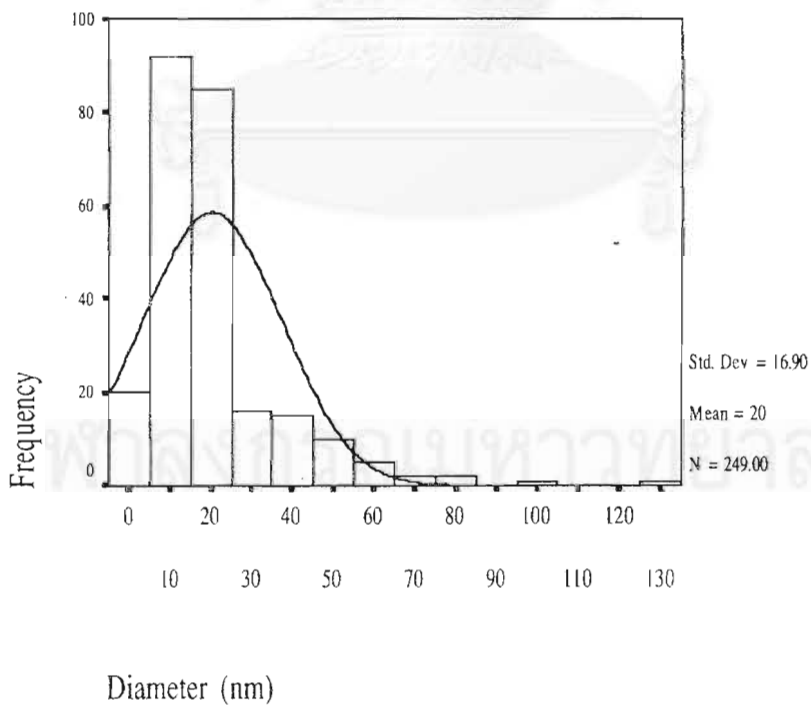


Figure 88 Droplet size histogram of ME8 after 6 months storage at temperature of 4°C

Table 54 Droplet size frequency of ME8 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	20	8.0	8.0	8.0
5.08	4	1.6	1.6	9.6
5.78	6	2.4	2.4	12.0
6.77	1	0.4	0.4	12.4
7.47	8	3.2	3.2	15.7
8.18	2	0.8	0.8	16.5
8.47	1	0.4	0.4	16.9
9.17	7	2.8	2.8	19.7
9.87	7	2.8	2.8	22.5
10.16	4	1.6	1.6	24.1
10.86	6	2.4	2.4	26.5
11.56	3	1.2	1.2	27.7
11.85	3	1.2	1.2	28.9
12.27	1	0.4	0.4	29.3
12.56	4	1.6	1.6	30.9
13.26	9	3.6	3.6	34.5
13.55	8	3.2	3.2	37.8
13.96	3	1.2	1.2	39.0
14.25	9	3.6	3.6	42.6
14.66	1	0.4	0.4	43.0
14.95	5	2.0	2.0	45.0
15.65	5	2.0	2.0	47.0
15.94	10	4.0	4.0	51.0
16.35	1	0.4	0.4	51.4
16.64	11	4.4	4.4	55.8
16.93	3	1.2	1.2	57.0
17.05	1	0.4	0.4	57.4
17.34	3	1.2	1.2	58.6
17.64	6	2.4	2.4	61.0
18.05	2	0.8	0.8	61.8
18.34	12	4.8	4.8	66.7
18.63	1	0.4	0.4	67.1
19.04	3	1.2	1.2	68.3
19.33	2	0.8	0.8	69.1
19.74	3	1.2	1.2	70.3
20.03	3	1.2	1.2	71.5
20.73	4	1.6	1.6	73.1
21.02	1	0.4	0.4	73.5
21.72	6	2.4	2.4	75.9
22.43	1	0.4	0.4	76.3
22.72	1	0.4	0.4	76.7
23.42	2	0.8	0.8	77.5
23.83	1	0.4	0.4	77.9
24.12	2	0.8	0.8	78.7
24.41	1	0.4	0.4	79.1
25.11	1	0.4	0.4	79.5
25.52	2	0.8	0.8	80.3
26.51	3	1.2	1.2	81.5
27.50	1	0.4	0.4	81.9
28.50	3	1.2	1.2	83.1
28.91	1	0.4	0.4	83.5
29.20	1	0.4	0.4	83.9
29.49	1	0.4	0.4	84.3
33.58	1	0.4	0.4	84.7
33.99	1	0.4	0.4	85.1
34.69	1	0.4	0.4	85.5
36.67	1	0.4	0.4	85.9
37.67	1	0.4	0.4	86.3
38.37	2	0.8	0.8	87.1
39.07	2	0.8	0.8	88.0
39.77	3	1.2	1.2	89.2
40.76	1	0.4	0.4	89.6
41.46	1	0.4	0.4	90.0
42.74	1	0.4	0.4	90.4
43.16	2	0.8	0.8	91.2
43.86	1	0.4	0.4	91.6
45.55	1	0.4	0.4	92.0
45.84	2	0.8	0.8	92.8
47.54	1	0.4	0.4	93.2
48.53	1	0.4	0.4	93.6
49.93	1	0.4	0.4	94.0
50.22	1	0.4	0.4	94.4
52.04	1	0.4	0.4	94.8
52.33	1	0.4	0.4	95.2
52.61	1	0.4	0.4	95.6
57.41	1	0.4	0.4	96.0
59.80	1	0.4	0.4	96.4
61.78	1	0.4	0.4	96.8
62.07	1	0.4	0.4	97.2
64.59	1	0.4	0.4	97.6
72.77	1	0.4	0.4	98.0
73.64	1	0.4	0.4	98.4
81.52	1	0.4	0.4	98.8
81.81	1	0.4	0.4	99.2
103.25	1	0.4	0.4	99.6
127.49	1	0.4	0.4	100.0
Total	249	100.0	100.0	

Table 55 Statistical data of microemulsion droplet size of ME8 after 6 months storage at temperature of 50°C

Statistics[a]

DIAMETER

N	Valid	47
	Missing	0
Mean		24.0115
Median		23.1255
Mode		23.42
Std. Deviation		13.4505
Minimum		3.39
Maximum		64.59
Sum		1128.54

a. FORMULAR = ME8-50C-M6 (F)

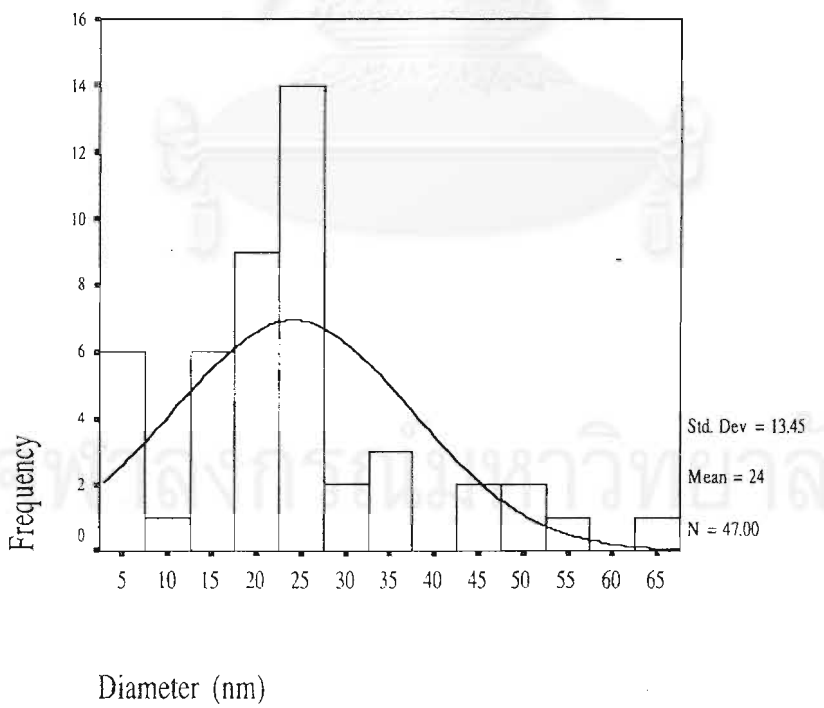


Figure 89 Droplet size histogram of ME8 after 6 months storage at temperature of 50°C

Table 56 Droplet size frequency of ME8 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	6.4	6.4	6.4
5.08	1	2.1	2.1	8.5
5.78	1	2.1	2.1	10.6
7.47	1	2.1	2.1	12.8
11.56	1	2.1	2.1	14.9
13.55	1	2.1	2.1	17.0
13.96	1	2.1	2.1	19.1
14.95	3	6.4	6.4	25.5
15.94	1	2.1	2.1	27.7
18.34	1	2.1	2.1	29.8
19.33	1	2.1	2.1	31.9
20.03	1	2.1	2.1	34.0
21.02	2	4.3	4.3	38.3
21.72	1	2.1	2.1	40.4
22.43	3	6.4	6.4	46.8
22.72	1	2.1	2.1	48.9
23.13	3	6.4	6.4	55.3
23.42	4	8.5	8.5	63.8
24.82	2	4.3	4.3	68.1
25.11	1	2.1	2.1	70.2
25.52	1	2.1	2.1	72.3
25.81	2	4.3	4.3	76.6
27.50	1	2.1	2.1	78.7
32.30	1	2.1	2.1	80.9
32.59	1	2.1	2.1	83.0
35.97	1	2.1	2.1	85.1
37.37	1	2.1	2.1	87.2
43.45	2	4.3	4.3	91.5
49.52	1	2.1	2.1	93.6
50.63	1	2.1	2.1	95.7
56.41	1	2.1	2.1	97.9
64.59	1	2.1	2.1	100.0
Total	47	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 57 Statistical data of microemulsion droplet size of freshly prepared ME9 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	183
	Missing	0
Mean		48.0009
Median		35.1718
Mode		11.73
Std. Deviation		45.2310
Minimum		11.73
Maximum		397.84
Sum		8784.17

a. FORMULAR = ME9-30C-M0 (A)

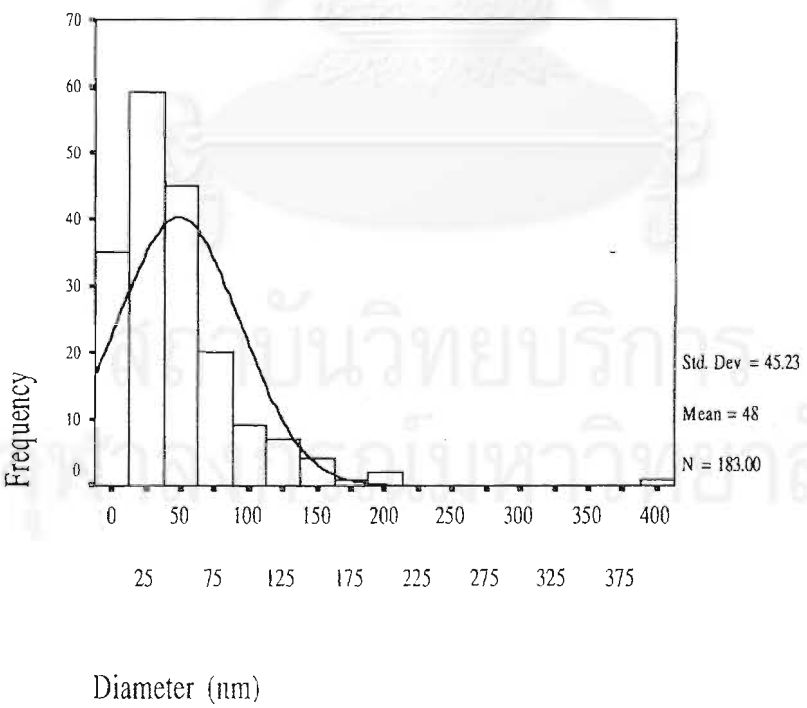


Figure 90 Droplet size histogram of freshly prepared ME9 storage at temperature of 30°C

Table 58 Droplet size frequency of freshly prepared ME9 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	35	19.1	19.1	19.1
17.59	11	6.0	6.0	25.1
20.01	10	5.5	5.5	30.6
23.45	6	3.3	3.3	33.9
25.87	8	4.4	4.4	38.3
28.30	2	1.1	1.1	39.3
29.31	3	1.6	1.6	41.0
31.74	12	6.6	6.6	47.5
34.16	3	1.6	1.6	49.2
35.17	4	2.2	2.2	51.4
37.60	4	2.2	2.2	53.6
40.03	5	2.7	2.7	56.3
41.03	2	1.1	1.1	57.4
42.46	1	0.5	0.5	57.9
43.46	6	3.3	3.3	61.2
45.89	5	2.7	2.7	63.9
46.89	1	0.5	0.5	64.5
48.32	3	1.6	1.6	66.1
49.32	4	2.2	2.2	68.3
51.75	4	2.2	2.2	70.5
54.18	1	0.5	0.5	71.0
55.18	1	0.5	0.5	71.6
57.61	3	1.6	1.6	73.2
60.04	2	1.1	1.1	74.3
61.05	3	1.6	1.6	76.0
63.47	4	2.2	2.2	78.1
65.90	2	1.1	1.1	79.2
68.33	1	0.5	0.5	79.8
69.33	2	1.1	1.1	80.9
70.76	1	0.5	0.5	81.4
71.76	1	0.5	0.5	82.0
74.19	1	0.5	0.5	82.5
75.20	2	1.1	1.1	83.6
80.05	2	1.1	1.1	84.7
82.48	1	0.5	0.5	85.2
83.49	1	0.5	0.5	85.8
85.92	2	1.1	1.1	86.9
88.34	1	0.5	0.5	87.4
92.78	1	0.5	0.5	88.0
93.20	1	0.5	0.5	88.5
95.21	2	1.1	1.1	89.6
96.63	1	0.5	0.5	90.2
97.64	1	0.5	0.5	90.7
103.50	1	0.5	0.5	91.3
107.35	1	0.5	0.5	91.8
113.21	1	0.5	0.5	92.3
114.22	1	0.5	0.5	92.9
118.66	1	0.5	0.5	93.4
120.08	1	0.5	0.5	94.0
122.09	1	0.5	0.5	94.5
132.81	2	1.1	1.1	95.6
137.66	1	0.5	0.5	96.2
141.10	1	0.5	0.5	96.7
141.51	1	0.5	0.5	97.3
150.81	1	0.5	0.5	97.8
171.83	1	0.5	0.5	98.4
196.28	1	0.5	0.5	98.9
198.71	1	0.5	0.5	99.5
397.84	1	0.5	0.5	100.0
Total	183	100.0	100.0	

Table 59 Statistical data of microemulsion droplet size of ME9 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	42
	Missing	0
Mean		34.4654
Median		29.1995
Mode		32.30
Std. Deviation		40.0683
Minimum		3.39
Maximum		277.40
Sum		1447.55

a. FORMULAR = ME9-30C-M6 (D)

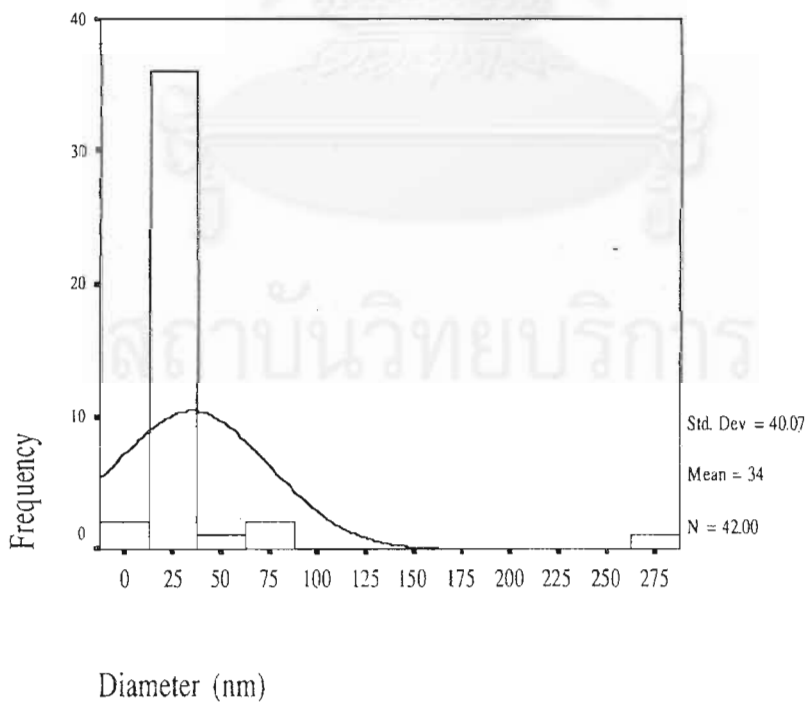


Figure 91 Droplet size histogram of ME9 after 6 months storage at temperature of 30°C

Table 60 Droplet size frequency of ME9 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	1	2.4	2.4	2.4
10.16	1	2.4	2.4	4.8
13.96	1	2.4	2.4	7.1
15.94	2	4.8	4.8	11.9
17.64	2	4.8	4.8	16.7
19.74	1	2.4	2.4	19.0
22.43	1	2.4	2.4	21.4
24.12	1	2.4	2.4	23.8
24.82	2	4.8	4.8	28.6
25.52	1	2.4	2.4	31.0
25.81	2	4.8	4.8	35.7
26.51	2	4.8	4.8	40.5
27.50	2	4.8	4.8	45.2
27.92	1	2.4	2.4	47.6
29.20	2	4.8	4.8	52.4
29.90	3	7.1	7.1	59.5
30.60	3	7.1	7.1	66.7
31.30	1	2.4	2.4	69.0
32.30	4	9.5	9.5	78.6
33.00	1	2.4	2.4	81.0
33.29	1	2.4	2.4	83.3
33.99	2	4.8	4.8	88.1
37.08	1	2.4	2.4	90.5
39.77	1	2.4	2.4	92.9
64.59	1	2.4	2.4	95.2
70.37	1	2.4	2.4	97.6
277.40	1	2.4	2.4	100.0
Total	42	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 61 Statistical data of microemulsion droplet size of ME9 after 6 months storage at temperature of 4°C

Statistics[b]

DIAMETER

N	Valid	11
	Missing	0
Mean		55.3003
Median		60.9127
Mode		9.87[a]
Std. Deviation		20.7804
Minimum		9.87
Maximum		80.65
Sum		608.30

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME9-4C-M6 (E)

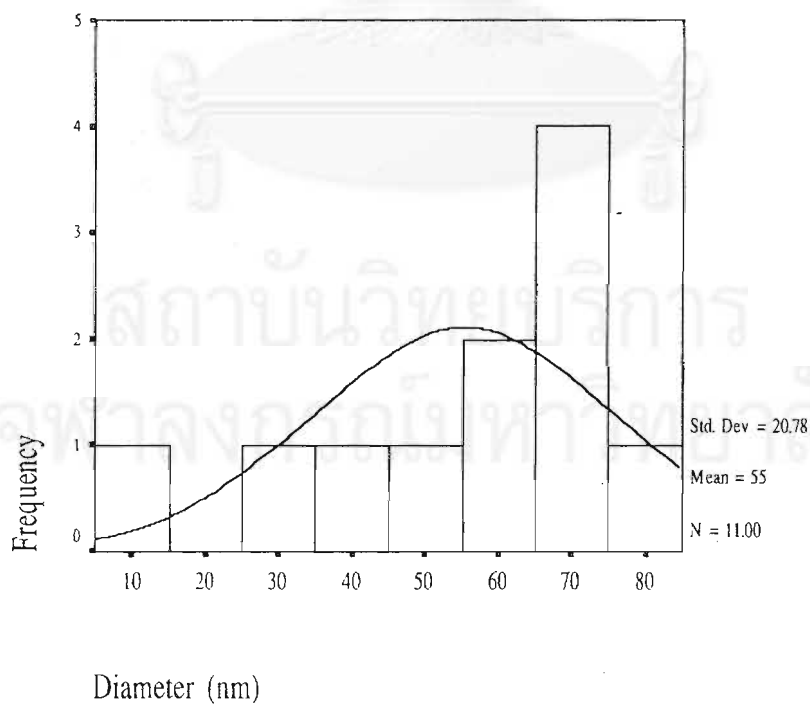


Figure 92 Droplet size histogram of ME9 after 6 months storage at temperature of 4°C

Table 62 Droplet size frequency of ME9 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
9.87	1	9.1	9.1	9.1
34.69	1	9.1	9.1	18.2
36.38	1	9.1	9.1	27.3
52.74	1	9.1	9.1	36.4
55.83	1	9.1	9.1	45.5
60.91	1	9.1	9.1	54.5
67.69	1	9.1	9.1	63.6
69.09	1	9.1	9.1	72.7
69.38	1	9.1	9.1	81.8
71.07	1	9.1	9.1	90.9
80.65	1	9.1	9.1	100.0
Total	11	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 63 Statistical data of microemulsion droplet size of ME9 after 6 months storage at temperature of 50°C

Statistics[b]

DIAMETER

N	Valid	62
	Missing	0
Mean		33.6446
Median		29.4048
Mode		3.39[a]
Std. Deviation		27.2998
Minimum		3.39
Maximum		193.36
Sum		2085.97

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME9-50C-M6 (F)

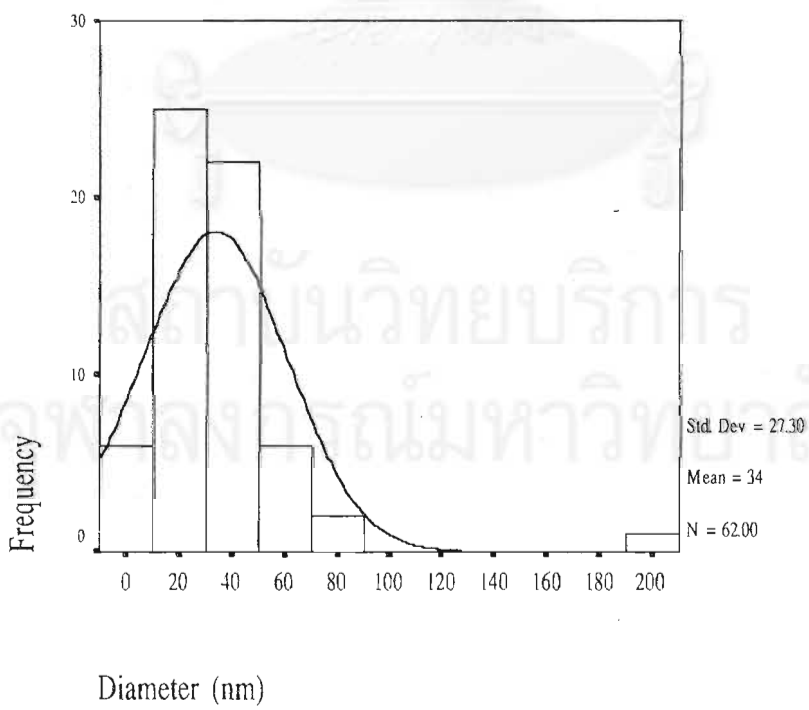


Figure 93 Droplet size histogram of ME9 after 6 months storage at temperature of 50°C

Table 64 Droplet size frequency of ME9 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	4.8	4.8	4.8
6.77	1	1.6	1.6	6.5
7.47	2	3.2	3.2	9.7
11.56	1	1.6	1.6	11.3
12.27	1	1.6	1.6	12.9
13.55	1	1.6	1.6	14.5
14.25	1	1.6	1.6	16.1
15.65	2	3.2	3.2	19.4
15.94	1	1.6	1.6	21.0
16.64	1	1.6	1.6	22.6
17.64	2	3.2	3.2	25.8
18.34	2	3.2	3.2	29.0
19.04	1	1.6	1.6	30.6
20.03	1	1.6	1.6	32.3
21.02	1	1.6	1.6	33.9
21.72	2	3.2	3.2	37.1
22.43	3	4.8	4.8	41.9
24.12	1	1.6	1.6	43.5
25.11	1	1.6	1.6	45.2
27.21	1	1.6	1.6	46.8
27.92	1	1.6	1.6	48.4
28.21	1	1.6	1.6	50.0
30.60	2	3.2	3.2	53.2
30.89	1	1.6	1.6	54.8
32.30	2	3.2	3.2	58.1
35.68	2	3.2	3.2	61.3
36.38	2	3.2	3.2	64.5
39.48	1	1.6	1.6	66.1
40.47	1	1.6	1.6	67.7
41.46	1	1.6	1.6	69.4
41.76	1	1.6	1.6	71.0
42.17	2	3.2	3.2	74.2
42.87	2	3.2	3.2	77.4
43.16	1	1.6	1.6	79.0
43.57	1	1.6	1.6	80.6
43.86	1	1.6	1.6	82.3
44.85	1	1.6	1.6	83.9
48.65	1	1.6	1.6	85.5
51.33	1	1.6	1.6	87.1
53.73	1	1.6	1.6	88.7
56.12	1	1.6	1.6	90.3
61.91	1	1.6	1.6	91.9
62.20	1	1.6	1.6	93.5
62.90	1	1.6	1.6	95.2
73.88	1	1.6	1.6	96.8
89.70	1	1.6	1.6	98.4
193.36	1	1.6	1.6	100.0
Total	62	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 65 Statistical data of microemulsion droplet size of freshly prepared ME10 storage at temperature of 30°C

Statistics

DIAMETER

N	Valid	54
	Missing	0
Mean		49.5143
Median		38.8134
Mode		11.73
Std. Deviation		45.3988
Minimum		11.73
Maximum		235.30
Sum		2673.77

a. FORMULAR = ME10-30C-M0 (A)

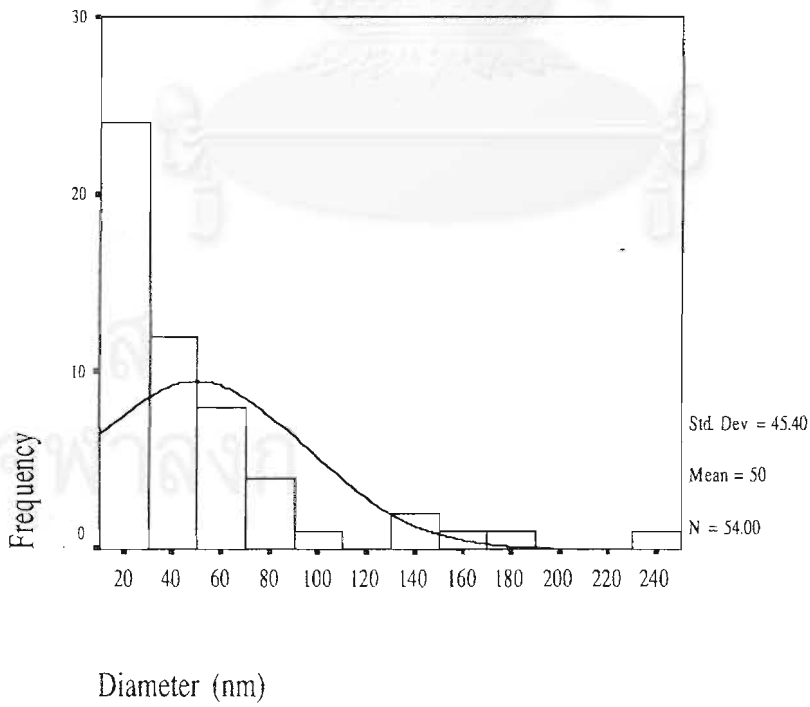


Figure 94 Droplet size histogram of freshly prepared ME10 storage at temperature of 30°C

Table 66 Droplet size frequency of freshly prepared ME10 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	9	16.7	16.7	16.7
17.59	4	7.4	7.4	24.1
20.01	1	1.9	1.9	25.9
23.45	3	5.6	5.6	31.5
25.87	5	9.3	9.3	40.7
29.31	2	3.7	3.7	44.4
31.74	1	1.9	1.9	46.3
35.17	1	1.9	1.9	48.1
37.60	1	1.9	1.9	50.0
40.03	2	3.7	3.7	53.7
43.46	3	5.6	5.6	59.3
45.89	2	3.7	3.7	63.0
46.89	2	3.7	3.7	66.7
51.75	2	3.7	3.7	70.4
52.75	1	1.9	1.9	72.2
54.18	1	1.9	1.9	74.1
55.18	1	1.9	1.9	75.9
57.61	1	1.9	1.9	77.8
60.04	1	1.9	1.9	79.6
63.47	1	1.9	1.9	81.5
77.62	1	1.9	1.9	83.3
84.49	1	1.9	1.9	85.2
86.92	1	1.9	1.9	87.0
89.35	1	1.9	1.9	88.9
91.78	1	1.9	1.9	90.7
132.81	1	1.9	1.9	92.6
135.24	1	1.9	1.9	94.4
166.97	1	1.9	1.9	96.3
171.83	1	1.9	1.9	98.1
235.30	1	1.9	1.9	100.0
Total	54	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 67 Statistical data of microemulsion droplet size of ME10 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	42
	Missing	0
Mean		16.2745
Median		13.2555
Mode		12.56
Std. Deviation		10.2533
Minimum		3.39
Maximum		57.82
Sum		683.53

a. FORMULAR = ME10-30C-M6 (D)

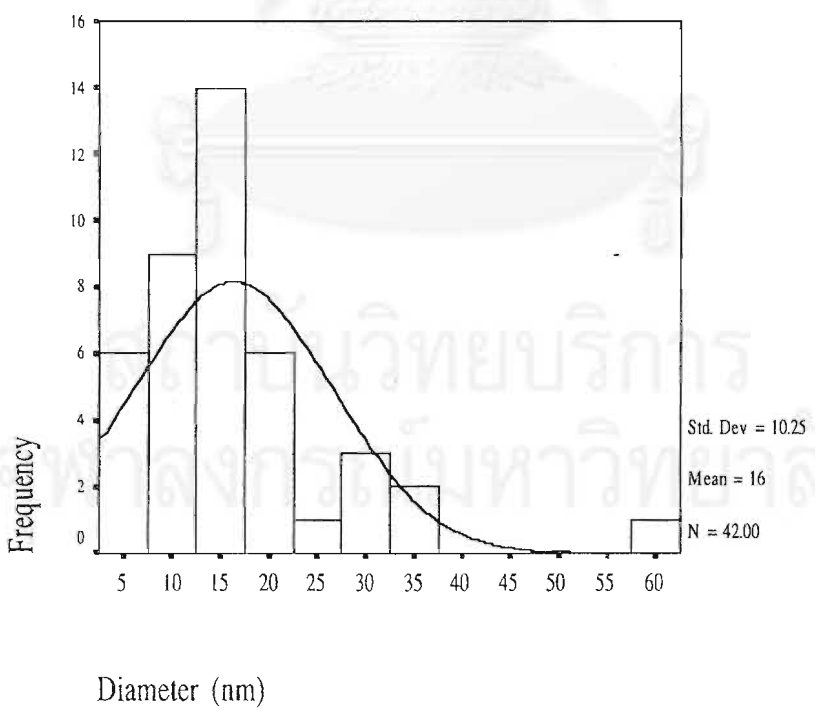


Figure 95 Droplet size histogram of ME10 after 6 months storage at temperature of 30°C

Table 68 Droplet size frequency of ME10 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	3	7.1	7.1	7.1
6.77	2	4.8	4.8	11.9
7.47	1	2.4	2.4	14.3
8.47	2	4.8	4.8	19.0
9.17	1	2.4	2.4	21.4
10.16	2	4.8	4.8	26.2
10.86	1	2.4	2.4	28.6
11.56	2	4.8	4.8	33.3
12.27	1	2.4	2.4	35.7
12.56	5	11.9	11.9	47.6
13.26	2	4.8	4.8	52.4
13.55	1	2.4	2.4	54.8
14.95	1	2.4	2.4	57.1
15.94	4	9.5	9.5	66.7
16.64	1	2.4	2.4	69.0
19.04	2	4.8	4.8	73.8
19.33	1	2.4	2.4	76.2
20.44	1	2.4	2.4	78.6
22.43	2	4.8	4.8	83.3
23.13	1	2.4	2.4	85.7
28.21	1	2.4	2.4	88.1
28.50	1	2.4	2.4	90.5
29.90	1	2.4	2.4	92.9
34.28	1	2.4	2.4	95.2
36.96	1	2.4	2.4	97.6
57.82	1	2.4	2.4	100.0
Total	42	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 69 Statistical data of microemulsion droplet size of ME10 after 6 months storage at temperature of 4°C

Statistics[b]

DIAMETER

N	Valid	11
	Missing	0
Mean		15.0136
Median		16.3514
Mode		6.77[a]
Std. Deviation		5.2378
Minimum		6.77
Maximum		22.72
Sum		165.15

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME10-4C-M6 (E)

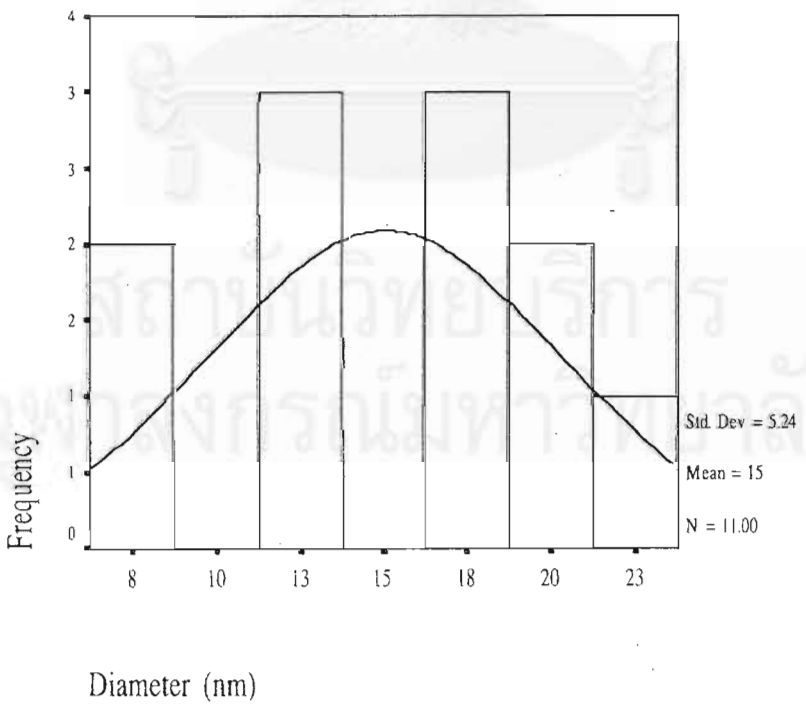


Figure 96 Droplet size histogram of ME10 after 6 months storage at temperature of 4°C

Table 70 Droplet size frequency of ME10 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
6.77	1	9.1	9.1	9.1
7.47	1	9.1	9.1	18.2
11.56	1	9.1	9.1	27.3
12.27	1	9.1	9.1	36.4
13.26	1	9.1	9.1	45.5
16.35	1	9.1	9.1	54.5
16.64	1	9.1	9.1	63.6
17.34	1	9.1	9.1	72.7
20.03	1	9.1	9.1	81.8
20.73	1	9.1	9.1	90.9
22.72	1	9.1	9.1	100.0
Total	11	100.0	100.0	

Table 71 Statistical data of microemulsion droplet size of ME10 after 6 months storage at temperature of 50°C

Statistics[b]

DIAMETER

N	Valid	33
	Missing	0
Mean		51.4928
Median		53.4386
Mode		16.64[a]
Std. Deviation		18.7323
Minimum		16.64
Maximum		85.73
Sum		1699.26

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME10-50C-M6 (F)

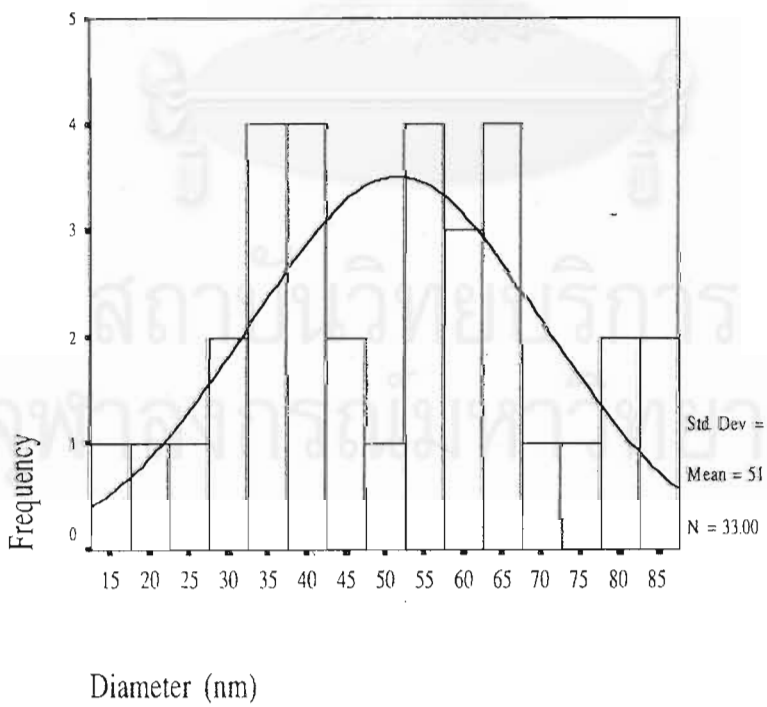


Figure 97 Droplet size histogram of ME10 after 6 months storage at temperature of 50°C

Table 72 Droplet size frequency of ME10 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
16.64	1	3.0	3.0	3.0
21.43	1	3.0	3.0	6.1
24.12	1	3.0	3.0	9.1
29.20	1	3.0	3.0	12.1
29.90	1	3.0	3.0	15.2
33.29	1	3.0	3.0	18.2
34.69	1	3.0	3.0	21.2
36.67	1	3.0	3.0	24.2
37.08	1	3.0	3.0	27.3
39.07	1	3.0	3.0	30.3
40.47	1	3.0	3.0	33.3
40.76	1	3.0	3.0	36.4
41.46	1	3.0	3.0	39.4
45.14	1	3.0	3.0	42.4
46.25	1	3.0	3.0	45.5
51.33	1	3.0	3.0	48.5
53.44	1	3.0	3.0	51.5
53.73	1	3.0	3.0	54.5
55.42	1	3.0	3.0	57.6
56.82	1	3.0	3.0	60.6
57.82	1	3.0	3.0	63.6
59.51	1	3.0	3.0	66.7
61.61	1	3.0	3.0	69.7
62.90	1	3.0	3.0	72.7
63.02	1	3.0	3.0	75.8
63.31	1	3.0	3.0	78.8
66.98	1	3.0	3.0	81.8
70.37	1	3.0	3.0	84.8
74.46	1	3.0	3.0	87.9
79.95	1	3.0	3.0	90.9
82.35	1	3.0	3.0	93.9
84.33	1	3.0	3.0	97.0
85.73	1	3.0	3.0	100.0
Total	33	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 73 Statistical data of microemulsion droplet size of freshly prepared ME11 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	163
	Missing	0
Mean		47.7117
Median		43.4605
Mode		11.73
Std. Deviation		26.9588
Minimum		11.73
Maximum		154.25
Sum		7777.01

a. FORMULAR = ME11-30C-M0 (A)

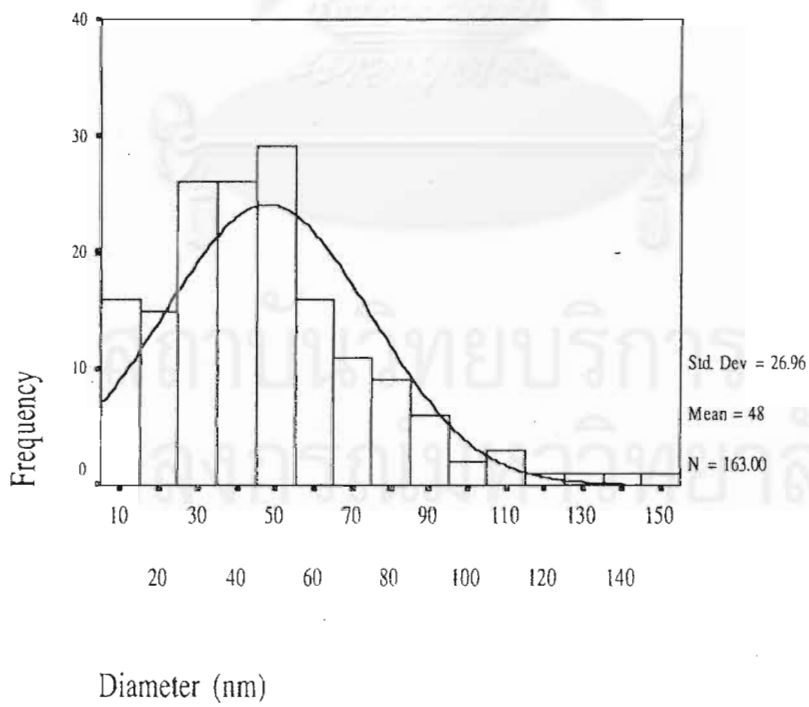


Figure 98 Droplet size histogram of freshly prepared ME11 storage at temperature of 30°C

Table 74 Droplet size frequency of freshly prepared ME11 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	16	9.8	9.8	9.8
17.59	4	2.5	2.5	12.3
20.01	9	5.5	5.5	17.8
23.45	2	1.2	1.2	19.0
25.87	9	5.5	5.5	24.5
28.30	1	0.6	0.6	25.2
31.74	10	6.1	6.1	31.3
34.16	6	3.7	3.7	35.0
35.17	5	3.1	3.1	38.0
37.60	6	3.7	3.7	41.7
40.03	5	3.1	3.1	44.8
41.03	1	0.6	0.6	45.4
42.46	4	2.5	2.5	47.9
43.46	5	3.1	3.1	50.9
45.89	4	2.5	2.5	53.4
46.89	5	3.1	3.1	56.4
48.32	1	0.6	0.6	57.1
49.32	6	3.7	3.7	60.7
51.75	10	6.1	6.1	66.9
54.18	3	1.8	1.8	68.7
55.18	4	2.5	2.5	71.2
57.61	1	0.6	0.6	71.8
59.04	1	0.6	0.6	72.4
60.04	1	0.6	0.6	73.0
61.05	4	2.5	2.5	75.5
62.47	2	1.2	1.2	76.7
63.47	3	1.8	1.8	78.5
65.90	3	1.8	1.8	80.4
69.33	5	3.1	3.1	83.4
70.76	1	0.6	0.6	84.0
71.76	1	0.6	0.6	84.7
72.77	1	0.6	0.6	85.3
75.20	1	0.6	0.6	85.9
77.62	2	1.2	1.2	87.1
78.63	1	0.6	0.6	87.7
80.05	1	0.6	0.6	88.3
81.06	1	0.6	0.6	89.0
83.49	3	1.8	1.8	90.8
85.92	2	1.2	1.2	92.0
89.35	2	1.2	1.2	93.3
90.77	1	0.6	0.6	93.9
94.20	1	0.6	0.6	94.5
97.64	1	0.6	0.6	95.1
103.50	1	0.6	0.6	95.7
109.36	2	1.2	1.2	96.9
110.78	1	0.6	0.6	97.5
115.22	1	0.6	0.6	98.2
128.37	1	0.6	0.6	98.8
135.24	1	0.6	0.6	99.4
154.25	1	0.6	0.6	100.0
Total	163	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 75 Statistical data of microemulsion droplet size of ME11 after 6 months storage at temperature of 30°C

Statistics[b]

DIAMETER

N	Valid	132
	Missing	0
Mean		27.1876
Median		24.8214
Mode		25.81[a]
Std. Deviation		13.1557
Minimum		3.39
Maximum		84.33
Sum		3588.76

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME11-30C-M6 (D)

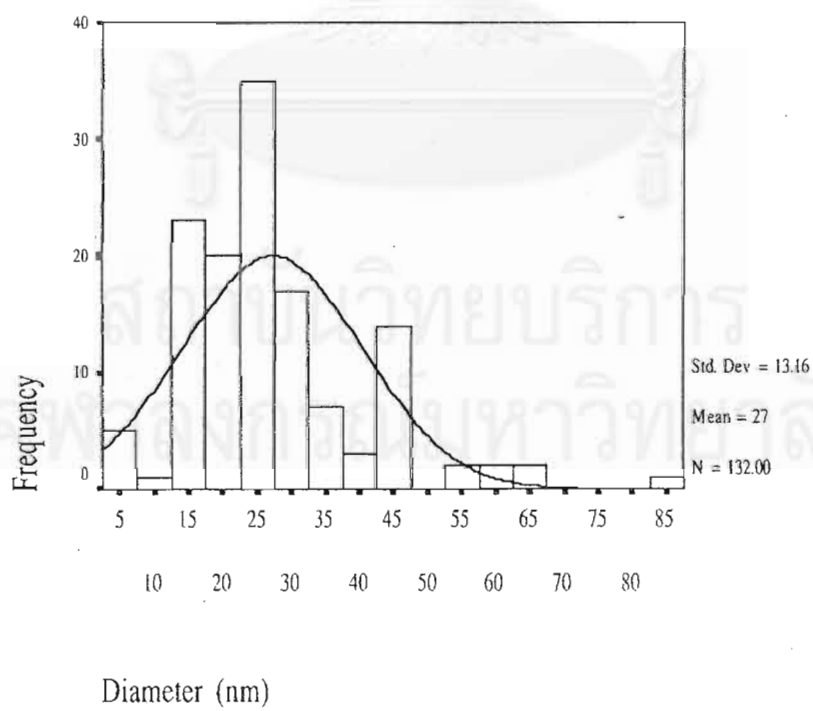


Figure 99 Droplet size histogram of ME11 after 6 months storage at temperature of 30°C

Table 76 Droplet size frequency of ME11 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	2	1.5	1.5	1.5
5.08	1	0.8	0.8	2.3
7.47	2	1.5	1.5	3.8
11.56	1	0.8	0.8	4.5
13.26	7	5.3	5.3	9.8
14.95	7	5.3	5.3	15.2
15.65	1	0.8	0.8	15.9
15.94	4	3.0	3.0	18.9
16.35	3	2.3	2.3	21.2
16.64	1	0.8	0.8	22.0
17.64	1	0.8	0.8	22.7
18.05	3	2.3	2.3	25.0
18.34	4	3.0	3.0	28.0
19.74	2	1.5	1.5	29.5
20.03	2	1.5	1.5	31.1
20.73	2	1.5	1.5	32.6
21.02	3	2.3	2.3	34.8
21.43	1	0.8	0.8	35.6
21.72	2	1.5	1.5	37.1
22.72	1	0.8	0.8	37.9
23.13	5	3.8	3.8	41.7
23.42	1	0.8	0.8	42.4
23.83	1	0.8	0.8	43.2
24.12	7	5.3	5.3	48.5
24.82	3	2.3	2.3	50.8
25.11	1	0.8	0.8	51.5
25.52	1	0.8	0.8	52.3
25.81	8	6.1	6.1	58.3
26.51	5	3.8	3.8	62.1
27.21	2	1.5	1.5	63.6
27.50	2	1.5	1.5	65.2
28.21	3	2.3	2.3	67.4
29.90	3	2.3	2.3	69.7
30.19	3	2.3	2.3	72.0
30.60	1	0.8	0.8	72.7
31.30	2	1.5	1.5	74.2
31.59	2	1.5	1.5	75.8
31.89	1	0.8	0.8	76.5
33.00	2	1.5	1.5	78.0
33.29	1	0.8	0.8	78.8
33.99	1	0.8	0.8	79.5
35.39	1	0.8	0.8	80.3
36.38	1	0.8	0.8	81.1
36.67	1	0.8	0.8	81.8
38.08	1	0.8	0.8	82.6
40.06	1	0.8	0.8	83.3
40.18	1	0.8	0.8	84.1
43.57	2	1.5	1.5	85.6
44.85	8	6.1	6.1	91.7
45.14	1	0.8	0.8	92.4
45.26	1	0.8	0.8	93.2
46.54	1	0.8	0.8	93.9
46.83	1	0.8	0.8	94.7
56.41	2	1.5	1.5	96.2
58.40	1	0.8	0.8	97.0
60.50	1	0.8	0.8	97.7
62.90	1	0.8	0.8	98.5
63.89	1	0.8	0.8	99.2
84.33	1	0.8	0.8	100.0
Total	132	100.0	100.0	

Table 77 Statistical data of microemulsion droplet size of ME11 after 6 months storage at temperature of 4°C

Statistics[b]

DIAMETER

N	Valid	52
	Missing	0
Mean		34.2878
Median		30.7475
Mode		25.81[a]
Std. Deviation		13.6461
Minimum		13.96
Maximum		102.25
Sum		1782.97

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME11-4C-M6 (E)

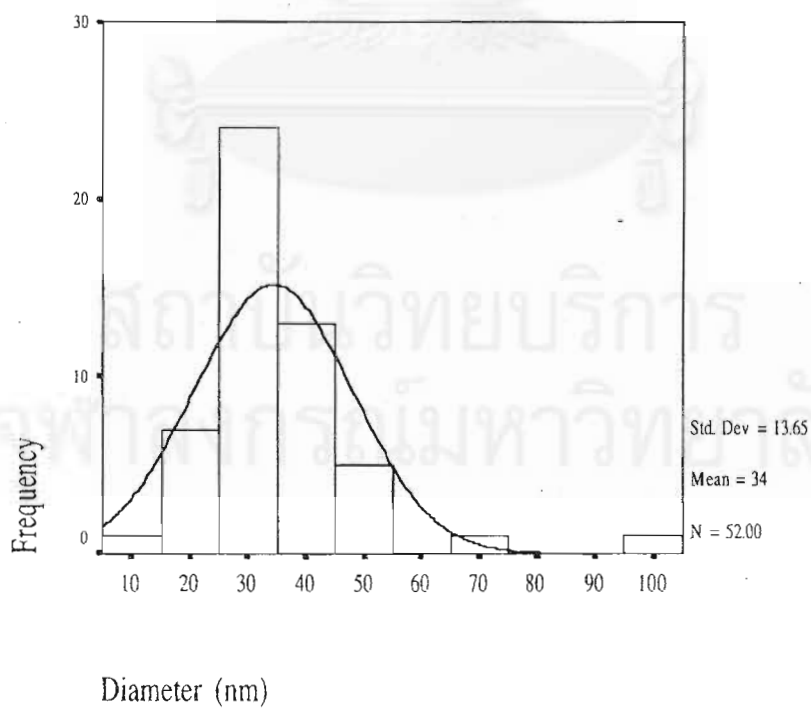


Figure 100 Droplet size histogram of ME11 after 6 months storage at temperature of 4°C

Table 78 Droplet size frequency of ME11 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
13.96	1	1.9	1.9	1.9
18.34	1	1.9	1.9	3.8
20.73	1	1.9	1.9	5.8
21.72	1	1.9	1.9	7.7
22.43	1	1.9	1.9	9.6
23.13	1	1.9	1.9	11.5
23.42	2	3.8	3.8	15.4
25.52	1	1.9	1.9	17.3
25.81	3	5.8	5.8	23.1
26.51	2	3.8	3.8	26.9
27.92	1	1.9	1.9	28.8
28.50	3	5.8	5.8	34.6
29.20	3	5.8	5.8	40.4
29.61	2	3.8	3.8	44.2
29.90	1	1.9	1.9	46.2
30.31	1	1.9	1.9	48.1
30.60	1	1.9	1.9	50.0
30.89	1	1.9	1.9	51.9
31.59	1	1.9	1.9	53.8
32.59	1	1.9	1.9	55.8
33.00	1	1.9	1.9	57.7
33.99	1	1.9	1.9	59.6
34.69	1	1.9	1.9	61.5
35.39	1	1.9	1.9	63.5
35.68	1	1.9	1.9	65.4
35.97	1	1.9	1.9	67.3
36.67	1	1.9	1.9	69.2
37.37	2	3.8	3.8	73.1
38.08	1	1.9	1.9	75.0
38.37	1	1.9	1.9	76.9
39.07	1	1.9	1.9	78.8
39.77	1	1.9	1.9	80.8
40.76	2	3.8	3.8	84.6
44.56	1	1.9	1.9	86.5
45.55	1	1.9	1.9	88.5
45.84	1	1.9	1.9	90.4
47.54	1	1.9	1.9	92.3
48.65	1	1.9	1.9	94.2
50.63	1	1.9	1.9	96.2
71.78	1	1.9	1.9	98.1
102.25	1	1.9	1.9	100.0
Total	52	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 79 Statistical data of microemulsion droplet size of ME11 after 6 months storage at temperature of 50°C

Statistics[a]

DIAMETER

N	Valid	159
	Missing	0
Mean		10.6720
Median		10.1595
Mode		3.39
Std. Deviation		5.6388
Minimum		3.39
Maximum		32.59
Sum		1696.85

a. FORMULAR = ME11-50C-M6 (F)

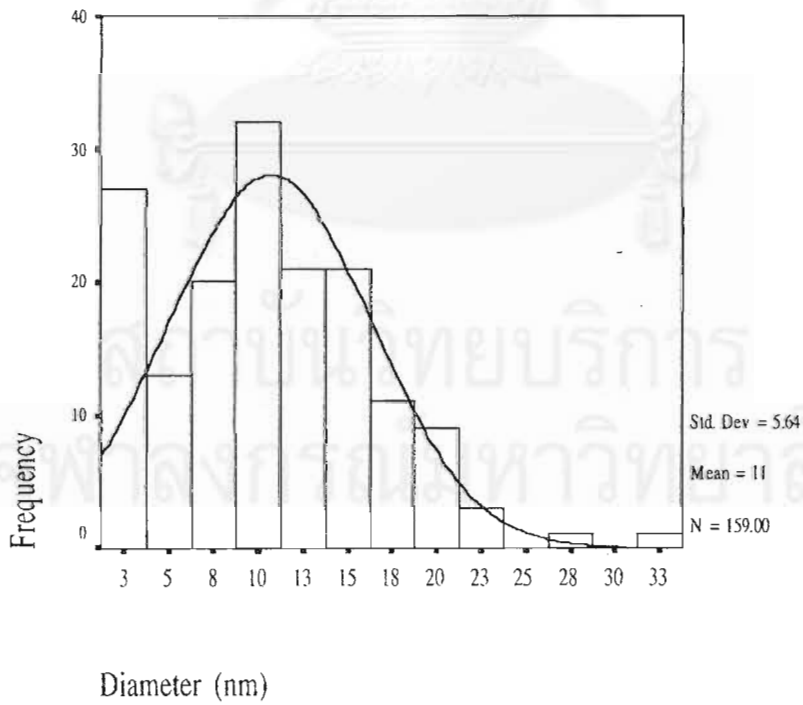


Figure 101 Droplet size histogram of ME11 after 6 months storage at temperature of 50°C

Table 80 Droplet size frequency of ME11 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	27	17.0	17.0	17.0
5.08	7	4.4	4.4	21.4
5.78	6	3.8	3.8	25.2
6.77	9	5.7	5.7	30.8
7.47	10	6.3	6.3	37.1
8.47	1	0.6	0.6	37.7
9.17	13	8.2	8.2	45.9
9.87	2	1.3	1.3	47.2
10.16	7	4.4	4.4	51.6
10.86	10	6.3	6.3	57.9
11.56	5	3.1	3.1	61.0
11.85	2	1.3	1.3	62.3
12.27	3	1.9	1.9	64.2
12.56	8	5.0	5.0	69.2
13.26	3	1.9	1.9	71.1
13.96	3	1.9	1.9	73.0
14.25	5	3.1	3.1	76.1
14.66	1	0.6	0.6	76.7
14.95	4	2.5	2.5	79.2
15.65	3	1.9	1.9	81.1
15.94	5	3.1	3.1	84.3
16.35	1	0.6	0.6	84.9
16.64	1	0.6	0.6	85.5
16.93	2	1.3	1.3	86.8
17.34	2	1.3	1.3	88.1
17.64	3	1.9	1.9	89.9
18.34	2	1.3	1.3	91.2
19.04	3	1.9	1.9	93.1
19.74	1	0.6	0.6	93.7
20.03	2	1.3	1.3	95.0
20.73	3	1.9	1.9	96.9
23.13	1	0.6	0.6	97.5
23.42	2	1.3	1.3	98.7
26.80	1	0.6	0.6	99.4
32.59	1	0.6	0.6	100.0
Total	159	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 81 Statistical data of microemulsion droplet size of ME12 after 6 months storage at temperature of 4°C

Statistics

DIAMETER

N	Valid	110
	Missing	0
Mean		31.3219
Median		31.7386
Mode		29.90
Std. Deviation		8.8567
Minimum		3.39
Maximum		57.82
Sum		3445.41

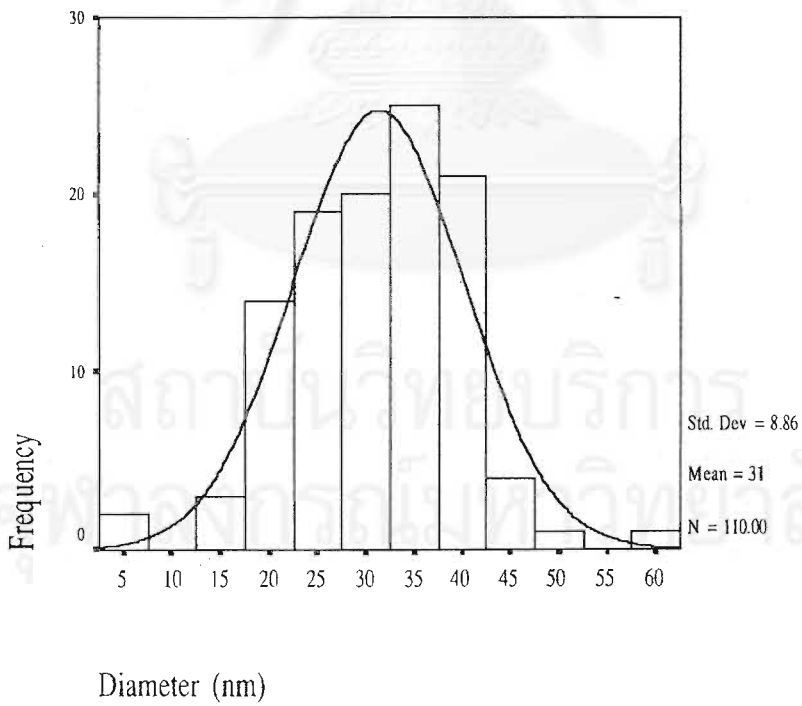


Figure 102 Droplet size histogram of ME12 after 6 months storage at temperature of 4°C

Table 82 Droplet size frequency of ME12 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	2	1.8	1.8	1.8
16.64	3	2.7	2.7	4.5
18.34	2	1.8	1.8	6.4
19.33	1	0.9	0.9	7.3
20.03	1	0.9	0.9	8.2
20.44	1	0.9	0.9	9.1
20.73	2	1.8	1.8	10.9
21.02	2	1.8	1.8	12.7
21.72	1	0.9	0.9	13.6
22.43	4	3.6	3.6	17.3
23.13	2	1.8	1.8	19.1
23.42	2	1.8	1.8	20.9
23.71	1	0.9	0.9	21.8
23.83	1	0.9	0.9	22.7
24.12	1	0.9	0.9	23.6
25.11	1	0.9	0.9	24.5
25.52	3	2.7	2.7	27.3
25.81	4	3.6	3.6	30.9
26.80	1	0.9	0.9	31.8
27.21	3	2.7	2.7	34.5
27.50	1	0.9	0.9	35.5
28.21	1	0.9	0.9	36.4
28.50	2	1.8	1.8	38.2
28.91	1	0.9	0.9	39.1
29.90	6	5.5	5.5	44.5
30.19	2	1.8	1.8	46.4
30.60	2	1.8	1.8	48.2
31.30	1	0.9	0.9	49.1
31.59	1	0.9	0.9	50.0
31.89	1	0.9	0.9	50.9
32.30	2	1.8	1.8	52.7
33.29	1	0.9	0.9	53.6
33.70	1	0.9	0.9	54.5
33.99	4	3.6	3.6	58.2
34.28	1	0.9	0.9	59.1
34.69	3	2.7	2.7	61.8
34.98	2	1.8	1.8	63.6
35.68	1	0.9	0.9	64.5
35.97	1	0.9	0.9	65.5
36.09	2	1.8	1.8	67.3
36.38	3	2.7	2.7	70.0
36.67	1	0.9	0.9	70.9
37.08	1	0.9	0.9	71.8
37.37	4	3.6	3.6	75.5
37.79	1	0.9	0.9	76.4
38.08	1	0.9	0.9	77.3
38.49	1	0.9	0.9	78.2
38.78	3	2.7	2.7	80.9
39.07	2	1.8	1.8	82.7
39.77	2	1.8	1.8	84.5
40.47	4	3.6	3.6	88.2
40.76	2	1.8	1.8	90.0
41.17	1	0.9	0.9	90.9
41.76	1	0.9	0.9	91.8
42.17	2	1.8	1.8	93.6
42.46	1	0.9	0.9	94.5
43.16	1	0.9	0.9	95.5
44.56	1	0.9	0.9	96.4
45.84	1	0.9	0.9	97.3
46.25	1	0.9	0.9	98.2
50.63	1	0.9	0.9	99.1
57.82	1	0.9	0.9	100.0
Total	110	100.0	100.0	

Table 83 Statistical data of microemulsion droplet size of freshly prepared ME13 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	57
	Missing	0
Mean		158.808
Median		120.079
Mode		37.60
Std. Deviation		121.031
Minimum		11.73
Maximum		472.45
Sum		9052.04

a. FORMULAR = ME13-30C-M0 (A)

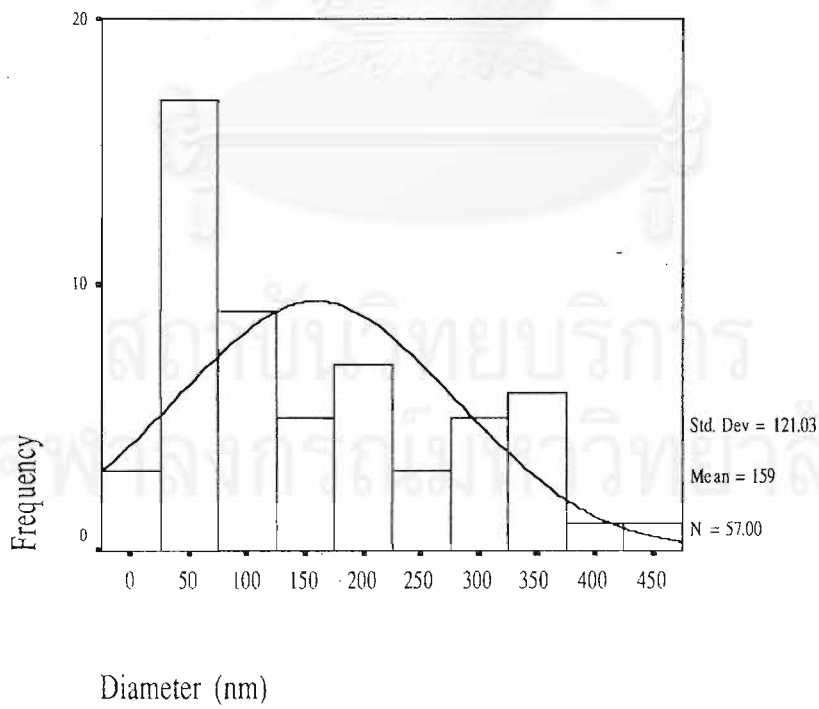


Figure 103 Droplet size histogram of freshly prepared ME13 storage at temperature of 30°C

Table 84 Droplet size frequency of freshly prepared ME13 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	1	1.8	1.8	1.8
17.59	1	1.8	1.8	3.5
23.45	1	1.8	1.8	5.3
25.87	1	1.8	1.8	7.0
29.31	1	1.8	1.8	8.8
31.74	1	1.8	1.8	10.5
37.60	3	5.3	5.3	15.8
40.03	1	1.8	1.8	17.5
43.46	2	3.5	3.5	21.1
48.32	1	1.8	1.8	22.8
49.32	1	1.8	1.8	24.6
51.75	1	1.8	1.8	26.3
57.61	2	3.5	3.5	29.8
62.47	1	1.8	1.8	31.6
69.33	2	3.5	3.5	35.1
75.20	1	1.8	1.8	36.8
77.62	1	1.8	1.8	38.6
83.49	2	3.5	3.5	42.1
85.92	2	3.5	3.5	45.6
91.78	1	1.8	1.8	47.4
120.08	2	3.5	3.5	50.9
125.94	1	1.8	1.8	52.6
157.68	1	1.8	1.8	54.4
163.54	1	1.8	1.8	56.1
174.26	2	3.5	3.5	59.6
183.55	1	1.8	1.8	61.4
188.41	1	1.8	1.8	63.2
195.28	1	1.8	1.8	64.9
202.56	1	1.8	1.8	66.7
208.42	1	1.8	1.8	68.4
216.71	1	1.8	1.8	70.2
223.58	1	1.8	1.8	71.9
248.45	1	1.8	1.8	73.7
268.46	1	1.8	1.8	75.4
274.32	1	1.8	1.8	77.2
277.76	1	1.8	1.8	78.9
282.61	1	1.8	1.8	80.7
283.62	1	1.8	1.8	82.5
288.48	1	1.8	1.8	84.2
309.91	1	1.8	1.8	86.0
326.07	1	1.8	1.8	87.7
327.50	1	1.8	1.8	89.5
331.94	1	1.8	1.8	91.2
346.09	2	3.5	3.5	94.7
362.67	1	1.8	1.8	96.5
422.71	1	1.8	1.8	98.2
472.45	1	1.8	1.8	100.0
Total	57	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 85 Statistical data of microemulsion droplet size of ME14 after 6 months storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	126
	Missing	0
Mean		12.2809
Median		9.1668
Mode		3.39
Std. Deviation		8.6080
Minimum		3.39
Maximum		52.04
Sum		1547.39

a. FORMULAR = ME14-30C-M6 (D)

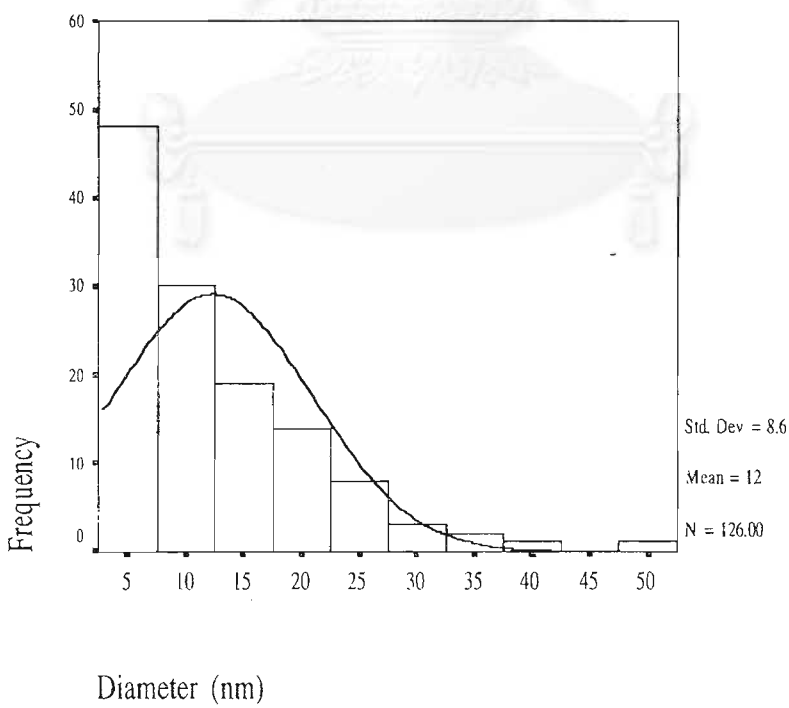


Figure 104 Droplet size histogram of ME14 after 6 months storage at temperature of 30°C

Table 86 Droplet size frequency of ME14 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	20	15.9	15.9	15.9
5.08	5	4.0	4.0	19.8
5.78	8	6.3	6.3	26.2
6.77	7	5.6	5.6	31.7
7.47	8	6.3	6.3	38.1
8.18	1	0.8	0.8	38.9
8.47	5	4.0	4.0	42.9
9.17	10	7.9	7.9	50.8
9.87	5	4.0	4.0	54.8
10.86	5	4.0	4.0	58.7
11.56	1	0.8	0.8	59.5
11.85	1	0.8	0.8	60.3
12.27	2	1.6	1.6	61.9
12.56	3	2.4	2.4	64.3
13.96	3	2.4	2.4	66.7
14.25	2	1.6	1.6	68.3
14.95	4	3.2	3.2	71.4
15.65	1	0.8	0.8	72.2
15.94	2	1.6	1.6	73.8
16.64	1	0.8	0.8	74.6
16.93	1	0.8	0.8	75.4
17.34	2	1.6	1.6	77.0
17.64	1	0.8	0.8	77.8
18.34	2	1.6	1.6	79.4
18.75	1	0.8	0.8	80.2
19.04	1	0.8	0.8	81.0
19.33	3	2.4	2.4	83.3
20.03	2	1.6	1.6	84.9
20.73	2	1.6	1.6	86.5
22.43	2	1.6	1.6	88.1
23.13	3	2.4	2.4	90.5
24.12	1	0.8	0.8	91.3
24.82	1	0.8	0.8	92.1
25.11	2	1.6	1.6	93.7
27.21	1	0.8	0.8	94.4
27.50	1	0.8	0.8	95.2
30.89	1	0.8	0.8	96.0
32.30	1	0.8	0.8	96.8
34.69	1	0.8	0.8	97.6
35.68	1	0.8	0.8	98.4
38.08	1	0.8	0.8	99.2
52.04	1	0.8	0.8	100.0
Total	126	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 87 Statistical data of microemulsion droplet size of freshly prepared ME14 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	20
	Missing	0
Mean		179.441
Median		178.195
Mode		131.80
Std. Deviation		92.0966
Minimum		51.75
Maximum		382.68
Sum		3588.83

a. FORMULAR = ME14-30C-M0 (A)

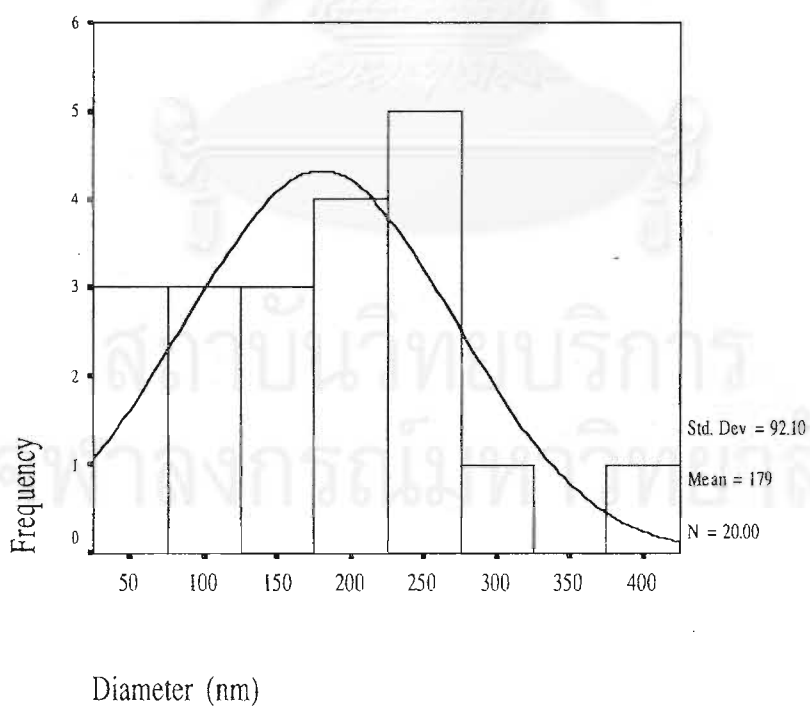


Figure 105 Droplet size histogram of freshly prepared ME14 storage at temperature of 30°C

Table 88 Droplet size frequency of freshly prepared ME14 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
51.75	1	5.0	5.0	5.0
56.60	1	5.0	5.0	10.0
65.90	1	5.0	5.0	15.0
80.05	1	5.0	5.0	20.0
86.92	1	5.0	5.0	25.0
103.50	1	5.0	5.0	30.0
131.80	2	10.0	10.0	40.0
136.66	1	5.0	5.0	45.0
175.26	1	5.0	5.0	50.0
181.13	1	5.0	5.0	55.0
214.28	1	5.0	5.0	60.0
222.57	1	5.0	5.0	65.0
240.16	1	5.0	5.0	70.0
246.02	1	5.0	5.0	75.0
254.31	1	5.0	5.0	80.0
265.03	1	5.0	5.0	85.0
272.90	1	5.0	5.0	90.0
289.48	1	5.0	5.0	95.0
382.68	1	5.0	5.0	100.0
Total	20	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 89 Statistical data of microemulsion droplet size of ME14 after 6 months storage at temperature of 4°C

Statistics[a]

DIAMETER

N	Valid	43
	Missing	0
Mean		23.7546
Median		20.0295
Mode		3.39
Std. Deviation		18.6937
Minimum		3.39
Maximum		78.96
Sum		1021.45

a. FORMULAR = ME14-4C-M6 (E)

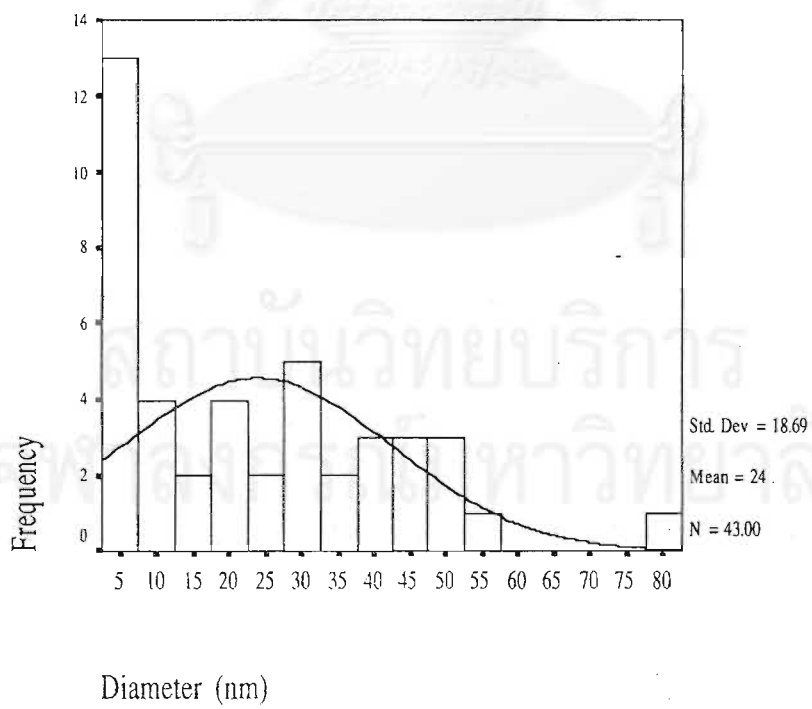


Figure 106 Droplet size histogram of ME14 after 6 months storage at temperature of 4°C

Table 90 Droplet size frequency of ME14 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	8	18.6	18.6	18.6
5.78	2	4.7	4.7	23.3
6.77	2	4.7	4.7	27.9
7.47	1	2.3	2.3	30.2
9.17	1	2.3	2.3	32.6
9.87	2	4.7	4.7	37.2
10.16	1	2.3	2.3	39.5
13.26	1	2.3	2.3	41.9
17.34	1	2.3	2.3	44.2
19.04	2	4.7	4.7	48.8
20.03	1	2.3	2.3	51.2
22.43	1	2.3	2.3	53.5
23.13	1	2.3	2.3	55.8
24.82	1	2.3	2.3	58.1
28.21	1	2.3	2.3	60.5
28.50	1	2.3	2.3	62.8
29.20	1	2.3	2.3	65.1
31.59	1	2.3	2.3	67.4
32.30	1	2.3	2.3	69.8
33.99	1	2.3	2.3	72.1
37.08	1	2.3	2.3	74.4
40.76	1	2.3	2.3	76.7
41.17	1	2.3	2.3	79.1
42.17	1	2.3	2.3	81.4
43.86	1	2.3	2.3	83.7
45.26	1	2.3	2.3	86.0
47.24	1	2.3	2.3	88.4
47.95	1	2.3	2.3	90.7
48.94	1	2.3	2.3	93.0
49.35	1	2.3	2.3	95.3
57.11	1	2.3	2.3	97.7
78.96	1	2.3	2.3	100.0
Total	43	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 91 Statistical data of microemulsion droplet size of ME14 after 6 months storage at temperature of 50°C

Statistics[b]

DIAMETER

N	Valid	33
	Missing	0
Mean		22.3737
Median		18.3368
Mode		17.34[a]
Std. Deviation		13.7205
Minimum		5.08
Maximum		56.41
Sum		738.33

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME14-50C-M6 (F)

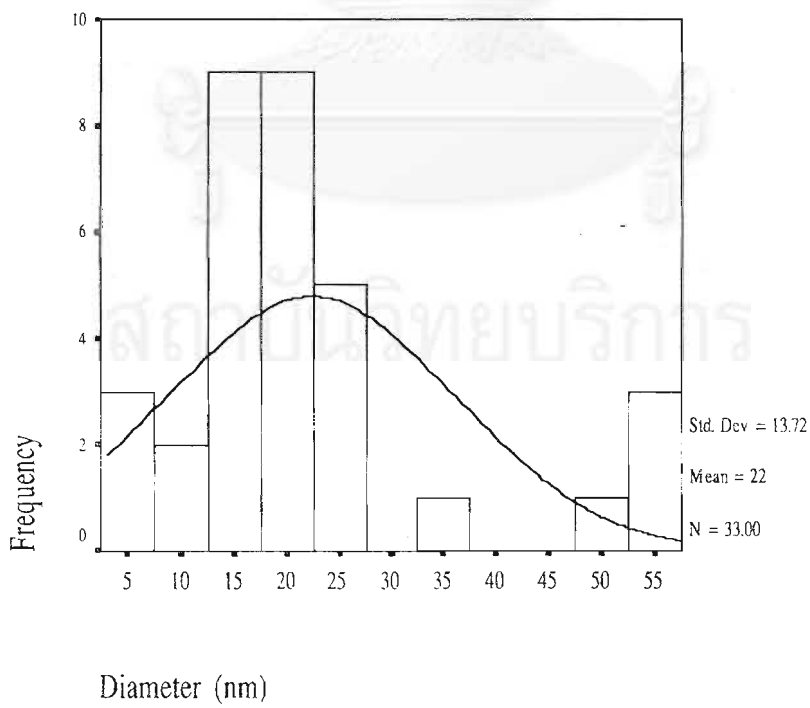


Figure 107 Droplet size histogram of ME14 after 6 months storage at temperature of 50°C

Table 92 Droplet size frequency of ME14 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
5.08	1	3.0	3.0	3.0
5.78	1	3.0	3.0	6.1
6.77	1	3.0	3.0	9.1
10.16	1	3.0	3.0	12.1
11.56	1	3.0	3.0	15.2
13.26	1	3.0	3.0	18.2
13.55	1	3.0	3.0	21.2
13.96	1	3.0	3.0	24.2
14.95	1	3.0	3.0	27.3
16.35	1	3.0	3.0	30.3
16.64	1	3.0	3.0	33.3
17.34	3	9.1	9.1	42.4
18.05	1	3.0	3.0	45.5
18.34	3	9.1	9.1	54.5
19.04	1	3.0	3.0	57.6
20.03	1	3.0	3.0	60.6
20.73	1	3.0	3.0	63.6
21.72	1	3.0	3.0	66.7
22.43	1	3.0	3.0	69.7
23.42	1	3.0	3.0	72.7
24.41	1	3.0	3.0	75.8
25.52	1	3.0	3.0	78.8
25.81	1	3.0	3.0	81.8
27.21	1	3.0	3.0	84.8
36.38	1	3.0	3.0	87.9
51.63	1	3.0	3.0	90.9
54.02	1	3.0	3.0	93.9
56.41	2	6.1	6.1	100.0
Total	33	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 93 Statistical data of microemulsion droplet size of freshly prepared ME15 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	204
	Missing	0
Mean		36.9915
Median		34.0168
Mode		22.92
Std. Deviation		17.7997
Minimum		4.24
Maximum		99.06
Sum		7546.26

a. FORMULAR = ME15-30C-M0 (A)

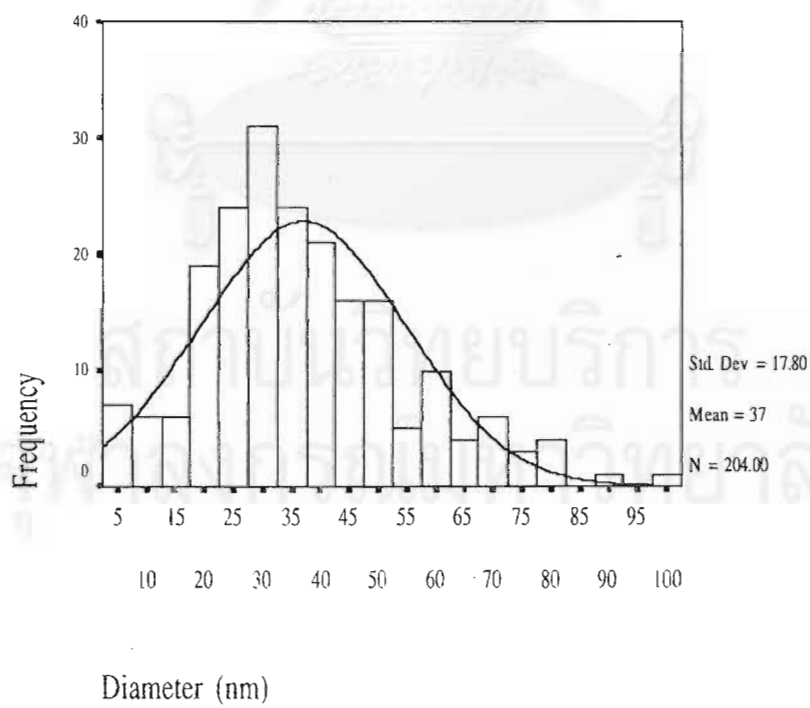


Figure 108 Droplet size histogram of freshly prepared ME15 storage at temperature of 30°C

Table 94 Droplet size frequency of freshly prepared ME15 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
4.24	5	2.5	2.5	2.5
6.35	1	0.5	0.5	2.9
7.23	1	0.5	0.5	3.4
8.47	1	0.5	0.5	3.9
9.35	4	2.0	2.0	5.9
11.46	1	0.5	0.5	6.4
13.58	1	0.5	0.5	6.9
14.82	2	1.0	1.0	7.8
16.57	2	1.0	1.0	8.8
16.93	1	0.5	0.5	9.3
17.81	5	2.5	2.5	11.8
18.69	1	0.5	0.5	12.3
19.57	2	1.0	1.0	13.2
19.93	5	2.5	2.5	15.7
20.44	1	0.5	0.5	16.2
20.80	2	1.0	1.0	17.2
21.68	3	1.5	1.5	18.6
22.56	3	1.5	1.5	20.1
22.92	8	3.9	3.9	24.0
23.80	3	1.5	1.5	25.5
24.16	1	0.5	0.5	26.0
25.04	3	1.5	1.5	27.5
25.55	1	0.5	0.5	27.9
25.92	3	1.5	1.5	29.4
27.15	2	1.0	1.0	30.4
27.67	1	0.5	0.5	30.9
28.03	2	1.0	1.0	31.9
28.39	1	0.5	0.5	32.4
28.91	4	2.0	2.0	34.3
29.27	3	1.5	1.5	35.8
30.15	5	2.5	2.5	38.2
30.51	2	1.0	1.0	39.2
31.03	5	2.5	2.5	41.7
31.39	1	0.5	0.5	42.2
31.90	3	1.5	1.5	43.6
32.26	4	2.0	2.0	45.6
32.78	2	1.0	1.0	46.6
33.14	3	1.5	1.5	48.0
33.50	1	0.5	0.5	48.5
34.02	4	2.0	2.0	50.5
34.38	4	2.0	2.0	52.5
35.26	3	1.5	1.5	53.9
35.62	1	0.5	0.5	54.4
36.14	1	0.5	0.5	54.9
36.50	3	1.5	1.5	56.4
37.01	1	0.5	0.5	56.9
37.37	1	0.5	0.5	57.4
37.74	2	1.0	1.0	58.3
38.25	4	2.0	2.0	60.3
38.61	1	0.5	0.5	60.8
39.13	1	0.5	0.5	61.3
39.49	1	0.5	0.5	61.8
40.01	1	0.5	0.5	62.3
40.37	1	0.5	0.5	62.7
40.73	2	1.0	1.0	63.7
41.25	2	1.0	1.0	64.7
41.61	1	0.5	0.5	65.2
41.97	1	0.5	0.5	65.7
42.12	2	1.0	1.0	66.7
42.49	2	1.0	1.0	67.6
42.85	1	0.5	0.5	68.1
43.36	1	0.5	0.5	68.6
43.72	1	0.5	0.5	69.1
44.24	2	1.0	1.0	70.1
44.60	4	2.0	2.0	72.1
45.48	2	1.0	1.0	73.0
45.84	2	1.0	1.0	74.0
45.99	1	0.5	0.5	74.5
46.72	2	1.0	1.0	75.5
47.60	3	1.5	1.5	77.0
47.96	1	0.5	0.5	77.5
48.47	2	1.0	1.0	78.4
48.83	1	0.5	0.5	78.9
49.71	2	1.0	1.0	79.9
50.08	1	0.5	0.5	80.4
50.59	3	1.5	1.5	81.9
51.47	1	0.5	0.5	82.4
51.83	2	1.0	1.0	83.3
53.07	1	0.5	0.5	83.8
53.58	2	1.0	1.0	84.8
53.94	1	0.5	0.5	85.3
55.34	1	0.5	0.5	85.8
57.82	1	0.5	0.5	86.3
58.18	1	0.5	0.5	86.8
58.69	1	0.5	0.5	87.3
59.93	2	1.0	1.0	88.2
61.54	1	0.5	0.5	88.7
61.69	1	0.5	0.5	89.2
62.41	3	1.5	1.5	90.7
62.93	1	0.5	0.5	91.2
64.53	1	0.5	0.5	91.7
66.80	1	0.5	0.5	92.2
67.16	1	0.5	0.5	92.6
67.52	1	0.5	0.5	93.1
68.04	2	1.0	1.0	94.1
69.64	1	0.5	0.5	94.6
71.03	1	0.5	0.5	95.1
71.39	1	0.5	0.5	95.6
72.63	2	1.0	1.0	96.6
77.38	1	0.5	0.5	97.1
77.74	2	1.0	1.0	98.0
78.98	1	0.5	0.5	98.5
81.25	1	0.5	0.5	99.0
92.20	1	0.5	0.5	99.5
99.06	1	0.5	0.5	100.0
Total	204	100.0	100.0	

Table 95 Statistical data of microemulsion droplet size of ME15 after 6 months storage at temperature of 30°C

Statistics[b]

DIAMETER

N	Valid	18
	Missing	0
Mean		79.7654
Median		73.1182
Mode		45.26[a]
Std. Deviation		25.0544
Minimum		45.26
Maximum		127.90
Sum		1435.78

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME15-30C-M6 (C)

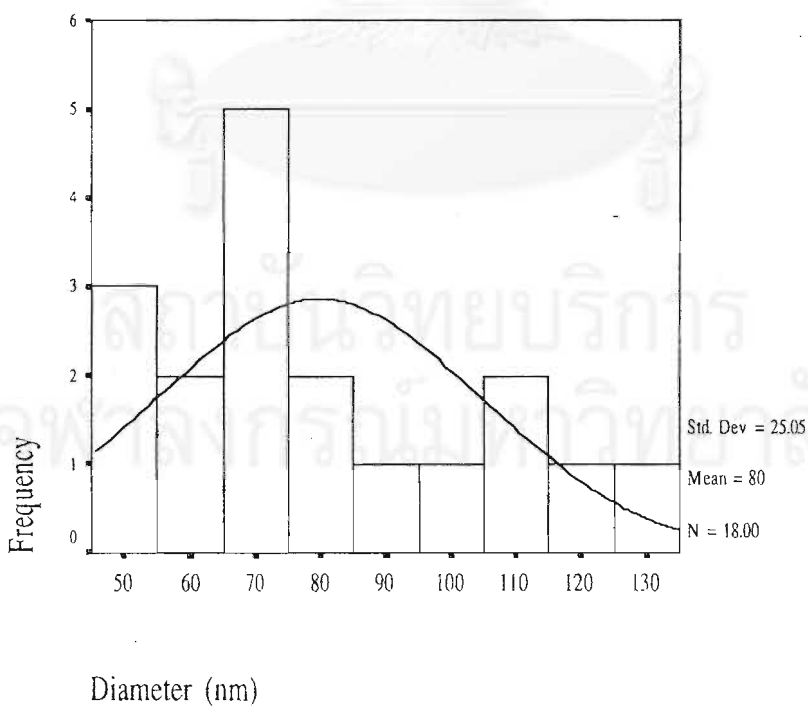


Figure 109 Droplet size histogram of ME15 after 6 months storage at temperature of 30°C

Table 96 Droplet size frequency of ME15 after 6 months storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
45.26	1	5.6	5.6	5.6
47.95	1	5.6	5.6	11.1
51.33	1	5.6	5.6	16.7
56.12	1	5.6	5.6	22.2
58.81	1	5.6	5.6	27.8
70.37	1	5.6	5.6	33.3
70.78	1	5.6	5.6	38.9
71.07	1	5.6	5.6	44.4
71.78	1	5.6	5.6	50.0
74.46	1	5.6	5.6	55.6
75.86	1	5.6	5.6	61.1
83.05	1	5.6	5.6	66.7
88.13	1	5.6	5.6	72.2
104.07	1	5.6	5.6	77.8
109.85	1	5.6	5.6	83.3
112.95	1	5.6	5.6	88.9
116.04	1	5.6	5.6	94.4
127.90	1	5.6	5.6	100.0
Total	18	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 97 Statistical data of microemulsion droplet size of ME15 after 6 months storage at temperature of 4°C

Statistics[a]

DIAMETER

N	Valid	47
	Missing	0
Mean		60.4721
Median		59.3600
Mode		37.72
Std. Deviation		28.9858
Minimum		8.97
Maximum		158.30
Sum		2842.19

a. FORMULAR = ME15-4C-M6 (E)

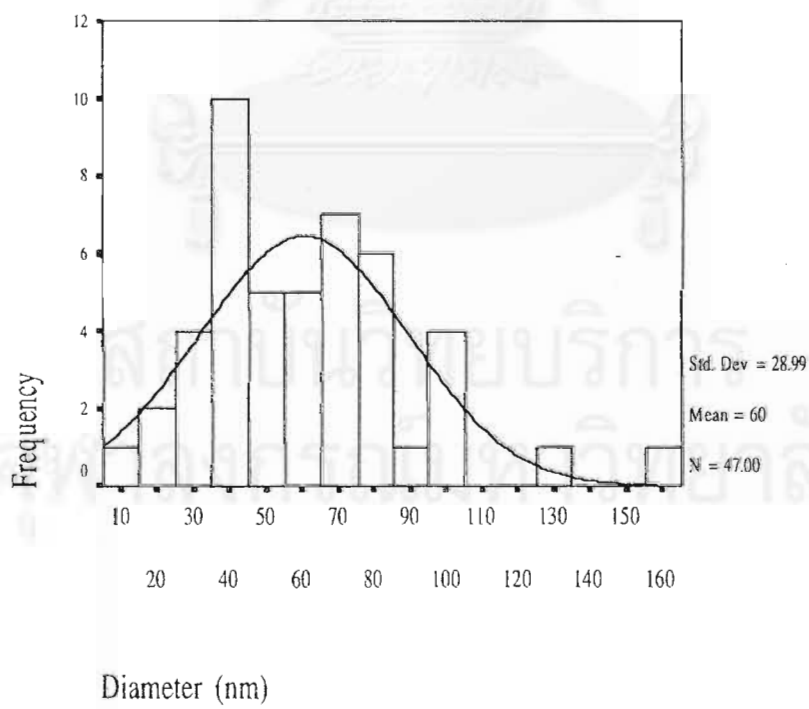


Figure 110 Droplet size histogram of ME15 after 6 months storage at temperature of 4°C

Table 98 Droplet size frequency of ME15 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
8.97	1	2.1	2.1	2.1
19.79	1	2.1	2.1	4.3
24.27	1	2.1	2.1	6.4
26.13	1	2.1	2.1	8.5
28.75	2	4.3	4.3	12.8
33.23	1	2.1	2.1	14.9
37.72	5	10.6	10.6	25.5
38.81	2	4.3	4.3	29.8
39.57	1	2.1	2.1	31.9
41.43	1	2.1	2.1	34.0
44.06	1	2.1	2.1	36.2
48.54	3	6.4	6.4	42.6
50.40	1	2.1	2.1	44.7
51.16	1	2.1	2.1	46.8
57.51	1	2.1	2.1	48.9
59.36	1	2.1	2.1	51.1
61.22	3	6.4	6.4	57.4
65.70	1	2.1	2.1	59.6
66.47	1	2.1	2.1	61.7
67.56	1	2.1	2.1	63.8
70.18	2	4.3	4.3	68.1
72.81	1	2.1	2.1	70.2
74.66	1	2.1	2.1	72.3
76.52	1	2.1	2.1	74.5
77.29	1	2.1	2.1	76.6
79.15	3	6.4	6.4	83.0
83.63	1	2.1	2.1	85.1
88.11	1	2.1	2.1	87.2
96.31	1	2.1	2.1	89.4
97.08	1	2.1	2.1	91.5
98.93	1	2.1	2.1	93.6
100.79	1	2.1	2.1	95.7
131.40	1	2.1	2.1	97.9
158.30	1	2.1	2.1	100.0
Total	47	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 99 Statistical data of microemulsion droplet size of ME15 after 6 months storage at temperature of 50°C

Statistics[b]

DIAMETER

N	Valid	14
	Missing	0
Mean		66.4418
Median		61.9039
Mode		46.25[a]
Std. Deviation		22.5425
Minimum		46.25
Maximum		121.29
Sum		930.19

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME15-50C-M6 (F)

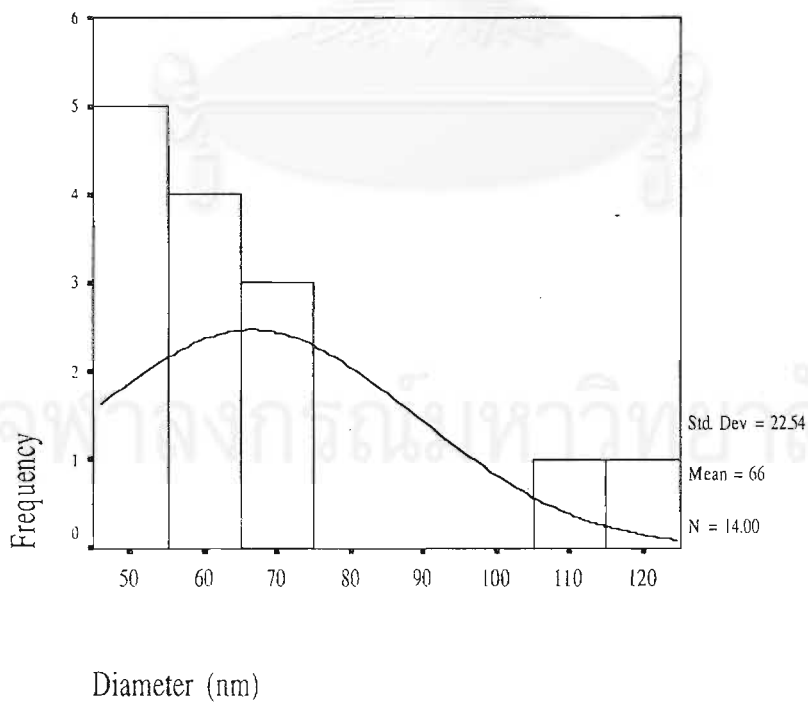


Figure 111 Droplet size histogram of ME15 after 6 months storage at temperature of 50°C

Table 100 Droplet size frequency of ME15 after 6 months storage at temperature of 50°C

	Frequency	Percent	Valid Percent	Cumulative Percent
46.25	1	7.1	7.1	7.1
46.54	1	7.1	7.1	14.3
52.33	1	7.1	7.1	21.4
53.03	1	7.1	7.1	28.6
54.43	1	7.1	7.1	35.7
55.83	1	7.1	7.1	42.9
61.20	1	7.1	7.1	50.0
62.61	1	7.1	7.1	57.1
63.19	1	7.1	7.1	64.3
65.29	1	7.1	7.1	71.4
67.28	1	7.1	7.1	78.6
68.68	1	7.1	7.1	85.7
112.25	1	7.1	7.1	92.9
121.29	1	7.1	7.1	100.0
Total	14	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 101 Statistical data of microemulsion droplet size of freshly prepared ME16 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	221
	Missing	0
Mean		48.3044
Median		42.9991
Mode		40.37
Std. Deviation		25.0411
Minimum		4.24
Maximum		159.36
Sum		10675.3

a. FORMULAR = ME16-30C-M0 (B)

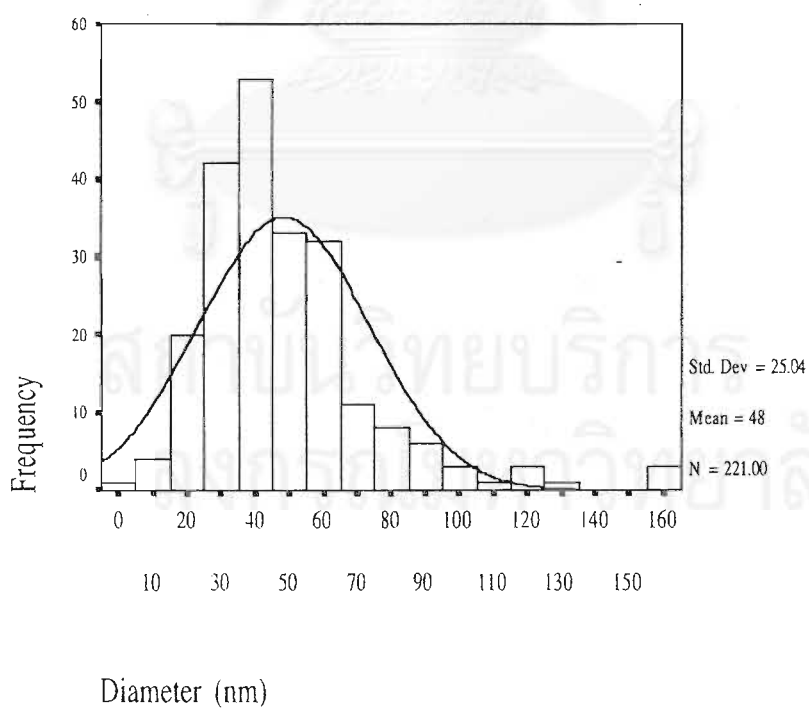


Figure 112 Droplet size histogram of freshly prepared ME16 storage at temperature of 30°C

Table 102 Droplet size frequency of freshly prepared ME16 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
4.24	1	0.5	0.5	0.5
9.35	1	0.5	0.5	0.9
13.58	1	0.5	0.5	1.4
14.46	1	0.5	0.5	1.8
14.82	1	0.5	0.5	2.3
15.69	4	1.8	1.8	4.1
16.57	1	0.5	0.5	4.5
17.81	1	0.5	0.5	5.0
18.69	1	0.5	0.5	5.4
19.93	1	0.5	0.5	5.9
21.68	2	0.9	0.9	6.8
22.56	1	0.5	0.5	7.2
22.92	3	1.4	1.4	8.6
23.80	2	0.9	0.9	9.5
24.16	4	1.8	1.8	11.3
25.04	2	0.9	0.9	12.2
25.40	1	0.5	0.5	12.7
25.92	2	0.9	0.9	13.6
26.79	1	0.5	0.5	14.0
27.15	2	0.9	0.9	14.9
27.67	2	0.9	0.9	15.8
28.03	4	1.8	1.8	17.6
28.54	2	0.9	0.9	18.6
28.91	4	1.8	1.8	20.4
29.27	1	0.5	0.5	20.8
30.15	4	1.8	1.8	22.6
30.51	2	0.9	0.9	23.5
31.03	2	0.9	0.9	24.4
31.90	2	0.9	0.9	25.3
32.26	3	1.4	1.4	26.7
33.14	5	2.3	2.3	29.0
33.50	1	0.5	0.5	29.4
34.02	1	0.5	0.5	29.9
34.38	1	0.5	0.5	30.3
35.26	6	2.7	2.7	33.0
36.14	2	0.9	0.9	33.9
36.86	1	0.5	0.5	34.4
37.01	1	0.5	0.5	34.8
37.37	4	1.8	1.8	36.7
37.74	1	0.5	0.5	37.1
38.25	2	0.9	0.9	38.0
38.61	1	0.5	0.5	38.5
39.13	2	0.9	0.9	39.4
39.49	3	1.4	1.4	40.7
39.86	2	0.9	0.9	41.6
40.37	7	3.2	3.2	44.8
41.25	2	0.9	0.9	45.7
41.61	1	0.5	0.5	46.2
42.49	5	2.3	2.3	48.4
42.85	2	0.9	0.9	49.3
43.00	2	0.9	0.9	50.2
43.36	3	1.4	1.4	51.6
43.72	1	0.5	0.5	52.0
44.09	1	0.5	0.5	52.5
44.24	3	1.4	1.4	53.8
44.60	1	0.5	0.5	54.3
45.12	1	0.5	0.5	54.8
45.48	2	0.9	0.9	55.7
46.72	2	0.9	0.9	56.6
47.08	1	0.5	0.5	57.0
47.60	5	2.3	2.3	59.3
48.47	1	0.5	0.5	59.7
48.83	1	0.5	0.5	60.2
49.35	1	0.5	0.5	60.6
49.71	1	0.5	0.5	61.1
50.08	1	0.5	0.5	61.5
50.23	1	0.5	0.5	62.0
51.32	1	0.5	0.5	62.4
51.47	3	1.4	1.4	63.8
51.83	1	0.5	0.5	64.3
52.34	1	0.5	0.5	64.7
52.71	1	0.5	0.5	65.2
53.07	1	0.5	0.5	65.6
53.58	3	1.4	1.4	67.0
54.31	1	0.5	0.5	67.4
54.46	1	0.5	0.5	67.9
54.82	3	1.4	1.4	69.2
55.19	4	1.8	1.8	71.0
55.34	1	0.5	0.5	71.5
55.70	3	1.4	1.4	72.9
56.06	1	0.5	0.5	73.3
56.58	5	2.3	2.3	75.6
56.94	1	0.5	0.5	76.0
57.45	1	0.5	0.5	76.5
57.82	2	0.9	0.9	77.4
58.18	1	0.5	0.5	77.8
58.69	2	0.9	0.9	78.7
59.05	1	0.5	0.5	79.2
59.42	1	0.5	0.5	79.6
59.93	2	0.9	0.9	80.5
60.30	2	0.9	0.9	81.4
60.81	1	0.5	0.5	81.9
61.17	1	0.5	0.5	82.4
62.05	2	0.9	0.9	83.3
62.93	1	0.5	0.5	83.7
65.41	2	0.9	0.9	84.6
66.28	2	0.9	0.9	85.5
68.04	1	0.5	0.5	86.0
68.40	1	0.5	0.5	86.4
70.15	1	0.5	0.5	86.9
71.39	2	0.9	0.9	87.8
72.63	1	0.5	0.5	88.2
74.90	1	0.5	0.5	88.7
75.26	1	0.5	0.5	89.1
77.38	1	0.5	0.5	89.6
80.01	1	0.5	0.5	90.0
80.22	1	0.5	0.5	90.5
80.37	1	0.5	0.5	91.0
81.98	1	0.5	0.5	91.4
82.49	1	0.5	0.5	91.9

Table 102 Droplet size frequency of freshly prepared ME16 storage at temperature of 30°C (cont.)

	Frequency	Percent	Valid Percent	Cumulative Percent
84.25	1	0.5	0.5	92.3
86.72	1	0.5	0.5	92.8
88.84	1	0.5	0.5	93.2
89.21	1	0.5	0.5	93.7
91.83	1	0.5	0.5	94.1
93.44	1	0.5	0.5	94.6
94.83	1	0.5	0.5	95.0
98.70	1	0.5	0.5	95.5
100.66	1	0.5	0.5	95.9
102.78	1	0.5	0.5	96.4
106.29	1	0.5	0.5	96.8
117.75	1	0.5	0.5	97.3
118.11	1	0.5	0.5	97.7
120.74	1	0.5	0.5	98.2
128.85	1	0.5	0.5	98.6
155.64	1	0.5	0.5	99.1
159.36	2	0.9	0.9	100.0
Total	221	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 103 Statistical data of microemulsion droplet size of ME16 after 6 months storage at temperature of 4°C

Statistics[a]

DIAMETER

N	Valid	39
	Missing	0
Mean		82.9480
Median		82.5151
Mode		30.60
Std. Deviation		39.5488
Minimum		30.60
Maximum		178.12
Sum		3234.97

a. FORMULAR = ME16-4C-M6 (E)

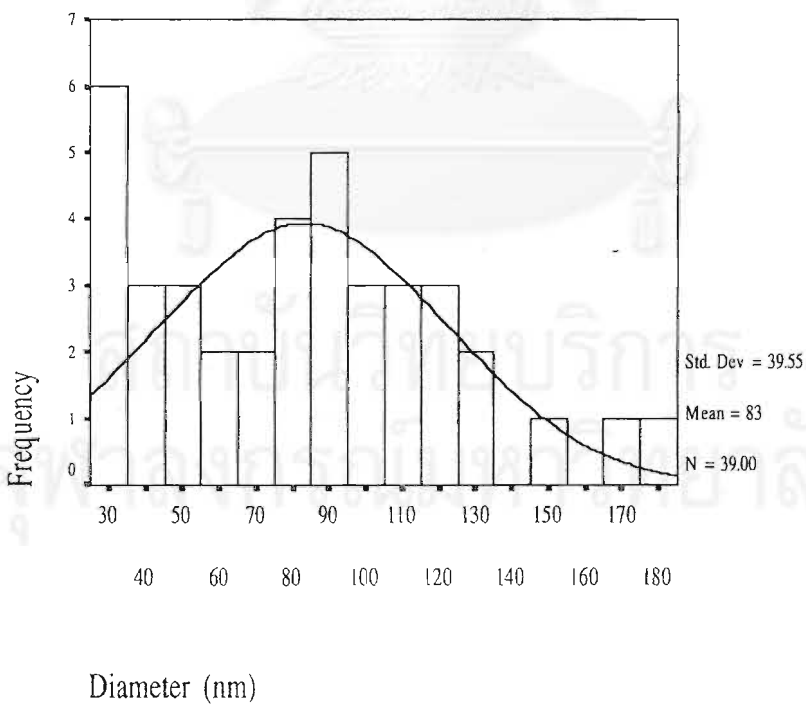


Figure 113 Droplet size histogram of ME16 after 6 months storage at temperature of 4°C

Table 104 Droplet size frequency of ME16 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
30.60	4	10.3	10.3	10.3
31.59	1	2.6	2.6	12.8
34.98	1	2.6	2.6	15.4
37.37	1	2.6	2.6	17.9
43.86	1	2.6	2.6	20.5
44.44	1	2.6	2.6	23.1
45.55	1	2.6	2.6	25.6
47.95	1	2.6	2.6	28.2
48.94	1	2.6	2.6	30.8
58.52	1	2.6	2.6	33.3
63.19	1	2.6	2.6	35.9
67.69	1	2.6	2.6	38.5
68.97	1	2.6	2.6	41.0
76.15	1	2.6	2.6	43.6
78.96	1	2.6	2.6	46.2
80.53	1	2.6	2.6	48.7
82.52	1	2.6	2.6	51.3
86.31	1	2.6	2.6	53.8
90.81	1	2.6	2.6	56.4
91.51	1	2.6	2.6	59.0
93.09	1	2.6	2.6	61.5
94.20	1	2.6	2.6	64.1
97.17	1	2.6	2.6	66.7
97.88	1	2.6	2.6	69.2
100.27	1	2.6	2.6	71.8
110.84	1	2.6	2.6	74.4
112.12	1	2.6	2.6	76.9
114.81	1	2.6	2.6	79.5
117.62	1	2.6	2.6	82.1
119.02	1	2.6	2.6	84.6
119.72	1	2.6	2.6	87.2
126.90	1	2.6	2.6	89.7
130.29	1	2.6	2.6	92.3
146.64	1	2.6	2.6	94.9
174.03	1	2.6	2.6	97.4
178.12	1	2.6	2.6	100.0
Total	39	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 105 Statistical data of microemulsion droplet size of freshly prepared ME17 storage at temperature of 30°C

Statistics[a]

DIAMETER

N	Valid	127
	Missing	0
Mean		37.1670
Median		33.1418
Mode		18.69
Std. Deviation		21.8321
Minimum		4.24
Maximum		110.01
Sum		4720.21

a. FOMULAR = ME17-30C-M0 (B)

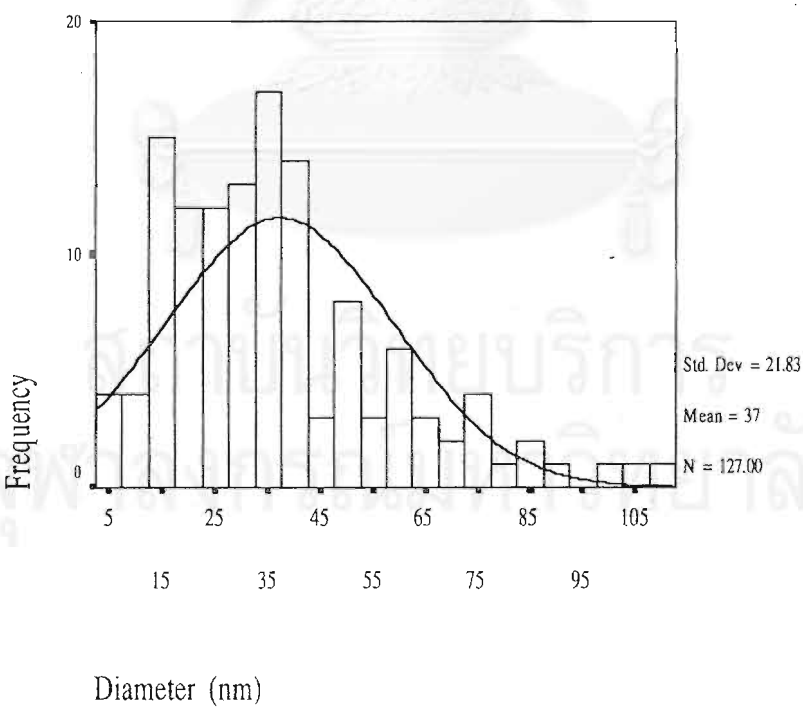


Figure 114 Droplet size histogram of freshly prepared ME17 storage at temperature of 30°C

Table 106 Droplet size frequency of freshly prepared ME17 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
4.24	2	1.6	1.6	1.6
6.35	1	0.8	0.8	2.4
7.23	1	0.8	0.8	3.1
8.47	1	0.8	0.8	3.9
9.35	3	2.4	2.4	6.3
12.70	2	1.6	1.6	7.9
13.58	3	2.4	2.4	10.2
14.46	3	2.4	2.4	12.6
15.33	1	0.8	0.8	13.4
15.69	1	0.8	0.8	14.2
16.57	4	3.1	3.1	17.3
17.45	1	0.8	0.8	18.1
17.81	2	1.6	1.6	19.7
18.69	5	3.9	3.9	23.6
19.57	1	0.8	0.8	24.4
19.93	1	0.8	0.8	25.2
20.80	1	0.8	0.8	26.0
21.68	1	0.8	0.8	26.8
22.04	1	0.8	0.8	27.6
22.56	2	1.6	1.6	29.1
22.92	1	0.8	0.8	29.9
23.80	2	1.6	1.6	31.5
25.55	1	0.8	0.8	32.3
25.92	3	2.4	2.4	34.6
26.28	1	0.8	0.8	35.4
27.15	2	1.6	1.6	37.0
28.03	3	2.4	2.4	39.4
28.39	2	1.6	1.6	40.9
29.27	2	1.6	1.6	42.5
30.15	1	0.8	0.8	43.3
30.51	1	0.8	0.8	44.1
31.03	1	0.8	0.8	44.9
32.26	3	2.4	2.4	47.2
33.14	4	3.1	3.1	50.4
34.38	4	3.1	3.1	53.5
35.26	3	2.4	2.4	55.9
35.62	3	2.4	2.4	58.3
37.01	1	0.8	0.8	59.1
37.37	2	1.6	1.6	60.6
38.25	2	1.6	1.6	62.2
39.49	2	1.6	1.6	63.8
40.37	3	2.4	2.4	66.1
40.73	2	1.6	1.6	67.7
41.25	1	0.8	0.8	68.5
41.61	2	1.6	1.6	70.1
42.49	2	1.6	1.6	71.7
43.36	1	0.8	0.8	72.4
44.60	1	0.8	0.8	73.2
46.36	1	0.8	0.8	74.0
47.60	1	0.8	0.8	74.8
48.47	1	0.8	0.8	75.6
51.32	1	0.8	0.8	76.4
51.47	1	0.8	0.8	77.2
51.83	4	3.1	3.1	80.3
53.58	1	0.8	0.8	81.1
57.30	2	1.6	1.6	82.7
57.82	1	0.8	0.8	83.5
59.42	1	0.8	0.8	84.3
60.30	1	0.8	0.8	85.0
61.17	1	0.8	0.8	85.8
62.05	1	0.8	0.8	86.6
62.41	1	0.8	0.8	87.4
63.65	1	0.8	0.8	88.2
67.16	2	1.6	1.6	89.8
67.52	2	1.6	1.6	91.3
73.51	1	0.8	0.8	92.1
73.87	1	0.8	0.8	92.9
75.48	1	0.8	0.8	93.7
76.50	1	0.8	0.8	94.5
77.74	1	0.8	0.8	95.3
82.70	1	0.8	0.8	96.1
83.22	1	0.8	0.8	96.9
91.32	1	0.8	0.8	97.6
99.43	1	0.8	0.8	98.4
106.29	1	0.8	0.8	99.2
110.01	1	0.8	0.8	100.0
Total	127	100.0	100.0	

Table 107 Statistical data of microemulsion droplet size of ME17 after 6 months storage at temperature of 4°C

Statistics[b]

DIAMETER

N	Valid	27
	Missing	0
Mean		37.1786
Median		29.8995
Mode		22.43[a]
Std. Deviation		29.6322
Minimum		10.16
Maximum		165.56
Sum		1003.82

a. Multiple modes exist. The smallest value is shown

b. FORMULAR = ME17-4C-M6 (E)

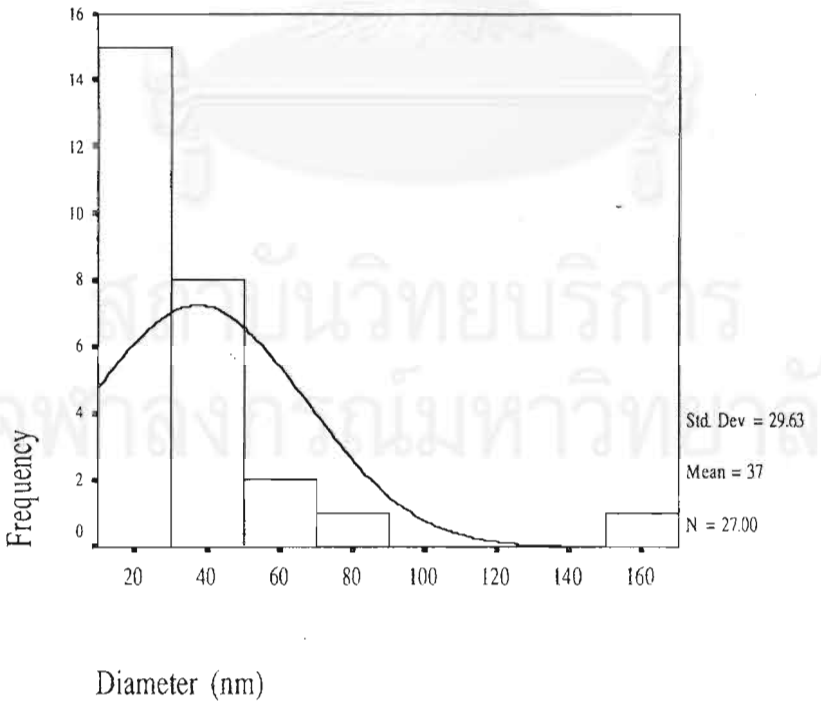


Figure 115 Droplet size histogram of ME17 after 6 months storage at temperature of 4°C

Table 108 Droplet size frequency of ME17 after 6 months storage at temperature of 4°C

	Frequency	Percent	Valid Percent	Cumulative Percent
10.16	1	3.7	3.7	3.7
17.64	1	3.7	3.7	7.4
18.34	1	3.7	3.7	11.1
19.04	1	3.7	3.7	14.8
20.73	1	3.7	3.7	18.5
22.43	2	7.4	7.4	25.9
24.82	1	3.7	3.7	29.6
25.11	1	3.7	3.7	33.3
25.81	2	7.4	7.4	40.7
26.51	1	3.7	3.7	44.4
28.91	1	3.7	3.7	48.1
29.90	2	7.4	7.4	55.6
32.30	1	3.7	3.7	59.3
33.70	1	3.7	3.7	63.0
33.99	1	3.7	3.7	66.7
34.69	1	3.7	3.7	70.4
37.37	2	7.4	7.4	77.8
45.26	1	3.7	3.7	81.5
48.65	1	3.7	3.7	85.2
50.34	1	3.7	3.7	88.9
51.04	1	3.7	3.7	92.6
86.02	1	3.7	3.7	96.3
165.56	1	3.7	3.7	100.0
Total	27	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 109 Statistical data of microemulsion droplet size of freshly prepared ME19 storage at temperature of 30°C

Statistics

DIAMETER

N	Valid	72
	Missing	0
Mean		73.1138
Median		47.1020
Mode		11.73
Std. Deviation		76.4218
Minimum		11.73
Maximum		314.35
Sum		5264.19

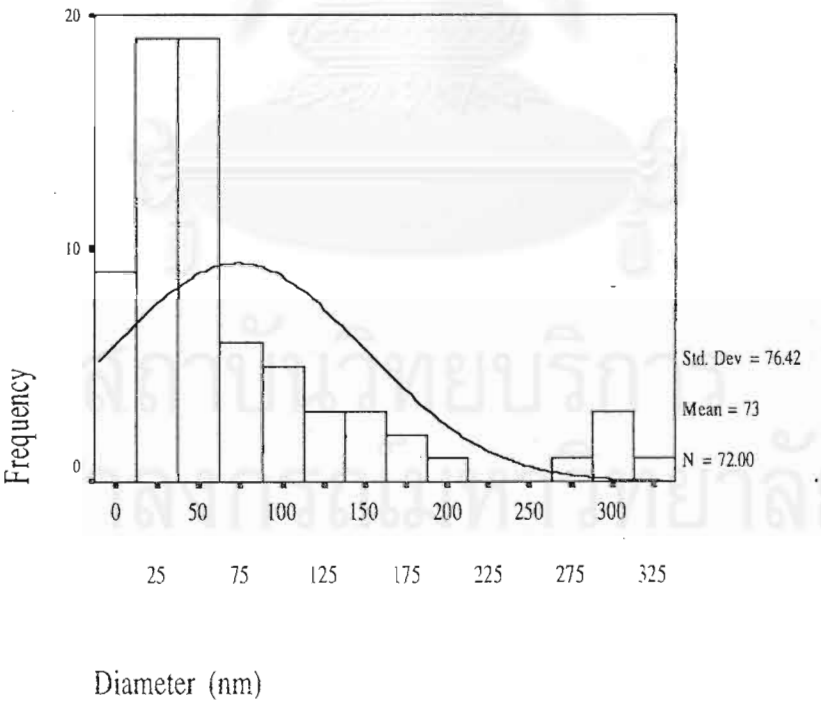


Figure 116 Droplet size histogram of freshly prepared ME19 storage at temperature of 30°C

Table 110 Droplet size frequency of freshly prepared ME19 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	9	12.5	12.5	12.5
17.59	7	9.7	9.7	22.2
23.45	4	5.6	5.6	27.8
25.87	4	5.6	5.6	33.3
34.16	1	1.4	1.4	34.7
35.17	2	2.8	2.8	37.5
36.59	1	1.4	1.4	38.9
37.60	1	1.4	1.4	40.3
40.03	2	2.8	2.8	43.1
41.03	2	2.8	2.8	45.8
43.46	2	2.8	2.8	48.6
45.89	1	1.4	1.4	50.0
48.32	1	1.4	1.4	51.4
49.32	1	1.4	1.4	52.8
50.74	1	1.4	1.4	54.2
51.75	1	1.4	1.4	55.6
53.17	1	1.4	1.4	56.9
54.18	1	1.4	1.4	58.3
55.18	2	2.8	2.8	61.1
56.60	1	1.4	1.4	62.5
61.05	2	2.8	2.8	65.3
63.47	2	2.8	2.8	68.1
65.90	2	2.8	2.8	70.8
72.77	1	1.4	1.4	72.2
75.20	1	1.4	1.4	73.6
95.21	1	1.4	1.4	75.0
97.64	1	1.4	1.4	76.4
100.07	2	2.8	2.8	79.2
109.36	1	1.4	1.4	80.6
115.22	1	1.4	1.4	81.9
123.51	1	1.4	1.4	83.3
126.95	1	1.4	1.4	84.7
137.66	1	1.4	1.4	86.1
149.39	1	1.4	1.4	87.5
149.81	1	1.4	1.4	88.9
171.83	1	1.4	1.4	90.3
185.98	1	1.4	1.4	91.7
207.00	1	1.4	1.4	93.1
269.47	1	1.4	1.4	94.4
294.34	1	1.4	1.4	95.8
305.05	1	1.4	1.4	97.2
308.49	1	1.4	1.4	98.6
314.35	1	1.4	1.4	100.0
Total	72	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 111 Statistical data of microemulsion droplet size of freshly prepared ME20 storage at temperature of 30°C

Statistics

DIAMETER

N	Valid	90
	Missing	0
Mean		58.3198
Median		46.3909
Mode		25.87
Std. Deviation		36.4341
Variance		1327.44
Minimum		11.73
Maximum		196.28
Sum		5248.78

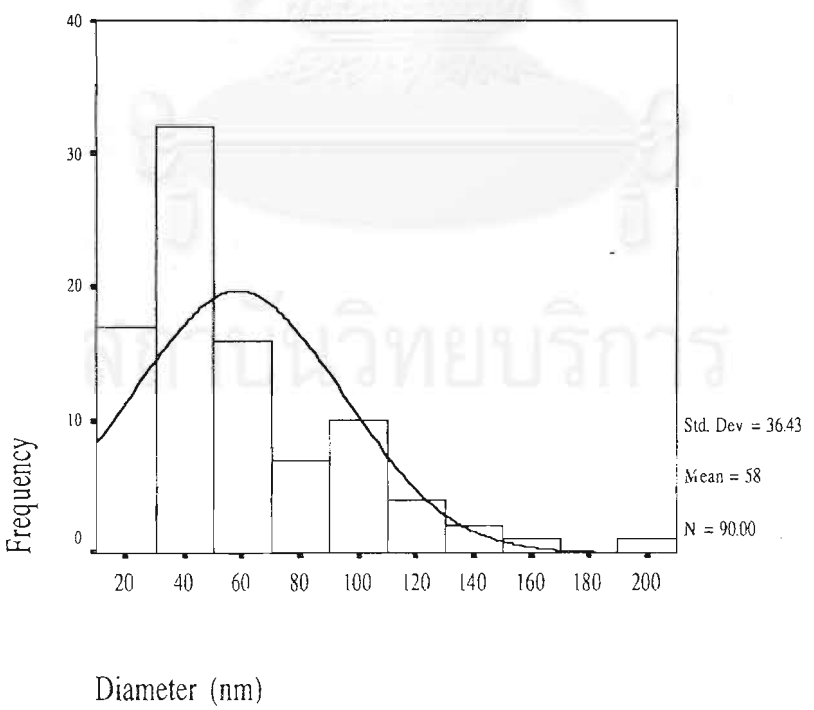


Figure 117 Droplet size histogram of freshly prepared ME20 storage at temperature of 30°C

Table 112 Droplet size frequency of freshly prepared ME20 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
11.73	5	5.6	5.6	
17.59	2	2.2	2.2	
20.01	1	1.1	1.1	
23.45	1	1.1	1.1	
25.87	7	7.8	7.8	
29.31	1	1.1	1.1	
31.74	4	4.4	4.4	
34.16	3	3.3	3.3	
35.17	4	4.4	4.4	
37.60	4	4.4	4.4	
40.03	4	4.4	4.4	
41.03	1	1.1	1.1	
42.46	1	1.1	1.1	
43.46	3	3.3	3.3	
45.89	4	4.4	4.4	
46.89	2	2.2	2.2	
49.32	2	2.2	2.2	
51.75	3	3.3	3.3	
54.18	1	1.1	1.1	
55.18	2	2.2	2.2	
56.60	1	1.1	1.1	
60.04	3	3.3	3.3	
61.05	1	1.1	1.1	
62.47	1	1.1	1.1	
63.47	2	2.2	2.2	
65.90	1	1.1	1.1	
66.91	1	1.1	1.1	
70.76	1	1.1	1.1	
80.05	1	1.1	1.1	
81.06	2	2.2	2.2	
83.49	1	1.1	1.1	
85.92	2	2.2	2.2	
91.78	1	1.1	1.1	
95.21	2	2.2	2.2	
96.63	2	2.2	2.2	
102.49	1	1.1	1.1	
103.50	2	2.2	2.2	
108.36	1	1.1	1.1	
109.36	1	1.1	1.1	
114.22	1	1.1	1.1	
116.65	1	1.1	1.1	
118.66	1	1.1	1.1	
122.51	1	1.1	1.1	
144.53	1	1.1	1.1	
149.39	1	1.1	1.1	
157.68	1	1.1	1.1	
196.28	1	1.1	1.1	
Total	90	100.0	100.0	

Table 113 Statistical data of microemulsion droplet size of freshly prepared ME23 storage at temperature of 30°C

Statistics

DIAMETER

N	Valid	72
	Missing	0
Mean		51.6692
Median		39.9573
Mode		8.97
Std. Deviation		37.5081
Minimum		8.97
Maximum		147.47
Sum		3720.18

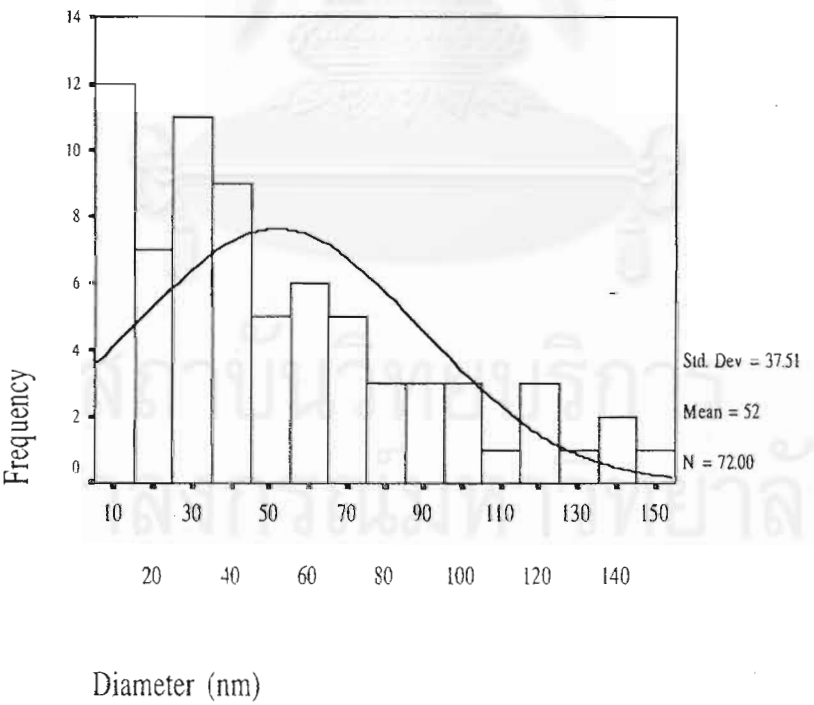


Figure 118 Droplet size histogram of freshly prepared ME23 storage at temperature of 30°C

Table 114 Droplet size frequency of freshly prepared ME23 storage at temperature of 30°C

	Frequency	Percent	Valid Percent	Cumulative Percent
8.97	9	12.5	12.5	12.5
13.45	3	4.2	4.2	16.7
15.30	1	1.4	1.4	18.1
17.93	3	4.2	4.2	22.2
19.79	2	2.8	2.8	25.0
22.41	1	1.4	1.4	26.4
26.13	1	1.4	1.4	27.8
26.90	3	4.2	4.2	31.9
28.75	2	2.8	2.8	34.7
30.61	1	1.4	1.4	36.1
33.23	4	5.6	5.6	41.7
35.09	1	1.4	1.4	43.1
36.95	2	2.8	2.8	45.8
37.72	1	1.4	1.4	47.2
39.57	2	2.8	2.8	50.0
40.34	1	1.4	1.4	51.4
42.20	2	2.8	2.8	54.2
45.91	1	1.4	1.4	55.6
46.68	1	1.4	1.4	56.9
48.54	1	1.4	1.4	58.3
50.40	1	1.4	1.4	59.7
54.88	1	1.4	1.4	61.1
55.65	1	1.4	1.4	62.5
56.74	1	1.4	1.4	63.9
57.51	1	1.4	1.4	65.3
59.36	2	2.8	2.8	68.1
63.07	1	1.4	1.4	69.4
66.47	2	2.8	2.8	72.2
68.33	1	1.4	1.4	73.6
70.95	1	1.4	1.4	75.0
72.81	1	1.4	1.4	76.4
79.15	1	1.4	1.4	77.8
81.00	1	1.4	1.4	79.2
81.77	1	1.4	1.4	80.6
87.03	1	1.4	1.4	81.9
93.36	1	1.4	1.4	83.3
94.45	1	1.4	1.4	84.7
98.93	1	1.4	1.4	86.1
103.42	1	1.4	1.4	87.5
104.50	1	1.4	1.4	88.9
107.90	1	1.4	1.4	90.3
116.87	1	1.4	1.4	91.7
121.35	1	1.4	1.4	93.1
123.20	1	1.4	1.4	94.4
129.86	1	1.4	1.4	95.8
135.88	1	1.4	1.4	97.2
140.36	1	1.4	1.4	98.6
147.47	1	1.4	1.4	100.0
Total	72	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 115 Statistical data of microemulsion droplet size of ME28

Statistics

DIAMETER

N	Valid	123
	Missing	0
Mean		112.225
Median		86.2500
Mode		13.45
Std. Deviation		84.2749
Minimum		13.45
Maximum		327.61
Sum		13803.7

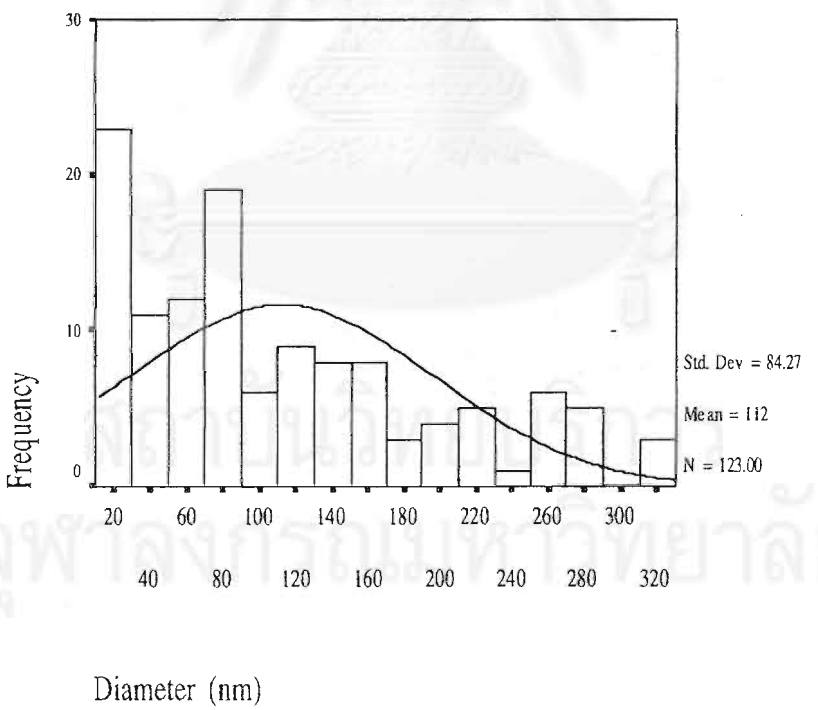


Figure 119 Droplet size histogram of ME28

Table 116 Droplet size frequency of ME28

	Frequency	Percent	Valid Percent	Cumulative Percent
13.45	13	10.6	10.6	10.6
20.17	3	2.4	2.4	13.0
22.95	4	3.3	3.3	16.3
29.68	3	2.4	2.4	18.7
32.46	1	0.8	0.8	19.5
33.62	1	0.8	0.8	20.3
39.18	1	0.8	0.8	21.1
40.34	2	1.6	1.6	22.8
43.12	1	0.8	0.8	23.6
45.91	1	0.8	0.8	24.4
48.69	2	1.6	1.6	26.0
49.85	2	1.6	1.6	27.6
52.63	1	0.8	0.8	28.5
53.79	1	0.8	0.8	29.3
55.42	1	0.8	0.8	30.1
56.57	2	1.6	1.6	31.7
59.36	2	1.6	1.6	33.3
60.51	1	0.8	0.8	34.1
66.08	2	1.6	1.6	35.8
68.86	2	1.6	1.6	37.4
70.02	2	1.6	1.6	39.0
71.65	1	0.8	0.8	39.8
72.80	3	2.4	2.4	42.3
75.59	1	0.8	0.8	43.1
76.74	1	0.8	0.8	43.9
78.37	1	0.8	0.8	44.7
79.53	4	3.3	3.3	48.0
82.31	1	0.8	0.8	48.8
83.46	1	0.8	0.8	49.6
86.25	3	2.4	2.4	52.0
89.03	1	0.8	0.8	52.8
92.97	1	0.8	0.8	53.7
94.60	2	1.6	1.6	55.3
95.76	1	0.8	0.8	56.1
97.39	1	0.8	0.8	56.9
102.48	1	0.8	0.8	57.7
111.99	2	1.6	1.6	59.3
118.71	1	0.8	0.8	60.2
119.87	1	0.8	0.8	61.0
121.49	1	0.8	0.8	61.8
125.43	2	1.6	1.6	63.4
128.22	2	1.6	1.6	65.0
133.79	1	0.8	0.8	65.9
134.94	1	0.8	0.8	66.7
141.66	1	0.8	0.8	67.5
144.45	2	1.6	1.6	69.1
145.60	1	0.8	0.8	69.9
148.39	2	1.6	1.6	71.5
151.17	1	0.8	0.8	72.4
152.33	1	0.8	0.8	73.2
155.11	1	0.8	0.8	74.0
157.90	1	0.8	0.8	74.8
164.62	2	1.6	1.6	76.4
166.25	1	0.8	0.8	77.2
169.71	1	0.8	0.8	78.0

	Frequency	Percent	Valid Percent	Cumulative Percent
171.34	1	0.8	0.8	78.9
174.13	1	0.8	0.8	79.7
183.64	1	0.8	0.8	80.5
199.39	1	0.8	0.8	81.3
201.02	2	1.6	1.6	82.9
203.81	1	0.8	0.8	83.7
210.53	1	0.8	0.8	84.6
214.47	1	0.8	0.8	85.4
217.25	1	0.8	0.8	86.2
225.13	1	0.8	0.8	87.0
227.91	1	0.8	0.8	87.8
246.93	1	0.8	0.8	88.6
252.50	1	0.8	0.8	89.4
254.81	1	0.8	0.8	90.2
256.44	1	0.8	0.8	91.1
261.53	1	0.8	0.8	91.9
265.95	1	0.8	0.8	92.7
269.89	1	0.8	0.8	93.5
272.67	1	0.8	0.8	94.3
279.39	1	0.8	0.8	95.1
282.18	1	0.8	0.8	95.9
284.48	1	0.8	0.8	96.7
287.27	1	0.8	0.8	97.6
316.95	1	0.8	0.8	98.4
318.10	1	0.8	0.8	99.2
327.61	1	0.8	0.8	100.0
Total	123	100.0	100.0	

Table 117 Statistical data of microemulsion droplet size of ME32

Statistics

DIAMETER

N	Valid	332
	Missing	0
Mean		70.5366
Median		72.8000
Mode		8.96
Std. Deviation		39.6750
Minimum		8.96
Maximum		190.73
Sum		23418.1

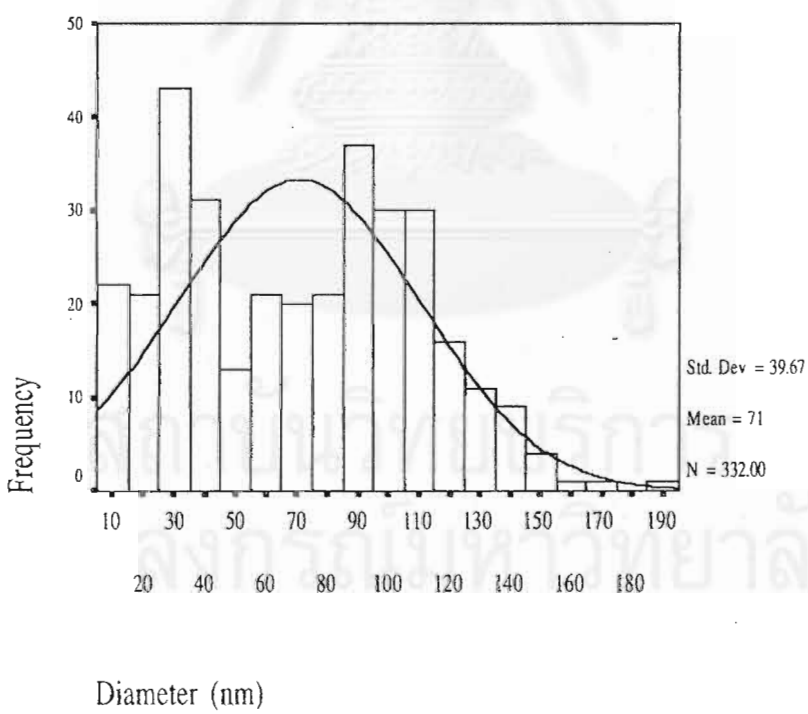


Figure 120 Droplet size histogram of ME32

Table 118 Droplet size frequency of ME32

	Frequency	Percent	Valid Percent	Cumulative Percent
8.96	21	6.3	6.3	6.3
13.45	1	0.3	0.3	6.6
15.30	7	2.1	2.1	8.7
17.93	3	0.9	0.9	9.6
19.78	7	2.1	2.1	11.7
22.41	1	0.3	0.3	12.0
24.26	3	0.9	0.9	13.0
26.12	4	1.2	1.2	14.2
26.89	14	4.2	4.2	18.4
28.75	9	2.7	2.7	21.1
30.60	10	3.0	3.0	24.1
32.46	2	0.6	0.6	24.7
33.23	4	1.2	1.2	25.9
35.09	4	1.2	1.2	27.1
35.85	6	1.8	1.8	28.9
36.94	2	0.6	0.6	29.5
38.80	1	0.3	0.3	29.8
39.57	5	1.5	1.5	31.3
41.42	4	1.2	1.2	32.5
42.19	6	1.8	1.8	34.3
44.05	2	0.6	0.6	34.9
44.82	1	0.3	0.3	35.2
45.14	1	0.3	0.3	35.5
45.90	1	0.3	0.3	35.8
46.67	1	0.3	0.3	36.1
48.53	3	0.9	0.9	37.0
50.39	1	0.3	0.3	37.3
52.24	1	0.3	0.3	37.7
53.01	1	0.3	0.3	38.0
54.87	4	1.2	1.2	39.2
55.64	1	0.3	0.3	39.5
56.73	3	0.9	0.9	40.4
57.49	3	0.9	0.9	41.3
59.35	3	0.9	0.9	42.2
61.21	7	2.1	2.1	44.3
63.83	3	0.9	0.9	45.2
64.92	1	0.3	0.3	45.5
65.69	2	0.6	0.6	46.1
68.31	3	0.9	0.9	47.0
70.17	5	1.5	1.5	48.5
72.03	4	1.2	1.2	49.7
72.80	2	0.6	0.6	50.3
73.88	2	0.6	0.6	50.9
74.65	2	0.6	0.6	51.5
76.51	2	0.6	0.6	52.1
79.14	10	3.0	3.0	55.1
80.99	3	0.9	0.9	56.0
81.76	1	0.3	0.3	56.3
82.85	2	0.6	0.6	56.9
83.62	1	0.3	0.3	57.2
84.39	1	0.3	0.3	57.5
84.70	1	0.3	0.3	57.8
85.47	11	3.3	3.3	61.1
86.24	1	0.3	0.3	61.4
87.33	2	0.6	0.6	62.0
88.10	2	0.6	0.6	62.7
89.95	2	0.6	0.6	63.3
91.81	10	3.0	3.0	66.3
92.58	2	0.6	0.6	66.9
93.67	3	0.9	0.9	67.8
94.44	4	1.2	1.2	69.0
96.29	7	2.1	2.1	71.1
97.06	1	0.3	0.3	71.4
98.15	4	1.2	1.2	72.6
98.92	4	1.2	1.2	73.8
100.77	4	1.2	1.2	75.0
102.63	4	1.2	1.2	76.2
103.40	4	1.2	1.2	77.4
104.49	2	0.6	0.6	78.0
105.25	7	2.1	2.1	80.1
107.11	2	0.6	0.6	80.7
108.97	2	0.6	0.6	81.3
109.74	10	3.0	3.0	84.3
110.83	1	0.3	0.3	84.6
111.59	6	1.8	1.8	86.4
113.45	2	0.6	0.6	87.0
115.31	1	0.3	0.3	87.3
116.08	2	0.6	0.6	88.0
117.93	1	0.3	0.3	88.3
118.70	2	0.6	0.6	88.9
119.47	1	0.3	0.3	89.2
119.79	1	0.3	0.3	89.5
120.56	1	0.3	0.3	89.8
121.33	1	0.3	0.3	90.1
122.41	3	0.9	0.9	91.0
124.27	3	0.9	0.9	91.9
126.89	1	0.3	0.3	92.2
128.75	2	0.6	0.6	92.8
129.52	1	0.3	0.3	93.1
129.84	1	0.3	0.3	93.4
130.29	1	0.3	0.3	93.7
131.38	2	0.6	0.6	94.3
133.23	2	0.6	0.6	94.9
134.77	1	0.3	0.3	95.2
135.86	1	0.3	0.3	95.5
136.95	1	0.3	0.3	95.8
137.72	2	0.6	0.6	96.4
139.57	1	0.3	0.3	96.7
140.34	1	0.3	0.3	97.0
142.20	1	0.3	0.3	97.3
142.97	1	0.3	0.3	97.6
144.82	1	0.3	0.3	97.9
145.91	1	0.3	0.3	98.2
153.02	2	0.6	0.6	98.8
154.87	1	0.3	0.3	99.1
158.59	1	0.3	0.3	99.4
170.94	1	0.3	0.3	99.7
190.73	1	0.3	0.3	100.0
Total	332	100.0	100.0	

Table 119 Statistical data of microemulsion droplet size of ME35

Statistics

DIAMETER

N	Valid	76
	Missing	0
Mean		37.5229
Median		14.9500
Mode		3.39
Std. Deviation		83.8618
Minimum		3.39
Maximum		526.50
Sum		2851.74

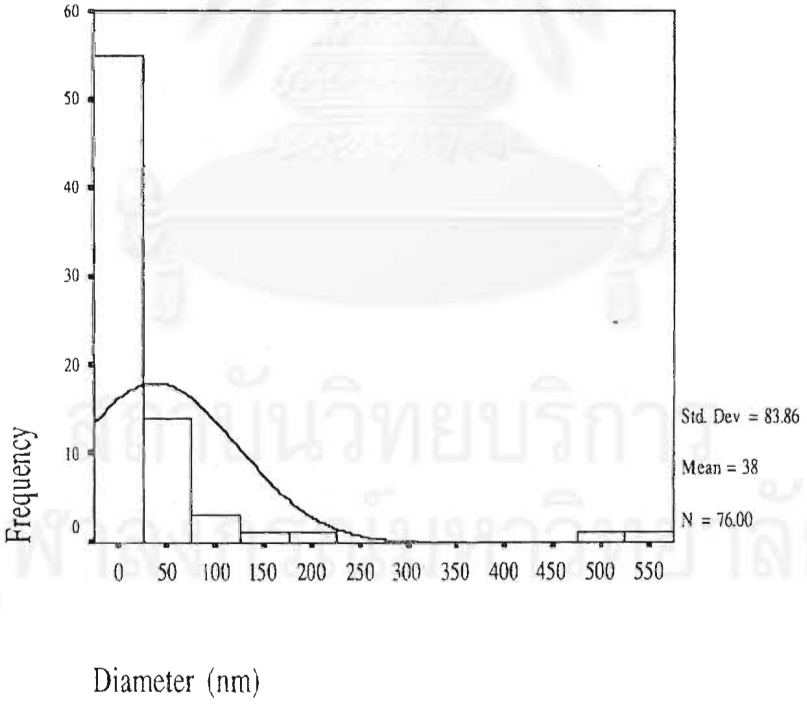


Figure 121 Droplet size histogram of ME35

Table 120 Droplet size frequency of ME35

	Frequency	Percent	Valid Percent	Cumulative Percent
3.39	8	10.5	10.5	10.5
5.08	3	3.9	3.9	14.5
5.78	2	2.6	2.6	17.1
6.77	4	5.3	5.3	22.4
7.47	3	3.9	3.9	26.3
8.47	3	3.9	3.9	30.3
9.17	1	1.3	1.3	31.6
9.87	1	1.3	1.3	32.9
10.16	2	2.6	2.6	35.5
10.86	1	1.3	1.3	36.8
11.56	1	1.3	1.3	38.2
11.85	1	1.3	1.3	39.5
12.26	1	1.3	1.3	40.8
12.55	2	2.6	2.6	43.4
13.55	2	2.6	2.6	46.1
14.25	2	2.6	2.6	48.7
14.95	2	2.6	2.6	51.3
15.65	1	1.3	1.3	52.6
16.64	2	2.6	2.6	55.3
17.63	1	1.3	1.3	56.6
18.33	1	1.3	1.3	57.9
19.33	1	1.3	1.3	59.2
20.03	3	3.9	3.9	63.2
20.73	1	1.3	1.3	64.5
21.43	1	1.3	1.3	65.8
22.42	1	1.3	1.3	67.1
22.71	1	1.3	1.3	68.4
23.12	2	2.6	2.6	71.1
24.12	1	1.3	1.3	72.4
25.10	1	1.3	1.3	73.7
25.81	1	1.3	1.3	75.0
27.50	1	1.3	1.3	76.3
31.30	1	1.3	1.3	77.6
33.57	1	1.3	1.3	78.9
34.69	1	1.3	1.3	80.3
35.67	1	1.3	1.3	81.6
42.45	1	1.3	1.3	82.9
44.84	1	1.3	1.3	84.2
46.24	1	1.3	1.3	85.5
50.91	1	1.3	1.3	86.8
53.02	1	1.3	1.3	88.2
56.11	1	1.3	1.3	89.5
60.08	1	1.3	1.3	90.8
86.30	1	1.3	1.3	92.1
96.58	1	1.3	1.3	93.4
115.90	1	1.3	1.3	94.7
131.55	1	1.3	1.3	96.1
192.33	1	1.3	1.3	97.4
488.02	1	1.3	1.3	98.7
526.50	1	1.3	1.3	100.0
Total	76	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

Table 121 Statistical data of microemulsion droplet size of ME35BSA

Statistics

DIAMETER

N	Valid	64
	Missing	0
Mean		52.2736
Median		44.0450
Mode		8.96
Std. Deviation		36.9596
Minimum		8.96
Maximum		187.01
Sum		3345.51

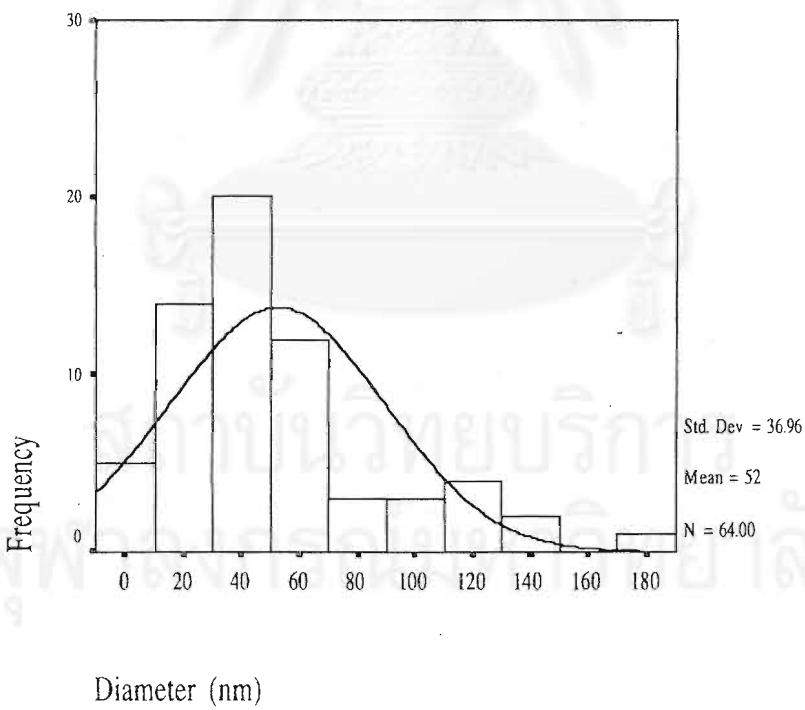


Figure 122 Droplet size histogram of ME35BSA

Table 122 Droplet size frequency of ME35BSA

	Frequency	Percent	Valid Percent	Cumulative Percent
8.96	5	7.8	7.8	7.8
15.30	1	1.6	1.6	9.4
17.93	3	4.7	4.7	14.1
19.78	3	4.7	4.7	18.8
24.26	2	3.1	3.1	21.9
26.12	3	4.7	4.7	26.6
28.75	2	3.1	3.1	29.7
30.60	3	4.7	4.7	34.4
35.09	1	1.6	1.6	35.9
35.85	1	1.6	1.6	37.5
37.71	2	3.1	3.1	40.6
39.57	1	1.6	1.6	42.2
41.42	3	4.7	4.7	46.9
42.19	2	3.1	3.1	50.0
45.90	2	3.1	3.1	53.1
46.67	3	4.7	4.7	57.8
47.76	1	1.6	1.6	59.4
49.62	1	1.6	1.6	60.9
50.39	3	4.7	4.7	65.6
51.16	1	1.6	1.6	67.2
59.35	3	4.7	4.7	71.9
63.83	1	1.6	1.6	73.4
64.60	1	1.6	1.6	75.0
65.69	1	1.6	1.6	76.6
66.46	1	1.6	1.6	78.1
68.31	1	1.6	1.6	79.7
72.03	1	1.6	1.6	81.3
74.65	2	3.1	3.1	84.4
98.92	1	1.6	1.6	85.9
100.77	1	1.6	1.6	87.5
105.25	1	1.6	1.6	89.1
113.45	1	1.6	1.6	90.6
116.08	2	3.1	3.1	93.8
124.27	1	1.6	1.6	95.3
131.38	1	1.6	1.6	96.9
148.53	1	1.6	1.6	98.4
187.01	1	1.6	1.6	100.0
Total	64	100.0	100.0	

	Frequency	Percent	Valid Percent	Cumulative Percent

APPENDIX D

Table 123 Data of calibration curve of buserelin acetate standard solution

Concentration (mcg/ml)	Peak area ratio									Mean	SD	%CV
	n1			n2			n3					
	BSA	CP	Ratio	BSA	CP	Ratio	BSA	CP	Ratio			
0.0000	0	0	0	0	0	0	0	0	0	0	0	0
1.0600	27477	240005	0.1145	24613	244133	0.1008	25726	238236	0.1080	0.1078	0.0068	6.34
2.6500	61748	236640	0.2609	61246	236729	0.2587	61395	235353	0.2609	0.2602	0.0013	0.48
5.3000	127601	235457	0.5419	126109	234722	0.5373	124062	235132	0.5276	0.5356	0.0073	1.36
10.6000	249438	237257	1.0513	250807	235387	1.0655	251623	235085	1.0703	1.0624	0.0099	0.93
21.2000	489629	231507	2.1150	490179	229545	2.1354	490526	229212	2.1401	2.1302	0.0134	0.63

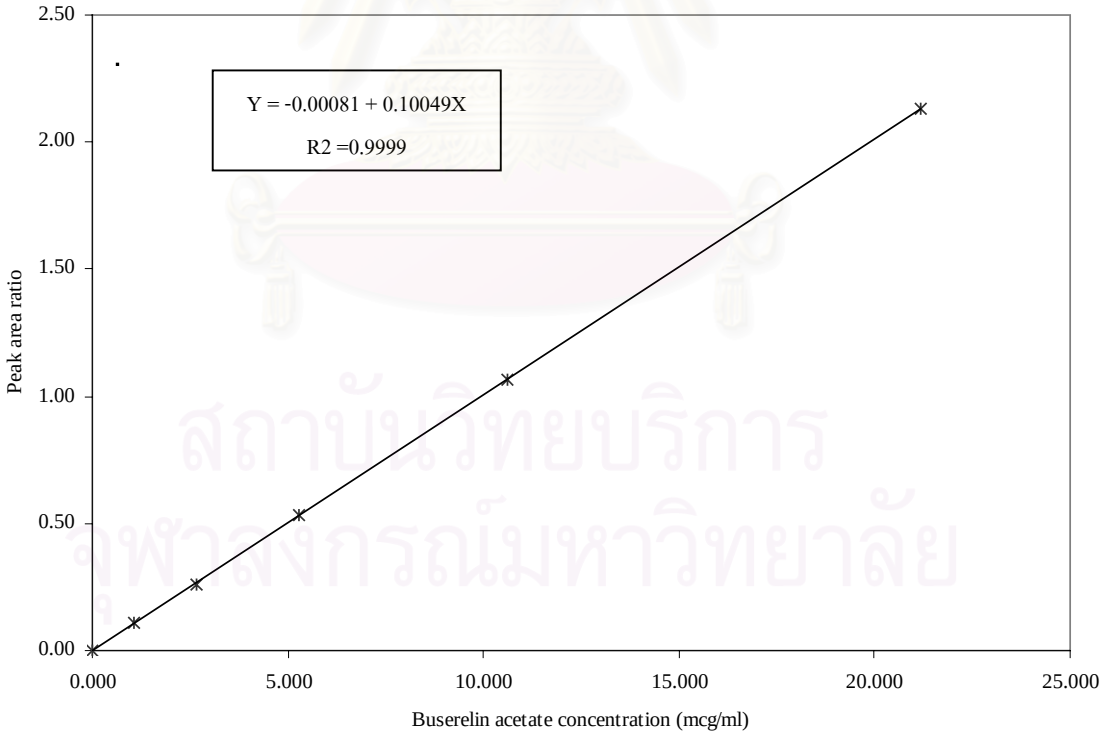


Figure 123 Calibration curve of buserelin acetate assayed by HPLC method

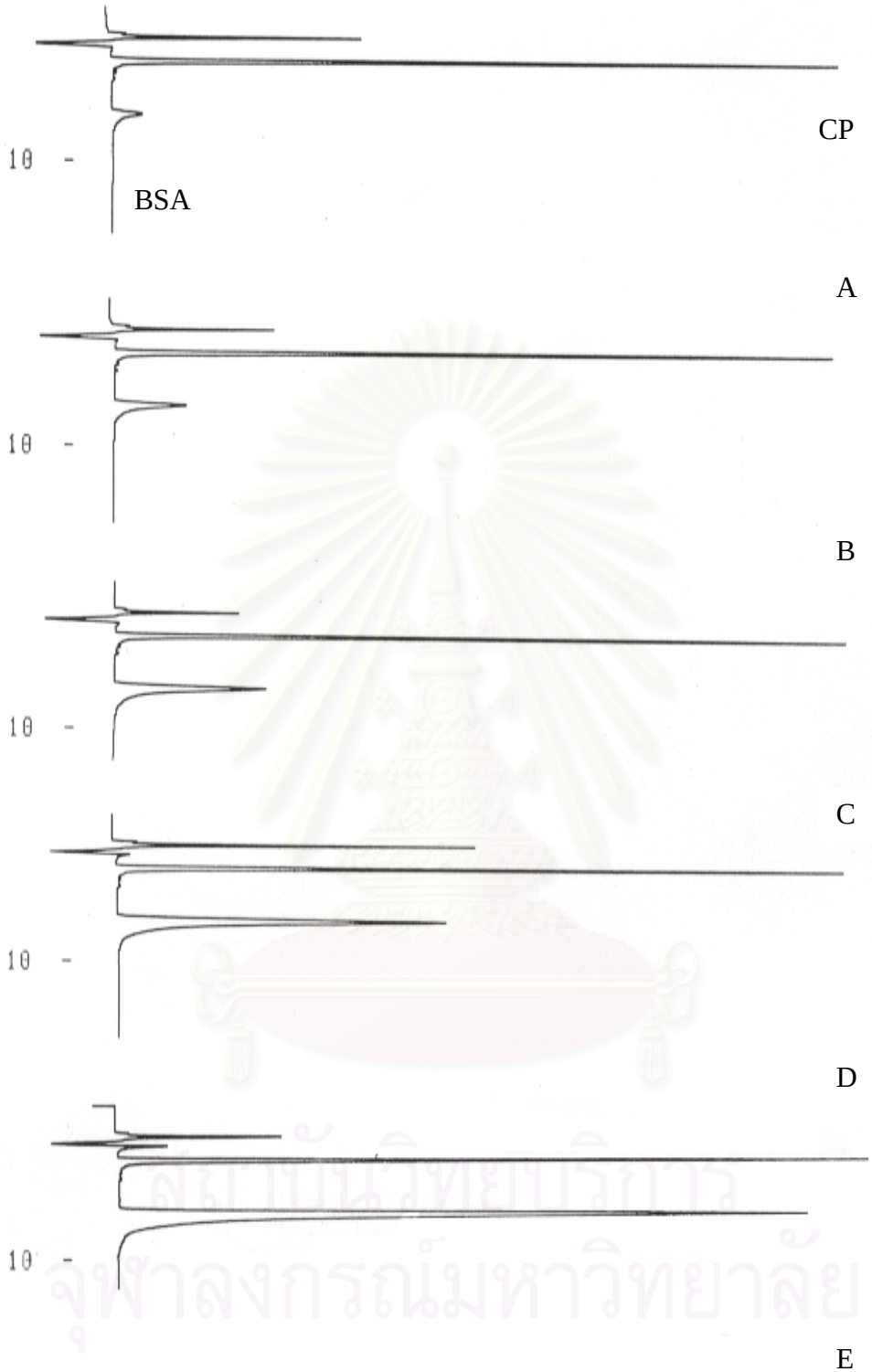


Figure 124 HPLC chromatograms of buserelin acetate calibration curve
Key: A=1 mg/ml, B=2.5 mg/ml, C=5 mg/ml, D=10 mg/ml, E=20 mg/ml

Table 124 Precision test of the analytical procedure for buserelin acetate at 5 and 10 μg/ml

Concentration	Within run precision				Between run precision			
(mcg)	Sample	BSA	CP	Ratio	Day	BSA	CP	Ratio
5.3000	N1	127601	235457	0.5419	D1	127601	235457	0.5419
	N2	126109	234722	0.5373	D2	147224	269737	0.5458
	N3	124062	235132	0.5276	D3	125939	233086	0.5403
	N4	110929	210011	0.5282	D4	128730	237769	0.5414
	N5	110027	206571	0.5326	D5	139343	245890	0.5667
	Mean			0.5335	Mean			0.5472
	SD			0.0061	SD			0.0111
	%CV			1.14%	%CV			2.02%
	Within run precision				Between run precision			
	Sample	BSA	CP	Ratio	Day	BSA	CP	Ratio
10.6000	N1	249438	237257	1.0513	D1	249438	237257	1.0513
	N2	250807	235387	1.0655	D2	216864	210039	1.0325
	N3	251623	235085	1.0703	D3	219172	213373	1.0272
	N4	234617	223124	1.0515	D4	247574	240209	1.0307
	N5	240019	224652	1.0684	D5	222490	217809	1.0215
	Mean			1.0614	Mean			1.0326
	SD			0.0093	SD			0.0113
	%CV			0.87%	%CV			1.09%

APPENDIX E

In vitro BSA release data

Table 125 Cumulative percent release of BSA from phosphate buffer pH 7.4

Time (day)	Cumulative % Drug Release				
	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	10.1083	9.9014	10.7810	10.2636	0.46
0.25	27.3395	29.0209	29.0933	28.4846	0.99
0.42	42.8076	41.9244	41.3874	42.0398	0.72
1	70.9949	70.7998	67.0645	69.6197	2.22
2	84.5963	84.6919	78.2305	82.5062	3.70
6	81.6137	81.0293	75.0718	79.2383	3.62
10	82.1548	81.2321	75.3613	79.5827	3.68
14	83.1280	82.0223	75.7330	80.2945	3.99
18	83.5409	82.3331	76.1123	80.6621	3.99
21	83.6381	82.5149	76.1422	80.7651	4.04

Table 126 Cumulative percent release of BSA from IPM-TW-PEG B 5/5 9% (ME26BSA)

Time (day)	Cumulative % Drug Release				
	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.5453	0.6424	0.4453	0.5443	0.10
0.25	1.5566	1.7034	1.2081	1.4893	0.25
0.42	3.8451	3.6321	2.9362	3.4711	0.48
1	9.5167	9.5027	8.0948	9.0381	0.82
2	18.5325	20.1709	16.2488	18.3174	1.97
6	28.7867	30.3355	24.5304	27.8842	3.01
10	30.5666	31.4585	27.0539	29.6930	2.33
14	29.8746	32.4596	27.3359	29.8900	2.56
18	30.3983	33.3247	28.1680	30.6303	2.59
21	30.5211	33.2617	27.8630	30.5486	2.70

Table 127 Cumulative percent release of BSA from IPM-TW-PEG C 5/5 9% (ME27BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	1.3471	1.1965	1.3919	1.3119	0.10
0.25	2.7751	2.4073	3.3074	2.8299	0.45
0.42	5.4721	4.5657	4.4604	4.8327	0.56
1	9.0005	6.3856	7.0693	7.4851	1.36
2	19.3329	18.0338	17.7206	18.3625	0.85
6	30.1872	26.6192	28.2028	28.3364	1.79
10	33.0772	29.1152	32.3828	31.5251	2.12
14	33.7906	29.5208	33.8406	32.3840	2.48
18	35.0060	29.7347	34.5286	33.0897	2.92
21	34.6046	29.5615	34.0685	32.7448	2.77

Table 128 Cumulative percent release of BSA from IPM-TW-PEG A 7/3 13% (ME28BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.8006	1.5711	0.9004	1.0907	0.42
0.25	4.4348	5.6461	4.7499	4.9436	0.63
0.42	11.1641	11.2597	11.8447	11.4228	0.37
1	24.9960	23.9925	28.4420	25.8102	2.33
2	37.7556	35.4488	41.4230	38.2091	3.01
6	49.0891	47.9943	53.6862	50.2565	3.02
10	51.1303	49.0783	53.0810	51.0965	2.00
14	53.1147	50.1773	54.0341	52.4421	2.01
18	53.2946	49.9648	53.4622	52.2405	1.97
21	53.0729	49.8589	53.4671	52.1330	1.98

Table 129 Cumulative percent release of BSA from IPM-TW-PEG C 7/3 9% (ME29BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.8720	0.4733	0.3300	0.5584	0.28
0.25	1.8769	0.9969	1.5646	1.4794	0.45
0.42	3.2560	2.5850	2.1363	2.6591	0.56
1	6.2787	5.7997	4.3664	5.4816	1.00
2	12.7375	12.7704	10.2020	11.9033	1.47
6	14.8362	16.3244	14.1825	15.1144	1.10
10	14.1085	15.3116	13.7729	14.3977	0.81
14	14.4044	15.2999	13.6632	14.4559	0.82
18	14.2972	15.4018	13.8790	14.5260	0.79
21	14.3021	15.5985	13.9045	14.6017	0.89

Table 130 Cumulative percent release of BSA from EO-TW-PEG B 7/3 9% (ME30BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.8144	0.5399	0.7171	0.6905	0.14
0.25	1.5570	2.2303	1.6426	1.8100	0.37
0.42	4.7546	4.4636	3.6454	4.2879	0.58
1	9.2531	9.6668	7.9269	8.9489	0.91
2	15.5111	15.7373	12.8307	14.6930	1.62
6	19.0724	19.8983	17.0640	18.6782	1.46
10	19.1817	19.9451	17.4720	18.8663	1.27
14	19.7934	20.4642	18.3515	19.5364	1.08
18	19.9306	20.1314	18.3380	19.4667	0.98
21	19.7938	20.1363	18.2035	19.3778	1.03

Table 131 Cumulative percent release of BSA from EO-TW-PEG C 7/3 9% (ME31BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	1.2753	1.4255	1.2282	1.3097	0.10
0.25	3.6432	3.2639	3.1461	3.3511	0.26
0.42	6.4845	5.3662	5.6564	5.8357	0.58
1	14.6135	12.7623	11.7967	13.0575	1.43
2	23.0623	21.4484	18.8861	21.1323	2.11
6	28.9458	29.1351	25.6393	27.9067	1.97
10	29.4019	29.1713	27.4465	28.6732	1.07
14	29.3478	29.8993	27.3231	28.8567	1.36
18	29.8928	30.7126	27.4282	29.3446	1.71
21	29.6585	30.3093	27.2865	29.0847	1.59

Table 132 Cumulative percent release of BSA from EO-TW-PG C 7/3 9% (ME32BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.6438	0.6228	0.5375	0.6014	0.06
0.25	2.2213	2.4332	2.5755	2.4100	0.18
0.42	4.9650	4.9916	5.2993	5.0853	0.19
1	8.7420	7.8682	9.8371	8.8158	0.99
2	17.0805	17.1572	20.2416	18.1598	1.80
6	33.8618	30.8531	32.9829	32.5659	1.55
10	42.1912	41.1367	44.2873	42.5384	1.60
14	44.9774	41.8230	46.6163	44.4722	2.44
18	46.6934	44.4283	49.0549	46.7255	2.31
21	47.4185	45.3909	49.9972	47.6022	2.31

Table 133 Cumulative percent release of BSA from MCT-TW-PG C 7/3 9% (ME33BSA)

Time (day)	Cumulative % Drug Release				
	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.7565	0.7276	0.8314	0.7718	0.05
0.25	1.6463	2.1362	1.9178	1.9001	0.25
0.42	2.8600	3.9091	3.1663	3.3118	0.54
1	10.7753	12.2805	10.8538	11.3032	0.85
2	20.7049	22.1787	26.1272	23.0036	2.80
6	33.2134	34.5100	36.8020	34.8418	1.82
10	33.2580	34.5828	37.9977	35.2795	2.45
14	33.8514	35.1362	37.7128	35.5668	1.97
18	34.3362	35.8270	37.9314	36.0316	1.81
21	34.4968	35.8686	38.0838	36.1497	1.81

Table 134 Cumulative percent release of BSA from MCT-TW-PEG C 7/3 9% (ME34BSA)

Time (day)	Cumulative % Drug Release				
	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.8407	1.1873	0.3458	0.7913	0.42
0.25	1.7988	3.5586	1.5895	2.3156	1.08
0.42	8.2422	8.1179	4.6518	7.0040	2.04
1	13.6157	11.4611	9.3002	11.4590	2.16
2	20.1658	16.5909	17.8850	18.2139	1.81
6	23.6884	21.0389	29.1439	24.6237	4.13
10	33.7546	32.6061	35.5641	33.9749	1.49
14	31.7707	29.6131	33.6185	31.6674	2.00
18	31.8792	29.6574	33.5959	31.7109	1.97
21	32.0929	29.8063	33.6595	31.8529	1.94

Table 135 Cumulative percent release of BSA from IPM-PC-PG A 5/5 9% (ME35BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.0069	0.0359	0.0359	0.0262	0.02
0.25	0.3091	0.6042	0.3355	0.4162	0.16
0.42	0.5496	0.6225	0.9709	0.7143	0.23
1	0.9583	1.0170	1.8376	1.2710	0.49
2	1.0558	1.3975	2.5796	1.6776	0.80
6	1.1153	2.6098	4.0377	2.5876	1.46
10	1.4928	3.0965	5.1453	3.2449	1.83
14	2.0983	5.4394	5.2691	4.2689	1.88
18	2.5136	6.5052	5.5269	4.8486	2.08
21	2.5252	6.9963	5.8088	5.1101	2.32

Table 136 Cumulative percent release of BSA from EO-PC-PG A 5/5 9% (ME36BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.0394	0.1253	0.0520	0.0722	0.05
0.25	0.0785	0.3468	0.2777	0.2343	0.14
0.42	0.3282	0.8696	0.4306	0.5428	0.29
1	0.6438	1.0074	0.8623	0.8378	0.18
2	0.6882	1.9282	1.0771	1.2312	0.63
6	1.0074	3.4421	1.9890	2.1462	1.22
10	2.1099	5.7686	4.7807	4.2198	1.89
14	5.6678	11.7472	7.5979	8.3377	3.11
18	7.5437	13.5746	9.0887	10.0690	3.13
21	7.7720	13.7270	9.3760	10.2917	3.08

Table 137 Cumulative percent release of BSA from IPM-PC-PG A 6/4 9% (ME37BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.0069	0.0068	0.0070	0.0069	0.00
0.25	0.0118	0.1191	0.1978	0.1096	0.09
0.42	0.4282	0.2720	0.6608	0.4537	0.20
1	1.1013	0.6414	0.6602	0.8010	0.26
2	1.3749	0.7339	1.2405	1.1164	0.34
6	1.7530	1.3386	2.7601	1.9506	0.73
10	2.1324	2.4159	4.5416	3.0300	1.32
14	2.9878	2.5746	4.7723	3.4449	1.17
18	3.1657	2.6283	4.8611	3.5517	1.17
21	3.0361	2.8232	5.0348	3.6314	1.22

Table 138 Cumulative percent release of BSA from EO-PC-PG A 6/4 9% (ME38BSA)

Time	Cumulative % Drug Release				
(day)	No 1	No 2	No 3	Mean	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.00
0.08	0.0330	0.0241	0.0329	0.0300	0.01
0.25	0.1024	0.0715	0.1764	0.1168	0.05
0.42	0.4421	0.2073	0.4189	0.3561	0.13
1	0.7044	0.6633	0.4842	0.6173	0.12
2	1.0218	1.4690	0.7726	1.0878	0.35
6	1.6488	2.5179	1.2386	1.8018	0.65
10	2.5656	4.4704	1.9931	3.0097	1.30
14	4.1632	5.2864	4.4373	4.6290	0.59
18	5.7623	5.4640	5.0709	5.4324	0.35
21	5.7599	5.5066	5.1351	5.4672	0.31

APPENDIX F

In vivo testosterone study

Table 139 Serum testosterone concentration (ng) from rabbits before treatment

Day	Testosterone (ng)										Mean	SD
	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10		
0	1.80	1.50	0.30	0.30	0.30	5.10	2.30	1.90	0.30	0.30	1.41	1.53
58	0.30	0.90	3.20	0.70	0.50	9.00	0.60	3.20	1.80	1.90	2.21	2.61
65	1.20	0.30	2.60	2.40	0.80	10.20	1.30	1.90	0.70	6.10	2.75	3.09
											2.12	

Table 140 Serum testosterone concentration (ng) of 20 rabbits used as benchmark for normal value

Testosterones (ng)										Mean	SD
1.20	0.30	2.60	2.40	0.80	10.20	1.30	1.90	0.70	6.10	2.57	2.40
3.40	0.30	2.80	5.30	1.70	1.90	2.30	0.30	4.10	1.70		

Table 141 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of blank microemulsion ME28 (injection on day 7 th)

Day	Testosterone (ng)					Mean	SD
	R-1	R-2	R-3	R-4	R-5		
0	1.20	0.30	2.60	2.40	0.80	1.46	1.00
10	0.30	5.00	0.30	2.60	5.00	2.64	2.35
13	1.90	3.60	7.00	0.60	5.90	3.80	2.67
17	0.40	1.20	2.30	0.60	0.60	1.02	0.78
20	0.80	0.40	0.30	0.30	1.10	0.58	0.36
31	1.10	1.70	1.00	1.90	1.80	1.50	0.42

Table 142 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of blank microemulsion ME32 (injection on day 7 th)

Day	Testosterone (ng)					Mean	SD
	R-1	R-2	R-3	R-4	R-5		
0	1.90	2.30	0.30	4.10	1.70	2.06	1.37
10	6.10	4.50	1.60	3.80	2.70	3.74	1.72
13	1.00	4.70	0.30	0.30	0.30	1.32	1.91
17	5.90	0.30	0.30	10.30	0.30	3.42	4.55
20	0.30	8.40	0.50	7.50	0.30	3.40	4.17
31	1.90	6.20	0.60	0.60	0.40	1.94	2.46

Table 143 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of blank microemulsion ME35 (injection on day 7 th)

Day	Testosterone (ng)					Mean	SD
	R-1	R-2	R-3	R-4	R-5		
0	10.20	1.30	1.90	0.70	6.10	4.04	4.04
10	0.80	1.80	3.40	5.20	3.00	2.84	1.67
13	0.50	0.10	3.70	1.80	1.80	1.58	1.41
17	0.60	8.20	1.70	0.30	1.20	2.40	3.29
20	8.30	4.90	3.20	0.80	6.60	4.76	2.92
31	1.80	0.80	0.70	5.40	0.50	1.84	2.05

Table 144 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of blank microemulsion ME36 (injection on day 7 th)

Day	Testosterone (ng)					Mean	SD
	R-1	R-2	R-3	R-4	R-5		
0	3.40	0.30	2.80	5.30	1.70	2.70	1.87
10	0.90	1.50	4.70	0.30	0.30	1.54	1.84
13	0.30	1.20	1.30	3.80	7.90	2.90	3.08
17	0.30	7.20	0.40	0.50	0.30	1.74	3.05
20	10.20	6.10	9.00	1.40	1.00	5.54	4.24
31	8.20	5.20	0.30	0.30	0.40	2.88	3.65

Table 145 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of 3.3 mg buserelin acetate buffer solution (PB) (injection on day 1 th)

Day	Testosterone (ng)					Mean	SD
	R-1	R-2	R-3	R-4	R-5		
0	1.10	1.80	8.20	0.40	0.60	2.42	3.28
4	3.70	3.50	2.50	2.20	1.60	2.70	0.89
7	1.90	2.90	7.40	1.10	1.50	2.96	2.57
11	2.40	3.10	4.70	3.60	0.80	2.92	1.45
17	0.90	1.70	8.80	0.50	3.40	3.06	3.40
23	0.40	0.70	0.90	0.30	3.10	1.08	1.15
30	2.50	2.80	0.30	1.10	6.20	2.58	2.27
37	2.70	2.50	0.80	1.90	1.60	1.90	0.76
44	2.10	0.90	0.30	6.60	1.90	2.36	2.48
59	4.60	2.60	0.50	8.10	8.50	4.86	3.46

Table 146 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of 3.3 mg buserelin acetate microemulsion(28BSA) (injection on day 1 th)

Day	Testosterone (ng)				Mean	SD
	R-1	R-2	R-3	R-4		
0	1.70	1.80	0.70	5.20	2.35	1.96
4	4.40	7.80	5.50	5.70	5.85	1.42
7	1.60	7.20	2.00	3.70	3.63	2.55
11	2.90	4.90	3.20	5.30	4.08	1.20
17	1.40	2.40	8.10	4.60	4.13	2.97
23	0.90	6.80	2.30	0.80	2.70	2.82
30	5.60	4.80	0.70	3.20	3.58	2.16
37	0.90	2.20	0.90	4.50	2.13	1.70
44	0.80	6.80	0.70	4.90	3.30	3.05
59	3.10	0.70	0.70	1.20	1.43	1.14

Table 147 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of 3.3 mg buserelin acetate microemulsion(32BSA) (injection on day 1 th)

Day	Testosterone (ng)				Mean	SD
	R-1	R-2	R-3	R-4		
0	1.00	0.80	0.30	0.60	0.68	0.30
4	5.70	5.60	4.00	0.60	3.98	2.38
7	1.80	1.70	3.50	2.30	2.33	0.83
11	1.40	0.90	0.30	1.00	0.90	0.45
17	2.90	0.90	2.00	1.20	1.75	0.90
23	1.20	2.00	0.50	1.10	1.20	0.62
30	4.10	0.60	0.40	0.50	1.40	1.80
37	3.00	0.70	0.80	3.90	2.10	1.60
44	6.40	3.30	4.70	0.70	3.78	2.41
59	2.40	0.70	5.80	1.40	2.58	2.26

Table 148 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of 3.3 mg buserelin acetate microemulsion(35BSA) (injection on day 1 th)

Day	Testosterone (ng)				Mean	SD
	R-1	R-2	R-3	R-4		
0	1.90	0.50	0.30	5.70	2.10	2.50
4	8.80	3.00	3.10	2.00	4.23	3.09
7	3.10	0.70	1.90	0.50	1.55	1.20
11	1.60	0.60	1.20	0.80	1.05	0.44
17	1.50	0.60	1.60	0.80	1.13	0.50
23	1.60	0.30	1.30	0.30	0.88	0.68
30	1.80	1.90	1.50	0.40	1.40	0.69
37	1.90	3.00	2.50	0.60	2.00	1.04
44	0.80	3.20	1.60	2.20	1.95	1.01
59	2.00	1.70	7.90	2.10	3.43	2.99

Table 149 Serum testosterone concentration (ng) from rabbits after subcutaneous injection of 3.3 mg buserelin acetate microemulsion(36BSA) (injection on day 1 th)

Day	Testosterone (ng)				Mean	SD
	R-1	R-2	R-3	R-4		
0	5.40	1.90	6.20	0.40	3.48	2.77
4	1.40	2.70	2.10	9.90	4.03	3.95
7	0.60	0.90	0.40	6.60	2.13	2.99
11	0.80	0.50	0.50	2.80	1.15	1.11
17	0.50	0.80	0.80	1.60	0.93	0.47
23	0.40	0.80	0.60	2.20	1.00	0.82
30	0.30	1.80	0.70	0.40	0.80	0.69
37	0.30	1.40	3.10	3.70	2.13	1.56
44	0.60	1.30	3.80	0.50	1.55	1.54
59	4.40	2.70	0.40	1.60	2.28	1.70

VITA

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