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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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DEVELOPMENT OF LIPID EMULSION FORMULATION FOR PARENTERAL NUTRITION

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การศึกษาการเตรียมอิมันชันไขมันสำหรับให้เป็นอาหารทางหลอดเลือดดำ โดยใช้น้ำมันสายโมเลกุลปานกลาง (น้ำมันเอ็มซีที) และน้ำมันสายโมเลกุลยาว (น้ำมันถั่วเหลือง) ในความเข้มข้นร้อยละ 5, 10 และ 20 ใช้สารอิมัลซิฟายเออร์ได้แก่ เลซิทินจากถั่วเหลือง สารลดแรงตึงผิวสังเคราะห์แบบไม่มีประจุ เช่น ครีมอฟอร์อีแอล, พอลอซาเมอร์ 188, โซลูทอลเอชเอส 15 และทวีน 80 ซึ่งใช้เพียงชนิดเดียวหรือใช้ร่วมกันระหว่างเลซิทินจากถั่วเหลืองกับสารลดแรงตึงผิวสังเคราะห์แบบไม่มีประจุ ศึกษาปัจจัยจากวิธีการเตรียมอันได้แก่ เวลาในการปั่นผสมด้วยเครื่องปั่นผสมความเร็วสูง, ความดันและจำนวนรอบในการผ่านสารเข้าสู่เครื่องปั่นผสมชนิดความดันสูง นำตำรับไปทำไร้เชื้อโดยใช้หม้อนึ่งอัดไอ ผลการศึกษาพบว่าตำรับที่ใช้ส่วนผสมของเลซิทินจากถั่วเหลืองกับทวีน 80 หรือ พอลอซาเมอร์ 188 สามารถเตรียมอิมัลชันไขมันที่คงตัวได้ ตำรับอิมัลชันไขมันที่ดีที่สุดในการศึกษานี้คือ 10% ฟาร์มาลิปิด ซึ่งประกอบด้วย น้ำมันถั่วเหลืองร้อยละ 10 เลซิทินจากถั่วเหลืองร้อยละ 1.71 และทวีน 80 ร้อยละ 1.29 เมื่อวัดเส้นผ่าศูนย์กลางร้อยละ 50 โดยปริมาตรของขนาดอนุภาคก่อนและหลังการทำไร้เชื้อมีค่า 0.31 และ 0.33 ไมโครเมตร ตามลำดับซึ่งไม่แตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 ค่าความเป็นกรด-ด่าง,ค่าออสโมแลลิตี และค่าความต่างศักย์ที่ผิวอนุภาคหลังการทำไร้เชื้อมีค่า 6.83, 337มิลลิออสโมลต่อกิโลกรัมน้ำ และ –33.52 มิลลิโวลท์ ตามลำดับ เมื่อเก็บไว้ที่อุณหภูมิห้องพบว่า ค่าความเป็นกรด-ด่างและค่าความต่างศักย์ที่ผิวอนุภาคมีค่าลดลงเล็กน้อย ในขณะที่เส้นผ่าศูนย์กลางร้อยละ 50 โดยปริมาตรของอนุภาคและค่าออสโมแลลิตีค่อนข้างคงที่

นำอิมันชันไขมันตำรับนี้และตำรับที่มีจำหน่ายในท้องตลาดได้แก่ 10%, 20% อินทราลิปิด, 10%, 20% ไลโปฟันดินเอ็มซีที/แอลซีที และ 10% ไลโปฟันดิน-เอส มาผสมร่วมกับสารละลายกรดอะมิโนและเดกซ์โทรสเพื่อเตรียมเป็นสารอาหารทั้งหมดที่ให้ทางหลอดเลือดดำ ศึกษาความคงตัวทางกายภาพและคุณสมบัติทางเคมีกายภาพของทุกสูตรทันทีที่เตรียมเสร็จใหม่และหลังจากเก็บไว้ที่อุณหภูมิห้องเป็นเวลา 24 ชั่วโมง พบว่าทุกตำรับมีความคงตัวดี มีความเป็นกรดอ่อนๆ มีค่าออสโมแลลิตีสูง ค่าความต่างศักย์ที่ผิวอนุภาคน้ำมันมีค่าลดลงเล็กน้อย เส้นผ่าศูนย์กลางร้อยละ 50 โดยปริมาตรของขนาดอนุภาคมีค่าใกล้เคียงกับของอิมันชันไขมันเริ่มต้น

ภาควิชา อาหารเคมี **ลายมือชื่อนิสิต**.....

สาขาวิชา อาหารเคมีและโภชนศาสตร์ทางการแพทย์ **ลายมือชื่ออาจารย์ที่ปรึกษา**.....

ปีการศึกษา 2543 **ลายมือชื่ออาจารย์ที่ปรึกษาร่วม**.....

4176587933 : MAJOR FOOD CHEMISTRY

KEY WORD: LIPID EMULSION / PARENTERAL NUTRITION / SOY LECITHIN / SYSTHETIC NONIONIC SURFACTANTS / HIGH PRESSURE HOMOGENIZER / STERILIZATION / PHYSICAL PROPERTIES / PHYSICOCHEMICAL PROPERTIES / TOTAL NUTRIENT ADMIXTURES

SUPINDA RUANGTHURAKIT : DEVELOPMENT OF LIPID EMULSION FORMULATION FOR PARENTERAL NUTRITION. THESIS ADVISOR : DR. WARANGKANA WARISNOICHAROEN, Ph.D. 184 pp. ISBN 974-346-417-4.

The preparation method of parenteral lipid emulsion was studied. The medium chain triglycerides (MCT oil) and long chain triglycerides (soybean oil) were used at concentrations of 5%, 10% and 20%. The emulsifiers used were soy lecithin, synthetic nonionic surfactants; namely, Cremophor®EL, poloxamer 188, Solutol®HS15 and Tween 80. The emulsifiers were used either alone or in combination between soy lecithin and synthetic nonionic surfactant. The methods of preparation were varied in homogenization time, pressure and cycles through high pressure homogenizer. The formulations were sterilized by autoclaving. The results indicated that formulations composed of the combination of soy lecithin and Tween 80 and soy lecithin and poloxamer 188 could form stable emulsion. The lipid emulsion containing 10% soybean oil emulsified by 1.71% soy lecithin and 1.29% Tween 80 was the best formulation and was called "10% Pharmalipid". The particle sizes (d(v,0.5)) of such formulation before and after autoclaving were 0.31 and 0.33 μm, respectively, which were insignificantly different ($p > 0.05$). The pH, osmolality and the value of zeta potential of the emulsion were 6.83, 337 mOsm/kg water and –33.52 millivolts, respectively. The pH and zeta potential were slightly decreased upon storage at room temperature while the d(v,0.5) and osmolality remain constant.

The developed emulsion and five commercial lipid emulsions; namely, 10% and 20% Intralipid®, 10% and 20% Lipofundin®MCT/LCT and 10% Lipofundin-S were used to prepare the total nutrient admixtures (TNA) by mixing with amino acid and dextrose solution. The physical stability and physicochemical properties of TNA were analyzed immediately after mixing and after storage at room temperature for 24 hours. The pH of TNA system was weakly acidic and remained constant throughout the period of study and the osmolality was slightly hyperosmotic. Moreover, the zeta potential was slightly decreased and the d(v,0.5) of emulsion in TNA systems remained close to that of the original lipid emulsion.

Department Food Chemistry Student Signature.....

Field of Study Food Chemistry and Medical Nutrition Advisor Signature.....

Academic Year 2000 Co-advisor Signature.....

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LIST OF ABBREVIATIONS

cm	=	centimeter (s)
CNS	=	central nervous system
^{o}C	=	degree celcius
d(4,3)	=	the volume weighted mean diameter
d(3,2)	=	the surface weighted mean diameter
d(v,0.1)	=	the diameter of particles of 10% volume percentile
d(v,0.5)	=	the diameter of particles of 50% volume percentile
d(v,0.9)	=	the diameter of particles of 90% volume percentile
EEG	=	electro-encephalogram
e.g.	=	exampli gratia (for example)
et al.	=	et alii (and others)
etc.	=	et cetera (and so on)
FAA	=	free fatty acid (s)
HDL	=	high density lipoprotein
HLB	=	hydrophilic-lipophilic balance
hr	=	hour (s)
i.e.	=	id est (that is)
LCT	=	long chain triglycerides
LD_{50}	=	lethal dose at 50%
LDL	=	low density lipoprotein
LE	=	soy lecithin
mcm	=	micrometer (s)
MCT	=	medium chain triglycerides
mg	=	milligram (s)

min	=	minute (s)
ml	=	milliliter (s)
mN/m	=	milliNeuton per meter
mo	=	month (s)
mOsm/kg	=	milliosmols per kilogram water
mPa s	=	milliPascal second
MW	=	molecular weight
N	=	normal
nm	=	nanometer (s)
No.	=	number of sample
o/w	=	oil in water emulsion
P188	=	poloxamer 188
PEG	=	polyethylene glycol
pH	=	the negative logarithm of the hydrogen ion concentration
psi	=	pound (s) per square inch
rpm	=	revolution (s) per minute
SB	=	soybean oil
SD	=	standard deviation
SEM	=	scanning electron microscopy
T80	=	Tween 80
TNA	=	total nutrient admixtures
TPN	=	total parenteral nutrition
v/v	=	volume by volume
wk	=	week (s)
w/v	=	weight by volume
w/w	=	weight by weight

μg	=	microgram (s)
μl	=	microliter (s)
μm	=	micrometer (s)
%	=	percentage
>	=	more than
<	=	less than
≤	=	equal or less than

CHAPTER I

INTRODUCTION

Lipid emulsions have been successfully used in parenteral nutrition since 1940 (Herman and Groves, 1992). They provide energy of 9 calories per grams and essential fatty acids, linoleic acid (omega 6) and α-linolenic acid (omega 3), for patients who are unable to consume or have abnormal gastrointestinal absorption (Hansrani, Davis, and Groves, 1983; Herman and Groves, 1992; Li and Caldwell, 1994; Washington and Davis, 1987).

The commercial products of lipid emulsion include Intralipid®, Lipofundin® and Liposyn®. The products normally containing 10, 20, or 30% w/v triglyceride oils from soybean, safflower, or cottonseed oils. Natural phospholipids are used to provide the optimum stabilization of the systems (Herman and Groves, 1992). However, the emulsion containing cottonseed oil has been withdrawn from parenteral use as the toxic side effects associated with contamination by trace quantities of gossypol (Hansrani et al., 1983; Herman and Groves, 1992). More recently mixtures of natural triglycerides with semisynthetic medium chain triglycerides from fractionated coconut oil have been employed (Herman and Groves, 1992).

Emulsions for parenteral nutrition are in the form of oil-in-water emulsions and have approximate size in the range of 0.4 to 1.0 μm which was the same size as natural chylomicrons (Chansiri et al., 1999; Hyltander, Sandström, and Lundholm, 1998). In addition, particle size should not be larger than 5 μm in order to avoid fat embolism in the vascular system

(Chansiri et al., 1999; Hansrani et al., 1983; Ishii, Sasaki, and Ogata, 1990). The risk is significantly greater in cases of neonates treated with parenteral lipid emulsions (Klang and Benita, 1998).

Lipid emulsions can be used as a part of total parenteral nutrition (TPN) by administration via peripheral vein while the higher osmotic solutions of amino acid and glucose are administered via central vein. Moreover, they can be directly added into amino acid and glucose solutions and the mixture is called the total nutrient admixtures (TNA) or three-in-one parenteral nutrition (Brown, Quercia, and Sigma, 1986). TNA can be administered via central or peripheral veins (Bradford, 1996). TNA is found to be a relatively safe and convenient method of TPN administration and also offers some advantages over TPN. TNA provides a comfortable way for patients given the TPN at home, a decrease in the waste of infusion set and a decrease in the incident of infection (Brown et al., 1986). However, the use of TNA sometimes seems to be limited which is possibly due to the uncertainty of the stability of the systems upon administration.

The natural emulsifier, lecithin, is widely used in parenteral lipid emulsions (Hansrani et al., 1983; Jumaa and Müller, 1998a; Krishna, Wood, and Sheth, 1998). Even lecithin is considered as the emulsifier of choice from its biocompatibility. It is considered not to be a very effective emulsifier (Hansrani et al., 1983; Herman and Groves, 1992). The emulsion produced using lecithin is sensitive to electrolyte and the change in pH. The use of nonionic surfactants are attempted to use for improving the stability of emulsion (Attwood and Florence, 1983). Their advantages include the less sensitivity to electrolytes and any change in pH and less expensive. It is also

found that the parenteral emulsions using nonionic surfactant had the slower removal rate from the blood stream compared to emulsion formed with lecithin (Attwood and Florence, 1983; Jumaa and Müller, 1998b).

Lipid emulsions could be produced by using a variety of pharmaceutical acceptable triglyceride oils and a series of nonionic surfactants. Cremophor EL, poloxamer 188, Solutol HS15, and Tween 80 are the synthetic nonionic surfactants which have already been approved by the various pharmacopoeias for parenteral administration (Klang and Benita, 1998; Jumaa and Müller, 1998b). Poloxamer 188 has no hemolytic effect to human blood cells in concentration up to 10% w/v solution (Wade and Weller, 1994). Tween 80 can be used in parenteral preparation in the range of 0.01 to 12% w/v and it is the most common and versatile solubilizing, wetting, and emulsifying agent (Nema, Washkuhn, and Brendel, 1997). Cremophor EL has no teratogenic or embryotoxic effects after administration to pregnant rats up to 10 % solution (Wade and Weller, 1994).

The widespread use of lipid emulsion in hospitalized patient has an effect on the requirement and the cost of treatment. The present study intends to produce the lipid emulsions from a variety of oils and emulsifiers. The chemicals used in the study are long chain triglycerides (soybean oil) and medium chain triglycerides (MCT oil). Soy lecithin is used as a natural emulsifier. Synthetic nonionic surfactants include Cremophor EL, poloxamer 188, Solutol HS15, and Tween 80. There are several factors that effect on the stability of emulsion including oil, emulsifiers and the manufacturing process (Hansrani et al., 1983; Jumaa and Müller, 1998b; Siekmann and Westesen, 1998; Washington, 1988). The study of physicochemical properties of the

prepared lipid emulsions such as the particle size, zeta potential, pH and osmolality may provide useful information to use as the guidelines for domestic manufacturing.

The well-known factors that affect on the stability of lipid emulsion in the TNA preparation are mainly the interaction with the composition in the system especially the electrolytes (Brown et al., 1986). The volume ratios of composition and the order of mixing may be important (Knutsen, Epps and McCormick, 1984). The only commercial TNA product available in Thailand can be prepared from adding 250 ml lipid emulsion (20% Intralipid®) into 750 ml solutions of dextrose, amino acid and some electrolytes. There are only a few studies investigated on stability of TNA using different volume ratios of the compositions and other available lipid emulsions. The effect of volume ratios of the composition in TNA and the stability of lipid emulsion in the presence of other nutrients are investigated. The results may be useful for development of lipid emulsions for parenteral nutrition purposes.

The objectives of the study

The aims of this study were as follows:

1. To study the factors involved in the formulation, and properties of lipid emulsions including types and amounts of ingredients, processing conditions (time, pressure, and number of cycle of homogenization) and steam sterilization.

2. To investigate the physical stability and physicochemical properties (osmolality, pH, particle size, and zeta potential) of lipid emulsion produced and scanning electron microscope.

3. To assess the possibility of using lipid emulsion for total nutrient admixtures by evaluating the change in physical stability and physicochemical properties of lipid emulsion.

CHAPTER II

LITERATURE REVIEW

1. Definition and terminology of lipid emulsions for parenteral nutrition

Emulsions are generally described as a heterogeneous system composed of two immiscible liquids normally oil and water where one phase is dispersed uniformly as droplets in the other. The disperse phase or internal phase presents as small droplets surrounded with continuous or external phase. Emulsifier or emulsifying agent is used to stabilize the system by decreasing the interfacial tension of the two phase by forming an interfacial film around the droplet of internal phase (Li and Caldwell, 1994; Rieger, 1986).

Typical emulsions contain droplet ranging from 0.1 to 100 μm in diameter hence they appear milky white liquid. Smaller droplets promote more stable systems while the larger droplets promotes the instability by fusion or coalescence. In term “macroemulsion” is sometimes employed to distinguish the ordinary emulsions defined above from “microemulsion”, which the dispersed droplets are of colloidal dimensions that have diameter less than 0.1 μm. Microemulsions differ from macroemulsions by their optical transparency, spontaneously forming and thermodynamic stability (Lund, 1994; Rieger, 1986; Swarbrick and Boylan, 1992).

It is almost universally accepted that the term emulsion should be limited to liquid in liquid system. The most common types of pharmaceutical or cosmetic emulsion include water as one of the phases and oil or lipid as the

other. If the oil is dispersed as droplets throughout the aqueous phase, the emulsion is termed oil-in-water (o/w) emulsion. If the oil is the continuous phase, the emulsion is the water-in-oil (w/o) type. All pharmaceutical emulsions designed for parenteral administration are o/w type (Swarbrick and Boylan, 1992).

Intravenous emulsions are required to meet pharmacopoeial requirements. The emulsions must be sterile, isotonic, non-pyrogenic, non-toxic, biodegradable and stable in both physical and chemical properties. Furthermore, the particle sizes of the droplets needs to be below 1 μm, and generally are in a range of 0.1-0.5 μm. The larger droplet size may cause fat embolism (Klang and Benita, 1998). For these reasons, a number of oils and emulsifiers available for use in such emulsions are very limited. The manufacturing techniques are of critical importance on both the size of oil droplets and the stability of the emulsion on storage (Bock et al., 1998).

2. Ideal properties of lipid emulsions for parenteral nutrition

The appropriate properties of lipid emulsions for parenteral nutrition shoule be: (i) small particle size normally less than 5 μm; (ii) less toxicity by the use of physiological compounds and the absence of toxic residues from the production process; (iii) ability to sterile by autoclaving; (iv) long term stability in aqueous dispersion with regard to coalescence; (v) production on large industrial scale to sufficiently supply to market; and (vi) acceptable cost to the health authorities of the customer countries (Burgess, 1990).

3. Excipient selection

For parenteral emulsions, the selection of the excipients and emulsion type is important. Specific attention should be given into two major ingredients in the emulsion formulation, the oils and emulsifiers. Potential toxicity, cost, and chemical incompatibilities, therapeutic response, stability and properties (i.e., particle size) of the formulation must be taken into account. Ingredient selection is often made by trial and error and the experience. A detailed description of the excipient specifications for parenteral emulsion was presented by Hansrani et al. (1983).

1. Oil

The choice of oil for parenteral emulsions is severely limited for reason of toxicity. In the past, the oil phases were based mainly on long chain triglycerides (LCT) from vegetable sources such as soybean, safflower, and cottonseed oils (Table 1.). The oils need to be purified and these oils are resistant to rancidity and show few chemical side effects. However, cottonseed oil emulsion, “Lipomul”, was withdrawn from parenteral use due to the following report of toxic side effects associated with contamination of the oil by trace quantities of grossypol (Hansrani et al., 1983).

The medium chain triglycerides (MCT) were used in parenteral emulsion formulation in combination with soybean oil as they are better energy sources for nutritional purposes (Swarbrick and Boylan, 1992). MCT obtained from hydrolysis of coconut oil and fractionated into free fatty acids containing the acyl chain length of 6 to 12 carbon atoms. The MCT are esterified with

glycerol and are 100 times more soluble in water than LCT (Hyltander et al., 1998). The energy provides from LCT oil is 9.0 calories per gram while MCT provides 8.3 calories per gram (Krummel, 1996). The fatty acid composition in MCT and LCT oil is shown in Table 2.

Table 1. Some commercial available lipid emulsions for parenteral administration (from Klang and Benita, 1998).

Trade name	Oil phase (%w/v)	Emulsifier (%w/v)	Additives(%w/v)
Intralipid	Soybean 10	Egg lecithin 1.2	Glycerol 2.25
	Soybean 20	Egg lecithin 1.2	Glycerol 2.25
Lipofundin-S	Soybean 10	Soy lecithin 0.75	Xylitol 2.5
	Soybean 20	Soy lecithin 1.2	Xylitol 2.5
Lipofundin	Soybean and MCT (1:1) 10	Egg lecithin 0.75	Glycerol 2.5
MCT/LCT	Soybean and MCT (1:1) 20	Egg lecithin 1.2	Glycerol 2.5
Liposyn	Safflower 10	Egg lecithin 1.2	Glycerol 2.5
	Safflower 20	Egg lecithin 1.2	Glycerol 2.5
Abbolipid	Safflower and soybean (1:1) 10	Egg lecithin 1.2	Glycerol 2.5
	Safflower and soybean (1:1) 20	Egg lecithin 1.2	Glycerol 2.5
Lipovenos	Soybean 10	Egg lecithin 1.2	Glycerol 2.5
	Soybean 20	Egg lecithin 1.2	Glycerol 2.5
Travemulsion	Soybean 10	Egg lecithin 1.2	Glycerol 2.5
	Soybean 20	Egg lecithin 1.2	Glycerol 2.5

Most of the commercial products are available at the oil concentrations of 10 or 20% w/v to meet differing energy requirements. The lipid emulsion containing 10% w/v oil provides 1.1 calories per ml and lipid emulsion containing 20% w/v oil provides 2.0 calories per ml. Normally the energy obtained from parenteral emulsion is approximately 10% of total energy. The calories of 2% to 4% obtained from parenteral emulsion should come from linoleic acid (omega-6) in soybean oil which is required to prevent

essential fatty acid deficiency. Maximum dose of fat should not exceed 2.5 g/kg /day (60% of total calories)(Bradford, 1996).

Table 2. The composition of fatty acids in MCT, soybean, and safflower oils (from Miller, 1954; Wade and Weller, 1994)

Fatty acid	Carbon : double bond of fatty acid	Amount of fatty acids (%)		
		MCT oil	Soybean oil	Safflower oil
Linoleic acid (omega-6)	C18:2	-	50-57	76-79
Linolenic acid (omega-3)	C18:3	-	5-10	0.04-0.13
Oleic acid	C18:1		17-26	14-17
Stearic acid	C18:0	-	3-6	3.1
Palmitic acid	C16:0	-	9-13	6.4
Capric acid	C10	23	-	-
Caprylic acid	C8	67	-	-
	More than C10	< 4	-	-
	Less than C8	< 6	-	-

Linoleic acid presents in the triglycerols of the soybean oil and also in the soybean lecithin. Long chain triglycerides are hydrolyzed by intestinal lipase before absorption so infusion of LCT leads to a relatively slow provision of energy. MCT are readily hydrolyzed and independent on lipase or bile salt hence, they are readily absorbed representing a more rapidly available source of energy (Table 3). Patients who have liver dysfunction should be administered lipid emulsion from MCT oil (Krummel, 1996). Continuous infusion of MCT and/or intake of high doses of MCT; however, it should be considered as MCT might increase total cholesterol and LDL cholesterol (Parnham, 1998).

Table 3. The characteristic of medium chain triglyceride and long chain triglyceride (from Kultida Chaijinda, 1998)

Characteristic	Medium chain triglyceride	Long chain triglyceride
1.Molecular structure	C6-12	C14-24
2.Energy (Cal/g)	8.2-8.4	9
3.Metabolism substance		
3.1 Pancreatic lipase	Independent	Dependent
3.2 Bile salt	Independent	Dependent
4.Chylomicron synthesis	No synthesis	Need
5.Essential fatty acid	No	Linoleic acid (omega-6) Linolenic acid (omega-3)
6.Fatty storage	Not stored in the liver	Stored in the liver
7.Ketogenesis promotion	Stimulate ketogenesis	No
8.Clearance	More rapidly than LCT	Slower than MCT
9.Hypertriglyceridemia level	May occur	Not occur
10.Source	Fractionated coconut oil	Soybean, safflower, sunflower, corn

2. Emulsifying agents

Emulsifying agents are used to promote emulsification at the time of manufacturing and to control stability during a shelf life that can vary from days for extemporaneously prepared emulsions to months or years for commercial preparations. The ideal emulsifying agents for pharmaceutical purposes should be stable, inert, non-toxic and non-irritant. It should be odorless, tasteless, colorless, effective and can be produce stable emulsions at low concentration of emulsifier (Lund, 1994; Swarbrick and Boylan, 1992).

The main functions of the emulsifying agents are decreasing the interfacial tension by forming a thin film at the oil/water interface, preventing

attachment of each particle, preventing flocculation and coalescence of the dispersed phase. It is important to use only the minimum concentration of the chosen emulsifying agent as any excess may result in the formation of the foam (Klang and Benita, 1998; Lund, 1994).

Emulsifying agents used in parenteral lipid emulsions were synthetic surface active agents and natural substances such as phospholipids. The nonionic surfactants are normally used to produce oil-in-water and water-in-oil emulsions for both external and internal administration. The advantages of nonionic surfactants include: (i) their resistance to the effects of electrolytes; (ii) their compatibility with other surfactants; (iii) unionization in acidic or basic condition; (iv) easily adjustment the value of hydrophilic and lipophilic balance (HLB) for emulsification efficiency; (v) very low toxicity; (vi) antibacterial activity; (vii) less impurities. A disadvantage of nonionic surfactants is possibly their tendency to bind or inactivate preservatives containing phenolic or carboxylic groups in the formulation (Attwood and Florence, 1983). The use of emulsifier is proposed to depend upon the hydrophilic-lipophilic balance (HLB) (Lund, 1994) (Table 4).

The HLB has been used as the basis for a more rational mean of selecting and classifying emulsifier. Many oils and waxy materials used in emulsions have “required HLB” value to form the stable emulsions. For example, castor oil has a required HLB value of 14 and soybean oil has a required HLB value of 8 (Krishna et al., 1998; Lund, 1994). HLB value can be adjusted to the required value by using a mixture of two surfactants, the lower HLB and the higher HLB value (Todd, 1973). Greater efficiency is often obtained by using a blend of surfactants instead of a single surfactant (Klang and Benita, 1998).

Table 4. The relationship between HLB numbers and surfactant properties (from Lund, 1994)

HLB range	Properties
0 – 3	Antifoaming agents
4 – 6	Emulsifying agents (water-in-oil)
7 – 9	Wetting agent
8 – 18	Emulsifying agent (oil-in-water)
13 – 15	Detergents
10 - 18	Solubilising agents

HLB values, however may not be precise parameter as the surfactants having the same HLB values may not produce emulsion in the same manner. The HLB values do not take into account the concentration of surfactants. It is often necessary to prepare a series of emulsions in order to determine the optimum concentration of emulsifiers used (Todd, 1973).

The examples of synthetic nonionic surfactants used in parenteral preparations are polyoxyethylene sorbitan (Tween), block copolymers of polyoxyethylene polyoxypropylene (poloxamer series), polyoxyethylene castor oil derivatives (Cremophor EL), and polyoxyethylene-660-(12)-hydroxystearate (Solutol HS15) (See Appendix A for properties of surfactants). These surfactants are already approved by the various pharmacopoeias for parenteral administration and can therefore be considered for parenteral emulsion formulations (Klang and Benita, 1998).

The natural emulsifying agents used in parenteral emulsion are phospholipids (lecithin) from egg-yolk and soybean sources. These phospholipids are the mixtures of two major components, phosphatidylcholine

and phosphatidylethanolamine (Table 5). Phosphatidylcholine shows surface activity and yields oil-in-water emulsions. The advantage of natural phospholipids over the synthetic surface active agents are an emulsion stabilized by some surface active are sometimes cracked or separated into its components by applying heat while emulsion containing natural phospholipids is considered to be more stable (Groves, 1988; Hansrani et al., 1983; Herman and Groves, 1992).

Table 5. Composition of egg lecithin and soy lecithin (modified from Othmer, 1995).

Phospholipids	Amount (%)	
	Egg lecithin	Soy lecithin
Phosphatidylcholine (PC)	69	21
Phosphatidylethanolamine (PE)	24	22
Sphingomyelin (SP)	2	trace
Phosphatidic acid (PA)	trace	10
Phosphatidylinositol (PI)	trace	19
Phosphatidylserine (PS)	3	1
Lyso-phosphatidylcholine (lyso-PC)	2	1
Lyso-phosphatidylethanolamine (lyso-PE)	2	1

Molecule of phospholipid consists of charged polar phosphate group which is attached to various polar entities such as choline or ethanolamine and the two hydrophobic moieties containing the fatty acid chains varying in length from C_{12} to C_{20}. (See Appendix A for properties). If one of lipid groups is hydrolyzed, the remaining phosphatidal components are called the lyso-compound, i.e., lyso-phosphatidylcholine (lyso-PC). The presence of lyso-PC in emulsion containing lecithin is responsible for emulsion

stability during long-term storage (Groves, 1988; Hansrani et al., 1983). However, it is found to be toxic to the red blood cell (Groves, 1988; Hermann and Groves, 1992; Siekmann and Westesen, 1998).

Most of the commercial parenteral lipid emulsions were normally stabilized by egg lecithin. Soy lecithins were less commomly used; however, they have the advantages over the egg lecithins in that they are obtained easily and less expensive. For the fatty acid composition, soy lecithins have higher content of polyunsaturated linoleic acid than egg lecithins (Table 6) and have linolenic acid compared with egg lecithins (Hansrani et al., 1983; Parnham, 1998).

Table 6. Fatty acid composition of soybean and egg lecithins (from Parnham, 1998)

Fatty acids	Carbon,double bond of fatty acids	Amount of fatty acid (%)	
		Soybean lecithin	Egg lecithin
Palmitic acid	C16:0	18.4	37.0
Stearic acid	C18:0	4.0	9.0
Oleic acid	C18:1	10.7	32.3
Linoleic acid	C18:2	58.0	16.7
Linolenic acid	C18:3	6.8	-
Arachidonic acid	C20:0	-	5.0
Others		2.1	-

The effect of lecithins in parenteral emulsions on serum lipoproteins has been previously reported. There were comparative investigations of Intralipid (containing egg lecithin) and Lipofundin-S (containing soy lecithin). The results indicated that Intralipid increased the

concentrations of very low density lipoproteins (VLDL) while Lipofundin-S decreased the concentration of VLDL resulting in the lower risk of hypercholesterolemia (Parnham, 1998).

Natural emulsifiers, lecithin, are derived from animal or vegetable sources. They are often complex, undefined, and variable in chemical compositions therefore they are thus subjected to considerable variation in emulsifying power. They are also susceptible to any microorganisms leading to rapid spoilage unless adequate preservatives are included (Brown et al., 1986).

Emulsions produced using a combination of emulsifiers are superior to those formed using a single emulsifier. The combination emulsifier can produce more flexible interfacial films necessary to form stable emulsion (Lundberg, 1994). In some studies, it was found that the emulsion containing lecithin as primary emulsifier and nonionic surfactant as secondary emulsifier had smaller particle size and more stability than emulsion containing only lecithin (Attwood and Florence, 1983; Jumaa and Müller, 1998b; Lundberg, 1994; Yamaguchi et al., 1995).

3. Other additives

The dispersion medium of parenteral lipid emulsion may contain one or more of the following additives: isotonicity agents; electrolytes; buffer; anti-flocculants and preservative.

Normally, parenteral emulsions have low osmotic pressure, therefore additives are needed to adjust the physiological tonicity. Glycerol is the most recommended isotonic agent. The osmolarity of emulsions should be in the range of 280–350 mOsm/L in order to prevent any hemolysis, pain, irritation, and tissue damage at the site of administration. However, xylitol and sorbitol are also being used as isotonic agents in parenteral emulsion (Hansrani et al., 1983; Jumaa and Müller, 1998a; Klang and Benita, 1998).

The pH of the system is adjusted to the desired value with either an aqueous solution of NaOH or HCL, depending on the value that should be reached. The pH of the emulsions is generally adjusted between 7 and 8 prior to sterilization to allow physiological compatibility and maintain emulsion physical integrity by minimizing hydrolysis of fatty acid ester, the MCT, LCT, and phospholipids. The pH of the emulsion is normally decreased after autoclaving or upon storage and the toxicity of emulsions could be correlated to free fatty acids levels in the product. The rate of free fatty acids production was minimal if the pH of the emulsion was between 6 and 7, after sterilization (Hansrani et al., 1983; Klang and Benita, 1998).

Oxidation can lead to unstable emulsions. Antioxidants are added in the formulation to minimize degradation. The suitable antioxidants are as follows: butylated hydroxy toluene (BHT)(0.00116 – 0.03 %w/v), butylated hydroxy anisole (BHA)(0.00028 – 0.03 %w/v), tocopherols (0.05 – 0.075 %w/v) (Nema et al., 1997). Furthermore, the explosure to oxygen during the manufacturing process should be avoided. This can be accomplished by purging the solvent system with filtered nitrogen during the manufacturing process, blanketing the product with filtered nitrogen or argon during the filling

operation, and displacing oxygen from the head space of the filled container with filtered nitrogen (Hansrani et al., 1983).

4. Preparation of lipid emulsions

There are many parameters which important in formulating parenteral lipid emulsions. All of these parameters can influence the particle size distributions of emulsions as follows: (i) emulsifier and other additives in aqueous phase or oil phase, (ii) process of high speed homogenization such as order of mixing of individual phases, homogenization temperature, intensity of homogenization, (iii) process of high pressure homogenization such as pressure and time used for homogenization (Bock et al., 1998).

To prepare the emulsion, the oil-soluble and water-soluble components are separately dissolved in either oil or water phase and a suitable emulsifier is selected. If it is necessary to melt or to heat components in either oil or water phase in order to maintain a fluid state, the phases should be brought to a similar temperature before mixing and emulsification (Lund, 1994). In Spalton's study he described that the water should be warmed to a temperature of a few degrees higher than that of the oil phase. The reason was to ensure that both phases were at approximately the same temperature when being mixed in order to avoid crystallization of the wax or waxes during a sudden lowering of temperature with the cold water (Spalton, 1959).

The method of emulsion preparation is normally the addition of the internal phase (dispersed phase) into the external phase (continuous phase) (Figure 1). Emulsifier and water-soluble components are dissolved into a

water phase and oil-soluble components are dissolved into an oil phase. The dispersed phase is gradually added to the continuous phase; often a more viscous primary concentrated emulsion is formed before the main bulk of the continuous phase is incorporated.

The important procedure for preparing emulsions was breaking up of the internal phase into external phase in order to form emulsion droplets by vigorously agitation. The coarse emulsion obtained is homogenized using high pressure homogenizer or microfluidizer to fine emulsion.

In contrary to the method described above, the external phase may be added into the internal phase to form emulsion. The continuous phase is gradually added to the disperse phase. If the disperse phase is in excess it will constitute the continuous phase of the first emulsion formed and then the phase inversion occurred to form the require type of emulsion. The problem of this method is in some cases phase inversion cannot be formed.

Descriptions of the different technical approaches for manufacturing submicron emulsions have been reported in the literature (Hansrani et al., 1983). It can be deduced that the conventional equipment (i.e., electric mixers and mechanical stirrers, etc.), should not only provide large droplet size but also a wide droplet size distribution (Klang and Benita, 1998). The ultrasonifier can make submicron emulsion in an average particle size of 0.97 μm with broad distribution (Chansiri et al., 1999). The homogeneous submicronized emulsion formulation can be manufactured only if high pressure homogenizers are used (Klang and Benita, 1998).

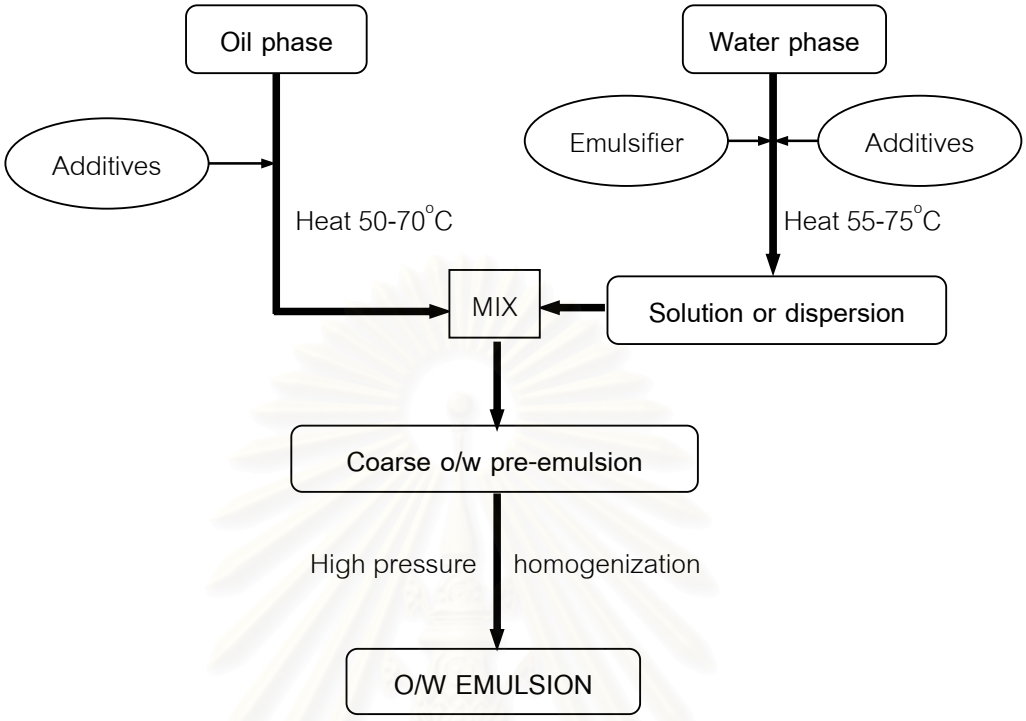


Figure 1. A schematic illustration of homogenization method.

High pressure homogenization is routinely used for the production of emulsions for parenteral administration. The required particle size can be achieved using various homogenizers or microfluidizers. Their ability on either laboratory or industrial scale must be acknowledged because the change of equipment during scale-up may affect the physical and chemical stability as well as the pharmacological efficacy.

In the homogenizer devices, the crude emulsion is forced under pressure through the annular space between a spring-loaded valve and the valve seat. The emulsion may be homogenized in appropriate pressure and number of times in order to achieve the required particle size. One type of

high pressure homogenizer is illustrated in Figure 2. However, a major concern of the homogenization process is the inevitable contamination produced from gasket materials, packing, and metal parts. These contaminants also originate from pumps and other metal surfaces. The new method which can minimize the effect is microfluidization.

Microfluidization has been successfully used to produce parenteral emulsion (Block, 1996). Microfluidizer processing is based on a submerged jet principle in which two fluidized streams interact at ultrahigh velocities in precisely defined microchannels within an interaction chamber. A combination of shear, turbulence, and cavitation forces results in the energy-efficient production of consistently fine droplets with a narrow size distribution. Diagram of the microfluidizer is illustrated in Figure 3 (Floyd and Jain, 1996).

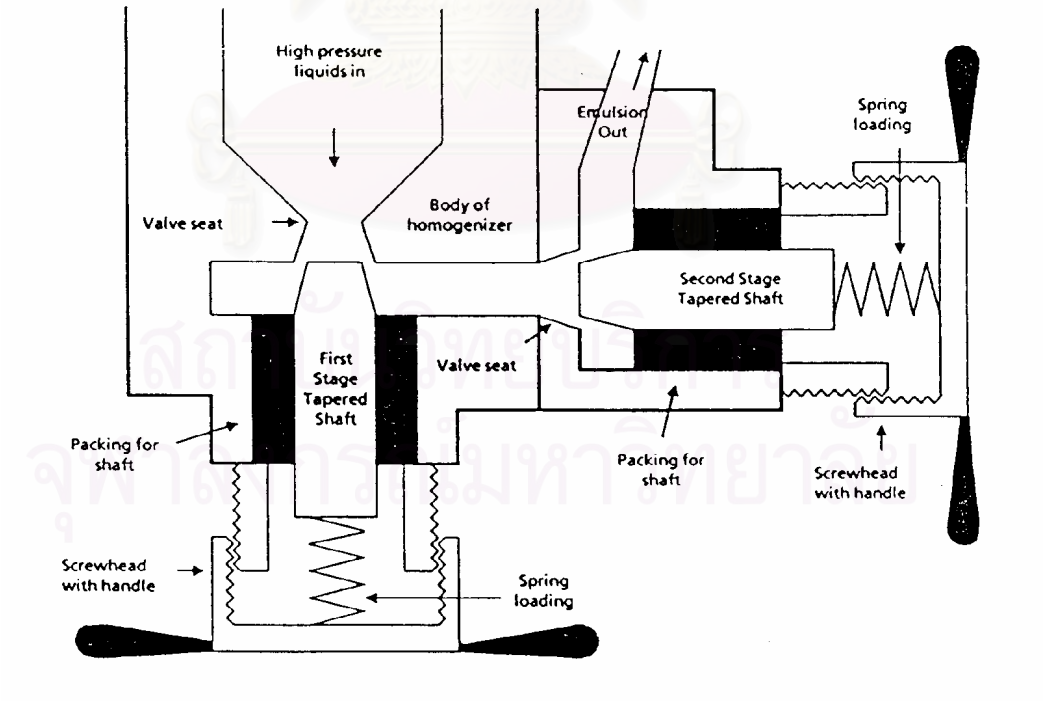


Figure 2. One type of single stage orifice for a high pressure homogenizer. (From Hansrani et al., 1983)

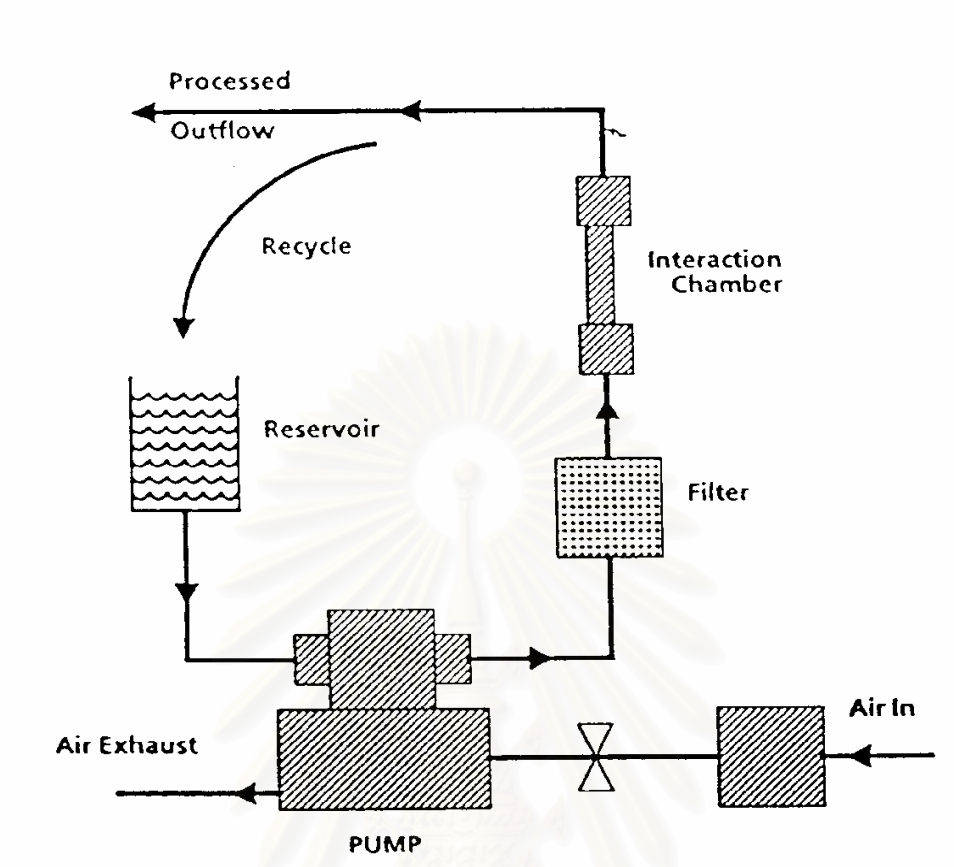


Figure 3. Diagram of the microfluidizer. (From Floyd and Jain, 1996)

5. Packing and sterilization of lipid emulsion for parenteral nutrition

The appropriate containers for intravenous emulsions should be type I (borosilicate) or type II (treated soda-lime) with butyl rubber or Teflon-faced rubber (Croce, Fisher, and Thomas, 1986). Plastic containers are not suitable for long-term storage of emulsion products because of oxygen and water vapor permeability and the extraction of oil-soluble plasticizers. An inert atmosphere, usually nitrogen, is essential for long-term stability. Sterilization conditions have to be carefully selected in order to avoid any degradation of thermolabile products. An essential requirement is a low initial bioburden of

the component parts of the emulsion in order to allow the absolute minimum of heat to be applied to the final product. The heating process may cause some hydrolytic breakdown especially from the lecithin resulting in the liberation of free fatty acid. The hydrolytic process will also occur more slowly during the shelf life of the product. The hydrolysis rate is at a minimum at a pH of around 6.5. For this reason, the initial pH is adjusted to above pH 8 by the addition of alkali in order to allow the pH of the product to drift slowly downward on storage (Groves, 1988; Hansrani et al., 1983).

The method of sterilization includes autoclaving at 121^{o}C with pressure at 15 psi for 15-20 minutes (Chansiri et al., 1999; Herman and Groves, 1992). An alternative method to sterile a complete emulsion is to sterilize the individual component and to assemble the product aseptically. This may be useful for lipid emulsion containing amino acids (Hansrani et al., 1983).

6. Properties and stability of lipid emulsion for parenteral nutrition

There are many properties of parenteral lipid emulsions necessary to evaluate and control.

1. Physical stability

Emulsions are promptly reverted to the original state of two separate liquids. Some of them are reversible such as creaming and flocculation while coalescence and separation are irreversible (Figure 4) (Nieuwenhuyzen, 1998).

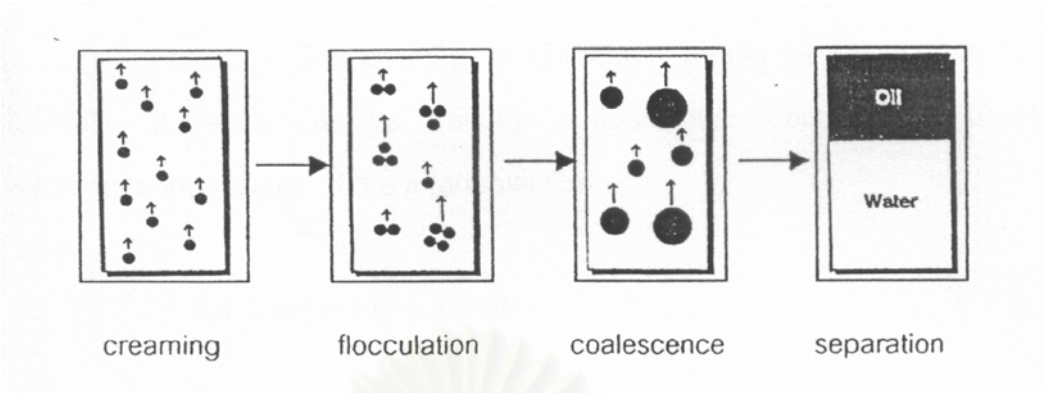


Figure 4. The physical instability of emulsions (from Nieuwenhuyzen, 1998)

1.1 Creaming

Creaming occurs when the dispersed oil globules move upwards and accumulate on the top under the influence of gravity to form a layer of more concentrated emulsion. An emulsion with creaming can usually be redispersed by gentle agitation. However, creaming is undesirable because the closeness of the droplets in the cream favors breakdown of the oil/water interface resulting in any coalescence of the droplets.

1.2 Flocculation

Flocculation is the clumping together of globules into loose aggregates. The aggregates can be redispersed by shaking as the interfacial films have not been destroyed.

1.3 Coalescence

The droplets of dispersed phase merge to form larger droplets. It begins with the drainage of liquid films around the oil droplets resulting in the rupture of the interfacial film.

1.4 Cracking or separation

Rupture of the interfacial film can lead to coalescence of the globules in the disperse phase. Coalescence may lead eventually to the complete and irreversible separation of the two phases; the term cracking is applied to such phase separation.

1.5 Phase inversion

It is the process by which the disperse phase of an emulsion becomes the continuous phase, and the continuous phase becomes the disperse phase. Phase inversion may occur by changing temperature, addition of a material that changes the solubility of the emulsifying agent (Lund, 1994; Swarbrick and Boylan, 1992).

2. Physicochemical properties

2.1 Particle size

The most significant characteristics of parenteral lipid emulsions are the size and the shape of the particles. Particle size has a direct effect on both stability and toxicity. Particle greater than 4 to 6 μm are known to increase the incidence of emboli and change the blood pressure. For intravenous emulsions, particles should be less than 5 μm in diameter

(Chansiri et al., 1999; Tian and Li, 1998). In some cases, particle size measurement is technically difficult because the particle sizes may extend beyond the limit of detection of any one given instrument. Thus, at least two complementary techniques should be employed. Many advanced instruments for determining particle size are available. For determinations of particle size below 1 μm, photon correlation spectroscopy (PCS) or quasielastic laser light scattering is useful (Chansiri et al., 1999; Ishii et al., 1990; Sjöström et al., 1993; Tian and Li, 1998; Westesen and Wehler, 1993). Electron microscopy, laser inspection system and coulter counter methods are used to determine the particle size larger than 1 μm. Transmission electron microscopy (TEM) is been particularly valuable for examination of particle size and shape of parenteral lipid emulsions. Freeze fractured TEM is the technique used to prepare sample observed under electron microscope (Westesen and Wehler, 1992; Westesen and Wehler, 1993). TEM pictures may indicate the validity of the assumption of spherical shape for the investigated systems. The particles were also characterized in polarized light by optical microscopy and by scanning electron microscopy (SEM) (Sjöström et al., 1993).

2.2 Zeta potential

The determination of zeta potential is great value for the development of parenteral lipid emulsions. The determination of the zeta potential of particle provides useful information of the sign and magnitude of the charge and its effect on the physical stability of the system (Rubino, 1990). The zeta potential is determined by measuring the migration velocity of the suspension particles with respect to the net effective charges on the surface, called electrophoretic mobility. A microelectrophoretic mobility apparatus, Zeta meter®, is used for measurement (Rubino, 1990). Furthermore, zeta

potential measurement can be performed using a dropler electrophoresis apparatus such as the Zetasizer® (Quintanar-Guerrero et al., 1998).

2.3 pH

The pH is important for maintainance of the desired particle size because of its effect on the surface charge of the particle (Chansiri et al., 1999). This parameter affects the physical and chemical stability.

2.4 Osmotic pressure

Osmolarity is the concentration expressed as moles of solute particles per litre of solution. The normal units of osmolarity are osmols and milliosmols. While osmolality is the concentration expressed as moles of solute particles per kilogram of water. Therefore, in dilute solutions osmolality is approximately equal to osmolarity. In most cases parenteral solutions are dilute solutions (Lund, 1994).

Osmotic pressure is a colligative property and therefore can be related to the relative molecular mass of the colloidal material (Reich, 1995). The osmotic property is determined by the gradient of some colligative properties, such as freezing point, boiling point, or pressure vapor. This property is important as it affects directly to the cells, especially red blood cells. Osmomat®O30-D is an instrument for determining the osmotic pressure using freezing point depression method.

2.5 Viscosity

Viscosity describes the resistance to flow with applied stress for a particular system; a more viscous system requires greater force or stress to make it flow at the same rate as a less viscous system. The emulsion viscosity can change due to concentration of ingredients, particle size, shape, and distribution. This parameter is directly used to describe the injectability of parenteral lipid emulsions. Many techniques could be used to measure this parameter. Capillary viscometers and the falling ball viscometers are simple instruments for measuring viscosity but only for Newtonian liquids. Rotational viscometers including the coaxial cylinder sensor systems (cup-and-bob viscometers) and cone-and-plate sensor systems are instruments for measuring either Newtonian or non-Newtonian liquids (Schramm, 1981).

3. Physicochemical parameters

These parameters are commonly used to assess the stability of emulsion. Firstly, the rate and extent of phase separation after aging of an emulsion may be observed visually or by measuring the volume of separated phase. Secondly, the zeta potential of emulsions can be measured by observing the movement of particles under the influence of electric current. The zeta potential is especially useful for assessing the flocculation since electrical charges on particles influence the rate of flocculation. If the instability is due to coalescence, the determination of the surface charges of particles may not be relevant for the prediction of the shelf life. Finally, the changes of the average particle size or the size distribution of droplets are important parameters for evaluating parenteral emulsions.

7. Assessment of shelf life of lipid emulsion for parenteral nutrition

1. Storage conditions

The final acceptance of an emulsion depends on stability, appearance, and functionality of the packaged product. Normally, the study on stability of emulsions should be performed in actually storage condition which takes a long time to study. However, there are no quick and sensitive methods for determining potential instability in an emulsion available except the stress conditions are employed (Block, 1996).

2. Stress conditions

Stress conditions are normally employed for evaluating the stability of emulsion. They are aging and temperature, centrifugation and agitation.

2.1 Aging and temperature

The routinely method to determine the shelf life of preparations are storing them for varying periods of time at temperatures higher than those normally encountered. It is important to realize that exposure to unrealistically high temperatures may produce meaningless results. It is clearly established that many emulsions may be perfectly stable at 40°C or 45°C, but cannot tolerate temperatures in excess of 55°C or 60°C even for a few hours. A particularly useful method of evaluating shelf life is cycling (heating and cooling) between two temperatures. Cycling system

should be conducted between 4°C and 45°C for 48 hours at each temperature and for 6-8 cycles (Lachman, Lieberman, and Kanig, 1976; Rieger, 1986). The normal effect of aging an emulsion at elevated temperature is the acceleration of the rate of coalescence or creaming which is usually coupled with change in viscosity. Most emulsions become less viscous at elevated temperature and more viscous at lower temperature. Freezing can damage an emulsion more than heating, since the solubility of the emulsifiers, both in the lipid and aqueous phases, is more sensitive to freezing than to modest warming. In addition, the formation of ice crystals develops pressure that can deform the spherical shape of emulsion droplets (Rieger, 1986).

2.2 Centrifugation

It is commonly accepted that shelf life under normal storage conditions can be predicted rapidly by observing the separation of the dispersed phase due to either creaming or coalescence when the emulsion is exposed to centrifugation. The difference of the gravity of the different phase accelerates emulsion separation. The stable emulsions should show no serious deterioration by centrifuging at 2,000 rpm to 3,000 rpm at room temperature (Rieger, 1986).

2.3 Agitation

Simple mechanical agitation can contribute to the energy in which two droplets impinge upon each other. It is rarely appreciated how useful the evaluation of an emulsion by agitation at or near room temperature can be. It was already noted that excessive shaking of an emulsion or excessive homogenization may interfere with the formation of emulsion and

agitation can also break emulsions (Rieger, 1986). The agitation program for emulsion is 24 to 48 hours on a reciprocating shaker (approximately 60 cycles per minute at room temperature and at 45°C).

8. Safety of lipid emulsion for parenteral nutrition

Factors affected the tolerability of lipid emulsion are listed in Table 7. Lipid emulsions are extremely well tolerated and can cause toxicity only at high doses (Parnham, 1998).

A high tolerance is required of lipid emulsions especially for parenteral use. Emulsion containing soybean oil has been infused into rats and dogs at amounts of 15 g/kg without toxic effects. The infusions of emulsion up to 75 g fat/day for 14 consecutive days were tolerated by humans. The major changes in plasma lipoproteins, total HDL and LDL cholesterol may occur after administration of normal doses of lipid emulsion. The patients in which lipid emulsions should not be administered are those with disorders of lipid metabolism. Low doses of lipid emulsion should be given to the newborn infants because of their poor ability to metabolize fat and the possible effects of high phosphatidylcholine concentrations on central nervous system (CNS). The use of MCT instead of LCT in fat emulsions can affect the tolerability of the emulsion. MCT are widely considered to have a neutral effect on serum cholesterol concentration because of their rapid metabolism (Bradford, 1996; Parnham, 1998). However, there are some studies stated that oral administration of MCT for 3 weeks leading to an increase in triacylglycerol, total cholesterol, and LDL cholesterol concentrations (Parnham, 1998).

Patients who have hyperlipidemia,hypertension may also be at risk when emulsions containing saturated fats or phospholipids or MCT are administered. Potential interactions between MCT and CNS depressant drugs should also be taken into account. The use of lipid emulsion might be limited for patients who have systemic lupus erythematosus (SLE) suffering from the antiphospholipid-antibody syndrome (Parnham, 1998).

Table 7. Factors affected the tolerability of lipid emulsions. (from Parnham, 1998)

Variable	Potential effect	Comment
Lysophospholipid contaminant	Hemolysis	Only at very high non-therapeutic doses
Degree of saturation of phospholipid/fat	Increase of total and LDL cholesterol; effects on membrane proteins	May occur on continuous infusion
Content of MCT	Increase in total and LDL cholesterol; CNS effects	May occur on continuous infusion and/or at high doses
Particle size	Pulmonary vascular damage	Infants appear to be susceptible

9. Definition of total nutrient admixtures

Intravenous lipid emulsions are traditionally administered separately from nutrient and other intravenous solutions to avoid unstable admixtures (Bettner and Stennett, 1986). A variety of terms or synonyms have been used to describe lipid-containing total parenteral nutrition admixtures including 3-in-1, all-in-one, and total nutrient admixtures (TNA). The development and use of stable lipid-containing TPN admixtures have several potential benefits (Bradford, 1996; Driscoll et al., 1986; Driscoll, 1997).

TNA become a more widely used therapeutic system to treat malnourished patients receiving parenteral nutrition since the approval of admixing intravenous lipid emulsions with various amino acid and dextrose solutions (Driscoll et al., 1986). TNA provides some advantages over classical TPN as follow:

(i) Fewer containers used resulting in reduced TPN preparation and set-up time for pharmacy and nursing staff

(ii) Fewer solution changes

(iii) Fewer administration sets

(iv) Single flow rates

(v) Less manipulation of the feed line with its attending risk

(vi) Increase in compliance of home patients

(vii) Increase tolerance of intravenous lipid emulsions in neonates when the fat is infused over a period of 24 hours (the recommendation of the maximum infusion time for lipid emulsions is 12 hours)

(viii) Ability to use in certain clinical situations (i.e., multiple trauma, excess CO_2 production from glucose overload, etc)

(ix) Possibility for stress patients who require less calories (Bettner and Stennett, 1986; Brown, Quercia, and Sigma, 1986; Bullock, Fitzgerald, and Walter, 1992).

Rollins et al. (1990) studied the safety and economic of TNA administration to infants and found that there were no significant differences between the groups receiving TNA and traditional TPN. The biochemical parameters between the two groups were no different while a hospital cost of group receiving TPN was higher than the group receiving TNA. They

concluded that the TNA administration to infant is safe, efficacious, and cost effective (Rollins et al., 1990). However, TNA may have several disadvantages in that in-line filters cannot be used due to the lipid component in the admixtures. They may have considerable waste of lipid emulsion as well as TPN components if complete admixtures are prepared for unstable patients with fluctuating needs (Rubin et al., 1993).

10. Composition of TNA systems

The TNA systems should be composed of all nutrients; carbohydrate, protein, fat, vitamins, and minerals at the amount required for the patients. The nutrients can be divided into 2 groups (Bradford, 1996; Burtis, Davis, and Martin, 1988).

1. Macronutrient

1.1 Carbohydrate

Carbohydrate for parenteral nutrition should be in form of solutions of monosaccharides (i.e., glucose, dextrose, and fructose) and sugar alcohols (i.e., sorbitol, glycerol, and xylitol). Dextrose monohydrate is the most suitable for use in parenteral nutrition as it is normally found in blood stream providing energy of 3.4 calories per gram while the sugar alcohols may cause lactic acidosis. Glucose solutions available in the market are in the concentration of 5-50 %. The glucose requirement for patient should be 20 g/kg/day (Bradford, 1996).

For total parenteral nutrition, the patient would receive dextrose solution in slightly high concentration and the TPN should be administered via central vein. If the concentration of dextrose in admixture is in a range of 10-15%, the TPN would have osmolality not more than 900 mOsm/kg water which can be administered via peripheral vein with no thrombophlebitis evidence (Bradford, 1996).

1.2 Protein

Protein for parenteral nutrition should compose of essential and non-essential crystalline amino acids in the appropriate proportion for patients requirement. Protein 1 gram provides energy of 4 calories. Approximately 15 to 20% of total energy intake should come from protein (Bradford, 1996). The steriled amino acid solutions are available in the concentration of 5-15%, with osmolality of 600-1,590 mOsm/kg, and pH values between 5.0-7.4 (Allwood and Kearney, 1998). The amino acid solutions can be divided for various therapeutic purposes into 3 formula: (i) mixed amino acids, (ii) high branched chain amino acids, and (iii) essential amino acids formula. The mixed amino acid solutions may contain carbohydrate solutions in form of dextrose, sorbitol, or glycerol and some minerals (Bradford, 1996). (See Appendix B for compositions).

1.3 Fat

Fat in the form of lipid emulsion provides energy at high level of 9 cal/g. Fat emulsion containing LCT can prevent essential fatty acid (linoleic acid and linolenic acid) deficiency. The total daily dose should not exceed 4 g/kg/day (Louie and Niemiec 1986). If patients do not receive fat for

2 weeks, they will have low blood level of essential fatty acids. If this situation continues for 3-4 weeks, the patients will show clinical sign of essential fatty acids deficiency such as loss of hair, desquamative dermatitis, and thrombocytopenia (Kultida Chaijinda, 1998). The prevention of essential fatty acids deficiency can be managed by administering lipid emulsion 2-3 times per week. Lipid emulsions can also deliver the fat-soluble vitamins into the body. Generally, lipid emulsions have isoosmotic pressure to blood vessel. The osmolality is in the range of 280-350 mOsm/kg water. So they can either administer at peripheral or central vein (Bradford, 1996). Normally, the daily requirement of fat is not more than 30% of total calories.

However, the cost of lipid emulsions are normally high, it should not lonely administer them to patients as the major energy. The major energy provided to patients should come from carbohydrate and fat.

2. Micronutrients

The quantity requirement of micronutrients for patients receiving TPN is recommended by American Medical Association (Table 8). The micronutrients include vitamins and minerals.

2.1 Vitamins

Vitamins are the important substances in metabolic pathway. Vitamins can be divided into two groups: water-soluble vitamins and fat-soluble vitamins (Table 8). The water-soluble vitamins are thiamine (B_1), riboflavin (B_2), niacin, pyridoxine (B_6), folate, cyanocobalamine (B_{12}), and

vitamin C. The body can eliminate them by urinary excretion. There have no toxic effect to the body. The fat-soluble vitamins are retinal (vitamin A), cholecalciferal (vitamin D), tocopherol (vitamin E), and phytomenadione (vitamin K). The over use of fat-soluble vitamins could collect in the liver and fat tissue and cause some toxic effect (Bradford, 1996; Kultida Chaijinda, 1998).

Table 8. The amount of micronutrients for administration with total parenteral nutrition for adult patients (from Bradford, 1996)

Vitamins			
Water-soluble		**Fat-soluble**	
B_1 (thiamine)	3.0 mg	A (retinal)	3,300 IU (1,000 μg)[a]
B_2 (riboflavin)	3.6 mg	D(cholecalciferol)	200 IU (5 μg)[b]
Niacin	40 mg	E (dl-α -tocopherol)	10 IU (10 mg)[c]
Pantothenic acid	15 mg	K (phytomenadione)	0.7-2 mg
B_6 (pyridoxine)	4 mg		
B_{12} (cyanocobalamine)	5 μg		
Biotin	60 μg		
Folate	400 μg		
C (ascorbic acid)	100 mg		
Trace elements		**Electrolytes**	
Zinc (Zn)	2.5-4.0μg	Sodium (Na^+)	60-100μg
Copper (Cu)	0.5-1.5μg	Potassium (K^+)	60-100μg
Cromium (Cr)	10-15 μg	Chloride (Cl^-)	60-100 mEq
Manganese (Mn)	0.15-0.8 μg	Calcium (Ca^{2+})	10-15 mEq*
Selenium (Se)	40-80μg	Magnesium (Mg^{2+})	8-20 mEq*
Molybdenum (Mo)	20-120 μg	Phosphorus ($H_2PO_4^-$)	20-45mmole*

[a] 700 μg retinol equivalents (RE) = 2,300 international units (IU)
[b] 10 μg cholecalciferol = 400 IU
[c] 7 mg dl-α -tocopherol = 7 IU
* = macroelements

2.2 Minerals

Minerals are macroelements and microelements which are important in metabolic pathway. Macroelements were required for the

body in at least 100 milligrams per day. They include sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), and phosphorus (P). Microelements (trace elements) were required for the body in a few milligrams per day. The trace elements are iron (Fe), zinc (Zn), copper (Cu), iodine (I), fluoride (F), manganese (Mn), chromium (Cr), molybdenum (Mo), and selenium (Se) (Bradford, 1996). (See Appendix B for commercial products).

3. Fluid needs

Maximum volumes of TPN rarely exceed 3.5 to 4.0 liters which typical prescriptions of 2 to 3 L/day. Over hydration is especially detrimental to the heart and kidneys (Bradford, 1996).

11. Preparation and storage of TNA systems

The method of preparation is important for stability of TNA. The compounding usually composes of 5-10% amino acids solution, 10-70% dextrose solution, and 10-20% lipid emulsion. The volume ratio of mixing is recommended to be 1:1:0.5 (or 1:1:1) of amino acid solution: dextrose solution : lipid emulsion, respectively (Trissel, 1998).

Brown et al. (1986) had different recommendation guidelines for the admixture. Amino acid solution (concentration of 8.5% or 10%), dextrose solution (concentration of 10% to 70%), and lipid emulsion (concentration of 10% or 20%) could be mixed in the volume ratios of: 2:1:1, 1:1:1 and 2:1:0.5 amino acid solution: dextrose solution: lipid emulsion. The final volume of TNA should be not less than 1.5 liter and final concentration of dextrose should be

between 10-23%. The order of mixing was suggested by firstly mixing electrolytes, vitamins, and trace elements to dextrose solution then phosphate solution was added to the amino acid solution (Figure 5). Finally, both of dextrose and amino acid solutions were combined with lipid emulsion at the same time. In the last step, the process should be done as quickly as possible. However, vitamins, electrolytes, and trace elements may be added during or after administrating amino acids and dextrose solutions (Brown et al., 1986). Calcium is recommended to add in TNA lastly in order to avoid precipitation with phosphate. They recommended that the exposure time including hanging time should be limited to 12 hours at room temperature. The prepared TNA should be stored under refrigeration and must be administered within 24 hours of preparation (Brown et al., 1986). However, they found TNA systems could be stored at least 7 days in the refrigerator without any instability.

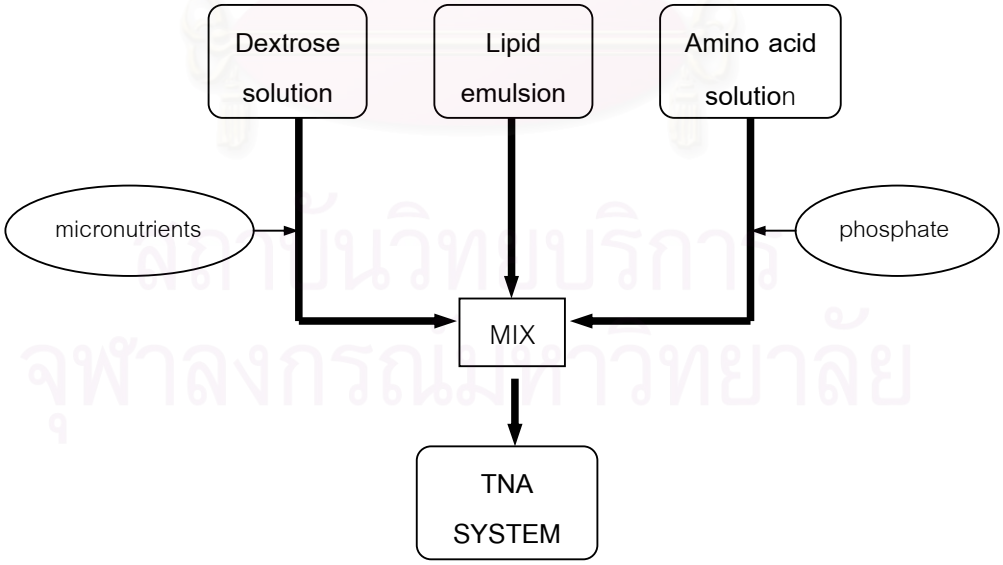


Figure 5. The schematic of TNA preparation (from Brown et al., 1986)

Some manufacturers (Travenol Laboratories) recommended the order of mixing in that the amino acid solution should be added to lipid emulsion and the dextrose solution is added lastly. Alternatively, amino acid and dextrose might be firstly mixed and then added to the lipid emulsion. They recommended that the final admixture could be refrigerated up to 48 hours then kept at room temperature for no more than 24 hours. Vitamins and other additives should be added immediately prior to administration (Sayeed et al., 1987).

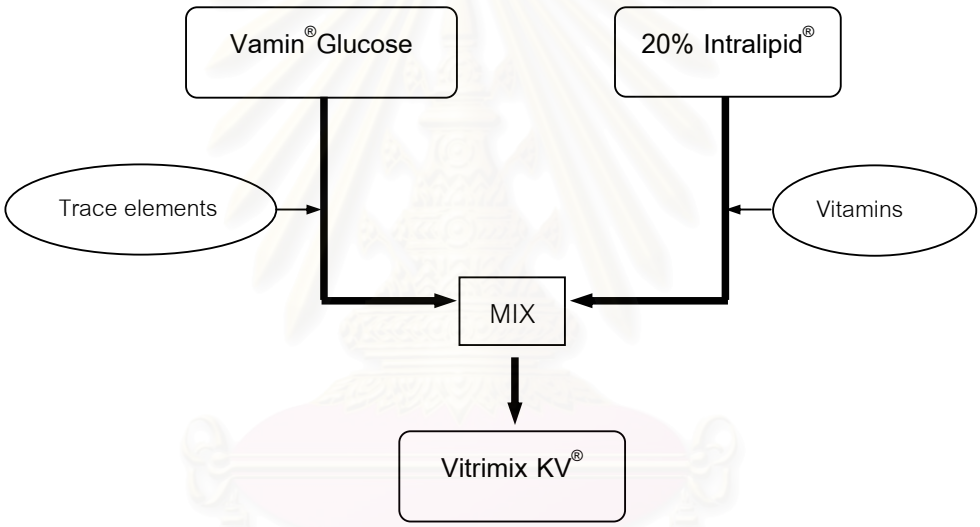


Figure 6. The schematic of Vitrimix KV® preparation (from Pharmacia & Upjohn Company)

Pharmacia & Upjohn company has recommendation guidelines for TNA system, Vitrimix KV®, composed of Vamin®Glucose (composed of 7% amino acid and 10% dextrose) and 20% Intralipid® in volume ratio of 3:1 Vamin®Glucose to IntralipidThe system was prepared by mixing lipid emulsion into Vamin®Glucose using transfer set. Additional, vitamins, electrolytes and trace elements might be added into the system (Figure 6).

12. Factors influence the stability of TNA

The TNA can undergo any instability influenced by other nutrient solutions (Mierzwa, 1994). The stability of TNA system is normally related to the stability of lipid emulsion.

1. Dextrose

Dextrose solution is acidic (pH 3.5 to 5.5) and can significantly decreases the pH of lipid emulsions and consequently the stability of lipid emulsion. The zeta potential that imparts high stability to lipid emulsion (i.e., -35 mV or greater) occurs in the pH range of 5 to 10. Some workers found that the addition of 25% dextrose solution to an equal volume of 10% Intralipid caused a decrease in the pH of the lipid from 7.0 to 3.45, and the particle size distribution of lipid emulsion significant changed after 48 hr at room temperature (Black and Popovich, 1981). Seventy-two hr after admixture a cream layer was formed at the top of emulsion and the electrostatic repulsive forces which contributed to lipid stability were reduced (Brown et al., 1986).

2. Amino acids

Amino acid solutions are generally considered safe to add to lipid emulsions. Amino acids, when mixed with lipids, appear to exert a protective effect against other additives. There are several mechanisms postulated for this protective effect (Allwood and Kearney, 1998; Brown et al., 1986).

a). Amino acids are thought to adsorb at the oil-water interface resulting in enhancing the mechanical barrier and reducing the opportunity for particles to aggregate and coalesce.

b). Amino acids have a buffering capacity which decreases the deleterious effects of low pH dextrose solutions. The higher the amino acid concentration, the greater the buffering capacity.

c). Enhancement of the mechanical barrier of emulsions droplets by a pH dependent ionic interaction between specific amino acids and the lipid emulsion.

3. Electrolytes, trace elements and vitamins

In lipid emulsion emulsified with anionic phospholipid, there is a collection of anions at the surface of the oil droplet, and then a much more diffuse layer in which there are varying concentrations of anions and cations. When electrolyte is introduced into the system, the system then has additional charged entities, and therefore the original condition changes. Hence, the total stability of the system may change especially for systems using ionic emulsifiers (Barnett, 1989; Brown et al., 1986)

The region of instability depends on the ion interactions and the valency of ions (monovalent, divalent, or trivalent ions). As the valency of the ions is increased, problems rapidly increase. The ionic strength can be simply calculated, and if it is increased, the instability of the system is increased. The electrolyte concentration also affects the zeta potential of the droplet. The

effects on zeta potential caused by changing the pH is considered. When additives are added into the TNA systems, one can produce changes of pH sometimes quite inadvertently (Barnett, 1989).

Addition of trace elements have been shown not to contribute significant degradation of TNA systems Vitamins have been shown to be stable in standard TPN and do not appear to contribute significantly for any degradation of TNA systems (Allwood and Kearney, 1998; Brown et al., 1986). Knutsen et al. (1984) reported that the multivitamins had no effect on the stability of TNA system. Visual examination of sample stored at 4^{o}C for one week showed that the emulsion was uniform with no flocculence.

CHAPTER III

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial sources and used as received. Distilled water was used throughout the experiments.

1. Lipids

1.1 Medium chain triglycerides (MCT® oil) (Mead Johnson & Company, USA, Lot No. MJH91)

1.2 Soybean oil (Sigma Chemical, St Louise, USA, Lot No. 98H0172)

2. Emulsifiers

2.1 Poloxamer 188 (Teric® PE68) (The East Asiatic Public, Co., Ltd., Thailand)

2.2 Polyethylene glycol 660 12-hydroxystearate (Solutol® HS-15) (BASF, Germany, Lot No. 671764)

2.3 Polyoxyl 35 castor oil (Cremophor® EL) (BASF, Germany, Lot No. 272030)

2.4 Soy lecithin (Lucas Mayer GMBH, Germany, Lot No. 199759)

2.5 Tween 80 (Polyoxethylene (20) sorbitan mono-oleate) (BDH Laboratory Supplies, England, Lot No. ZA2088516649)

3. Additives

3.1 Glycerin (BDH Laboratory Supplies, England, Lot No. K23624360708)

3.2 Sodium hydroxide (Mallinckrodt, Mexico, Lot No. B348098)

4. Commercial parenteral nutritions

4.1 Addamel®N (Pharmacia & Upjohn, Sweden, Batch No. 22085-52)

4.2 Intralipid®10% (Pharmacia & Upjohn, Sweden, Batch No. 2193951)

4.3 Intralipid®20% (Pharmacia & Upjohn, Sweden, Batch No.20161A51)

4.4 Lipofundin®MCT/LCT10% (B.Braun, Germany, Batch No. 8093A81)

4.5 Lipofundin®MCT/LCT20% (B.Braun, Germany, Batch No. 9202A81)

4.6 Lipofundin-S®10% (B.Braun, Germany, Batch No. 9075A81)

4.7 OMVI® injection (Otsuka Pharmaceutical Co., Ltd., Japan, Lot No. 6L74MV1)

4.8 Vamin®Glucose (Pharmacia & Upjohn, Sweden, Batch No. 9655701)

Equipments

1. Analytical balance (Model 264, Oertling, England)
2. Autoclave (Model No. 1941x, Ashcroft, USA)
3. Cryoscopic osmometer (Model Osmomat® O30-D, Gonotec, Germany)
4. Hot air oven (Model 1811530000202, WTB binder, Germany)
5. High pressure homogenizer (Model Emulsiflex® C5, Avestin, Canada)
6. High speed homogenizer (Model D-7801, Ystral, Germany)
7. Laminar flow (Model VS-124, Holten Laminar-Air, Denmark)
8. Magnetic stirrer (Model MR 2002, Heidolph, Germany)
9. Particle size analyzer (Mastersizer® S long bed Ver. 2.11, Malvern Instruments Ltd., Malvern, UK)
10. pH meter (Model Φ 50 pH, Beckman, USA)
11. Scanning electron microscope (Model JSM-5410LV, JOEL®, Japan)
12. Zeta meter® 3.0+ (Model ZM 3UG, Zeta meter Inc., USA)
13. Vial type I glass (USP, APA Industries Co., Ltd., Lot No. 32430224)
14. Viscometer (Model Rotovisco® RV20, Haake, Germany)

Methods

1. Formulation of lipid emulsions

1.1 Preparation of lipid emulsions containing lecithin as an emulsifier.

In the experiment, the study was designed to investigate various parameters such as oils, emulsifiers, and the process of manufacturing. The conditions used in emulsion preparation were the influence of homogenization time by high speed homogenizer, level of pressure and cycles of high pressure homogenization using Emulsiflex® C5. It was noted that emulsion in the present study was prepared in % w/w. The volume of 1 g emulsion was approximately 1 ml calculated from the densities of the chemicals used which were shown in appendix A. To study the effect of oils, the oils used were either MCT oil or soybean oil at initial concentration of 5%. The ingredients used in formulation were listed in Table 9.

Table 9. The composition of lipid emulsion using soy lecithin

Chemicals		Concentration (%)
Oil	MCT or soybean	5
Emulsifier	soy lecithin	1 or 2 or 3
Tonicity adjustor	glycerol	2.5

Lipid emulsions were prepared using 5% oil, various concentrations (1-3%) of lecithin, 2.5% glycerol and distilled water was used to adjust to the final weight normally 80 g). The lecithin was dispersed in the water phase which were glycerol and distilled water and the water phase was

heated to 55°C to prevent the degradation of lecithin. The oil phase which was preheated to 50°C was then added to the water phase. The high speed homogenizer was used to form coarse emulsions at the speed of 4,000 rpm for different periods of time, 5 or 10 minutes. The coarse emulsions were then homogenized to gain fine emulsions using Emulsiflex® C5 operating at different pressure, 15,000 or 20,000 psi, for different homogenization cycles, 5 or 10 cycles. The pH of the resulting emulsions was adjusted to 8.0 using 0.1 N sodium hydroxide solutions. Then the emulsion was filled into 20 ml vials, purged with nitrogen gas for a few seconds before sealing with rubber caps and aluminum ring. Lipid emulsion was then sterilized using steam autoclave at 121°C, 15 psi for 15 minutes.

1.2 Preparation of lipid emulsions containing non-ionic surfactants.

The non-ionic surfactant was used in emulsion preparation instead of lecithin. The non-ionic surfactants used in the present study were normally formulated in parenteral formulations which were Cremophor EL, poloxamer 188, Solutol HS 15, or Tween 80 and used at a concentration range of 1% to 3%.

These lipid emulsions were prepared using 5% MCT oil or soybean oil, various concentrations (1-3%) of non-ionic surfactant, and 2.5% glycerin. Distilled water was used to adjust the final weight. The emulsifier was dissolved in the water phase and the solution was then heated to 75°C. The oil phase which was preheated to 70°C and then added to the water phase. The high speed homogenizer was used to prepare coarse emulsions at the speed of 4,000 rpm for different period of times, 5 or 10 minutes. The coarse emulsions were then homogenized to gain fine emulsions using

Emulsiflex®C5 operating at different pressure, 15,000 or 20,000 psi, for different homogenization cycles, 5 or 10 cycles. The pH of the resulting emulsions was then adjusted to 8.0 using 0.1 N sodium hydroxide solutions. Then the emulsion was filled into 20 ml vials, purged with nitrogen gas for a few minutes before sealing with rubber caps and aluminum ring. Lipid emulsion was then steriled using steam autoclave at 121°C, 15 psi for 15 minutes.

1.3 Preparation of lipid emulsions containing soy lecithin and co-emulsifiers.

Soy lecithin was used as primary emulsifier and non-ionic surfactant was used as co-emulsifiers (Cremophor EL, poloxamer 188, Solutol HS 15, or Tween 80). The formulation consisted of oil 5%, glycerol 2.5%, and the various weight ratios of lecithin to co-emulsifier (Table 10).

Table 10. Composition of emulsifier and co-emulsifier in lipid emulsion

Emulsifier	Total emulsifier concentration (%)	
	2	3
Soy lecithin	1	2
Co-emulsifier	1	1
Ratio of soy lecithin to co-emulsifier	1 : 1	2 : 1

Lipid emulsion was prepared by heating the water phase consisting of soy lecithin, nonionic surfactant, and glycerol to 55°C. The oil phase heated to 50°C was then added to the water phase. The coarse emulsion was formed by using high speed homogenizer at the speed of 4,000 rpm for 5 minutes. The coarse emulsions were then homogenized to gain fine

emulsions using Emulsiflex®C5 operating at 10,000 psi for 5 cycles. The pH of the resulting emulsions was adjusted to 8.0, purged with nitrogen gas and then sterile using steam autoclave at 121°C, 15 psi for 15 minutes. Nonionic surfactant which provided the best emulsion was chosen to be optimized for the concentration used in the formulation. Emulsion was prepared using constant lecithin concentration of 2 % with varying amounts (0.5, 1, 1.5 and 2%) of co-emulsifier

1.4 Optimization of total emulsifier concentration

The best co-emulsifier concentration obtained was then optimized for total emulsifier concentration by preparing emulsions with varying amounts of total emulsifier (1, 2, 3, 4 and 5%).

2. Stability testing

After being sterilized, the lipid emulsions were cooled to room temperature and visually observed for any instability (i.e., coalescence,oil separation). If there were no sign of instability, the stable formulations were then further investigated for long term stability after storage at room temperature for at least 4 weeks. Moreover, the samples were visually observed under the accelerated conditions (heating and cooling cycle), by storing the samples at 4°C for 48 hr and at 45°C for 48 hr for 6 cycles.

For the lipid emulsions prepared in 1.1 and 1.2 was investigated for physical stability by visual observation to select the appropriate conditions of manufacturing.

The preparations prepared in 1.3 were determined for the physical stability and the appropriate lipid emulsion was chosen for further studies.

3.Determination of physicochemical properties of lipid emulsions.

The formulations which remained stable after sterilization were investigated for physicochemical properties. The samples were freshly prepared and studied by the following procedures. The formulation found to be the most stable and had the suitable properties was used as a candidate for preparation of the total nutrient admixtures (described later).

3.1 Determination of particle size

Laser particle sizer (Mastersizer®) (with 300RF mm range lens, 2.40 mm beam length) was used to determine the particle size of lipid emulsion. The sample was automatically diluted with purified water. Particle size was analyzed by the curve plotted between particle diameter versus percentage volume of particles. The diameter of particles was reported at 10%, 50%, and 90% volume percentile, d(v,0.1), d(v,0.5), and d(v,0.9), respectively. The d(v,0.5) was used as the average particle size. The data obtained was the average of three determinations.

3.2 Determination of zeta potential

The zeta potential of the preparations was determined by microelectrophoresis using the Zeta meter® system 3.0+ with a fused quartz and teflon electrophoresis cell equipped with a cylindrical molybdenum anode

and platinum rod cathode. A 200-μl sample was diluted in 50-ml water. Before beginning to track the particles, the specific conductance of each sample was measured for selecting an appropriate voltage, which did not cause thermal overtone. About 60 particles were tracked for each sample. The zeta potential was automatically calculated by the Zeta meter® system 3.0+. These results were all normalized with respect to zeta potential of –49±2 millivolts for colloidal silica (Minusil®) standard solution. The measurements were made at room temperature.

3.3 Measurement of Osmolality

The osmolality of preparation was determined before and after sterilization by using freezing point depression methods (Osmomat O30-D). A 50 μl sample was filled in osmolality probe and sample was freezed and became crystal ice. Osmomat O30-D was calibrated to zero using distilled water.

3.4 Measurement of pH

The pH of preparations before and after sterilization was measured at room temperature using a pH meter. The equipment was calibrated at pH 4, 7, and 10 using Fisher standard buffer.

3.5 Scanning electron microscopy

The appearance of lipid particle of selected lipid emulsion was studied by a scanning electron microscope. The specimen was obtained by

cryo-fixation technique. Dispersion of lipid emulsion was cryofixed under standard conditions, using Balzer-type specimen support plates immersed in liquid nitrogen (-140^{o}C). The sample was immediately observed by the scanning electron microscope under the temperature of -140^{o}C by circulating liquid nitrogen through a jacket surrounding the instrument.

3.6 Measurement of viscosity

Haake viscometer® was used to determine the viscosity of lipid emulsions at room temperature. The sample was loaded and the shear rate was increased from 0 s^{-1} to 1000 s^{-1} in one minute. The shear rate was maintained at 1000 s^{-1} for two minutes. The last step was one minute to decrease the shear rate back to 0 s^{-1}. The complete cycle took four minutes to shear a sample. The viscosity at shear rate of 1000 s^{-1} was used to compare the viscosity of lipid emulsions.

4. Preparation of total nutrient admixtures

To study the preparation and physicochemical properties of total nutrient admixtures. A mixture of amino acids and dextrose solutions was the commercial product called Vamin®Glucose (see appendix B for composition). The lipid emulsion used was the best formulation chosen from the physical stability and physicochemical properties determined previously. The commercially available lipid emulsions were also studied.

The total nutrient admixtures were prepared aseptically in laminar air flow unit. Vamin®Glucose and lipid emulsion were mixed at the different volume ratio, 2:1, 3:1, and 4:1 (Table 11.).

The commercial lipid emulsions were 10% and 20% Intralipid®, 10% and 20% Lipofundin®MCT/LCT, and 10% Lipofundin-S®. The 30 ml TNA made of Vamin®Glucose and freshly prepared lipid emulsion or 20% Intralipid in volume ratio 3:1 were mixed with 0.12 ml vitamins (OMVI® injection) and 0.30 ml trace elements (Addamel®N). (See appendix B for composition of OMVI and Addamel-N).

It was noted that the addition of trace elements was initially added into the Vamin®Glucose while vitamins was added into lipid emulsion according to the recommendation of manufacturer of commercial TNA (Vitrimix KV®). Finally, lipid emulsion was added into Vamin®Glucose solution. The mixtures were swirled to obtained the homogeneous solution.

Table 11. Compositions of total nutrient admixtures

Volume ratio of Vamin®Glucose to lipid emulsion	Vamin®Glucose (ml)	Lipid emulsion (ml)	Total volume of TNA (ml)
2 : 1	20.00	10.00	30.00
3 : 1	22.22	7.78	30.00
4 : 1	24.00	6.00	30.00

For the TNA preparation, the sterile condition was ensured by agar plate sampling in laminar flow hood at 3 positions: the inner most, the middle, and

the edge of laminar flow hood. The agar plates were made of soybean-casein digest medium. The TNA prepared was also tested for the microbial contamination by culturing in the medium in order to ensure the aseptic technique was acheived.

5. Physical stability and physicochemical properties studies.

Physical stability and physicochemical properties; namely, the particle size, pH, osmolality and zeta potential, of the TNA were investigated within 24 hr after preparation using the same methods as previously described.

6. Analysis of the results

The physicochemical properties; particle size, pH, osmolality and zeta potential were reported as the mean value from 3 determination. The values of particle size and zeta potential of emulsion were compared statistically by paired-T test at 95% confidential interval ($p \leq 0.05$). The differences between the emulsion before and after mixing with other nutrients at various volume ratios were tested by Analysis of Variance ANOVA at $p \leq 0.05$ The computer program used for statistic calculation was SPSS for windows version 9.0.

CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of lipid emulsions without co-emulsifier

Since the lipid emulsion is used for parenteral administration, the small particle size of lipid emulsion (normally < 1 μm) is required in order to prevent the occurrence of fat embolism. This makes some difficulties for the production of parenteral lipid emulsion. The optimized conditions used in the preparation reported were more highly dependent upon the type of equipments and the materials used (Hansrani et al., 1983; Washington, 1988). In screening experiments, the aim of which was to investigate the possible influential and interacting factors. The experimental factors that would like to study include homogenizing time, the pressure, recycle times for homogenization and the emulsifiers used. These factors were investigated in the present study. The lipid emulsions were made using either soy lecithin or synthetic nonionic surfactants as a sole emulsifier.

1.1 Lipid emulsions stabilized by soy lecithin

Soy lecithin was primarily used as the emulsifier of choice for parenteral nutrition. Soybean oil and soy lecithin at the concentrations of 5% and 1%, respectively, were used to prepare emulsions. Glycerol at the concentration of 2.5% was used for isotonic adjustment. The effect of various conditions used for the preparation of emulsions studied included homogenizing time, the pressure, and recycle times for homogenization. The

water phase contained soy lecithin and glycerol were heated at the temperature below 55°C to ensure no any degradation of the phospholipid (Jumaa and Müller, 1998a). Bock et al. (1998) reported that the high temperature promoted the hydrolysis of lecithin to lysolecithin.

In this study the homogenizing time was 5 min and 10 min to obtain coarse emulsion (Table 12). The coarse emulsion was passed through the high pressure homogenizer the pressure of 15,000 psi and 20,000 psi, the cycles of 5 and 10 cycles were chosen.

Table 12. The conditions used in lipid emulsion preparation.

Homogenizing time (min)	5				10			
Pressure (psi)	15,000		20,000		15,000		20,000	
Recycle times (cycle)	5	10	5	10	5	10	5	10

For preparing emulsion containing 5% soybean oil and 1% soy lecithin, the experiment was performed initially using the homogenizing time for 5 min, pressure at 15,000 psi, and 5 recycle times. The result showed that lipid emulsions were stable before autoclaving. After autoclaved at 121°C for 15 min, the emulsion exhibited oil separation. Even increased the cycles from 5 to 10 cycles, the same result was obtained. The effect of pressure was studied by increasing the pressure from 15,000 psi to 20,000 psi using the homogenizing cycle of either 5 or 10 cycles and the same result was obtained. Even increasing the homogenizing time from 5 to 10 min with varying the pressure and recycle times of homogenizer the results were in the same trend in that none of the factors had any effects on the emulsion formation. Using

the MCT oil instead of soybean oil and the same condition of praparation, the results were similar.

The amount of emulsifiers used (1%) might be insufficient to emulsify oil droplets. So further step was to increase the lecithin concentrations. Upon increasing the concentration of soy lecithin from 1 to 2 and 3%, the results showed that emulsions were stable only before autoclaving regardless of the homogenization time (5 or 10 min), pressure (15,000 or 20,000 psi) and homogenization cycle (5 or 10 cycles) used. After autoclaved, the emulsion exhibited coalescence. The reason might be the high temperature produced high kinetic energy and could affect the emulsifier film. The oil droplets would coalesce and increased in droplet size which could be markedly observed (Chansiri et al., 1999). Moreover, the preparation containing 2% soy lecithin was also prepared by using homogenization time of 10 min and the pressure of 5,000 psi or 10,000 psi for 5 cycles, the results showed that the emulsions were stable only before autoclaving but became unstable after autoclaving.

Lecithin used to stabilize emulsion was reported to hydrolyse and produced free fatty acid after autoclaving, thereby lowering the pH of the whole system. The hydrolysis of lecithins has been associated with an increase in droplet zeta potential which might increase the repulsive forces of oil droplet. However, the change might not be significant if lecithin contained nonionic fraction, PC, as main components (Chansiri et al., 1999; Herman and Groves, 1992; Washington and Davis, 1987).

The effect of the condition used in the emulsion praparation has been reported in some studies. Jumaa and Müller (1998b) prepared emulsion using Ultra Turrax as homogenizer and homogenizing time at 3 min. They found that the emulsion could be produced with good stability. In the study of Chanana and Sheth (1993), they varied the time of homogenization of pre-emulsion and found that no further reduction of particle size was obtained after mixing for 5 min by propeller mixer. In the study of Krishna et al. (1998), they varied the homogenizing time at 3, 6, and 9 min and they found that increasing the mixing time beyond 3 min did not result in a further decrease in particle size. In the study of Washington and Davis (1988), they optimized the pressure and recycle times for the emulsion preparation using Microfluidizer. Emulsions were prepared using 5%, 10%, and 20% soybean oil with 1.2% egg lecithin. They found that when using the pressure greater than 8,000 psi a droplet diameter corresponding to the commercial products (10% and 20% Intralipid®) was obtained. Moreover, the smallest droplet diameter of emulsions was obtained when passed 4 homogenizing cycles at the same pressure. In the study of Bock et al. (1998), they investigated the effect of high pressure homogenization parameters. Emulsions containing 10% (or 20%) soybean oil and soy lecithin 0.75% (or 1.5%), respectively. They varied the pressure used from 4,800 to 13,200 psi and recycle times from 1 to 10 cycles. They examined the average particle size (D_{50}) and found that the higher the pressure used, the lower the D_{50} values and the more recycle times, the more narrow particle size distribution was obtained (Bock et al., 1998). The lowest values were seen following ten cycles at 13,200 psi. In Chansiri et al. (1999), they formulated 20% emulsion containing Miglyol 812 (MCT) and olive oil at the weight ratio of 1:1 using 1.2% egg lecithin as emulsifier. Pre-emulsion was passed through Microfluidizer at the pressure of 20,000 psi at 5

cycles. The average particle size was obtained in between 0.28-0.40 μm. Unfortunately, in the present study, the autoclaved emulsion made of soy lecithin were unstable even using the high pressure and recycles time for homogenization. The different homogenizer used might have effect on the stability of emulsion; however, the only type of high pressure homogenizer available was used throughout the study.

The purity of emulsifier might be an important factor. Lecithin normally contained various types of phospholipid and the phosphatidylcholine (PC) was the major ingredient which was responsible for emulsifying properties. The lecithin which had high amounts of PC tended to be a better emulsifier. Soy lecithin composed of 20% PC (L-α-Phosphatidylcholine®) was used to prepare lipid emulsion system in order to compare the influence of purity of lecithin in emulsifying properties. The result was found that, the formulation was affected by the purity of lecithin in that the emulsion prepared using soy lecithin containing 20% PC underwent flocculation and coalescence more easily than that containing 93% PC. However, in some studies the mixtures of phospholipids were found to be more effective for emulsifying than lecithin containing only PC because lecithin was a complex mixtures of uncharged and negatively charged phosphatides. When it adhered on the droplets, their surface layer tended to be negatively charged. Electrostatic repulsion would occur and prevented the particles from approaching closely enough to overcome the effective van der Waals for the attractive forces, thus stabilizing the oil droplet against interparticular attachments or coalescence (Hansrani et al., 1983). Pure phosphatidylcholine was known to tend to form bilayer structure in aqueous solutions and was therefore classified as an ineffective oil-in-water emulsifier (Kan et al., 1999).

1.2 Lipid emulsions stabilized by synthetic nonionic surfactants

The 5% oil and 1% nonionic surfactant (Cremophor EL, poloxamer 188, Solutol HS15, or Tween 80) were used to prepare lipid emulsions using the same conditions as lipid emulsions stabilized by soy lecithin. From the results, stable emulsion were unable to be prepared both before and after autoclaving. It should be estimated that the low emulsifier concentration were insufficient for emulsion formation. These results were similar to Jumaa and Müller (1998b) studies. They found that the poor stability of the emulsions when amount of nonionic surfactant was lower than 2% could be attribute to an insufficient amount of emulsifier to properly coat the oil droplets.

The preparation containing 5% soybean oil emulsified by 2% (or 3%) poloxamer 188 and the condition used were homogenizing time 10 min, pressure 20,000 psi for 5 cycles and the preparation containing 5% soybean oil emulsified by 3% poloxamer 188 and homogenizing time for 5 min (or 10 min), pressure 20,000 psi for 5 (or 10) cycles could form stable emulsion before autoclaving but became separated after autoclaving. The emulsion was stable before autoclaving because the proportion of large droplets decreased as the homogenization pressure and recycle times increased (Bock et al., 1998; Washington and Davis, 1988). The increase in temperature during autoclaving raised the kinetic energy of droplets leading to breakdown of emulsion film and droplet coalescence (Rieger, 1986). For other emulsions containing emulsifiers at higher amount (2% or 3%), the results were similar to formulation with 1% surfactant. Swarbrick and Boylan (1992) stated that, the important repulsion in nonionic surfactants are steric and hydration forces.

The hydrated polyoxyethylene chains extend into the continuous phase to provide steric stabilization and the hydrophobic propylene oxide portion is anchored onto the droplet surface to form a strong protecting layer against coalescence. When the emulsion received high temperature from autoclaving, the chains of nonionic polyether surfactant were dehydrated so the oil droplets approached together and became coalescent. Müller, Mäder, and Gohla (2000) described that the autoclaving temperature might affect the polymer adsorption layer to partially collapsed leading to an insufficient stabilization of particle aggregation. Moreover, the preparation containing 3% nonionic surfactant (Cremophor EL, poloxamer 188, Solutol HS15 or Tween 80) was also prepared by using pressure of 5,000 and 10,000 psi for 10 cycles at the homogenization time of 10 min. The results found that they could not form stable emulsion before autoclaving.

The effect of acyl chain length of the triglyceride oils has been investigated. When MCT oil was used instead of soybean oil, it was found that stable emulsion could not be prepared both before and after autoclaving with all surfactants and conditions used. The results obtained were similar to Jumaa and Müller (1998b) studied. The results indicated that the main factors that might affect the formation of stable emulsion were emulsifiers and oil used.

Jumaa and Müller (1998b) investigated the effect of autoclaving on the stability of emulsion with different oil phases and different nonionic surfactants. It was found that a combination of oil phase consisting of castor oil and soybean oil (or MCT oil) at the ratio of 1:1 emulsified with poloxamer 188 yield fine particle size. These emulsions did not show a significant

change in their droplet size upon autoclaving and showed good stability. In contrast, emulsions prepared using other nonionic surfactants such as Cremophor EL, Solutol HS15, or Tween 80 showed an increase in droplet size upon autoclaving. Poloxamer 188 was also more resistant to undergo dehydration at high temperature during autoclaving which resulted in more stable film and prevented the coalescence of the oil droplets upon autoclaving. While Solutol H15, Cremophor EL, and Tween 80 showed a greatest change in dehydration at the sterilization temperature and this lead to breakdown of the film around the oil droplets. The partial coalescence resulted in a sudden increase in the particle size. Soybean or MCT oil emulsified with only poloxamer 188 undergo changes in particle size upon autoclaving. These results could be explained on the basis of the interfacial tension properties of castor oil and poloxamer 188. The castor oil itself could remarkably decrease the interfacial tension and could prevent coalescence of the oil particles (Jumaa and Müller, 1998b).

In this study, some emulsion was prepared using the short chain triglycerides as tributyrin and isopropyl myristate instead of MCT and LCT oils. The results were similar to the findings of Jumaa and Müller (1998b) in that neither of them could form stable emulsion. They also found that tributyrate emulsified with poloxamer 188 shown phase separation at once after autoclaving. Floyd and Jain (1996) supported that short chain triglycerides have been investigated with little success.

2. Preparation of lipid emulsions stabilized by soy lecithin and co-emulsifier

From the previous experiments, lipid emulsions were not stable when soy lecithin or nonionic surfactants were used as a sole emulsifier. The homogenizing time, homogenizing pressure, and number of cycles passed through high pressure homogenizer showed an effect on emulsification in systems containing P188 as emulsifiers. However, the type of emulsifier and oil used were important. The sterilization decreased the stability of emulsions formed before autoclaving and none of the systems could be stable after autoclaving. Preparation of lipid emulsion containing lecithin-surfactant mixture was studied as stability of the system might be improved as they can form close pack mixed film and provide electrostatic and steric repulsion. Soy lecithin was used as a major or primary emulsifier and non-ionic surfactants were used as co-emulsifiers. The concentrations of lecithin were chosen at 1 and 2%. The concentration of co-emulsifier used was 1%. The oil phase used were MCT and soybean oil at various concentrations 5, 10, and 20%. The composition of the system is shown in Figure 7. In the previous experiments, the preparation containing 5% soybean oil emulsified by 2% soy lecithin could form stable emulsion before autoclaving in various pressures (5,000, 10,000, 15,000 and 20,000 psi), homogenization time 10 min and number of cycle as 10 cycles. In present experiment, it should be considered that the combination of emulsifier might be easily form stable emulsion than single emulsifier, hence the conditions were selected, lipid emulsion containing soy lecithin and co-emulsifier were prepared and the conditions used were homogenizing time for 5 min, homogenizing pressure at 10,000 psi, and number of recycle for 5 cycles. However, the optimum condition was

suggested to have low energy but produce the suitable stable products (Krishna et al., 1998).

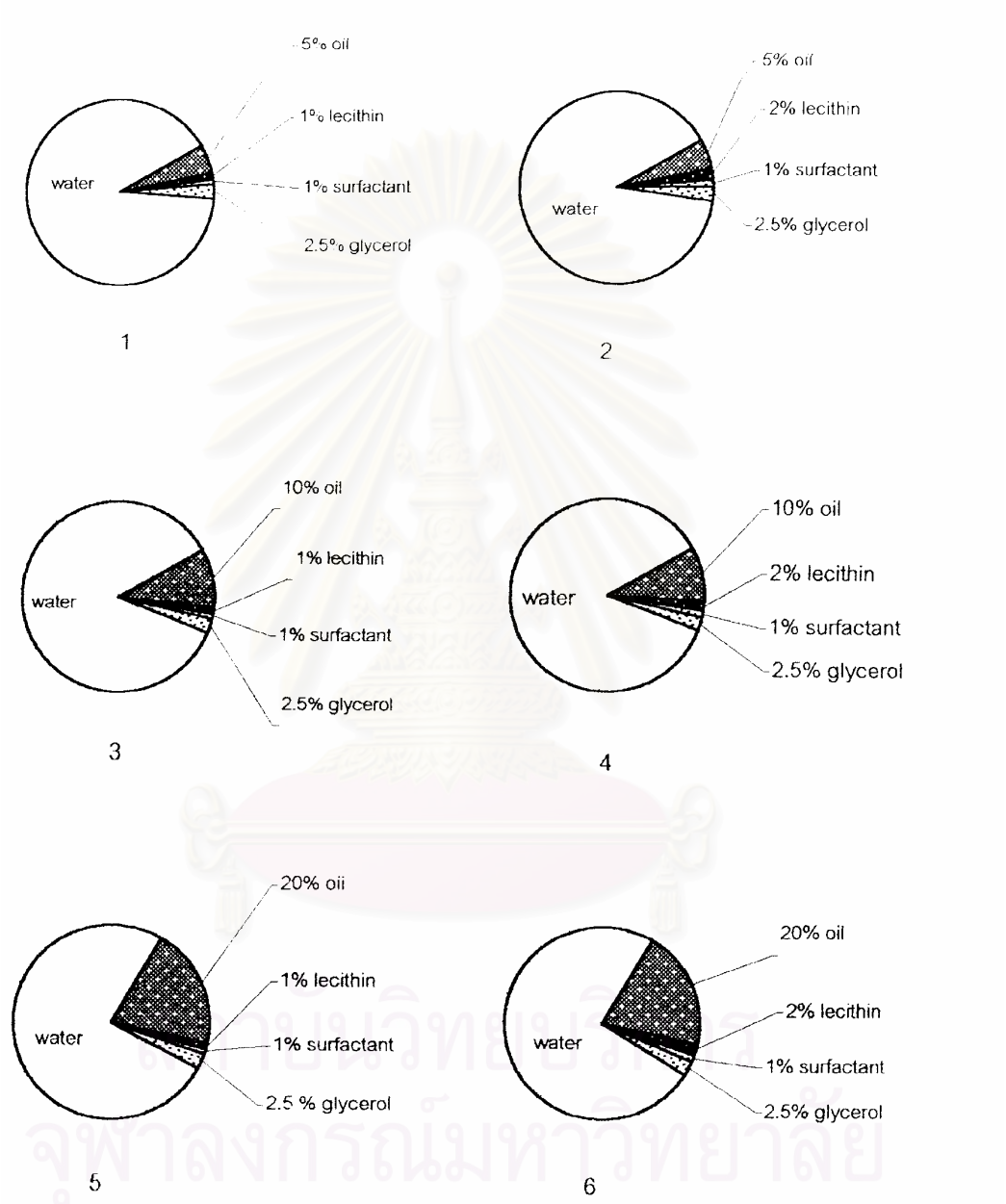


Figure 7. The composition of the lipid emulsion system stabilized by soy lecithin and co-emulsifier:

From the result, the use of secondary emulsifier seemed to improve the emulsifier efficiency in that some emulsion systems were stable both before and after autoclaving (Table 13). The systems were emulsions containing 5% soybean oil emulsified with 1% soy lecithin and either 1% Tween 80 or 1% poloxamer 188. Others were emulsions containing 5% soybean oil emulsified by 2% lecithin and either 1% Tween 80 or 1% poloxamer 188. Increasing soybean oil concentration to 10 % emulsion stabilized with soy lecithin 1% and Tween80 1% and emulsion emulsified with 2% soy lecithin and either 1% Tween80 or 1% poloxamer188 as co-emulsifier were stable both before and after autoclaving. Moreover. Emulsion containing 20% w/w soybean oil emulsified with 2% soy lecithin and 1% Tween 80 was stable before and after autoclaving. It could imply that in some cases, the primary emulsifier did not provide enough stable formulation as the weak, thin film around the droplet was easily broken after autoclaving. The secondary emulsifier was needed to enhance the physical stability of the emulsion (Klang and Benita, 1998; Swarbrick and Boylan, 1992). The system contained a mixture of emulsifiers could form stronger interfacial film and were superior to those formed using a single emulsifier (Lund, 1994). Jumaa and Müller (1998b) suggested that nonionic surfactants, i.e., Cremophor EL, poloxamer 188, Solutol HS15, and Tween 80, were usually combined with phospholipids to improve stability of the surfactant layer. A close-packed mixed film was obtained by combination of emulsifiers which confered steric stability to the dispersed droplets. A nonionic surfactant polymer, poloxamer 188, has been proposed to have more steric effect resulting from the structure of polymer; however, this experiment showed that poloxamer 188 was a weak co-emulsifier. It could help lecithin to form stable emulsion. The results found were the same trend as Lundberg's (1994)study.

Table 13. The formation of lipid emulsions containing soybean oil and various types of co-emulsifier.

Composition					Visual observation	
Oil (%)	Soy lecithin (%)	Co-emulsifier (%)	Ratio of lecithin to co-emulsifier	Total emulsifier (%)	Before autoclaving	After autoclaving
5	1	1% Tween 80	1:1	2	White fluid dispersion	White fluid dispersion
5	1	1% poloxamer 188	1:1	2	White fluid dispersion	White fluid dispersion
5	1	1% Cremophor®EL	1:1	2	Unstable	Unstable
5	1	1% Solutol®HS15	1:1	2	Unstable	Unstable
5	2	1% Tween 80	2:1	3	White fluid dispersion	White fluid dispersion
5	2	1% poloxamer 188	2:1	3	White fluid dispersion	White fluid dispersion
5	2	1% Cremophor®EL	2:1	3	White fluid dispersion	Unstable
5	2	1% Solutol®HS15	2:1	3	Unstable	Unstable
10	1	1% Tween 80	1:1	2	White fluid dispersion	White fluid dispersion
10	1	1% poloxamer 188	1:1	2	Unstable	Unstable
10	1	1% Cremophor®EL	1:1	2	Unstable	Unstable
10	1	1% Solutol®HS15	1:1	2	Unstable	Unstable
10	2	1% Tween 80	2:1	3	White fluid dispersion	White fluid dispersion
10	2	1% poloxamer 188	2:1	3	White fluid dispersion	White fluid dispersion
10	2	1% Cremophor®EL	2:1	3	Unstable	Unstable
10	2	1% Solutol®HS15	2:1	3	Unstable	Unstable
20	1	1% Tween 80	1:1	2	Unstable	Unstable
20	1	1% poloxamer 188	1:1	2	Unstable	Unstable
20	1	1% Cremophor®EL	1:1	2	Unstable	Unstable
20	1	1% Solutol®HS15	1:1	2	Unstable	Unstable
20	2	1% Tween 80	2:1	3	White fluid dispersion	White fluid dispersion
20	2	1% poloxamer 188	2:1	3	Unstable	Unstable
20	2	1% Cremophor®EL	2:1	3	Unstable	Unstable
20	2	1% Solutol®HS15	2:1	3	Unstable	Unstable

Unstable = coalescence or phase separation (cracked)

Table 14. The formation of lipid emulsions containing MCT oil and various types of co-emulsifier.

Composition					Visual observation	
Oil (%)	Soy lecithin (%)	Co-emulsifier (%)	Ratio of lecithin to co-emulsifier	Total emulsifier (%)	Before autoclaving	After autoclaving
5	1	1% Tween 80	1:1	2	Blue-white fluid dispersion	Blue-white fluid dispersion
5	1	1% poloxamer 188	1:1	2	White fluid dispersion	White fluid dispersion
5	1	1% Cremophor®EL	1:1	2	Unstable	Unstable
5	1	1% Solutol®HS15	1:1	2	Unstable	Unstable
5	2	1% Tween 80	2:1	3	White fluid dispersion	Unstable
5	2	1% poloxamer 188	2:1	3	White fluid dispersion	Unstable
5	2	1% Cremophor®EL	2:1	3	Unstable	Unstable
5	2	1% Solutol®HS15	2:1	3	Unstable	Unstable
10	1	1% Tween 80	1:1	2	Unstable	Unstable
10	1	1% poloxamer 188	1:1	2	Unstable	Unstable
10	1	1% Cremophor®EL	1:1	2	Unstable	Unstable
10	1	1% Solutol®HS15	1:1	2	Unstable	Unstable
10	2	1% Tween 80	2:1	3	Unstable	Unstable
10	2	1% poloxamer 188	2:1	3	Unstable	Unstable
10	2	1% Cremophor®EL	2:1	3	Unstable	Unstable
10	2	1% Solutol®HS15	2:1	3	Unstable	Unstable

Unstable = coalescence or phase separation (cracked)

Formulations using Cremophor EL and Solutol HS15 as co-emulsifiers at any ratios of soy lecithin to co-emulsifier were unable to form stable emulsion before and after autoclaving using either MCT or soybean oil. The exception was the formulation of 5% MCT emulsified with 2% lecithin and 1% Solutol®HS15 which could form stable emulsion before autoclaving but cracked after autoclaving. The reason is it might be that heat exposure following steam sterilization can alter the hydrophilicity of emulsifier resulting in final phase separation. This reason was supported by Buszello et al. (2000) and Klang and Benita (1998) they described that at high temperature nonionic surfactant, i.e., Solutol HS15 became more and more dehydrated and tended to leave the interfacial layer.

Upon increasing concentration of soybean oil to 20%, emulsion could form when 2% soy lecithin and 1% Tween 80 were used. The result was possibly due to sufficient amount of lecithin to emulsify oil droplet and Tween 80 could form a strong complex film. But this formula maintained the stability only 1 day after autoclaving. The emulsion containing 5% MCT oil could make the stable emulsion when stabilized by 1% soy lecithin and 1% Tween80 (or 1% poloxamer188) (Table 14).

From the results, there were some differences in the formulation of emulsion using MCT and soybean oil when using the mixture of emulsifiers. It was possibly due to the interfacial tension of nature of oil (Jumaa and Müller, 1998b). The other reason was MCT has more ability to take up water than LCT. If other variables are kept constant, the more MCT the more oil droplets occurred in the system. It also needs more emulsifiers. If the ratio of lecithin to nonionic surfactant is still 2:1, thus less emulsifiers will be located at the

surface of oil droplets. When the MCT oil droplets occurred, they tended to be easier flocculated and coalesce when compared to soybean oil.

The results showed that some nonionic surfactants were effective co-emulsifier. Cremophor EL and Solutol HS-15 reduced the emulsifying properties of soy lecithin. In contrary, Tween 80 and poloxamer 188 proved to pose favorable properties as co-emulsifiers in combination with soy lecithin. The findings were in the same trend as Lundberg (1994). Moreover, Tween 80 seemed to be the preferable co-emulsifiers for the formation of parenteral lipid emulsions.

2.1 Physical stability of the emulsions on storage interval

Only the preparations which were stable after autoclaving were further examined for the physical stability upon storage at room temperature ($30\pm2^{\circ}C$) for at least 4 weeks. The stability at accelerated condition described in page 53 was also studied. The results are shown in Table 15.

The systems containing 5% soybean oil stabilized by 1% poloxamer 188 and 1% or 2% lecithin were stable at room temperature for at least 4 weeks. Using the same amount of oil, system emulsified by 2% soy lecithin and 1% Tween 80 were stable at room temperature for at least 4 weeks. System containing 10% soybean oil emulsified by 2% soy lecithin and 1% Tween80 or 1% poloxamer 188 were stable at room temperature for at least 1 week. The preparation containing 20% soybean oil emulsified with 2% lecithin and 1% Tween80 remained stable within 24 hours. This result was similar to Kan et al. (1999) in that oil/water beyond 10% emulsified by Tween 80 and egg lecithin led to an enlargement in particle size and were found less

stable. It is possibly due to the higher amount of oil which might required higher amount of emulsifier to stabilize oil droplets. Interestingly, the system containing 5% MCT emulsified by 1% lecithin and 1% Tween 80 showed the higher stability of the systems in that it was stable at room temperature for 12 weeks. The accelerated condition might be used for determination of the stability upon storage as the more stable system could pass more cycles of accelerated condition. The preparation tested by accelerated condition found that all preparation could not pass this testing so they were not described further. Exception for the preparation containing 5% MCT oil emulsified by 1 % soy lecithin and 1% Tween 80 could pass 6 cycles of accelerated test. Hence, the systems containing 10% soybean oil with 2% soy lecithin and 1% Tween 80 which passed higher cycle was expected to have higher stability over systems consisting of 1% poloxamer 188 as co-emulsifier at the same amount of oil as the former could pass more cycles of the test.

Table 15. The physical stability of the emulsions containing soybean or MCT oil at various weight ratios of soy lecithin to co-emulsifier at room temperature and at accelerated condition.

Composition	Visual observation					
	24 hr	1 wk	4wk	8 wk	12 wk	Accelerated. test (cycles)
5SB+1LE+1T80	ST	ST	UN	-	-	3
5SB+1LE+1P188	ST	ST	ST	UN	-	3
5SB+2LE+1T80	ST	ST	ST	UN	-	3
5SB+2LE+1P188	ST	ST	ST	UN	-	3
10SB+1LE+1T80	ST	UN	-	-	-	1
10SB+2LE+1T80	ST	ST	UN	-	-	3
10SB+2LE+1P188	ST	ST	UN	-	-	2
20SB+2LE+1T80	ST	UN	-	-	-	1
5MCT+1LE+1T80	ST	ST	ST	ST	ST	6
5MCT+1LE+1P188	ST	ST	ST	UN	-	1

ST = stable; UN = unstable; - = not examine

SB = soybean oil; LE = soy lecithin; T80 = Tween 80; P188 = poloxamer 188

2.2 Physicochemical properties of the emulsions

2.2.1 Particle size

The particle sizes of stable emulsion both before and after autoclaving were determined by laser particle sizer. In order to select the suitable emulsion, the d(v,0.5) or average particle size was used for comparing the droplet size in each preparation. The results are shown in Table 16 and Table 17.

Nonionic surfactants stabilized emulsion by the method called steric stabilization from two forces: osmotic forces and entropies effects.

(i) Osmotic (solvation) forces: nonionic surfactants usually contained the polyethylene chain or hydrophilic polymer chain as the hydrophilic portions. When two droplets come in close contact, the polymer chain would overlap and the region became more concentrate. This led to the osmotic gradient resulting in the dilution of the overlap area by water molecules and the solution forces occurred which pushed the droplets apart.

(ii) Another force or mechanism was called “The entropic effects”. When the polymer chain overlapped, the entropy of the system was lost. This resulted in thermodynamically unfavourable condition which forced the droplets to be separated (Attwood and Florence, 1983).

All preparations that could form stable emulsion both before and after autoclaving provided the particle size in submicron. The emulsifier concentration of 2% lecithin and 1% Tween 80 stabilized 5% soybean oil could produce the smallest particles after autoclaving. The

d(v,0.5) of almost all of the formulations were not significantly different in sizes both before and after autoclaving as tested by paired-T test. Exception for the particle size of formulations containing 5% soybean oil emulsified by 2% lecithin and 1% Tween 80 was significantly different from that before autoclaving ($p \leq 0.05$). The differences; however, were less than 30%, so the high temperature during steam sterilization did not have much effect on the particle size of the formulations produced. Groves and Herman (1992) stated that sterilized emulsion was somewhat different from the unsterilized starting material. For example, the amounts of the lyso-PC and lyso-PE compounds were significantly increased, as there were the free fatty acids presented in the system. However, the particle size of the disperse phase droplets remained unchanged or decreased after sterilization, and there was some evidence that the stability of these emulsions was enhanced on sterilization.

The effect of storage period on the particle size was found that most of formulations were unstable after storage for 4 weeks and the coalescence was observed. However, the results showed that some emulsions (preparation containing 5% soybean oil emulsified by 2% soy lecithin and 1% Tween 80 or 1% poloxamer 188 and 5% MCT oil emulsified by 2% soy lecithin and 1% poloxamer 188) had significant differences in particle size after storage compared to the same systems observed 24 hours after autoclaving. These differences; however, was not large. Exception for the formulation containing 5% MCT oil emulsified by 1% soy lecithin and 1% Tween 80 could be stable for at least 12 weeks and the particle size was significantly decreased after 12 weeks storage. The particle size of this preparation on accelerated test was not significantly difference from 24 hours after autoclaving.

Table 16. The particle size of emulsions containing soybean oil and various emulsifiers at room temperature and at accelerated condition.

Composition	Mean particle size[a], μm						
	Before autoclaving	After autoclaving					Accelerated test
		24 hr	1 wk	4 wk	8 wk	12 wk	
5SB+1LE+1T80	0.31±0.01	0.32±0.01	0.32±0.02	UN	UN	UN	UN
5SB+1LE+1P188	0.30±0.02	0.32±0.02	0.35±0.01	0.34±0.00	UN	UN	UN
5SB+2LE+1T80	0.33±0.01*	0.24±0.02	0.29±0.01*	0.33±0.00*	UN	UN	UN
5SB+2LE+1P188	0.31±0.01	0.31±0.00	0.33±0.01*	0.33±0.01*	UN	UN	UN
10SB+1LE+1T80	0.32±0.01	0.33±0.01	UN	UN	UN	UN	UN
10SB+2LE+1T80	0.32±0.01	0.33±0.02	0.34±0.01	UN	UN	UN	UN
10SB+2LE+1P188	0.33±0.01	0.33±0.00	0.33±0.01	UN	UN	UN	UN
20SB+2LE+1T80	0.33±0.01	0.33±0.01	UN	UN	UN	UN	UN

[a] = mean±SD, n=3; UN = unstable
* = significantly different at $p \leq 0.05$ (compared with 24 hours after autoclaving)

Table 17. The particle size of emulsions containing MCT oil and various emulsifiers at room temperature and at accelerated condition.

Composition	Mean particle size[a], μm						
	Before autoclaving	After autoclaving					Accelerated test
		24 hr	1 wk	4 wk	8 wk	12 wk	
5MCT+1LE+1T80	0.27±0.02	0.31±0.01	0.30±0.02	0.30±0.01	0.31±0.01	0.18±0.01*	0.33±0.03
5MCT+1LE+1P188	0.33±0.00	0.33±0.01	0.30±0.01*	0.29±0.01*	UN	UN	UN

[a] = mean±SD, n=3 ; UN = unstable
* = significantly different at $p \leq 0.05$ (compared with 24 hours after autoclaving)

The systems containing higher amount of soybean oil, 10% and 20%, had particle sizes in the range of 0.32-0.34 μm. The sizes were approximately the same regardless of autoclaving and the storage time.

2.2.2 pH and osmolality

The pH values of stable emulsion containing 5, 10, and 20% soybean oil and 5% MCT oil are shown in Table 18, 19. All preparations

were pH adjusted to 8.0 before autoclaving. After autoclaving the pH of all formulations slowly decreased with time to weakly acidic. The lowest pH, 5.26, was found in the formulation containing 5% MCT oil emulsified by 1% soy lecithin and 1% Tween 80 stored at room temperature for 3 months. It was possibly due to the hydrolysis of some lipid in the emulsions leading to the formation of free fatty acids which gradually reduced the pH of the system (Hansrani et al., 1983; Herman and Groves, 1992). The final pH of the formulation was suggested to be in the range of 6-6.5 as the rate of degradation seemed to be minimum (Groves, 1988). It was noted that accelerated condition was not affected the pH of this preparation.

The osmolalities of all emulsions examined were rather constant (Table 18, 19). All preparations had osmolalities between 293-390 mOsm/kg water. Compared the osmolalities of emulsions using soybean oil with MCT oil at the same amount of oil with the same type and amount of emulsifiers. Emulsions containing MCT oil have slightly lower osmolalities than soybean oil. The osmolality of the preparation containing 5% MCT oil emulsified by 1% soy lecithin and 1% Tween 80 which passed accelerated condition was similar to preparation stored at room temperature. The results indicated that the preparations containing higher amount of oil had higher osmolality. So, emulsions contained 20% soybean oil had the highest osmolality. The osmolality seemed to be independent on the storage time. The range of osmolality values of the 10% and 20% commercial parenteral lipid emulsions were in between 300-380 mOsm/kg water. It could imply that the osmolality of the formulations in this study were in the same range of the commercial products.

Table 18. The pH, osmolality and zeta potential of lipid emulsions containing soybean oil.

Composition	Condition	pH[a]	Osmolality[a] (mOsm/kg water)	Zeta potential[a] (millivolts)
5SB+1LE+1T80	Before autoclaving	8.0±0	308±2	-27.94±0.54
	24 hr after autoclaving	6.8±0.1	306±1	-28.72±0.51
	1 wk after autoclaving	6.6±0.1	306±3	-28.46±1.11
5SB+1LE+1P188	Before autoclaving	8.0±0	300±7	-31.22±0.82
	24 hr after autoclaving	6.7±0.1	300±7	-31.19±0.56
	1 wk after autoclaving	6.3±0.1	300±7	-32.74±0.35
	1 mo after autoclaving	6.5±0.1	296±5	-28.74±1.49
5SB+2LE+1T80	Before autoclaving	8.0±0.0	294±5	-28.18±0.15
	24 hr after autoclaving	6.6±0.2	294±7	-27.96±0.66
	1 wk after autoclaving	6.3±0.0	294±6	-31.51±0.17*
	1 mo after autoclaving	6.5±0.1	293±9	-24.53±1.00*
5SB+2LE+1P188	Before autoclaving	8.1±0.0	311±0	-25.22±0.81*
	24 hr after autoclaving	6.6±0.2	315±2	-29.61±0.43
	1 wk after autoclaving	6.3±0.0	313±3	-29.90±0.45
	1 mo after autoclaving	6.4±0.1	311±7	-24.85±1.12*
10SB+1LE+1T80	Before autoclaving	8.0±0.0	320±7	-32.81±0.45
	24 hr after autoclaving	6.6±0.1	321±6	-31.18±0.58
10SB+2LE+1T80	Before autoclaving	8.1±0.1	342±4	-28.19±0.62
	24 hr after autoclaving	7.2±0.1	343±2	-29.99±0.84
	1 wk after autoclaving	7.4±0.1	340±3	-29.48±0.81
10SB+2LE+1P188	Before autoclaving	8.0±0	342±2	-28.43±0.46
	24 hr after autoclaving	7.1±0	342±2	-28.95±0.49
	1 wk after autoclaving	7.3±0.2	337±1	-28.39±0.83
20SB+2LE+1T80	Before autoclaving	8.0±0.1	389±7	-35.09±1.09
	24 hr after autoclaving	6.7±0.2	390±5	-35.14±0.52

[a] = mean±SD, n=3

* = significantly different at $p \leq 0.05$ (compared with 24 hours after autoclaving)

Table 19. The pH, osmolality and zeta potential of lipid emulsions containing MCT oil.

Composition	Condition	PH[a]	Osmolality[a] (mOsm/kg water)	Zeta potential[a] (millivolts)
5MCT+1LE+1T80	Before autoclaving	8.0±0.1	303±5	-29.63±0.15
	24 hr after autoclaving	6.6±0	299±8	-29.95±0.89
	1 wk after autoclaving	6.8±0	299±8	-28.20±0.59
	1 mo after autoclaving	6.7±0.2	305±4	-29.88±0.74
	2 mo after autoclaving	5.7±0.1	304±8	-28.78±1.95
	3 mo after autoclaving	5.3±0.1	302±7	-29.70±0.35
	Accelerated test	6.6±0.1	298±0	-22.38±1.43*
5MCT+1LE+1P188	Before autoclaving	8.0±0	298±4	-30.38±0.47
	24 hr after autoclaving	6.5±0.1	299±5	-30.86±0.69
	1 wk after autoclaving	6.7±0.1	300±8	-30.22±0.44
	1 mo after autoclaving	6.8±0.2	300±9	-30.39±1.22

[a] = mean±SD, n=3

* = significantly different at $p \leq 0.05$ (compared with 24 hours after autoclaving)

2.2.3 Zeta potential

All preparations had the negative zeta potential from negative charge of some phospholipid in lecithin. The high values of zeta potential of more than –30 mV is desirable in most of the emulsions prepared in order to ensure a high energy barrier which caused repulsion of adjacent droplets resulting in the formation of stable emulsions (Klang and Benita, 1998). Before the measurement of zeta potential, the standard Minusil® solution was used to calibrate for the precision and accuracy. The zeta potential of the standard Minusil® solution measured was –48±2 mV which was near the value recommended, –49±2 mV. Autoclaved emulsions containing soybean oil seemed to increase the zeta potential to more negative value after storage which was possibly due to the hydrolysis of the lecithin resulting in the

pH lowering of the bulk medium and more negative charge at the interfacial area of the droplet (Herman and Groves, 1992; Yamaguchi et al., 1995). (Table 18 and Table 19). The zeta potential values were approximately the same or slightly increase after 1 week. In contrast to the system stored for a month which showed a decrease in zeta potential. This was possibly due to the removal of lecithin from the interfacial area of the droplet resulting in less negative charge.

The systems using MCT oil showed that the values of zeta potential were rather constant (Table 19) regardless of the effect of autoclaving or the storage time after autoclaving. But the preparation which passed accelerated test showed a significantly decrease in zeta potential.

Emulsifiers can stabilize the emulsion droplet not only by the formation of a mechanical barrier, but also by producing an electrical repulsive of surface charges. The surface charge of the droplets was produced by the ionization of interfacial film-forming component which showed enormous effect when the ionic emulsifiers were used. The zeta potential of an emulsion droplet was dependent upon the extent of ionization of the emulsifying agent. The ionization extent of some phospholipids comprised in lecithin was markedly pH-dependent. A mixture of phospholipids should provide some advantages for the formulation studies. They comprised of phosphatidylcholine and negatively charged phospholipids such as phosphatidylethanolamine which caused the electrostatic repulsive force for stabilizing the system. In addition, other components such as cholesterol might affect the interfacial film-charge extent (Klang and Benita, 1998).

From the present study, soybean oil was better than MCT oil because it could form stable emulsion when increasing the amount of oil to 10% or 20%. The 10% soybean oil emulsion had more long-term stability than 20% soybean oil in the same emulsifiers used. The 5% oil emulsion had the longest stability but the concentration of 5% oil was not sufficient to use as parenteral nutrition. Tween 80 seemed to be the best co-emulsifier as the stable autoclaved emulsion could be obtained with various oil concentrations and it was the only co-emulsifier which could form stable emulsion composed of 20% soybean oil. In all further experiments, the efficiency of the emulsification process was mainly quantitated by measurement of the particle size. To evaluate the effect of the ratio of soy lecithin to Tween 80, a series of samples were prepared with varying ratios of soy lecithin to Tween 80 and concentrations of surfactant.

2.3 Optimization of the emulsion containing the soy lecithin and Tween 80

The formulation containing 10% soybean oil emulsified with 2% soy lecithin and 1% Tween 80 was chosen to study for optimizing concentration of Tween80 needed in the emulsifier system. The emulsion could contain high amount of oil (10%) and was stable enough for investigation of the physicochemical properties. A series of emulsions was prepared with increasing amounts of Tween 80 from 0.5 to 2.0 at constant lecithin concentration of 2 % (Table 20).

Table 20. The physical stability of emulsions containing 10% soybean oil stabilized by various amounts of Tween 80 as co-emulsifier.

Ratio of lecithin to Tween 80	Total emulsifier concentration (%)	Visual observation	
		Before autoclaving	After autoclaving
2:0.5	2.5	Stable	Unstable
2:1	3.0	Stable	Stable
2:1.5	3.5	Stable	Stable
2:2	4.0	Stable	Stable

Unstable = coalescence

2.3.1 Physical stability

The physical stability of the preparations are shown in Table 20. Most of them were stable before and after autoclaving except for the system containing total emulsifier concentration of 2.5% which was coalesce after autoclaving. It might be due to the amount of Tween 80 was not enough to form a strong complex film with soy lecithin. The formulation combining 2:1.5 and 2:2 weight ratio of soy lecithin to Tween 80 were stable for at least one month.

2.3.2 Particle size, pH, osmolality and zeta potential

The effect of increasing concentration of Tween80 on the particle size of autoclaved emulsions which were stable as shown in Table 21. The particle sizes of all preparations before and after autoclaving were not significantly different as tested by paired-T test. The emulsions were measured after stored for a week and the results were found that there were no significant difference between formulations kept for 24 hours and 1 week.

Table 21. The mean particle size, pH, osmolality and zeta potential of emulsions containing 2% soy lecithin and various amounts of Tween 80.

Ratio of lecithin to Tween 80	Condition	Physicochemical properties[a]			
		Mean particle size (μm)	pH	Osmolality (mOsm/kg water)	Zeta potential (mV)
2:1	a	0.32±0.01	8.06±0.06	342±4	-28.19±0.62
	b	0.33±0.01	7.22±0.05	343±2	-29.99±0.84
	c	0.34±0.01	7.43±0.10	340±3	-29.48±0.81
2:1.5	a	0.29±0.03	8.06±0.05	332±1	-31.67±0.41
	b	0.31±0.02	6.70±0.31	335±1	-31.79±1.67
	c	0.33±0.02	6.74±0.09	332±1	-32.71±0.48
	d	0.31±0.02	6.54±0.09	333±1	-31.64±0.81
2:2	a	0.33±0.01	8.01±0.02	337±3	-32.45±0.90
	b	0.30±0.01	6.75±0.05	334±2	-30.98±0.72
	c	0.32±0.02	6.71±0.05	332±5	-31.10±0.71

[a] = mean±SD, n=3

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature

The pH values, osmolality and zeta potential of all preparations are shown in Table 21. The pH of all preparations were adjusted to 8 before autoclaving. The pH values of all autoclaved preparations slowly decreased with storage time as previously described in 2.2.2. Osmolalities of all preparations were rather constant with the values in between 332-343 mOsm/kg water water.

Zeta potentials of formulation with various weight ratio of soy lecithin to Tween 80 were in approximately ranged from –28 to –32 mV. The zeta potential values of some systems were slightly increased after autoclaving insignificant different.

From the results, it showed that the weight ratio of soy lecithin to Tween 80 had no significant effect on the physicochemical properties of the emulsions. However, the systems containing soy lecithin to Tween 80 at the ratio of 2:1.5 and 2:2 were more stable but the former was selected for further studies as it contained less emulsifiers.

2.4 Optimization of total emulsifier concentration

The next parameter investigated was the total amount of the emulsifier systems used to stabilize the emulsion containing 10% soybean oil. Emulsions were prepared with varying amounts of total emulsifiers but the same ratio of soy lecithin to Tween 80, 2:1.5 (Table 22).

Table 22. Concentration of emulsifiers and physical stability of emulsions containing 10 % soybean oil at weight ratio of 2:1.5 soy lecithin to Tween 80

Total concentration of emulsifier (%)	Soy lecithin (%)	Tween 80 (%)	Visual observation	
			Before autoclaving	After autoclaving
1	0.57	0.43	Stable	Unstable
2	1.14	0.86	Stable	Unstable
3	1.71	1.29	Stable	Stable
3.5	2.00	1.50	Stable	Stable
4	2.29	1.71	Stable	Stable
5	2.86	2.14	Stable	Stable

Unstable = coalescence

2.4.1 Physical stability

All preparations were stable before autoclaving. When passed through autoclaving, the lipid emulsion which had total concentration of emulsifier at 1% and 2% were unstable (Table 22). It is possibly due to the insufficient amount of emulsifier to emulsify oil droplets. Emulsions stabilized by emulsifier at the total concentration not less than 3% could form stable emulsion both before and after autoclaving. It was noted that total emulsifier concentration of 3.5% was previously studied.

2.4.2 Particle size, pH, osmolality and zeta potential

The effect of total emulsifier concentration on the particle size of emulsions is shown in Table 23. In the same system, there were significant differences in particle size of non-autoclaved and autoclaved emulsions containing total concentration of emulsifier at 4% and 5%. This difference was negligible in emulsions with total concentration of 3% and 3.5%.

The pH values of all stable preparations were slightly decreased during the period of time as previously described in 2.2.2. The pH values of autoclaved emulsions were in between 6.54 and 6.97. The osmolalities were not affected by from autoclaving and the storage time. The osmolality of the systems was rather constant for all storage period.

The zeta potential tend to increase in negativity during storage. Upon autoclaving, the zeta potential seemed to increase in the system containing 3% emulsifiers. While zeta potential in other systems were

rather constant. The long-term storage had small effect on zeta potential of all emulsions studied as the values were slightly decreased after being stored for a month compared to the values obtained after storage 24 hours.

Table 23. The mean particle size, pH, osmolality and zeta potential of emulsions containing 10 % soybean oil at weight ratio of 2:1.5 soy lecithin to Tween 80

Total concentration of emulsifier (%)	Condition	Physicochemical properties[a]			
		Mean particle size (μm)	pH	Osmolality (mOsm/kg water)	Zeta potential (mV)
3	a	0.31±0.02	8.01±0.01	337±2	-32.12±0.34*
	b	0.33±0.01	6.83±0.07	337±0	-33.52±0.06
	c	0.34±0.01	6.86±0.08	337±2	-31.77±0.45*
	d	0.34±0.01	6.65±0.08	335±1	-31.68±0.08*
3.5	a	0.29±0.03	8.06±0.05	332±1	-31.67±0.41
	b	0.31±0.02	6.70±0.31	335±1	-31.79±1.67
	c	0.33±0.02	6.74±0.09	332±1	-32.71±0.48
	d	0.31±0.02	6.54±0.09	333±1	-31.64±0.81
4	a	0.31±0.01*	8.03±0.03	333±1	-33.12±0.54
	b	0.32±0.01	6.96±0.04	334±0	-33.00±1.19
	c	0.32±0.01	6.97±0.06	336±2	-32.41±0.48
5	a	0.23±0.01*	8.08±0.11	343±2	-31.95±0.10
	b	0.28±0.02	6.79±0.02	344±1	-31.63±0.76
	c	0.32±0.01*	6.96±0.04	346±1	-32.14±0.85

[a] = mean±SD, n=3

* = significantly different at $p \leq 0.05$ (compared with 24 hours after autoclaving)
a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature

From the results, the physical stability and physicochemical properties of all preparations were not much different. So the suitable emulsion was chosen to be the formulation using the lowest total

concentration of emulsifier which was 3% at the lecithin-to-Tween 80 weight ratio of 2:1.5. The total emulsifier concentration of more than 3% might contained excess emulsifier left in the formulation.

Finally, the most suitable preparation was 10% soybean oil emulsified with 1.71% soy lecithin and 1.29% Tween 80. The formulation was named 10% Pharmalipid. The characteristic of this formulation is shown in Table 24. The scanning electron microscope shown that the emulsion droplets were spherical in shape and rather monodisperse (Figure 8). It was noted that 10 g Pharmalipid was approximately to 9.98 ml. The total energy of this preparation was calculated by summation of the energy obtained from soybean oil and soy lecithin (9 cal/g) and glycerol (4.32 cal/g).

Table 24. The composition and characteristic of formulated parenteral lipid emulsion (10% Pharmalipid).

Composition	Amount (g)
Soybean oil	10
Soy lecithin	1.71
Tween 80	1.29
Glycerol	2.5
Distilled water	qs to 100 g
Characteristic	**Value**
Mean particle size* (μm)	0.33 ± 0.01
pH*	6.8 ± 0.1
Osmolality* (mOsm/kg water water)	337
Zeta potential* (millivolts)	-33.52 ± 0.06
Calculated Total energy (Cal/L)	1,162

* = examined at 24 hours after autoclaving

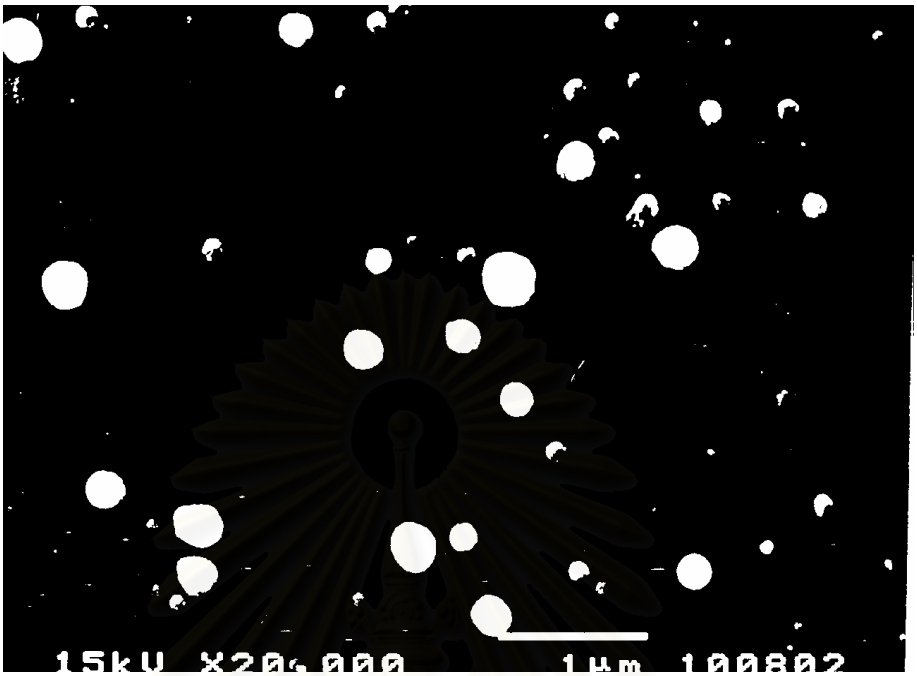


Figure 8. The particle size and shape of 10% Pharmalipid examined by scanning electron microscope

The 10% Pharmalipid was also examined for the rheological property. The emulsion had Newtonian flow and the viscosity value of 10% Pharmalipid was 1.46±0.02 mPa s. This value was higher than the commercial product, 10% Intralipid®, containing the same amount of oil. Which had the viscosity of 0.94±0.13 mPa s. It was possibly due to 10% Pharmalipid contained higher concentration of emulsifier, 3%, compared to emulsifier concentration of 1.2% of 10% Intralipid. Swarbrick (1995) described that if the emulsifier concentration increased, the viscosity of the system increased.

3. Preparation and characterization of total nutrient admixtures

Lipid emulsion was mixed with the known amount of dextrose and amino acid solutions to make the total nutrient admixtures (TNA). The lipid emulsions used to form TNA were the commercial products namely, 10% and 20% Intralipid®, 10% and 20% Lipofundin®MCT/LCT, 10% Lipofundin-S®, and the prepared formulation (10% Pharmalipid). It was noted that commercial TNA (Vitrimix KV®) composed of Vamin®Glucose and 20% Intralipid® at the volume ratio of 3:1. The TNA preparations were stored at room temperature and in the refrigerator.

3.1 Physical stability of total nutrient admixtures

TNA preparations with an opaque, nonreflecting surface were considered stable. When the systems were left undisturbed, there were no creaming, clear-yellow oil droplets (coalescence), precipitation, or separation occurred. From the result, TNA could be formed with every volume ratios of Vamin®Glucose to lipid emulsion used which were 2:1, 3:1 and 4:1. For the systems stored in refrigerator, a very thin, white cream layer appeared on the surface within 24 hours. The cream layer might be the result of the differences in density between the oil and the denser aqueous solution containing dextrose, amino acids, and electrolytes. The results found that all TNA preparations were stable and no instability observed within 24 hrs. However, when the temperature system increased to room temperature, the cream layer disappeared and no oil droplets on the surface were observed for all preparations after gentle agitation. These finding were previously reported by Parry, Harrie, and McIntoch-Lowe (1986) and Sayeed et al. (1987). The particles in the cream layer might have a slightly larger droplet size which

increased the possibility of coalescence and eventually oil separation. It has been recommended that the particle size of lipid emulsion should be smaller than the diameter of the capillaries or similar to naturally occurring chylomicrons which were less than 1.0 μm in order to prevent the occurance of fat embolism upon administration (Kawilarang, Georghiou, and Groves, 1980). Then the cream layer observed on systems containing solutions of amino acid and glucose (Vamin® Glucose) and 20% lipid emulsion (20% Intralipid®) at volume ratios of 2:1, 3:1, and 4:1 was determined for the droplet sizes. The mean particle size of the systems were 0.34, 0.34, and 0.36 μm, respectively and were not significant difference from pure lipid emulsion, so the TNA kept in refrigerator was promising for parenteral use.

Dextrose is acidic solution (pH 3.5 to 5.5) and can significantly decreases the pH of lipid emulsions and consequently the stability of lipid emulsion. The zeta potential that imparts high stability to lipid emulsion (i.e., -35 mV or greater) occurs in the pH range of 5 to 10. Some workers found that the addition of 25% dextrose solution to an equal volume of 10% Intralipid caused a decrease in the pH of the lipid from 7.0 to 3.45, and the particle size distribution of lipid emulsion significant changed after 48 hr at room temperature (Black and Popovich, 1981). Seventy-two hours after admixture a cream layer was formed at the top of emulsion and the electrostatic repulsive forces which contributed to lipid stability were reduced (Brown et al., 1986).

Amino acid solutions are generally considered safe to add to lipid emulsions. Amino acids, when mixed with lipids, appear to exert a protective effect against other additives. There are several mechanisms

postulated for this protective effect (Allwood and Kearney, 1998; Brown et al., 1986).

a). Amino acids are thought to adsorb at the oil-water interface resulting in enhancing the mechanical barrier and reducing the opportunity for particles to aggregate and coalesce.

b). Amino acids have a buffering capacity which decreases the deleterious effects of low pH dextrose solutions. The higher the amino acid concentration, the greater the buffering capacity.

c). Enhancement of the mechanical barrier of emulsions droplets by a pH dependent ionic interaction between specific amino acids and the lipid emulsion.

3.2 Physicochemical properties of total nutrient admixtures

3.2.1 Particle size

The average particle size of commercial lipid emulsions were examined before TNA preparation. The compositions of commercial lipid emulsions are listed in Table b2 (in Appendix B). The mean particle size provided by manufacturer were in the range of 0.3-0.4 μm for 10% and 20% Lipofundin®MCT/LCT, and 0.3 μm for 10% Lipofundin-S®. The results from present study found the mean particle sizes of 10% and 20% Lipofundin®MCT/LCT and 10% Lipofundin-S® were 0.29, 0.30 and 0.29 μm, respectively (Table 25). The particle size of 10% and 20% Lipofundin®MCT/LCT studied by Müller and Heinemann (1993) were 0.29 and 0.29 μm, respectively. The mean particle sizes of 10% and 20% Intralipid® were 0.32 and 0.33 μm, respectively. Ishii et al. (1990) reported the mean

particle size of 0.25 and 0.41 μm for 10% and 20% Intralipid®, respectively. It was noted that the average particle size of 10% lipid emulsion was slightly less than the values found for the 20% lipid emulsion (Müller and Heinemann, 1994). The freshly prepared 10% Pharmalipid was found to be 0.30 μm.

Table 25. The change in particle size of lipid emulsion after being mixed with Vamin®Glucose at 0 and 24 hrs at room temperature

Lipid emulsion (LE)	Mean particle size[a] (μm)						
	Plain LE	Volume ratio of Vamin®Glucose to lipid emulsion					
		2:1		3:1		4:1	
		0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
10% Intralipid®	0.32±0.00	0.33±0.01	0.33±0.01	0.33±0.00	0.32±0.01	0.33±0.01	0.33±0.00
10% Lipofundin®MCT/LCT	0.29±0.00	0.29±0.01	0.29±0.00	0.30±0.00*	0.29±0.01	0.29±0.00	0.30±0.01
10% Lipofundin-S®	0.29±0.01	0.33±0.01*	0.34±0.01*	0.33±0.00*	0.34±0.00*	0.34±0.00*	0.34±0.00*
10% Pharmalipid	0.30±0.02	0.29±0.01	0.31±0.01	0.31±0.01	0.33±0.01	0.32±0.01	0.33±0.01
20% Intralipid®	0.33±0.01	0.33±0.00	0.34±0.01	0.32±0.01	0.32±0.01	0.33±0.00	0.33±0.00
20% Lipofundin®MCT/LCT	0.30±0.00	0.31±0.00	0.31±0.00	0.31±0.00	0.31±0.00	0.31±0.00	0.32±0.00

[a] = mean±SD, n = 3

* = significantly different at $p \leq 0.05$ compared with pure lipid emulsion

The particle sizes of lipid emulsion after mixing immediately at volume ratios of Vamin®Glucose to lipid emulsion were not significantly different ($p>0.05$) from those without Vamin®Glucose. Exception for the system of Vamin®Glucose to 10% Lipofundin MCT/LCT at volume ratios of 3:1 after mixing immediately and 10% Lipofundin-S in all volume ratios were significantly different ($p\leq0.05$). Moreover, no significant differenced in particle size were observed between freshly prepared TNA and TNA stored at room temperature for 24 hrs. From the results, the different formulations of lipid emulsion had the particle size in the same range (0.29-0.34 μm). Moreover, the effect of volume ratio of Vamin®Glucose to lipid emulsion on particle size

observed within 24 hrs was not significant even the ratio of the commercial TNA (Vitrimix KV®) which was 3:1.

Table 26. The effect of solutions of trace element (Addamel-N®) and vitamin (OMVI®) on the mean particle size of lipid emulsion in TNA systems composed of 20% Intralipid® (or 10% Pharmalipid) at the volume ratio of 3:1 after being mixed with Vamin®Glucose at 0 and 24 hrs at room temperature.

Lipid emulsion	Mean particle size[a] (μm)		
	Pure lipid emulsion	Volume ratio of Vamin®Glucose to lipid emulsion 3:1	
		0 hr	24 hr
20% Intralipid®	0.33±0.01	0.32±0.01	0.32±0.01
20% Intralipid® + Addamel-N and OMVI	-	0.32±0.01	0.32±0.01
10% Pharmalipid	0.30±0.01	0.31±0.01	0.33±0.01
10% Pharmalipid + Addamel-N and OMVI	-	0.29±0.03	0.32±0.03

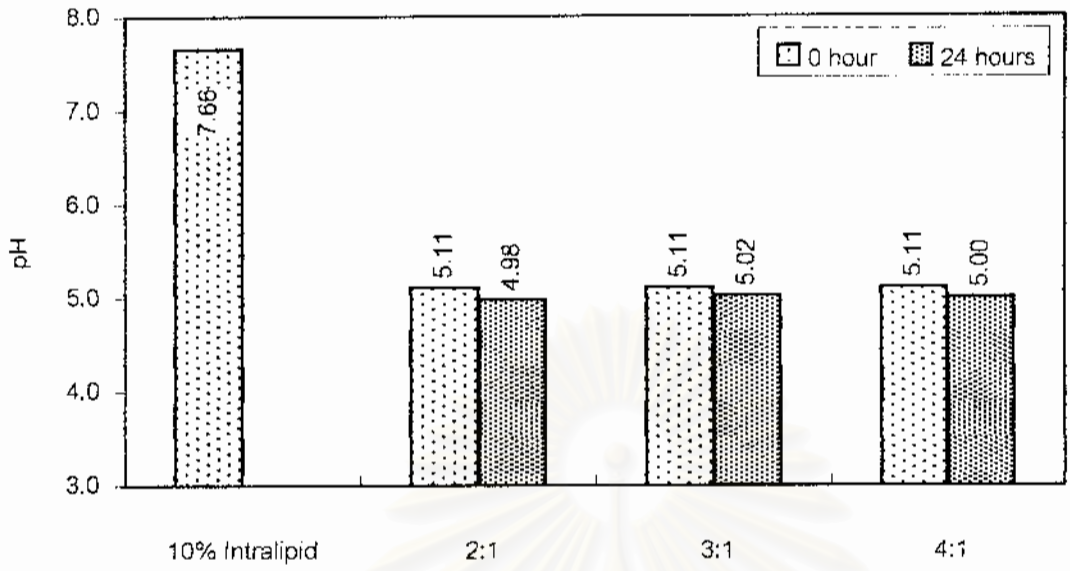
[a] = mean±SD, n = 3

The change in particle size of lipid emulsion in TNA preparation was expected to be the effect of electrolytes in Vamin®Glucose (See Table b3 in Appendix B for composition of Vamin®Glucose). Electrolytes especially the divalent or trivalent cations were known to neutralize the charge of the emulsion droplet causing less stability of emulsion (Allwood and Kearney, 1998; Driscoll, 1997). There was no obvious effect of electrolytes on particle size and stability of commercial emulsions made of only lecithin and prepared emulsion (10% Pharmalipid) made of lecithin and nonionic surfactant (Table 26). It was possibly due to that amino acids were adsorbed at the o/w interface resulting in enhancing the mechanical barrier and reducing the opportunity for particles to aggregate and coalesce. The use of nonionic surfactants as co-emulsifiers might provide advantages over charged surfactants as their less sensitivity to any electrolytes and pH (Attwood and Florence, 1983). The effect of trace elements and vitamins on particle size of

emulsion in TNA was also studied. Ten ml of trace elements (Addamel-N®) and 4 ml of vitamins (OMVI®) were added into 1 liter TNA. It was noted that the amount of trace elements and vitamins added was the same as the recommendation by the manufacturer of Vitrimix KV®. The findings (Table 28) showed that trace elements and vitamins caused no significant changes in particle size. The results were in agreement with the studies of Allwood and Kearney. (1998) and Rollins et al. (1992).

3.2.2 pH and osmolality

The values of pH of all TNAs were less than lipid emulsions, 6.5-8.0, because of the acidity of dextrose and amino acid in solutions (Figure 9, 10, 11). So, the pH of TNA was predicted to be the same as Vamin®Glucose which was approximately 5.2. A slight decrease in pH of TNA stored for 24 hrs was found in the admixtures composed of 10% Intralipid® or 10% Lipofundin®MCT/LCT at all volume ratios. The result was similar to Parry et al. (1986). The pH of all TNA preparations was in the range of 4.98 to 5.19, regardless of the volume ratio use. Hansrani et al. (1983) and Brown et al. (1986) have recommended that the pH of admixtures should remain above 6.0 in order to minimize free fatty acid formation and to stabilize the emulsion. However, it was slightly different from the manufacturer's suggestion in that the TNA systems should have pH in the range of 5.4 to 6.5. The effect of trace elements and vitamins on pH of TNA has been studied in TNA composed of Vamin®Glucose and 20% Intralipid® (or 10% Pharmalipid) at the same volume ratio (3:1). The same pH values of TNA were obtained. The pH of Addamel-N® was reported by the manufacturer to be 2.2. However, a more decrease in pH of TNA was not observed as a very small amount of Addamel-N® was added.



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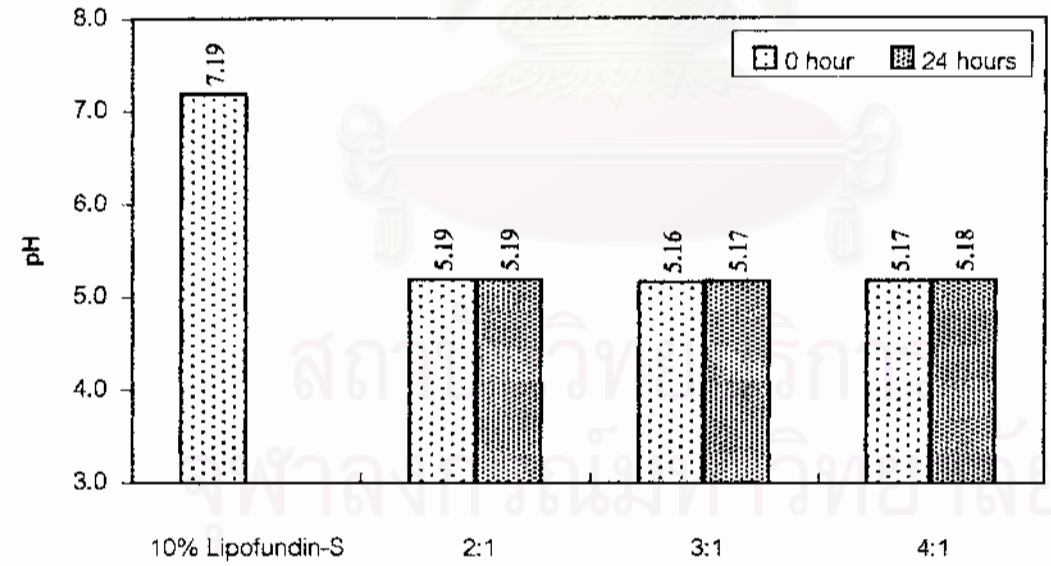
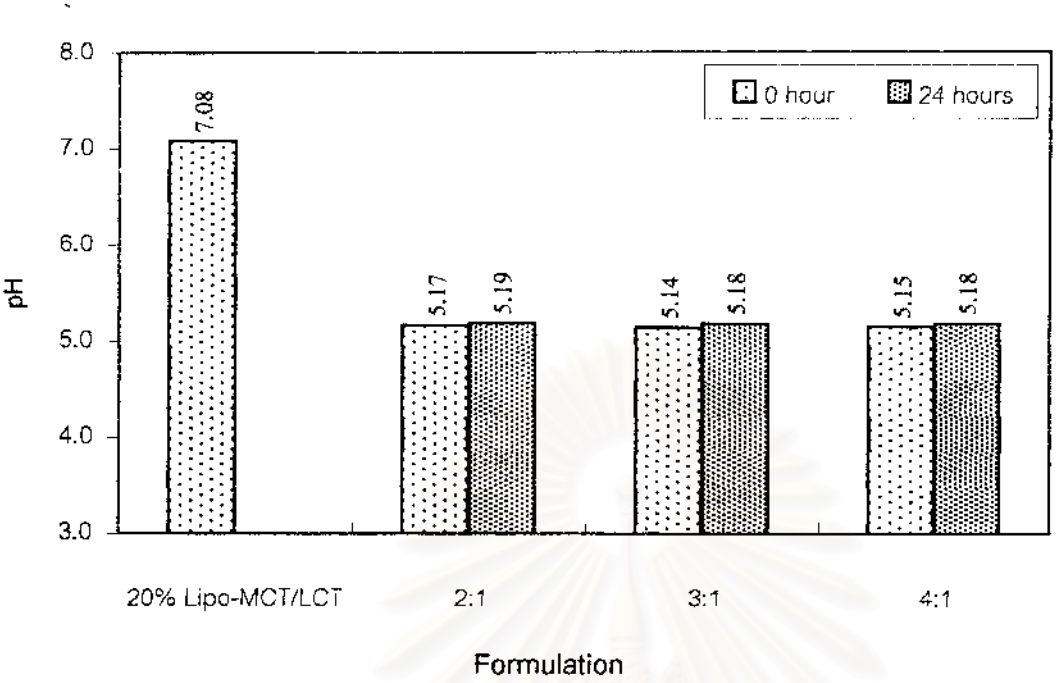


(b)

Figure 9. The change in pH of TNA system composed of different emulsions at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Intralipid® as lipid emulsion; (b) 10% Lipofundin-S® as lipid emulsion.



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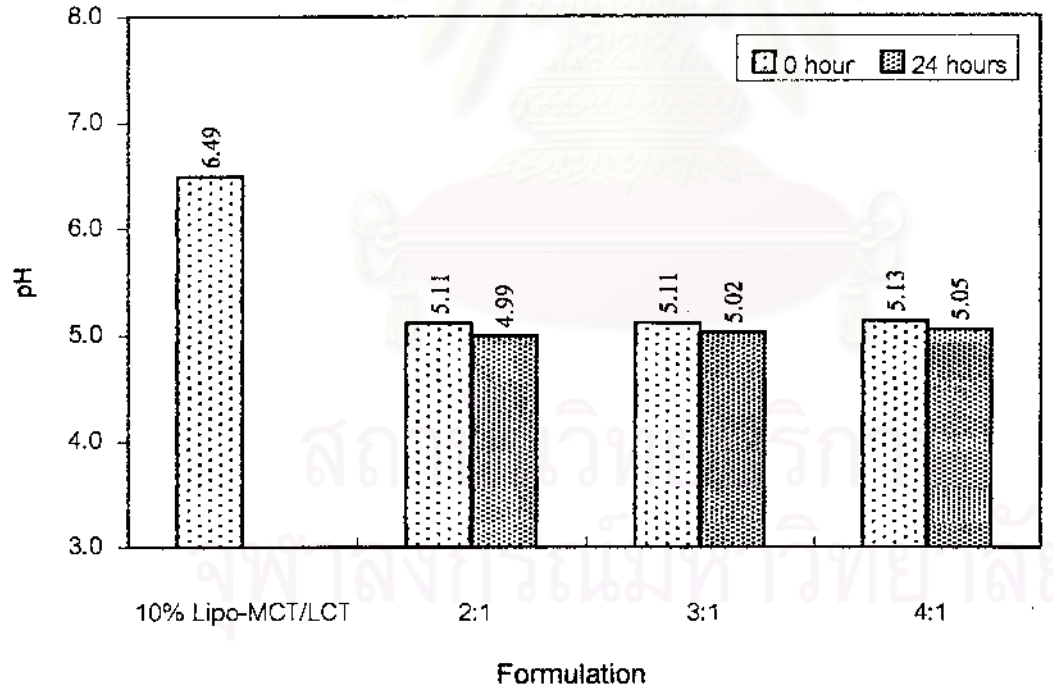
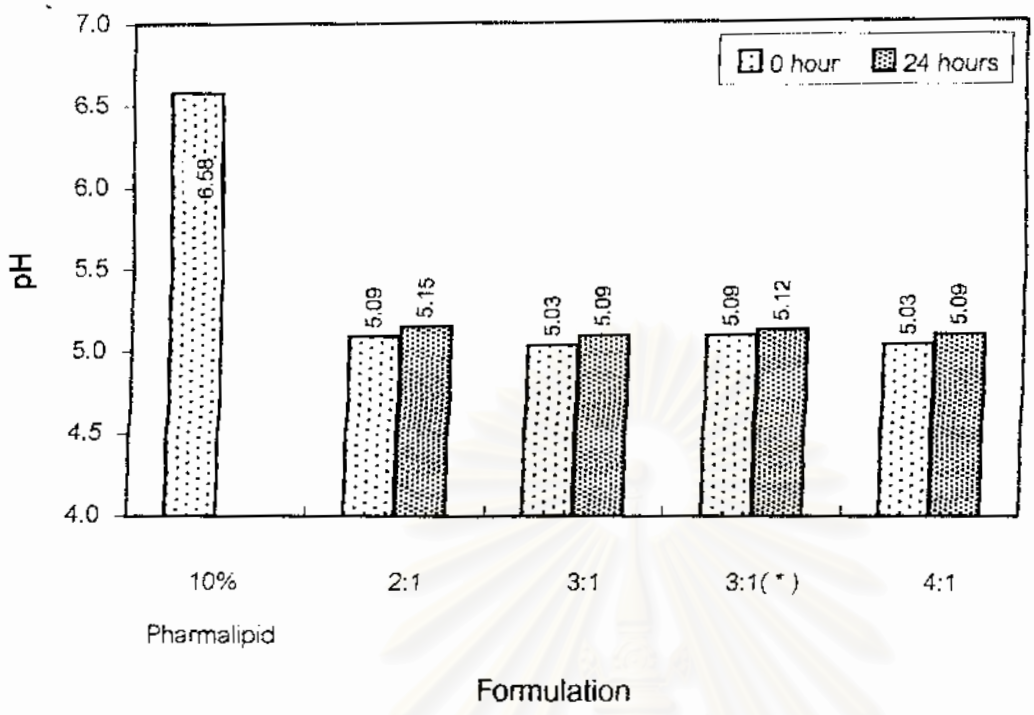


(b)

Figure 10. The change in pH of TNA system composed of different emulsions at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Lipofundin MCT/LCT® as lipid emulsion; (b) 20% Lipofundin MCT/LCT® as lipid emulsion.



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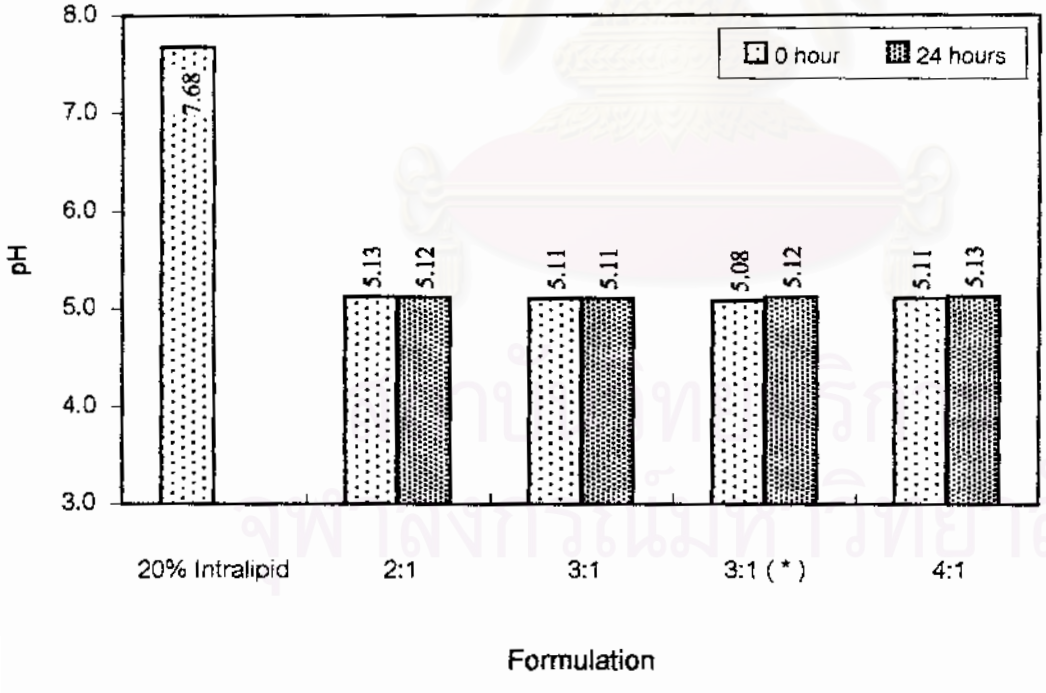
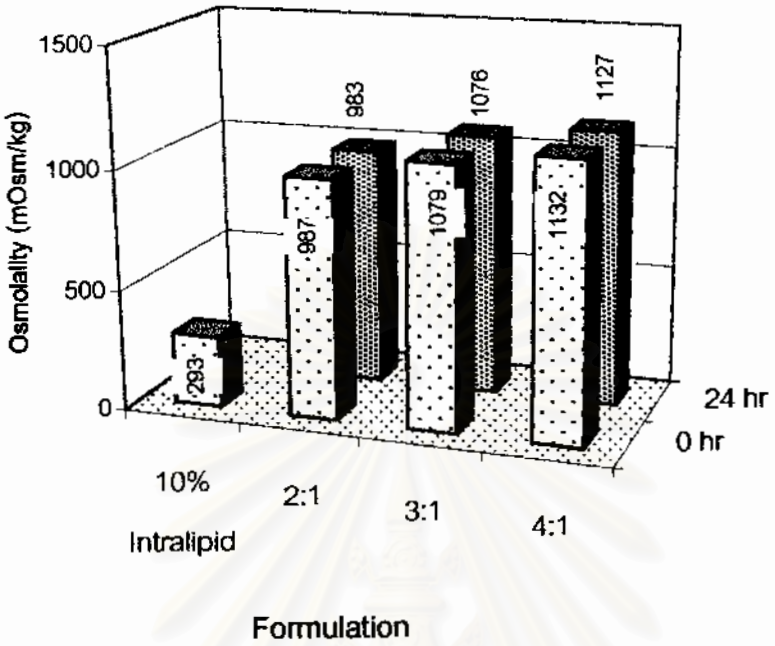
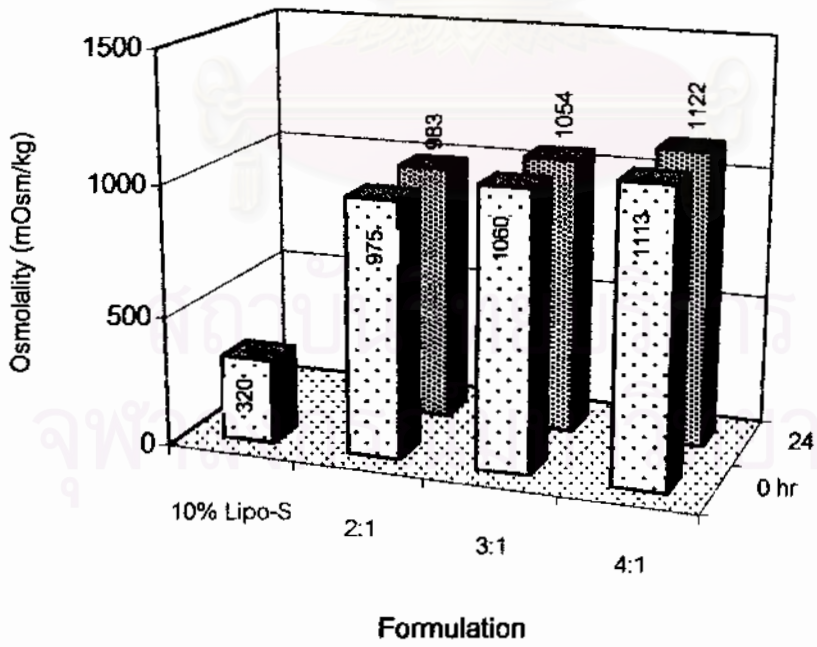


(b)

Figure 11. The change in pH of TNA system composed of different emulsions at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Pharmalipid as lipid emulsion; (b) 20% Intralipid® as lipid emulsion (* = added Addamel-N® and OMVI®)

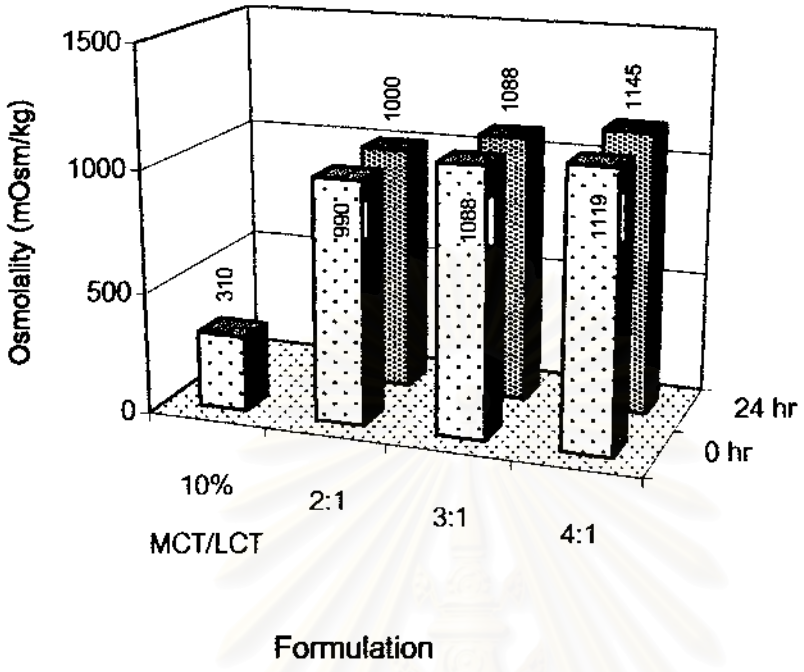


(a)



(b)

Figure 12. The change in osmolality of TNA system composed of different emulsion at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Intralipid® as lipid emulsion; (b) 10% Lipofundin-S® as lipid emulsion.



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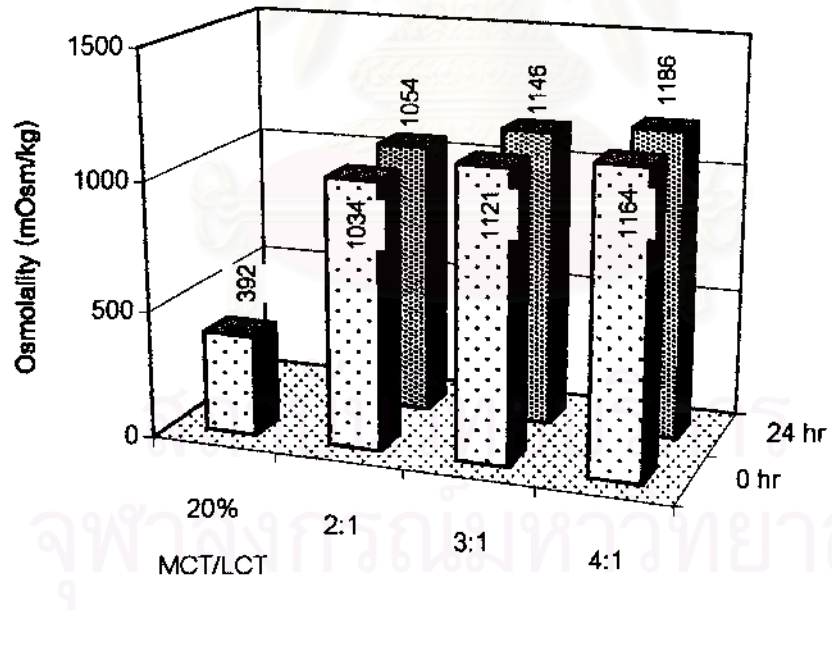
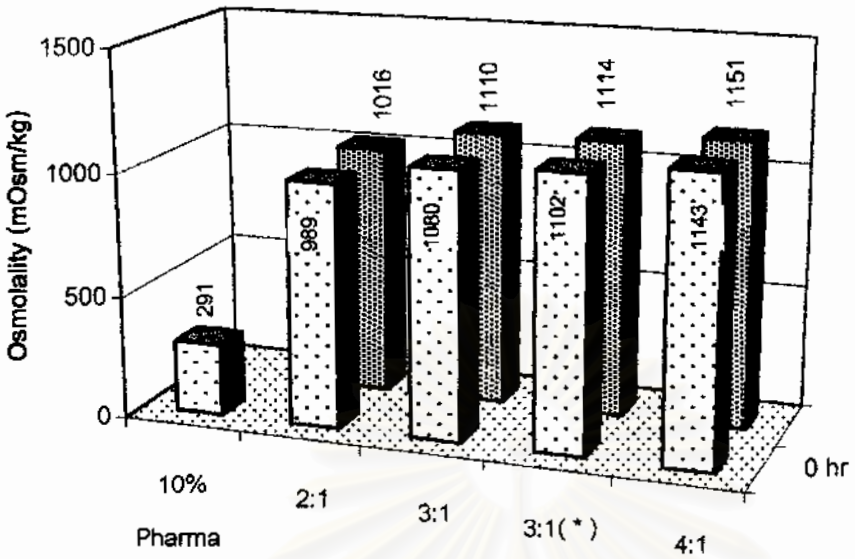


(b)

Figure 13. The change in osmolality of TNA system composed of different emulsion at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Lipofundin MCT/LCT® as lipid emulsion; (b) 20% Lipofundin MCT/LCT® as lipid emulsion.



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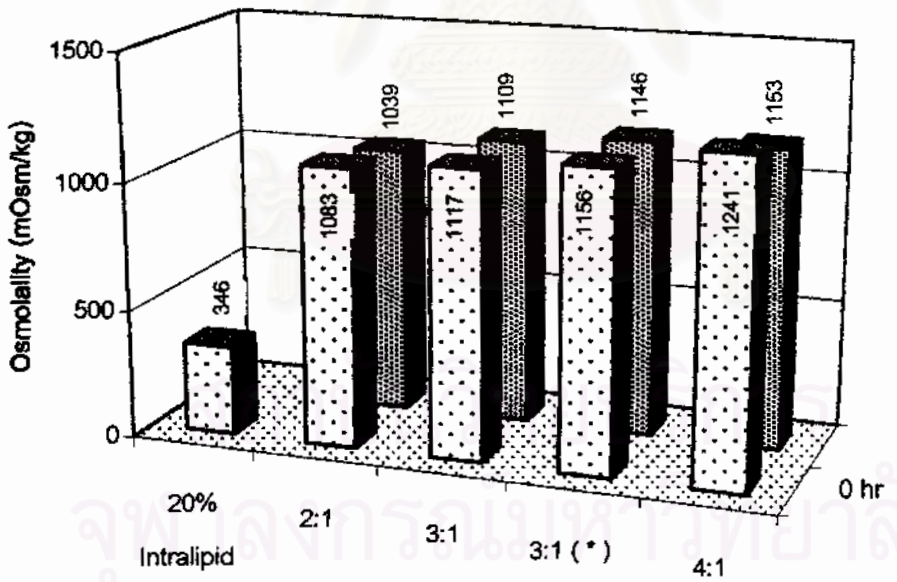

(b)

Figure 14. The change in osmolality of TNA system composed of different emulsion at various volume ratios of Vamin®Glucose to lipid emulsion: (a) 10% Pharmalipid as lipid emulsion; (b) 20% Intralipid® as lipid emulsion (* = added Addamel-N® and OMVI®)

The values of osmolality of TNA were shown as the relative hyperosmolality compared to the osmolality of lipid emulsion (291-392 mOsm/kg water). The Vamin®Glucose had high osmolality approximately 1,350 mOsm/kg water (reported by the manufacturer). The osmolalities of all TNA had a small change during the study period and the values were in between 975-1,186 mOsm/kg water (Figures 12, 13, 14). The results were similar to the studies of Parry et al. (1986) and Sayeed et al. (1987) in that the osmolality of the admixtures was considered to be stable. Trace element and vitamin caused slightly higher values of osmolalities. The osmolality of Addamel-N® as reported by manufacturer was 1,900 mOsm/kg water.

3.2.3 Zeta potential

The effect of electrolytes and amino acids on zeta potential of lipid emulsion was examined. The results showed no significant differences found in systems using all commercial lipid emulsions at the volume ratios of 2:1, 3:1 and 4:1 after immediate mixing compared to lipid emulsion in the absence of Vamin®Glucose. The significant decrease in zeta potential was observed in TNA using 10% Pharmalipid at volume ratio of 3:1 and 4:1 after immediate mixing (Table 27, 28). A decrease in zeta potential of lipid emulsion was possibly due to the presence of cationic electrolytes especially divalent cations (Ca^{2+}, Mg^{2+}) in Vamin®Glucose. Increasing the volume ratios might cause a decrease in zeta potential as the amount of cationic electrolytes increased. However, it was true for only TNA using 10% Pharmalipid. The prepared emulsion, 10% Pharmalipid, seemed to be the most sensitive to electrolytes presented which perhaps caused instability of the TNA.

Table 27. The zeta potential of emulsion in TNA system at various volume ratios of Vamin®Glucose to lipid emulsion

Lipid emulsion (LE)	The zeta potential[a] (millivolts)						
	Pure lipid emulsion	Volume ratio of Vamin®Glucose to lipid emulsion					
		2:1		3:1		4:1	
		0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
10% Intralipid®	-44.92±0.36	-44.71±0.41	-43.93±1.2	-44.18±0.95	-42.05±0.73*	-44.98±0.60	-42.41±0.72*
10% Lipofundin®MCT/LCT	-39.23±0.74	-39.13±0.12	-36.65±0.75*	-38.59±0.60	-35.86±0.36*	-39.24±0.34	-38.87±0.31
10% Lipofundin-S®	-41.55±0.61	-41.78±0.64	-40.38±0.23	-41.70±0.78	-41.02±0.30	-41.56±0.28	-40.03±0.64
10% Pharmalipid	-36.14±0.70	-35.07±0.70	-26.94±1.81*	-26.33±0.35*	-25.72±0.78*	-26.991±1.56*	-28.80±0.73*
20% Intralipid®	-44.04±0.91	-44.48±0.25	-44.09±0.47	-43.97±0.26	-42.89±1.07	-44.68±0.79	-42.50±0.68
20% Lipofundin®MCT/LCT	-38.98±0.12	-40.21±0.47	-37.92±0.58*	-39.20±0.18	-38.17±0.64	-39.78±0.60	-39.65±0.28

[a] = mean±SD, n = 3

* = significantly different at $p \leq 0.05$ compared with pure lipid emulsion.

Table 28. The effect of solutions of trace element (Addamel-N®) and vitamin (OMVI®) on the zeta potential of lipid emulsion in TNA systems at the volume ratio of 3:1 Vamin®Glucose to lipid emulsion at 0 and 24 hrs at room temperature.

Lipid emulsion	The zeta potential[a] (millivolts)		
	Pure lipid emulsion	Volume ratio of Vamin®Glucose to lipid emulsion 3:1	
		0 hr	24 hr
20% Intralipid®	-44.04±0.91	-43.97±0.26	-42.89±1.07
20% Intralipid® + Addamel-N and OMVI	-	-43.22±0.32	-40.68±0.89
10% Pharmalipid	-36.14±0.70	-26.33±0.35	-25.72±0.78
10% Pharmalipid + Addamel-N and OMVI	-	-25.08±0.41	-29.91±0.84*

[a] = mean±SD, n = 3

* = significantly different at at $p \leq 0.05$ compared between A and B of each emulsion

The zeta potential of all TNA kept for 24 hours were likely to decrease except for the commercial TNA, systems with 20% Intralipid® which the zeta potential was rather constant. Addition of trace elements and vitamins in TNA with 10% Pharmalipid at the volume ratio of 3:1 seemed to slightly decrease the values of zeta potential compared to that without trace elements/vitamins at the same volume ratio. Surprisingly, the value of zeta potential of the systems stored for 24 hrs was increased. Such increase might imply higher stability of the emulsions upon adding electrolytes as the higher in electrostatic repulsive forces.

3.3 Nutritional value of total nutrient admixtures

The energy provided by the nutrients in TNA prepared with Vamin®Glucose and from the previous results, the 10% Pharmalipid showed promising properties to be used as lipid emulsion alone or in TNA preparation.

The composition of 1 liter TNA containing 10% Pharmalipid was calculated and tabulated in Table 29. The composition of 1 liter Vitrimix KV® was indicated by Pharmacia & Upjohn.

Table 29. Composition of 1 liter TNA composed of 10% Pharmalipid at the volume ratio of 3:1 Vamin®Glucose to 10% Pharmalipid compared to Vitrimix KV®.

Composition	TNA (10% Pharmalipid)	Vitrimix KV®
	Amount (g/L)[a]	Amount (g/L)[b]
Amino acids	53	53
Glucose (anhydrous)	75	75
Soybean oil	25	50
Soy phospholipids	4.28	3
Glycerol	6.25	5.6
Content of amino acids		
L-Alanine	2.2	2.2
L-Arginine	2.5	2.5
L-Aspartic acid	3.1	3.1
L-Cysteine/L-Cystine	1.1	1.1
L-Glutamic acid	6.8	6.8
Glycine	1.6	1.6
L-Histidine	1.8	1.8
L-Isoleucine	2.9	2.9
L-Leucine	4.0	4.0
L-Lysine	2.9	2.9
L-Methionie	1.4	1.4
L-Phenylalanine	4.1	4.1
L-Proline	6.1	6.1
L-Serline	5.6	5.6
L-Threonine	2.2	2.2
L-Tryptophan	0.75	0.75
L-Tyrosine	0.38	0.38
L-Valine	3.2	3.2
Content of electrolytes		
Sodium	38	38
Potassium	15	15
Calcium	1.9	1.9
Magnesium	1.1	1.1
Chloride	38	38
Sulphate	1.1	1.1
Characteristic	Value[c]	Value[b]
pH	5.03	5.20
Osmolality (mOsm/kg water)	1,080	1,130

[a] = calculated

[b] = from Pharmacia & Upjohn

[c] = from measurement of freshly prepared TNA

Table 30. The calculated energy provided by 1 liter of TNA systems composed of Vamin®Glucose to 20% Intralipid® and Vamin®Glucose to 10% Pharmalipid at the volume ratio of 3:1.

Properties	20% Intralipid®			10% Pharmalipid		
	Ratio of Vamin®Glucose to lipid emulsion					
	2:1	3:1[a]	4:1	2:1	3:1	4:1
Component (mL)						
Vamin®Glucose	667	750	800	667	750	800
Lipid emulsion	333	250	200	333	250	200
Total calories (Cal/L)	1,100	1,000	920	821	778	752
Non-protein calories (Cal/L)	942	800	732	663	600	564
Total nitrogen (g/L)	6.3	7.1	7.5	6.3	7.1	7.5
Ratio of non-protein cal to nitrogen (g)	150:1	114:1	98:1	105:1	84:1	75:1
Energy distribution (%)						
Protein	15	18	20	19	23	25
Fat	60	50	44	47	37	31
Carbohydrate	25	32	36	34	40	44

[a] = commercial TNA system called Vitrimix KV®

The TNA or so called all-in-one product could be administered via either peripheral or central vein. The amount of preparation depended on the requirement of supplementation. The total energy distribution in daily requirement for TPN should come from protein 15-20%, fat not more than 30% and carbohydrate approximate to 50-55% (Bradford, 1996).

Energy provided by 1 liter TNA systems was calculated by summation the energy provided by glucose and amino acid in Vamin®Glucose (4 cal/g) and lipid emulsion (1.16 cal/ml for 10% Pharmalipid and 2 cal/ml for 20% Intralipid®) (Table 30). The TNA systems composed of Vamin®Glucose to 20% Intralipid® at the volume ratio of 3:1 (Vitrimix KV®) had the energy

distribution of 20%, 30% and 50% provided by protein, carbohydrate and fat, respectively. TNA composed of Vamin®Glucose to 10% Pharmalipid at the same volume ratio had the energy distribution of 23%, 40% and 37% from protein, carbohydrate and fat, respectively. It was considered that these preparations provided rather high amount of fat which might cause hyperlipidemia. Alteration the volume ratio of TNA system to 4:1 may be required for decreasing the amount of fat. The present study showed that the volume ratio of 4:1 has the suitable physical stability and physicochemical properties. On the other hand, it should also be considered the ratio of non-protein calories to nitrogen which would imply the utilization of the regimen in that the system which had non-protein calories to nitrogen ratio more than 100:1 was appropriate for non-stress patient while the value equal or less than 100:1 was appropriate for stress patient, i.e., sepsis and hypermetabolic state (Winkler and Manchester, 1996).

CHAPTER V

CONCLUSION

The stable and biocompatible lipid emulsion having particle size with an average diameter of less than 1 μm was considered for the purposes of parenteral nutrition. Lipid emulsions made of natural phospholipids as emulsifiers were sometimes unstable as emulsifying properties was low and mostly dependent upon the purity of lecithin and impurities involved. In the present study, an attempt to improve the emulsification efficiency using synthetic nonionic surfactants normally used for the parenteral formulations.

According to the results, neither soy lecithin nor nonionic surfactants could form stable autoclaved emulsions. The time for producing the coarse emulsion, the homogenization pressure and cycles used in the manufacturing process were not the important factors to produce the stable emulsion using all emulsifiers used in the study. However, the types and concentration of oil and surfactants used seemed to be more critical.

Using only one emulsifier, natural or synthetic nonionic surfactant, the stability of emulsion was poor. It was possible due to a single emulsifier was not effective to reduce interfacial tension of oil or could not form strong film barrier to prevent coalescence. The other reason was the degradation of fatty acid normally in lecithin during autoclaving resulting in less emulsifying properties to form stable film around the oil droplets.

The emulsification process was dependent not only on the capacity of the emulsifier but also the properties of the oil phase. From the results, MCT oil more hardly penetrate into the interfacial film of surfactant to form stable lipid emulsion than the soybean oil.

Using the combination of emulsifiers, soy lecithin as primary emulsifier and synthetic nonionic surfactant as co-emulsifier, a more stable emulsion could be made. The results indicated that the emulsifying properties of soy lecithin were improved with the addition of a suitable type and amount of co-emulsifier. The nonionic surfactant added might possibly increase the steric stabilization of the emulsion system. Tween 80 was found to be more effective than other synthetic nonionic surfactants; namely, Cremophor EL, poloxamer 188 and Solutol HS15. The systems were able to be autoclaved without any instability and were stable upon storage at room temperature. Concentration of co-emulsifier did not affect the pH, zeta potential and osmolality of formulation because of its unionization property. However, the particle sizes tend to become smaller as the concentration of Tween 80 increased. The pH of autoclaved emulsions decreased which was possibly due to the hydrolysis of soy phospholipid. In contrast, the zeta potential became more negative following autoclaving as the increasing of fatty acid concentration and the redistribution of the phospholipid from the aqueous phase. In the present study, the optimum lipid emulsion containing 10% soybean oil was stabilized by 1.71% soy lecithin and 1.29% Tween 80 with 2.5 % glycerol for tonicity adjustment. The emulsion was named “10% Pharmalipid”. The emulsion was stable at least 4 weeks and the physicochemical properties were suitable for parenteral use (pH = 6.83, osmolality = 337 mOsm/kg water, average particle

size = 0.33 μm and zeta potential = -33.52 mV). Providing the energy of 1,162 Cal/L and was further investigated for TNA preparation.

For TNA prepartion, Vamin®Glucose contained amino acid and glucose solutions with some electrolytes were prepared in various volume ratios of Vamin®Glucose to lipid emulsion and were determined for the stability and the change in physicochemical properties at the time interval suggested for administration (24 hours). The results exhibited that TNA could be formed using 10% Pharmalipid and a range of commercial lipid emulsions (10% and 20% Intralipid, 10% and 20% Lipofundin MCT/LCT and 10% Lipofundin-S) at all volume ratios of Vamin®Glucose to lipid emulsion. The finding might provide alternative TNA systems for the patients with different nutrient requirement. The pH of the system was weakly acidic (approximately 5) while the osmolality was hyperosmotic (approximately 975-1,186 mOsm/kg water). The change in particle size of each lipid emulsion with and without Vamin®Glucose were slightly different. However, zeta potential of lipid emulsion with Vamin®Glucose was more obviously change in that some of them showed decreasing values of zeta potentials. The electrolytes present in Vamin®Glucose could neutralize the anionic species of some phospholipids constituted in soy lecithin. The trace elements and vitamins were also investigated and the decrease in zeta potential was found in TNA composed of 20% Intralipid while pH, osmolality and particle size were rather constant.

From the results, the developed lipid emulsion, 10% Pharmalipid, showed a promising stability and properties to use for parenteral administration. However, the presence of other nutrients could obviously change the values of zeta potential which might affect the systems.

Suggestion for further study

The further studies are needed to develop the producing technique and investigate for another sterilization technique for improving long-term stability of 10% Pharmalipid and to determine the chemical composition in emulsion including amount of oil, amount of free fatty acids which occurred during the storage period in lipid emulsion preparation. The amount of macronutrients and micronutrients in TNA should be also investigated. *In vivo* study should be investigated further in the animal in order to determine the possibility to use in human body.

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APPENDICES

APPENDIX A

DETAILS OF SOME SUBSTANCES

1. **Medium chain triglycerides** (Louie and Niemiec, 1986; Wade and Weller, 1994)

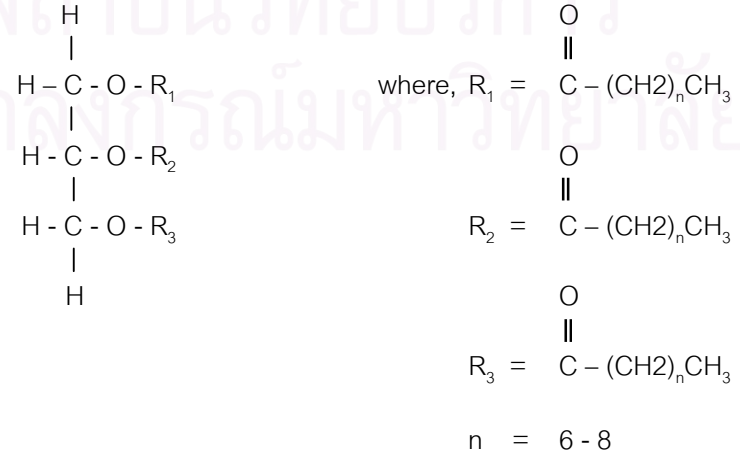
1.1 Chemical name

Medium chain triglycerides

1.2 Empirical formula

Described in the PhEur 1993, medium chain triglycerides are the fixed oil extracted from the hard, dried fraction of the 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 acids, of which not less than 95% are the saturated fatty acids octanoic (caprylic) acid and decanoic (capric) acid.

1.3 Chemical formula



1.4 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.

1.5 Solubility

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

1.6 Typical properties

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

1.7 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%).

1.8 Safety

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

2. Soybean oil (Louie and Niemiec, 1986; Wade and Weller, 1994)

2.1 Chemical name

Soybean oil

2.2 Appearance

Soybean oil is a pale yellow colored, odorless or almost odorless liquid, with a bland taste.

2.3 Solubility

Soybean oil is practically insoluble in ethanol (95%) and water, miscible with carbon disulfide, chloroform, ether and petroleum spirit (boiling range 40-60^{o}C)

2.4 Typical properties

Autoignition temperature	: 445^{o}C
Density	: 0.916-0.922 g/cm^{3} at 25^{o}C
Energy provide	: 9 Cal/g
Flash point	: 282^{o}C
Freezing point	: -10 to -16^{o}C
Interfacial tension	: 50 mN/m at 20^{o}C
Surface tension	: 25 mN/m at 20^{o}C
Viscosity	: 50.09 mPa s at 25^{o}C

2.5 Purity

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

2.6 Safety

Soybean oil is widely used intramuscularly as a drug vehicle, or as a component of emulsions used in parenteral nutrition regimens; it is also consumed as an edible oil. Generally, soybean oil is regarded as an essentially nontoxic and nonirritant material. However, serious adverse reactions of soybean oil emulsions administered parenterally have been reported. These conclude cases of hypersensitivity, CNS reactions and fat embolism.

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

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

3. Poloxamer 188 (Wade and Weller, 1994)

3.1 Chemical name

α-hydro-ω-hydroxypoly-(oxyethylene)-poly-(oxypropylene)-poly-(oxyethylene) block copolymer.

3.2 Molecular weight

7,680 – 9,510 g/mole

3.3 Chemical structure

$$HO(C_2H_4O)_a(\underset{CH_3}{\underset{|}{CH}}-CH_2O)_b(CH_2CH_2O)_a H$$

Where *a* in the general formula given above average value of 75 and *b* given the average value of 30

3.4 Appearance

Poloxamer 188 is a white, waxy, free-flowing prilled granules or flakes; practically tasteless and odorless.

3.5 Solubility

Poloxamer 188 is soluble in water, dilute acids and ethyl alcohol; slightly soluble in toluene and xylene; insoluble in propylene glycol, perchloroethylene, glycerin, mineral oil, vegetable oil, and liquid paraffin.

3.6 Typical properties

Antimicrobial action	: supports mold growth in aqueous solution.
Cloud point	: more than 100^{o}C (in 1 and 10% solution).
HLB value	: about 29
Interfacial tension	: 0.1% = 19.8 mN/m at 25^{o}C
	0.01% = 24.0 mN/m at 25^{o}C
Melting point	: 52^{o}C
pH	: between 6.0 to 7.4 (2.5% w/v)
Specific gravity	: about 1.06 g/cm^3 at 25^{o}C

Surface tension : 0.1% = 50.3 mN/m at 25°C

0.01% = 51.2 mN/m at 25°C

Viscosity : 1,000 mPa s at 77°C as a melt

3.7 Safety

Poloxamer 188 can be used in parenteral preparations, which generally nontoxic and nonirritant substance. Poloxamer 188 is not metabolized in the body.

LD_{50} (mice,IV): 5.5 g/kg at 5% solution

LD_{50} (rats,IV): 3.95 g/kg at 5% solution

There is no hemolysis effect on human blood cells observed over 18 hours at 25°C when tested with poloxamer 188 at the concentration of 0.001% to 10% w/v.

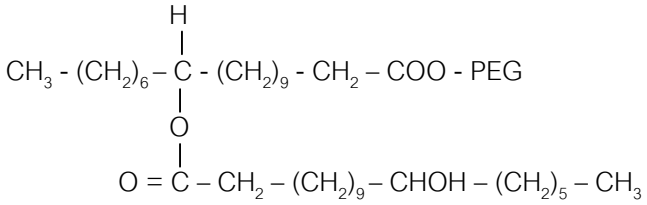
4. Polyethylene glycol 660 12- hydroxystearate (Solutol® HS15) (Wade and Weller, 1994)

4.1 Chemical name

Polyethylene glycol-660-(12)- hydroxystearate

4.2 Chemical structure

Apart from free polyethylene glycol and its mono-esters, di-esters are also detectable. NMR analysis has provided clues to one possible structure of the di-ester.



4.3 Composition

Solutol®HS15 is composed of poly glycolester of 12-hydroxystrearic acid (70%) as hydrophobic part and polyethylene glycol (30%) as hydrophilic part.

4.4 Appearance

This substance is a white-yellowish paste at room temperature that becomes liquid at 30°C.

4.5 Solubility

Solutol®HS15 dissolves in water, ethanol and 2-propanol to form clear solutions.

4.6 Typical properties

Solidification point	: 25-30°C
Saponification number	: 53-63
pH (10% in water)	: 6-7

4.7 Safety

The acute toxicity data are determined on different species of animals.

LD_{50} (mouse, IV)	: >= 3.16 ml/kg

LD_{50} (rabbit, IV) : > 1.0 g/kg, < 1.4 g/kg

LD_{50} (dog, IV) : >= 3.1 g/kg

LD_{50} (rat, IV) : > 1.0 g/kg, < 1.47 g/kg

5. Polyoxyl 35 castor oil (Cremophor® EL) (Wade and Weller, 1994)

5.1 Chemical name

Polyoxyl 35 castor oil; Polyoxyethylene glycerol triricinoleat 35

5.2 Empirical formula

Polyoxyl 35 castor oil has the hydrophobic constituents comprised of about 83% of the total mixture. The main component is glycerol polyethylene glycol ricinoleate. Other hydrophobic constituents include fatty acid esters of polyethylene glycol along with some unchanged castor oil. The hydrophilic part (17%) consists of polyethylene glycols and glycerol ethoxylates.

5.3 Appearance

Cremophor® EL is a pale yellow, oily liquid that is clear at temperatures above 30°C. It has a slight but characteristic odor and can be completely liquefied by heating to 26°C.

5.4 Solubility

Cremophor® EL forms clear solutions in water. It is also soluble in ethyl alcohol, n-propyl alcohol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene.

5.5 Typical properties

Cloud point	: 72.5°C (at 1% solution)
Density	: 1.05-1.06 g/cm^3 at 25°C
HLB value	: 12-14
pH value	: 6-8 (10% aqueous solution)
Melting point	: residual solids liquefy at 19-20°C

5.6 Safety

There have been reports of anaphylactic reactions in animals and humans after parenteral administration of pharmaceutical products containing Cremophor® EL.

6. Soy lecithin (Louie and Niemiec, 1986; Wade and Weller, 1994)

6.1 Chemical name

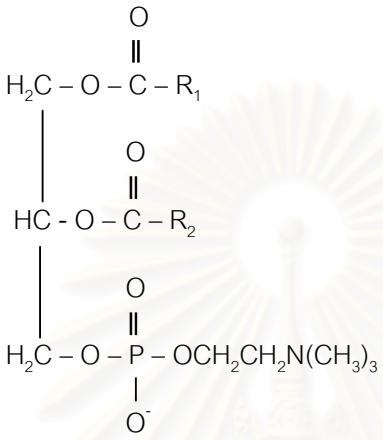
The chemical nomenclature and CAS registry numbering of lecithin is complex. The commercially available lecithin is used in cosmetics, pharmaceuticals and food products. Complex mixture of phospholipids and other material may be referred to some literature sources as 1,2-diacyl-*sn*-glycero-3-phosphocholine (phosphatidylcholine). This material is the principal constituent of soy lecithin and has the same CAS registry number.

6.2 Molecular formula

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidylcholine, phosphatidylethanolamine,

phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids and carbohydrates.

6.3 Chemical structure



Where, R_1 and R_2 are fatty acid which may be different or identical

α - Phosphatidylcholine

The structure above shows phosphatidylcholine, the principal component of soy lecithin, in its α - form. In the β - form the phosphorus containing group and the R_2 group exchange positions.

6.4 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.

6.5 Solubility

Lecithin is soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbon, mineral oil and fatty acids. It is practically insoluble in cold vegetable and animal oils, polar solvents and water.

6.6 Typical properties

Energy provide : 9 Cal/g

HLB : approximately 7

Isoelectric point : approximately 3.5

pH : approximately 6.6

6.7 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 doses of up to 80 g per day have been used therapeutically in the treatment of tardive dyskinesia. It has been accepted as an additive in parenteral preparations.

7. Tween80 (Wade and Weller, 1994)

7.1 Chemical name

Polyoxyethylene 20 sorbitan monooleate

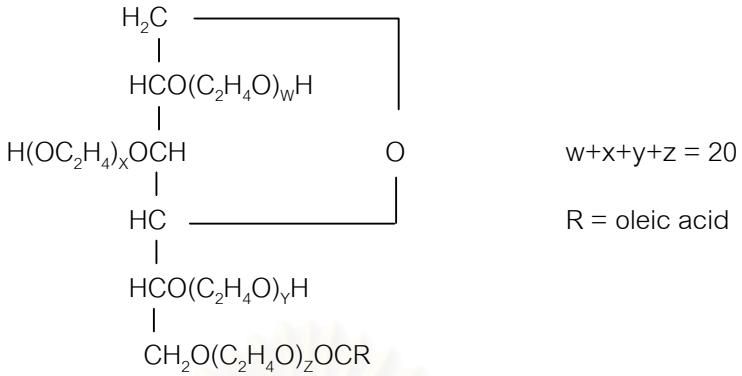
7.2 Molecular formula

$C_{64}H_{124}O_{26}$

7.3 Molecular weight

1310 g/mole

7.4 Chemical structure



7.5 Appearance

Tween80 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

7.6 Solubility

Tween80 is miscible with water, alcohol, dehydrate alcohol, ethyl acetate, and methyl alcohol; practically insoluble in liquid paraffin and fixed oils.

7.7 Safety

Tween80 is widely used in cosmetics, food products, parenteral and topical pharmaceutical formulations and is generally well tolerated, practically non-irritating and of very low toxicity. The WHO has set an estimated acceptable daily intake for tween80, calculated as total polysorbate esters, at up to 25 mg/kg.

8. Glycerin (Louie and Niemiec, 1986; Wade and Weller, 1994)

8.1 Chemical name

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

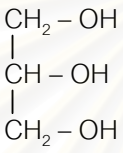
8.2 Molecular formula

$C_3H_8O_3$

8.3 Molecular weight

92.09 g/mole

8.4 Chemical structure



8.5 Appearance

Glycerin is a clear, colorless, odorless, syrupy and hygroscopic liquid

8.6 Solubility

Glycerin is miscible with water, alcohol and methanol. One part of glycerin dissolves 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.

8.7 Typical properties

Energy provide	: 4.32 Cal/g
Melting point	: 17.9^{o}C
Hygroscopicity	: medium to high
Relative density	: 1.258-1.263 g/cm^3 at 25^{o}C

Surface tension	: 63.4 mN/m at 20°C
Viscosity	: 1,490 mPa s at 20°C
	954 mPa s at 25°C
Osmolarity	: 2.6% v/v solution is iso-osmotic with serum

8.8 Safety

Glycerin in 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.

APPENDIX B

Commercial products for parenteral nutrition

Table b1. Dextrose solution

Trade name	Concentration of dextrose (% w/v)	Caloric density (Cal/L)	Osmolarity (mOsm/L)
D5W	5	170	253
D10W	10	340	505
D15W	15	510	758
D20W	20	680	1010
D25W	25	850	1330
D50W	50	1700	2525

Dextrose monohydrate provide energy 3.4 Cal/g

Table b2. Fat emulsions

Composition	Intralipid (Pharmacia)		Lipofundin-S (B Braun)	Lipofundin MCT/LCT (B Braun)		Lipovenos (Fresenius)	
	10%	20%	10%	10%	20%	10%	20%
Soybean oil (g/L)	100	200	100	50	100	100	200
MCT oil (g/L)	-	-	-	50	100	-	-
Egg lecithin (g/L)	12	12	-	7.5	12	12	12
Soy lecithin (g/L)	-	-	7.5	-	-	-	-
Glycerol (g/L)	22	22	-	25	25	25	25
Xylitol (g/L)	-	-	25	-	-	-	-
α -tocopherol (mg/L)	-	-	-	85$\pm$20	170$\pm$40	-	-
pH	7.5	7.5	7.8	6.5-8.5	6.5-8.5	7-8.5	7-8.7
Osmolarity (mOsm/L)	300	350	340	345	380	310	360
Calories (Cal/L)	1100	2000	1068	1022	1908	1100	2000
Particle size (μm)	< 1	< 1	0.3	0.3-0.4	0.3-0.4	< 1	< 1
Stroage ($^{\circ}$C)	2-8	2-8	< 25	< 25	< 25	2-8	2-8

Soybean oil provides energy 9 Cal/g; MCT oil provides energy 8.2-8.4 Cal/g

Table b3. Composition of (1,000 ml) Vamin®Glucose composed of 7% amino acid, 10% dextrose and some electrolytes

Vamin®Glucose	
Content of amino acids[a] and glucose	Amount (g/L)
L-Alanine	3.0
L-Arginine	3.3
L-Aspartic acid	4.1
L-Cysteine/L-Cystine	1.4
L-Glutamic acid	9.0
Glycine	2.1
L-Histidine	2.4
L-Isoleucine	3.9
L-Leucine	5.3
L-Lysine	3.9
L-Methionie	1.9
L-Phenylalanine	5.5
L-Proline	8.1
L-Serline	7.5
L-Threonine	3.0
L-Tryptophan	1.0
L-Tyrosine	0.5
L-Valine	4.3
Total nitrogen	9.4
Glucose anhydrous	100
Content of electrolytes	Amount (m mol/L)
Sodium	50
Potassium	20
Calcium	2.5
Magnesium	1.5
Chloride	55
Characteristic	Value
pH	5.2
Osmolarity (mOsm/L)	1.350
Calories (Cal/L))	650

[a] = composed of 41% essential amino acid, 19.2% branched chain amino acid and 9.3% aromatic amino acid

Table b4. Amino acid solutions

Composition	Aminoleban (Ostsuka) 8%	Aminofunfin (Pharmacia) L300 2.5%	Aminofunfin (Pharmacia) L600 5%	Aminoplasma (Berli Jucker) L-5	Aminoplasma (Berli Jucker) L-10	Aminosol (Ostsuka) 5%	Aminosol (Ostsuka) 10%	Amiparen (Ostsuka) 5%	Amiparen (Ostsuka) 10%	Aminosterile KE (Fresenius) 10%	Aminosterile N-HEPA (Fresenius) 8%
Total amino acid (g/L)	79.9	25.3	50.5	51.5	103.0	50	100	52.1	104.3	100	80
% Essential AA	54.1	26.9	26.7	39.4	39.4	45.6	45.6	60.3	60.8	41.0	64.6
% Branched chain AA	35.5	10.5	10.4	18.3	18.3	18.8	18.8	28.8	28.8		42.0
% Aromatic AA	2.1	5.3	5.3	6.7	6.7	7.2	7.2	8.6	8.6		(Fischer's ratio
Total nitrogen (g/L)	12.1	3.8	7.6	8.0	16.0	8.0	16.0	15.7	15.7		50.0)[a]
Carbohydrate (g/L)											
Sorbitol	-	25	50	100	100	100	100	-	-	-	-
Xylitol	-	25	50	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-	-	-	-
Electrolyte											
Sodium (mEq/L)	14	40.5	40	47	47	48	48	1	2	-	-
Potassium (mEq/L)	-	30	30	25	25	25	25	-	-	-	-
Chloride (mEq/L)	94	7	14	31	62	31	62	-	-	-	-
Phosphate (mM/L)	-	-	-	9	9	9	9	-	-	-	-
Calcium (mEq/L)	-	-	-	-	-	-	-	-	-	-	-
Magnesium (mEq/L)	-	10	10	2.6	2.6	5	5	-	-	-	-
Acetate (mEq/L)	-	10	10	59	59	59	59	60	120	-	-
Malate (mM/L)	-	22.5	15	7.5	7.5	7.5	7.5	-	-	8.95	48
pH	5.8	7.3	-	-		-	6-6.5	-	-	-	-
Osmolarity (mOsm/L)	886	600	1100	1140	1590	1140	1590	-	-	1006	770
Calories (Cal/L)	320	300	600	600	800	600	800	200	400	400	328

[a] Fisher's ratio = Molar ratio BCAA/Tyrosine + Phynylalanine

Essential amino acid: L-Leucine*, L-Isoleucine*, L-Lysine, L-Methionine, L-Phynylalanine**, L-Threonine, L-Valine*, L-Tryptophan**

Non essential amino acid: L-Alanine, L-Arginine, L-Aspartic acid, L-Cysteine, Glycine, Glutamic acid, L-Histidine, L-Proline, L-Serline,L-Tyrosine**

(* Branched chain amino acid; ** Aromatic amino acid)

Table b5. Vitamin solutions

Vitamins	OMVI[a] 1&2 Ostsuka (4 ml)	Pancebrin Eli Lilly (10 ml)	Soluvit N Pharmacia (10 ml)	Vitalipid N adult Pharmacia (10 ml)	Metaplex Takeda (10 ml)
A (retonol) (IU)	3300	5000	-	3300	-
D (cholecalciferol) (IU)	200	500	-	200	-
E (dl-α -tocopherol) (IU)	10	1	-	10	-
K (phytomenadione) (mg)	2	-	-	0.15	-
B_1 (thiamine) (mg)	3	5	3.2	-	100
B_2 (riboflavin) (mg)	3.6	1	3.6	-	5
Niacin (mg)	40	10	40	-	50
Pantothenic acid (mg)	15	1.5	15	-	5
B_6 (pyridoxine) (mg)	4	1.5	4	-	5
B_{12} (cyanocobalamine) (μg)	5	-	5	-	-
Biotin (μg)	60	-	60	-	-
Folate (μg)	400	-	400	-	0.5
C (ascorbic acid) (mg)	100	30	100	-	-

[a] OMVI composed of 2 formulations: Formulation 1 = water soluble vitamins (lyophilized powder)
Formulation 2 = fat soluble vitamins (solution)

Table b6. Trace element solution

Components	Addamel N Pharmacia (10 ml)	Ped-El Pharmacia (20 ml)
Zn (μg)	650	9.75
Cu (μg)	130	4.9
Fe (μg)	112	28
Mn (μg)	27.5	13.75
Cr (μg)	1.04	-
Se (μg)	3.16	-
Mo (μg)	1.92	-
F (μg)	95	14.25
I (μg)	12.7	1.27
Ca^{2+} (mg)*	-	6
Mg^{2+} (mg)*	-	0.6
$H_2PO_4^-$ (mg)*	-	2.3
Cl^- (mg)*	-	12.4

* = macroelement

Table b7. The nutritional value and energy provided from 1 liter of TNA systems composed of Vamin®Glucose and 10% and 20% lipid emulsion at different volume ratios

Lipid emulsion (LE)	Ratio of	Component (ml)		Energy distribution (%)			Total Calories	Non-protein	Total nitrogen	Ratio of non-protein
	VG to LE	VG[a]	LE	AA[b]	Dex[c]	Fat	(Cal/L)	calories (Cal/L)	(g/L)	calories to nitrogen (g)
10% Intralipid®	2:1	667	333	20	46	34	800	642	6.3	102:1
	3:1	750	250	23	36	41	763	585	7.1	82:1
	4:1	800	200	25	30	45	740	552	7.5	74:1
10% Lipofundin®MCT/LCT	2:1	667	333	20	45	35	774	616	6.3	98:1
	3:1	750	250	24	36	40	743	565	7.1	80:1
	4:1	800	200	26	29	45	724	536	7.5	71:1
10% Lipofundin-S®	2:1	667	333	20	45	35	789	631	6.3	100:1
	3:1	750	250	24	35	41	755	577	7.1	81:1
	4:1	800	200	26	29	45	734	546	7.5	73:1
10% Pharmalipid	2:1	667	333	19	47	34	821	663	6.3	105:1
	3:1	750	250	23	37	40	778	600	7.1	84:1
	4:1	800	200	25	31	44	752	564	7.5	75:1
20% Intralipid®	2:1	667	333	15	60	25	1100	942	6.3	150:1
	3:1	750	250	18	50	32	1000	800	7.1	114:1
	4:1	800	200	20	44	36	920	732	7.5	98:1
20% Lipofundin MCT/LCT®	2:1	667	333	15	59	26	1069	911	6.3	145:1
	3:1	750	250	18	49	33	965	787	7.1	111:1
	4:1	800	200	21	42	37	902	714	7.5	95:1

[a] VG = Vamin®Glucose; [b] AA = amino acids; [c] Dex = dextrose

APPENDIX C

Particle size determination of lipid emulsion

The particle size of lipid emulsion was determined by Mastersizer S. It is a range of laser scattering based particle sizers (Mastersizer particle size analyzer, Instrumental manual). The results reported by them are a number of fundamental concepts as:

- The result is volume based.
- The result is expressed in terms of equivalent spheres.
- The analyzed distribution is a set of size classes which the representative diameter ($\bar{d}$) for each class is taken to be the geometric mean of the size band limits:

$$\bar{d} = \sqrt{d_{i-1} d_i}$$

The result from the analysis is the relative distribution of volume of particles in the range of size classes. From this basic result the statistics of the distribution are calculated. Moreover, the span and uniformity are calculated for describing the distribution of the particles. The span gives a description of the width of the distribution which is independent of the median size. The uniformity is a measure of the absolute deviations from the median.

The derived diameters are defined as:

$$D\,[m, n] = \left[\frac{\sum V_i d_i^{m-3}}{\sum V d_i^{n-3}} \right]^{\frac{1}{m-n}}$$

Where

V_i is the relative volume in class *i* with mean class diameter of d_i.

m and *n* are integer values which describe the type of derived diameter.

d[4,3] is the volume weighted mean.

d[3,2] is the surface weight mean.

d[v,0.1] is the volume at the 10^{th} percentile of particle.

d[v,0.5] is the volume at the 50^{th} percentile of particle.

d[v,0.9] is the volume at the 90^{th} percentile of particle.

The results of determination of lipid emulsion and TNA systems are in Tables c1-c5 and Figures c1-c102. These data were average from three determinations.

The observation of the formulations in all tables and figures stands for the composition used in the systems. For example, formulation 5SB+1LE+1T80 refer to formulation containing 5% soybean oil emulsified with 1% soy lecithin and 1% Tween 80. Formulation 5MCT+1LE+1P188 refer to formulation containing 5% MCT oil emulsified with 1% soy lecithin and 1% poloxamer 188.

Table c1. Particle size of lipid emulsion containing soybean oil and various type and amount of emulsifiers before and after autoclaving

Formulation		Volume particle size (mcm)									Figure[a]
		d(V,0.1)			d(V,0.5)			d(V,0.9)			
		sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
5SB+1LE+1T80	a)	0.20	0.20	0.24	0.31	0.30	0.32	0.51	0.49	0.49	c1
	b)	0.21	0.22	0.23	0.31	0.33	0.33	0.57	0.47	0.55	c2
	c)	0.14	0.20	0.18	0.34	0.31	0.30	0.57	0.58	0.53	c3
5SB+1LE+1P188	a)	0.22	0.25	0.24	0.28	0.31	0.31	0.37	0.42	0.45	c4
	b)	0.19	0.17	0.19	0.30	0.33	0.33	0.59	0.53	0.59	c5
	c)	0.20	0.20	0.19	0.35	0.35	0.34	0.62	0.62	0.61	c6
	d)	0.21	0.21	0.21	0.34	0.34	0.34	0.57	0.57	0.57	c7
5SB+2LE+1T80	a)	0.25	0.25	0.25	0.32	0.33	0.33	0.49	0.47	0.52	c8
	b)	0.13	0.18	0.13	0.26	0.24	0.23	0.34	0.34	0.32	c9
	c)	0.18	0.19	0.15	0.30	0.28	0.29	0.47	0.48	0.49	c10
	d)	0.21	0.21	0.21	0.33	0.33	0.33	0.52	0.50	0.50	c11
5SB+2LE+1P188	a)	0.19	0.21	0.20	0.31	0.31	0.30	0.50	0.48	0.48	c12
	b)	0.24	0.23	0.24	0.30	0.30	0.30	0.39	0.42	0.40	c13
	c)	0.20	0.22	0.20	0.32	0.32	0.34	0.51	0.54	0.51	c14
	d)	0.23	0.22	0.22	0.34	0.33	0.33	0.55	0.50	0.50	c15
10SB+1LE+1T80	a)	0.21	0.18	0.18	0.32	0.31	0.32	0.48	0.51	0.54	c16
	b)	0.18	0.21	0.20	0.33	0.33	0.32	0.52	0.52	0.55	c17
10SB+2LE+1T80	a)	0.21	0.20	0.18	0.32	0.32	0.31	0.50	0.49	0.53	c18
	b)	0.20	0.21	0.22	0.32	0.33	0.35	0.50	0.55	0.53	c19
	c)	0.20	0.20	0.21	0.33	0.34	0.34	0.58	0.55	0.60	c20
10SB+2LE+1P188	a)	0.20	0.21	0.21	0.32	0.33	0.33	0.51	0.55	0.55	c21
	b)	0.22	0.22	0.22	0.33	0.33	0.33	0.52	0.52	0.52	c22
	c)	0.21	0.21	0.21	0.34	0.34	0.32	0.51	0.54	0.54	c23
20SB+2LE+1T80	a)	0.19	0.19	0.20	0.33	0.33	0.34	0.57	0.58	0.61	c24
	b)	0.21	0.20	0.21	0.33	0.33	0.34	0.58	0.58	0.60	c25

[a] = d(v,0.5) of sample 1

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature

Table c1 (cont.). Particle size of lipid emulsion containing soybean oil and various type and amount of emulsifiers before and after autoclaving

Formulation		Volume particle size (mcm)									Figure[a]
		d(V,0.1)			d(V,0.5)			d(V,0.9)			
		sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
10SB+2LE+1.5T80	a)	0.17	0.18	0.11	0.31	0.30	0.25	0.51	0.50	0.49	c26
	b)	0.22	0.19	0.20	0.33	0.31	0.30	0.51	0.50	0.51	c27
	c)	0.21	0.22	0.19	0.33	0.34	0.31	0.52	0.56	0.53	c28
	d)	0.20	0.23	0.19	0.33	0.30	0.30	0.48	0.51	0.48	c29
10SB+2LE+2T80	a)	0.21	0.23	0.20	0.33	0.33	0.32	0.50	0.52	0.52	c30
	b)	0.21	0.18	0.16	0.31	0.30	0.30	0.46	0.49	0.55	c31
	c)	0.20	0.21	0.21	0.31	0.32	0.34	0.51	0.46	0.53	c32
10SB+1.71LE+1.29T80	a)	0.15	0.19	0.14	0.33	0.31	0.30	0.60	0.56	0.59	c33
	b)	0.21	0.21	0.21	0.33	0.33	0.34	0.57	0.53	0.58	c34
	c)	0.20	0.21	0.20	0.33	0.34	0.35	0.58	0.56	0.55	c35
	d)	0.20	0.20	0.23	0.34	0.35	0.33	0.62	0.56	0.59	c36
10SB+2.29LE+1.71T80	a)	0.19	0.17	0.14	0.31	0.31	0.30	0.51	0.53	0.56	c37
	b)	0.21	0.22	0.22	0.33	0.32	0.32	0.52	0.49	0.49	c38
	c)	0.20	0.19	0.19	0.31	0.32	0.32	0.52	0.55	0.53	c39
10SB+2.86LE+2.14T80	a)	0.10	0.10	0.10	0.23	0.23	0.24	0.48	0.49	0.50	c40
	b)	0.15	0.18	0.19	0.27	0.28	0.30	0.49	0.51	0.47	c41
	c)	0.21	0.20	0.20	0.32	0.31	0.32	0.47	0.48	0.51	c42

[a] = d(v,0.5) of sample 1

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature

Table c2. Particle size of lipid emulsion containing MCT oil and various type and amount of emulsifiers before and after autoclaving

Formulation		Volume particle size (mcm)									Figure[a]
		d(V,0.1)			d(V,0.5)			d(V,0.9)			
		sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
5MCT+1LE+1T80	a)	0.12	0.14	0.11	0.28	0.25	0.27	0.52	0.52	0.49	c43
	b)	0.16	0.16	0.17	0.30	0.31	0.32	0.57	0.54	0.56	c44
	c)	0.18	0.15	0.17	0.30	0.29	0.32	0.50	0.53	0.57	c45
	d)	0.17	0.18	0.17	0.30	0.31	0.30	0.55	0.56	0.54	c46
	e)	0.16	0.17	0.16	0.32	0.31	0.31	0.53	0.55	0.54	c47
	f)	0.08	0.08	0.08	0.19	0.18	0.18	0.39	0.39	0.38	c48
	g)	0.18	0.19	0.16	0.35	0.34	0.30	0.64	0.65	0.56	c49
5MCT+1LE+1P188	a)	0.20	0.20	0.20	0.33	0.33	0.33	0.59	0.57	0.58	c50
	b)	0.23	0.25	0.23	0.33	0.34	0.33	0.52	0.51	0.52	c51
	c)	0.23	0.22	0.22	0.30	0.30	0.29	0.41	0.40	0.40	c52
	d)	0.22	0.22	0.21	0.29	0.28	0.29	0.40	0.38	0.38	c53

[a] = d(v,0.5) of sample 1

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature, e): after autoclaving and storage for 2 months at room temperature, f): after autoclaving and storage for 3 month at room temperature, g): after autoclaving and storage in accelerated condition

Table c3. Particle size of 10% and 20% commercial lipid emulsion and 10% Pharmalipid

Formulation	Volume particle size (mcm)									Figure[a]
	d(V,0.1)			d(V,0.5)			d(V,0.9)			
	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
10% Intralipid	0.24	0.24	0.24	0.32	0.32	0.32	0.44	0.44	0.44	c54
10% Lipofundin MCT/LCT	0.20	0.20	0.21	0.29	0.29	0.29	0.39	0.39	0.39	c55
10% Lipofundi-S	0.23	0.22	0.22	0.30	0.29	0.29	0.41	0.40	0.40	c56
10% Pharmalipid	0.21	0.17	0.17	0.30	0.30	0.31	0.44	0.50	0.53	c57
20% Intralipid	0.23	0.24	0.22	0.34	0.34	0.32	0.56	0.54	0.51	c58
20% Lipofundin MCT/LCT	0.23	0.23	0.23	0.30	0.30	0.30	0.41	0.41	0.41	c59

[a] = d(v,0.5) of sample 1

Table c4. Particle size of TNA system at 0 and 24 hour at room temperature

Formulation at volume ratios of VG to LE		Volume particle size (mcm)									Figure[a]
		d(V,0.1)			d(V,0.5)			d(V,0.9)			
		sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
VG:10% Intralipid = 2:1	h)	0.22	0.23	0.22	0.34	0.32	0.34	0.51	0.50	0.51	c60
	I)	0.21	0.23	0.23	0.32	0.33	0.33	0.52	0.51	0.51	c61
VG:10% Intralipid = 3:1	h)	0.23	0.23	0.23	0.33	0.33	0.33	0.50	0.50	0.50	c62
	I)	0.21	0.21	0.22	0.32	0.33	0.33	0.53	0.54	0.52	c63
VG:10% Intralipid = 4:1	h)	0.23	0.23	0.23	0.32	0.33	0.33	0.49	0.50	0.50	c64
	I)	0.22	0.22	0.23	0.33	0.33	0.33	0.53	0.54	0.52	c65
VG:10% Lipofundin MCT/LCT = 2:1	h)	0.22	0.22	0.22	0.29	0.30	0.29	0.39	0.40	0.39	c66
	I)	0.22	0.22	0.22	0.29	0.29	0.29	0.39	0.39	0.40	c67
VG:10% Lipofundin MCT/LCT = 3:1	h)	0.22	0.22	0.22	0.30	0.30	0.30	0.40	0.40	0.40	c68
	I)	0.23	0.21	0.21	0.30	0.29	0.28	0.40	0.39	0.36	c69
VG:10% Lipofundin MCT/LCT = 4:1	h)	0.22	0.21	0.21	0.29	0.29	0.29	0.40	0.39	0.39	c70
	I)	0.23	0.23	0.22	0.30	0.30	0.29	0.40	0.40	0.40	c71
VG:10% Lipofundin-S = 2:1	h)	0.23	0.23	0.23	0.33	0.33	0.32	0.48	0.48	0.49	c72
	I)	0.22	0.22	0.24	0.33	0.33	0.35	0.55	0.55	0.58	c73
VG:10% Lipofundin-S = 3:1	h)	0.23	0.23	0.23	0.33	0.33	0.33	0.51	0.51	0.51	c74
	I)	0.22	0.22	0.22	0.34	0.34	0.34	0.58	0.58	0.58	c75
VG:10% Lipofundin-S = 4:1	h)	0.23	0.23	0.23	0.34	0.34	0.34	0.54	0.54	0.54	c76
	I)	0.22	0.22	0.22	0.34	0.34	0.34	0.59	0.59	0.59	c77
VG:10% Pharmalipid = 2:1	h)	0.17	0.16	0.19	0.29	0.29	0.30	0.49	0.50	0.50	c78
	I)	0.22	0.19	0.17	0.32	0.32	0.30	0.47	0.54	0.51	c79
VG:10% Pharmalipid = 3:1	h)	0.19	0.23	0.17	0.31	0.32	0.30	0.49	0.47	0.51	c80
	I)	0.21	0.22	0.21	0.33	0.32	0.33	0.55	0.50	0.54	c81
VG:10% Pharmalipid = 3:1(++)	h)	0.17	0.23	0.14	0.29	0.32	0.27	0.48	0.50	0.47	c82
	I)	0.22	0.21	0.16	0.33	0.34	0.29	0.53	0.53	0.49	c83
VG:10% Pharmalipid = 4:1	h)	0.24	0.23	0.22	0.32	0.32	0.31	0.50	0.50	0.50	c84
	I)	0.24	0.21	0.21	0.33	0.33	0.32	0.46	0.51	0.51	c85

[a] = d(v,0.5) of sample 1
VG = Vamin® Glucose
(++) = added Addamel_N and OMVI
h): immediately after mixing (0 hour), I): 24 hours

Table c4 (cont.). Particle size of TNA system at 0 and 24 hours at room temperature

Formulation at volume ratios of VG to LE		Volume particle size (mcm)									Figure[a]
		d(V,0.1)			d(V,0.5)			d(V,0.9)			
		sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
VG:20% Intralipid = 2:1	h)	0.22	0.22	0.22	0.33	0.33	0.33	0.56	0.53	0.53	c86
	I)	0.21	0.21	0.23	0.33	0.33	0.35	0.59	0.60	0.60	c87
VG:20% Intralipid = 3:1	h)	0.21	0.22	0.22	0.33	0.32	0.32	0.55	0.52	0.52	c88
	I)	0.18	0.20	0.20	0.31	0.32	0.32	0.55	0.56	0.56	c89
VG:20% Intralipid = 3:1 (++)	h)	0.20	0.19	0.22	0.33	0.31	0.32	0.57	0.51	0.48	c90
	I)	0.20	0.19	0.19	0.33	0.31	0.32	0.57	0.52	0.55	c91
VG:20% Intralipid = 4:1	h)	0.22	0.21	0.21	0.32	0.32	0.32	0.52	0.52	0.52	c92
	I)	0.21	0.22	0.21	0.33	0.33	0.33	0.56	0.56	0.56	c93
VG:20% Lipofundin MCT/LCT = 2:1	h)	0.24	0.24	0.24	0.31	0.31	0.31	0.41	0.41	0.41	c94
	I)	0.24	0.24	0.24	0.31	0.31	0.31	0.41	0.41	0.41	c95
VG:20% Lipofundin MCT/LCT = 3:1	h)	0.24	0.24	0.24	0.31	0.31	0.31	0.41	0.41	0.42	c96
	I)	0.24	0.24	0.24	0.31	0.31	0.31	0.41	0.41	0.41	c97
VG:20% Lipofundin MCT/LCT = 4:1	h)	0.24	0.24	0.24	0.31	0.31	0.31	0.41	0.41	0.41	c98
	I)	0.25	0.25	0.25	0.32	0.32	0.32	0.42	0.42	0.42	c99

[a] = d(v,0.5) of sample 1
VG = Vamin®Glucose
(++) = added Addamel_N and OMVI
h): immediately after mixing (0 hour), I): after mixing for 24 hours

Table c5. Particle size of observed cream layer of TNA stored in refrigerator compose of Vamin®Glucose and 20% Intralipid® at volume ratio of 2:1, 3:1 and 4:1 after 24 hours storage

Formulation at volume ratios of VG to LE	Volume particle size (mcm)									Figure[a]
	d(V,0.1)			d(V,0.5)			d(V,0.9)			
	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	
VG:20% Intralipid = 2:1	0.22	0.22	0.22	0.34	0.34	0.34	0.57	0.57	0.57	c100
VG:20% Intralipid = 3:1	0.22	0.22	0.22	0.34	0.34	0.35	0.59	0.59	0.62	c101
VG:20% Intralipid = 4:1	0.24	0.22	0.22	0.37	0.35	0.35	0.64	0.62	0.62	c102

[a] = d(v,0.5) of sample 1

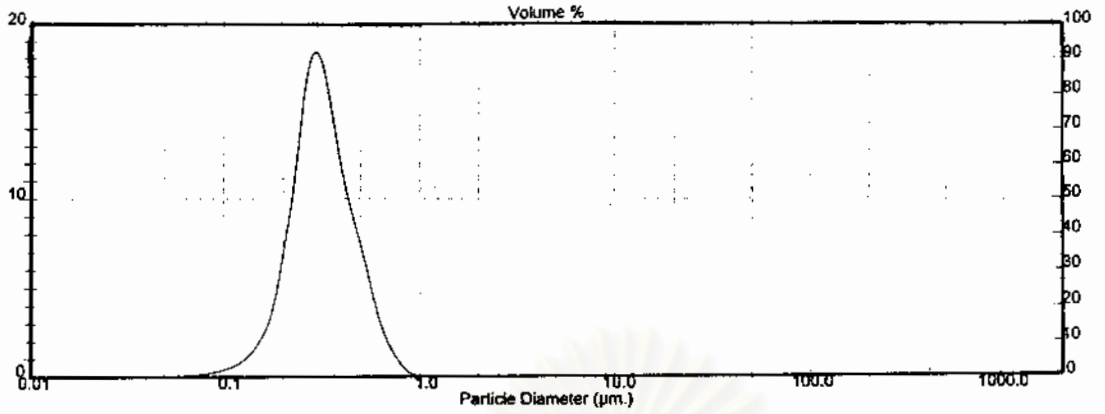


Figure c1. Particle size distribution of formulation 5SB+1LE+1T80 before autoclaving

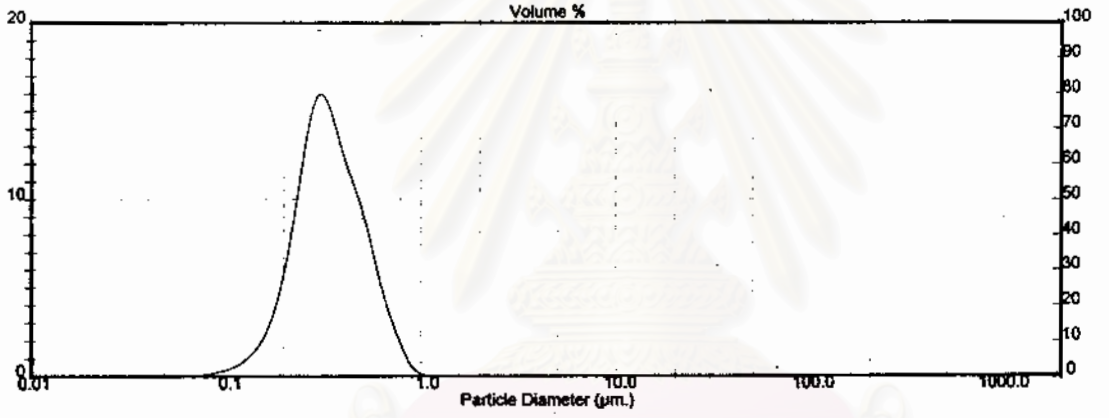


Figure c2. Particle size distribution of formulation 5SB+1LE+1T80 after autoclaving and storage for 24 hours at room temperature

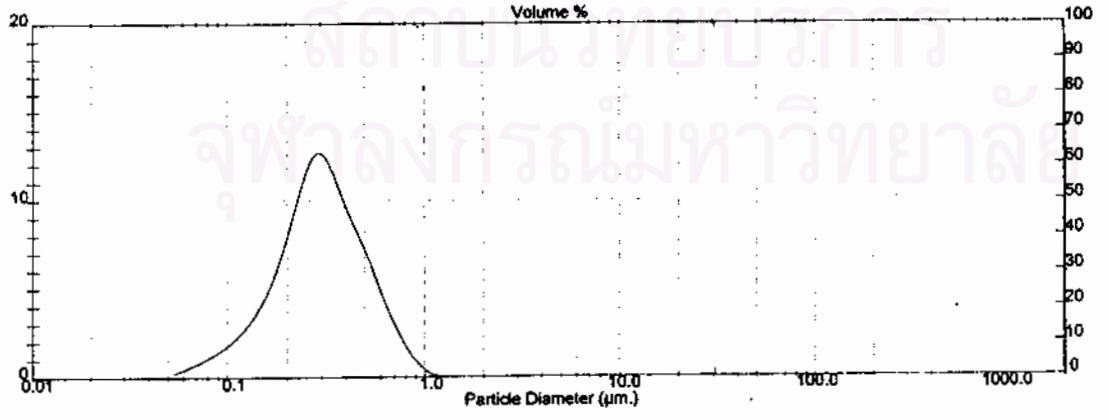


Figure c3. Particle size distribution of formulation 5SB+1LE+1T80 after autoclaving and storage for 7 days at room temperature

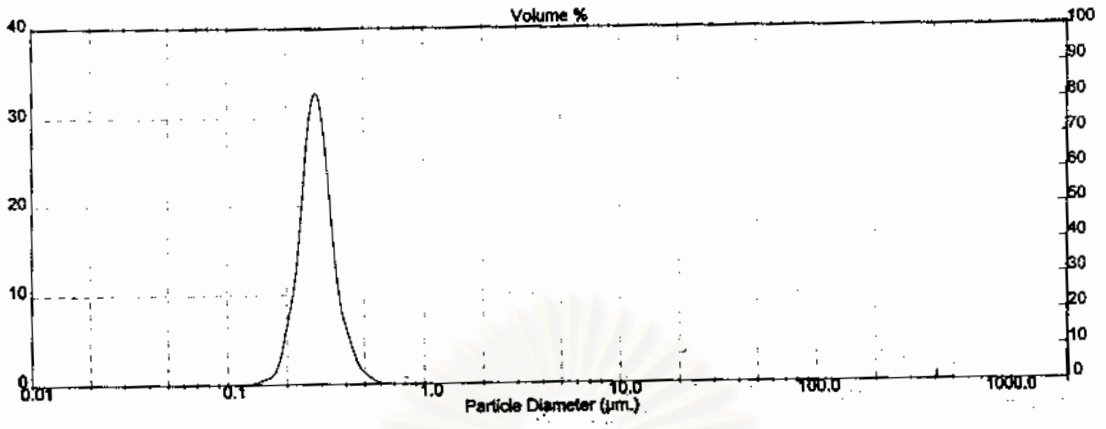


Figure c4. Particle size distribution of formulation 5SB+1LE+1P188 before autoclaving

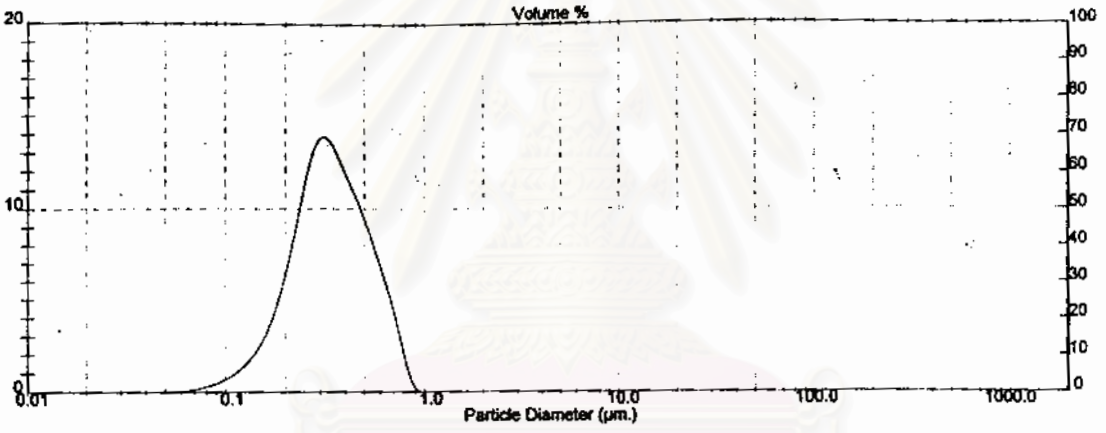


Figure c5. Particle size distribution of formulation 5SB+1LE+1P188 after autoclaving and storage for 24 hours at room temperature

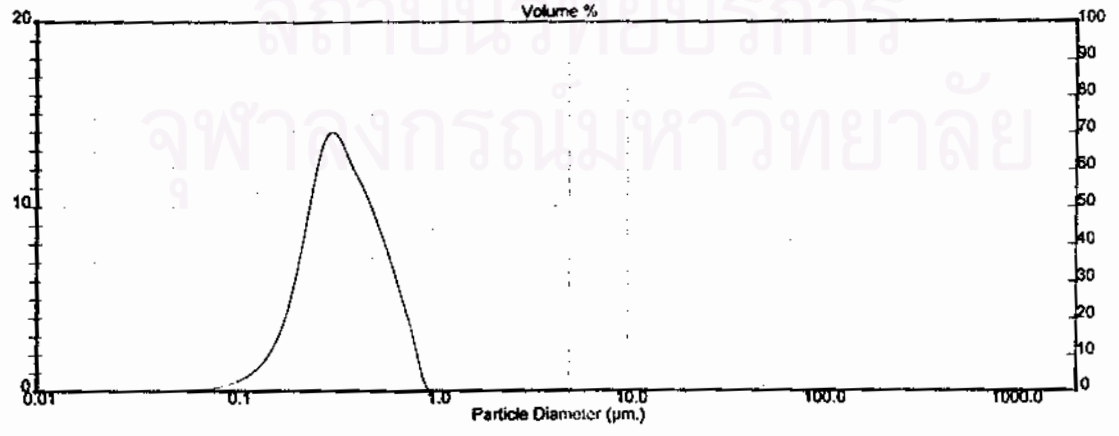


Figure c6. Particle size distribution of formulation 5SB+1LE+1P188 after autoclaving and storage for 7 days at room temperature

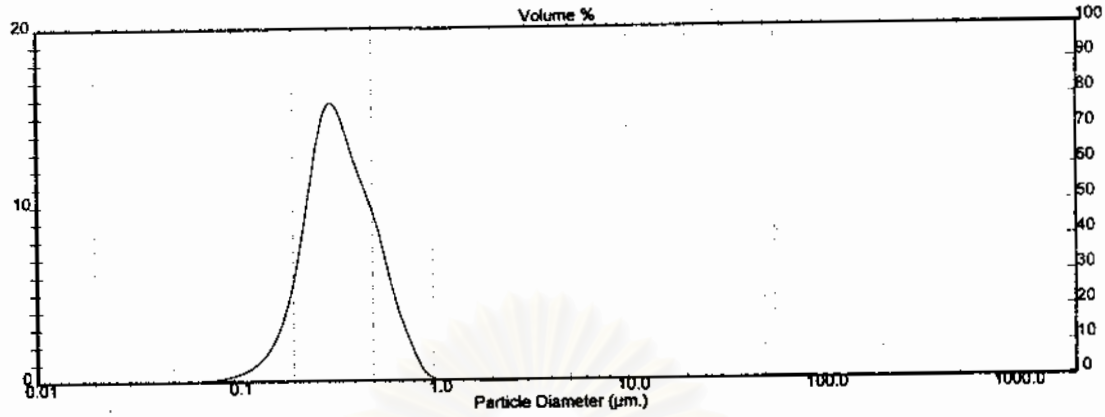


Figure c7. Particle size distribution of formulation 5SB+1LE+1P188 after autoclaving and storage for 1 month at room temperature

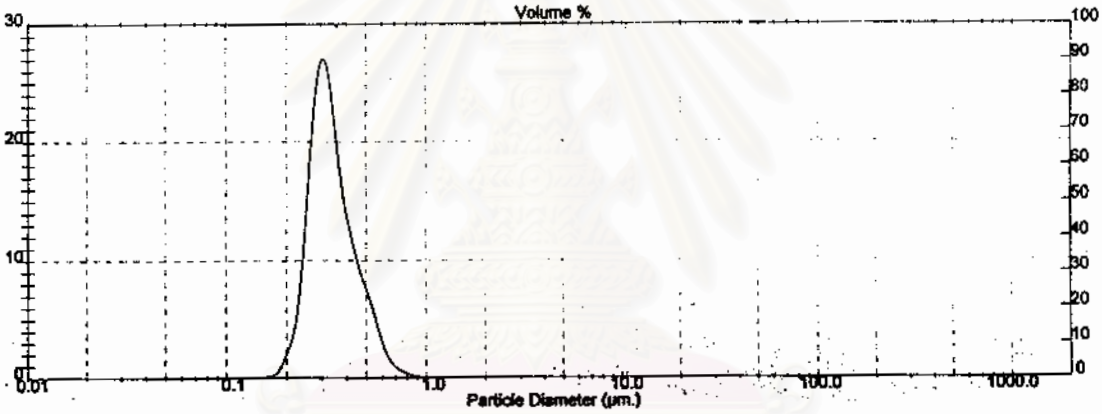


Figure c8. Particle size distribution of formulation 5SB+2LE+1T80 before autoclaving

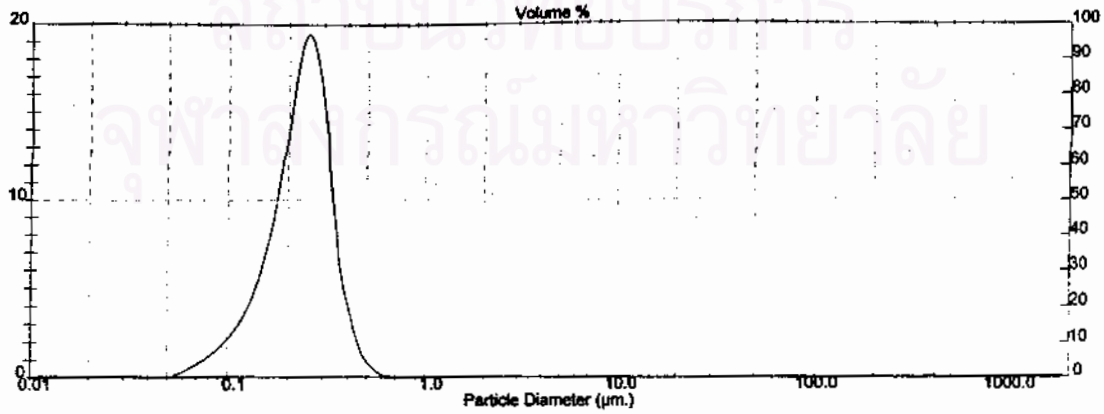


Figure c9. Particle size distribution of formulation 5SB+2LE+1T80 after autoclaving and storage for 24 hours at room temperature

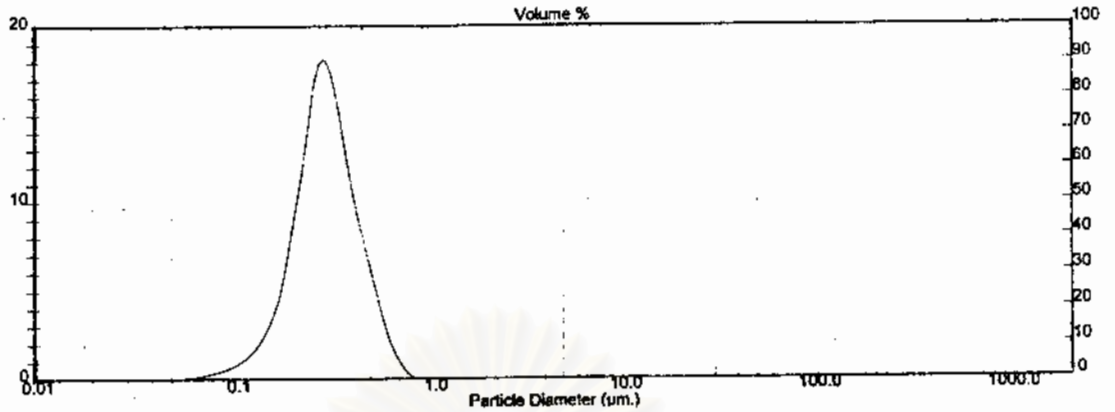


Figure c10. Particle size distribution of formulation 5SB+2LE+1T80 after autoclaving and storage for 7 days at room temperature

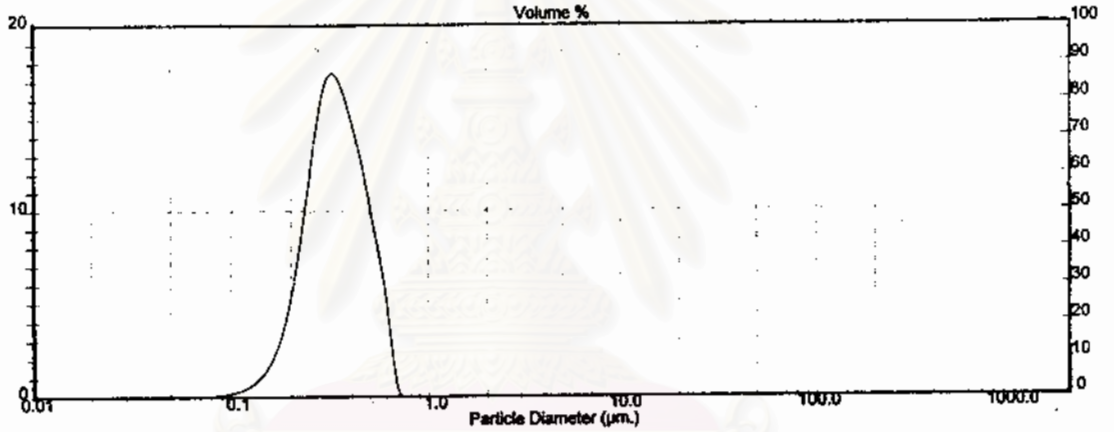


Figure c11. Particle size distribution of formulation 5SB+2LE+1T80 after autoclaving and storage for 1 month at room temperature

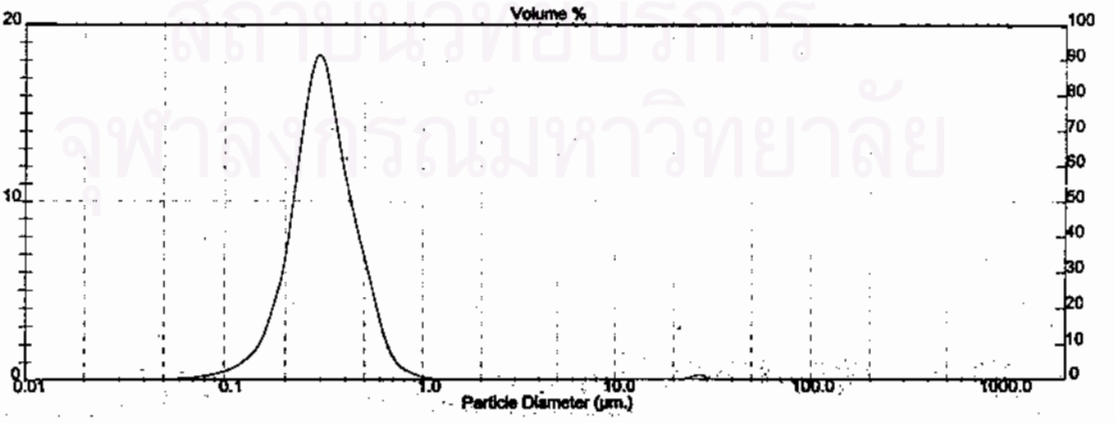


Figure c12. Particle size distribution of formulation 5SB+2LE+1P188 before autoclaving

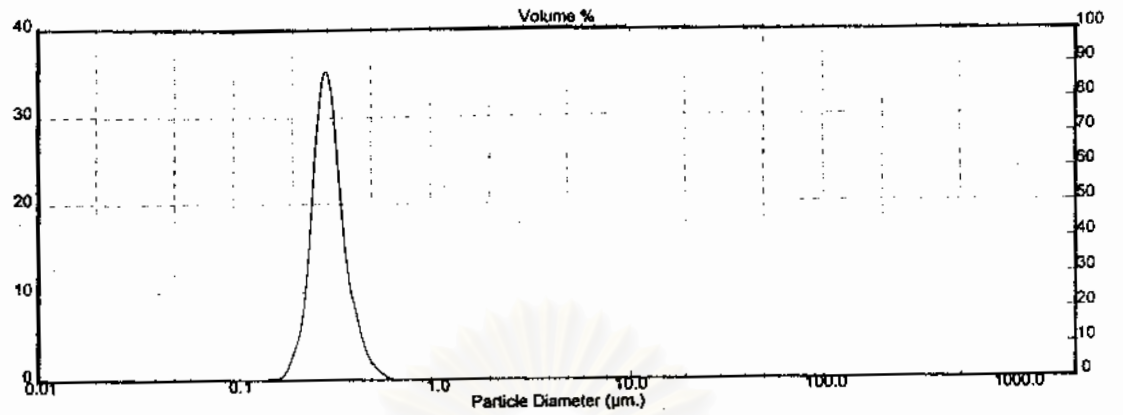


Figure c13. Particle size distribution of formulation 5SB+2LE+1P188 after autoclaving and storage for 24 hours at room temperature

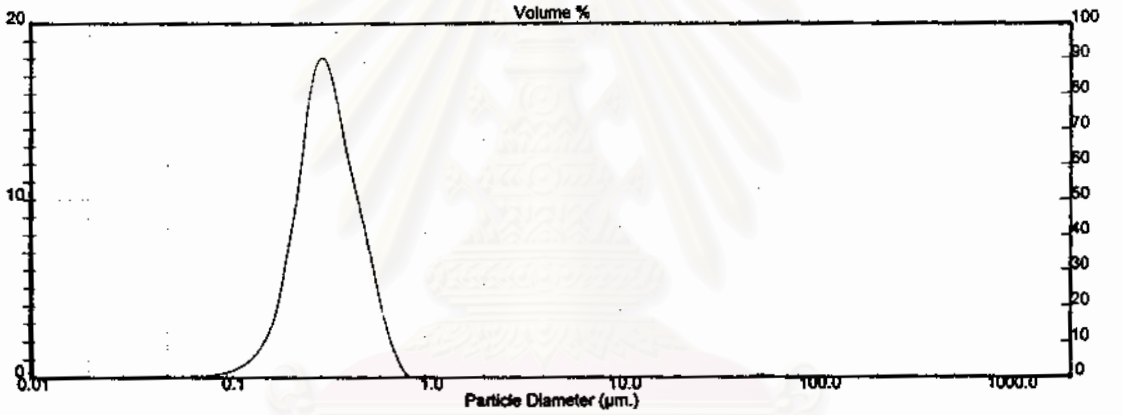


Figure c14. Particle size distribution of formulation 5SB+2LE+1P188 after autoclaving and storage for 7 days at room temperature

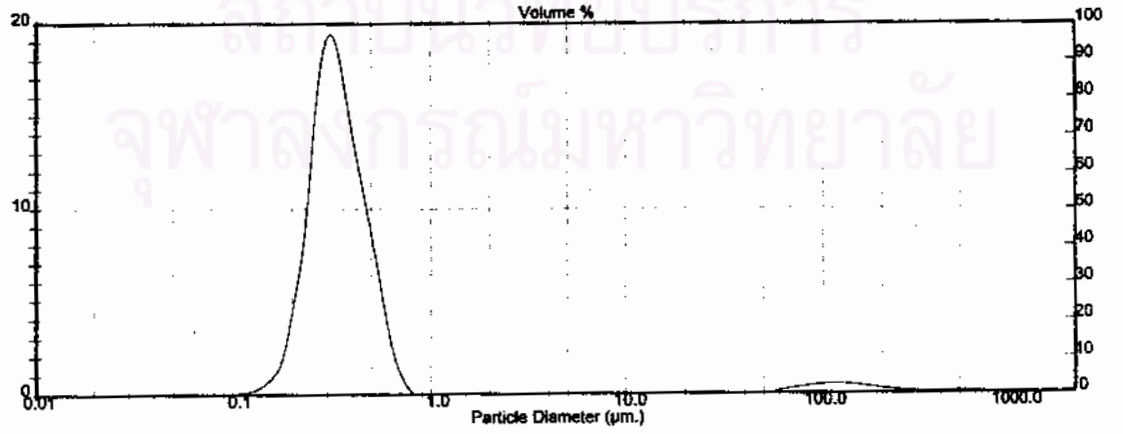


Figure c15. Particle size distribution of formulation 5SB+2LE+1P188 after autoclaving and storage for 1 month at room temperature

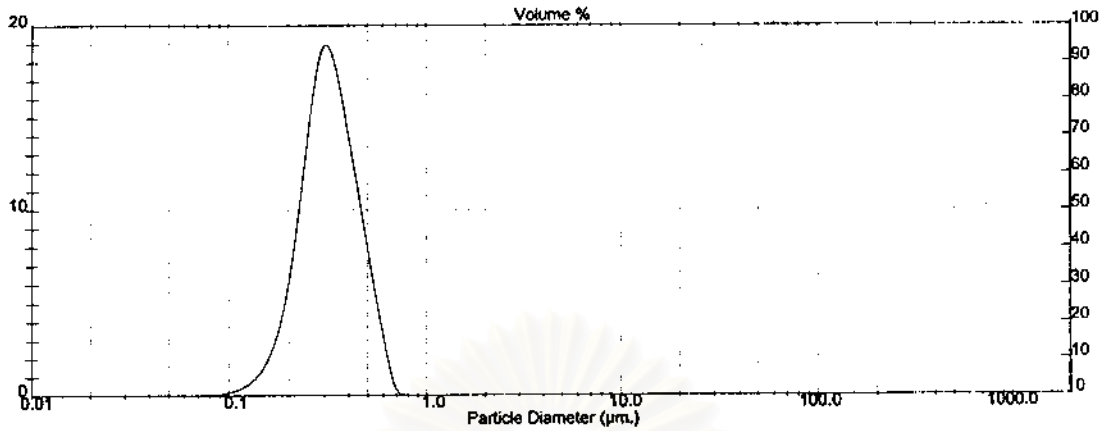


Figure c16. Particle size distribution of formulation 10SB+1LE+1T80 before autoclaving

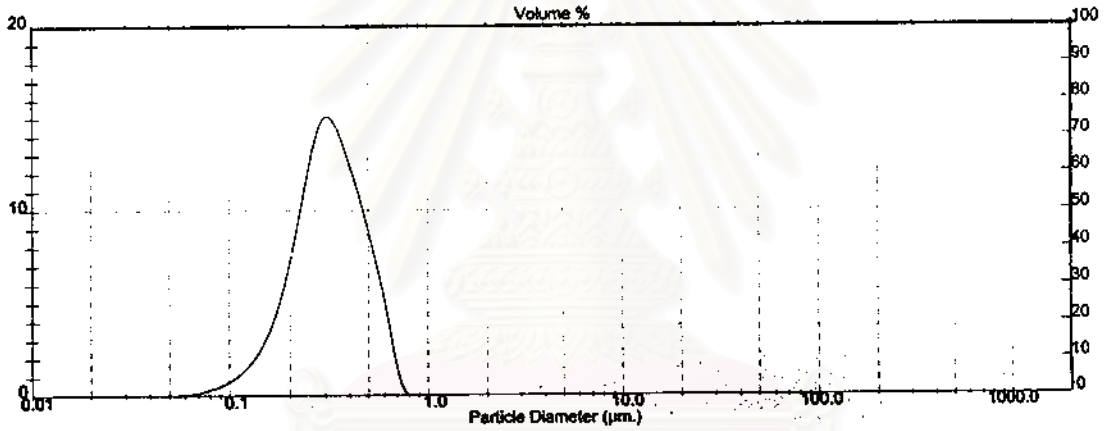


Figure c17. Particle size distribution of formulation 10SB+1LE+1T80 after autoclaving and storage for 24 hours at room temperature

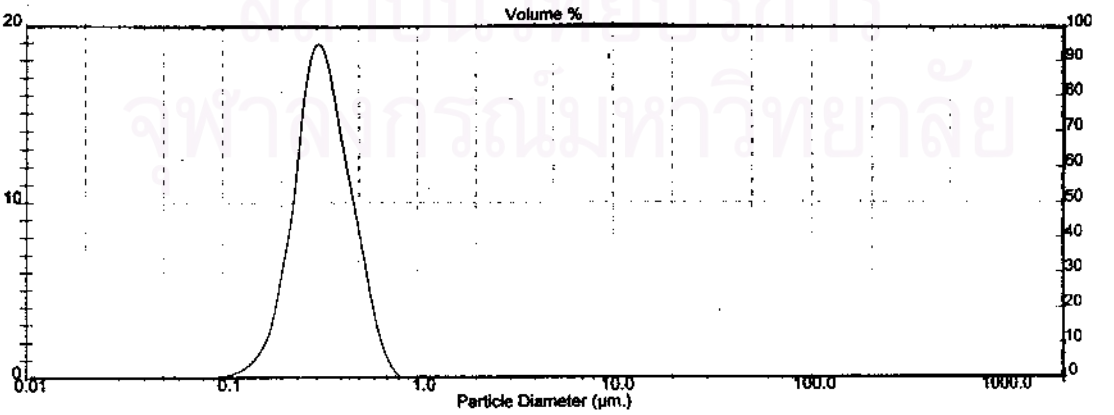


Figure c18. Particle size distribution of formulation 10SB+2LE+1T80 before autoclaving

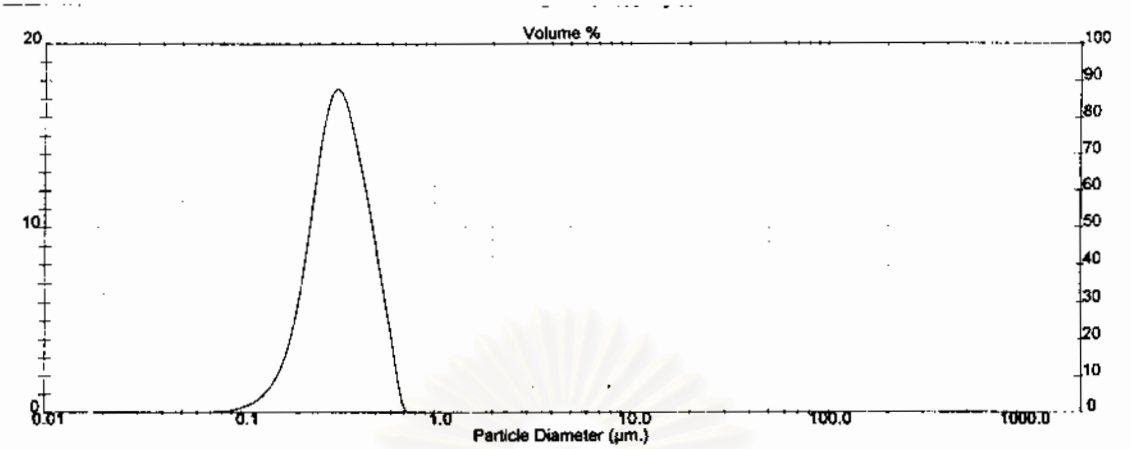


Figure C19. Particle size distribution of formulation 10SB+2LE+1T80 after autoclaving and storage for 24 hours at room temperature

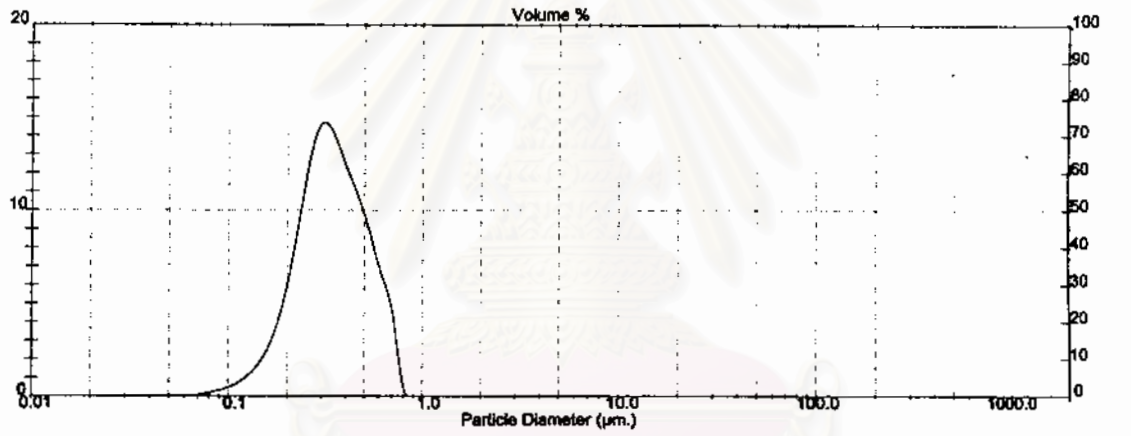


Figure c20. Particle size distribution of formulation 10SB+2LE+1T80 after autoclaving and storage for 7 days at room temperature

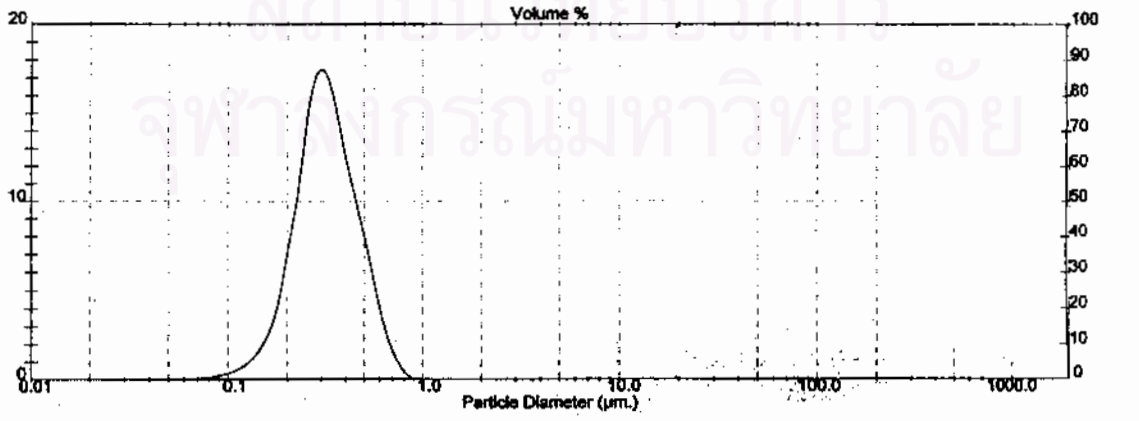


Figure c21. Particle size distribution of formulation 10SB+2LE+1P188 before autoclaving

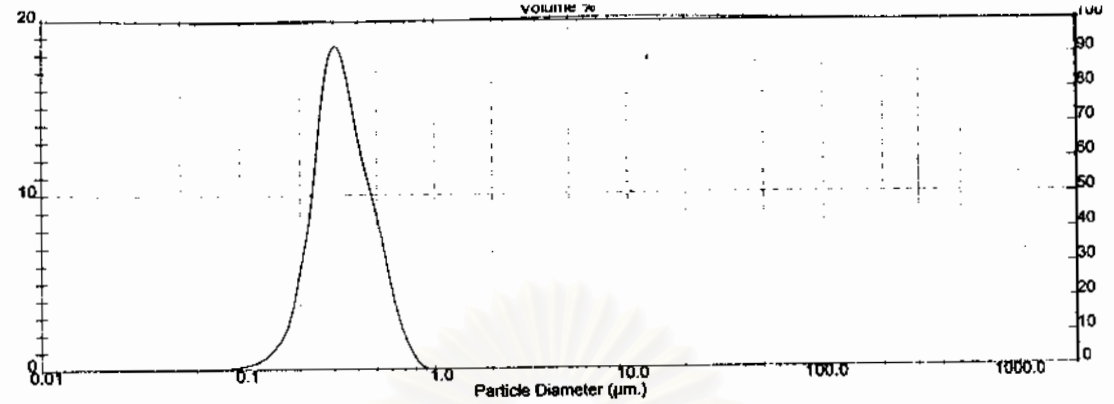


Figure c22. Particle size distribution of formulation 10SB+2LE+1P188 after autoclaving and storage for 24 hours at room temperature

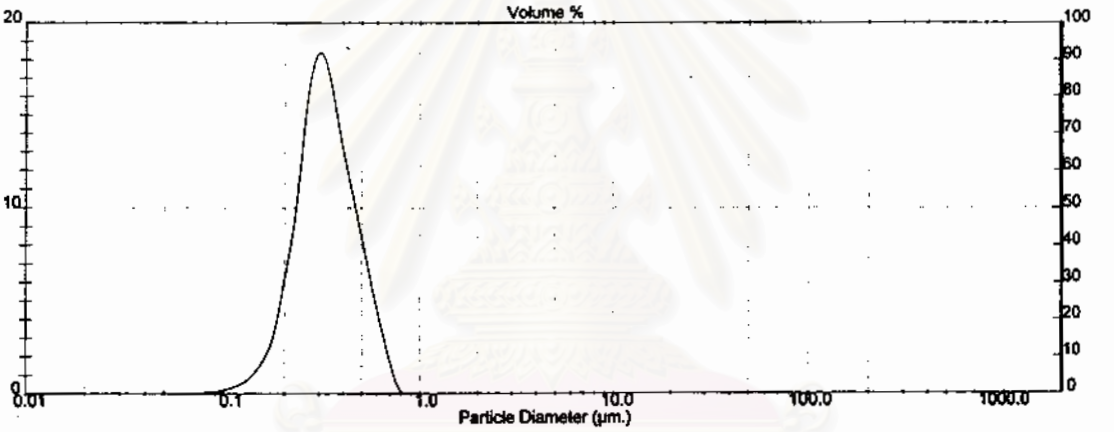


Figure c23. Particle size distribution of formulation 10SB+2LE+1P188 after autoclaving and storage for 7 days at room temperature

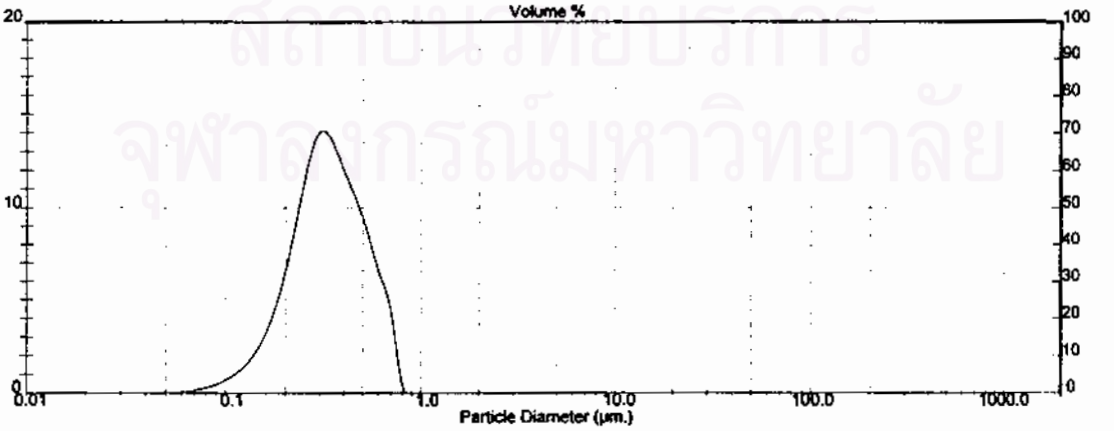


Figure c24. Particle size distribution of formulation 20SB+2LE+1T80 before autoclaving

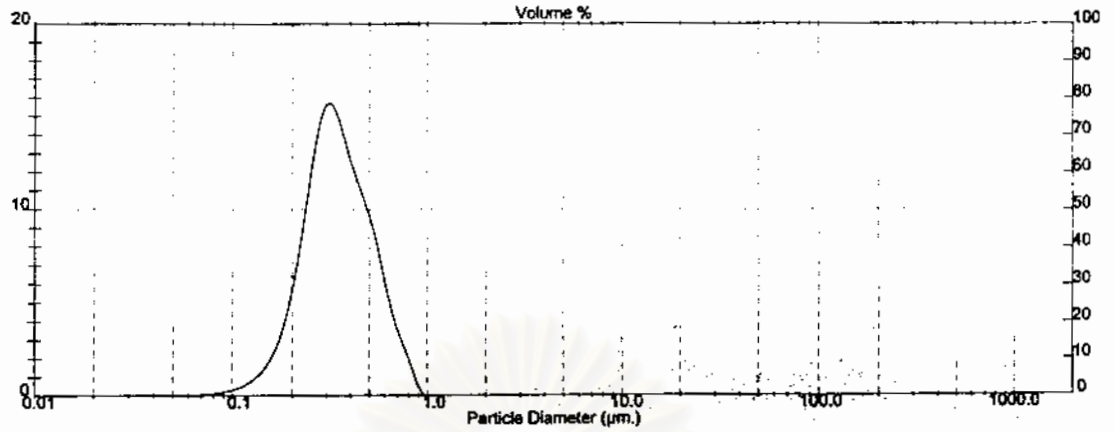


Figure c25. Particle size distribution of formulation 20SB+2LE+1T80 after autoclaving and storage for 24 hours at room temperature

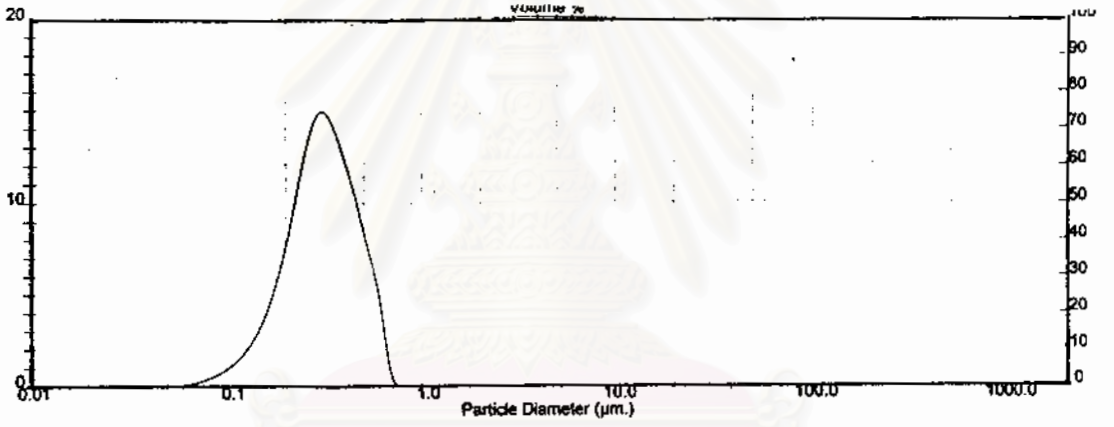


Figure c26. Particle size distribution of formulation 10SB+2LE+1.5T80 before autoclaving

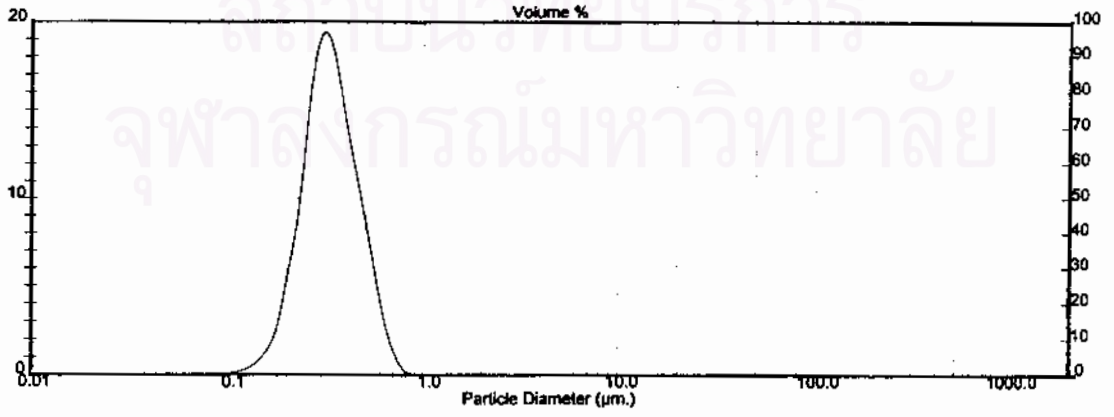


Figure c27. Particle size distribution of formulation 10SB+2LE+1.5T80 after autoclaving and storage for 24 hours at room temperature

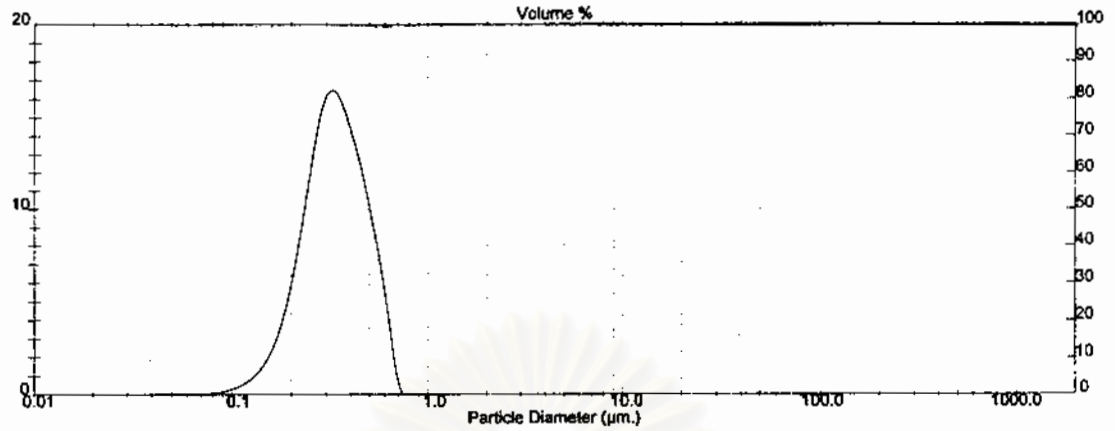


Figure c28. Particle size distribution of formulation 10SB+2LE+1.5T80 after autoclaving and storage for 7 days at room temperature

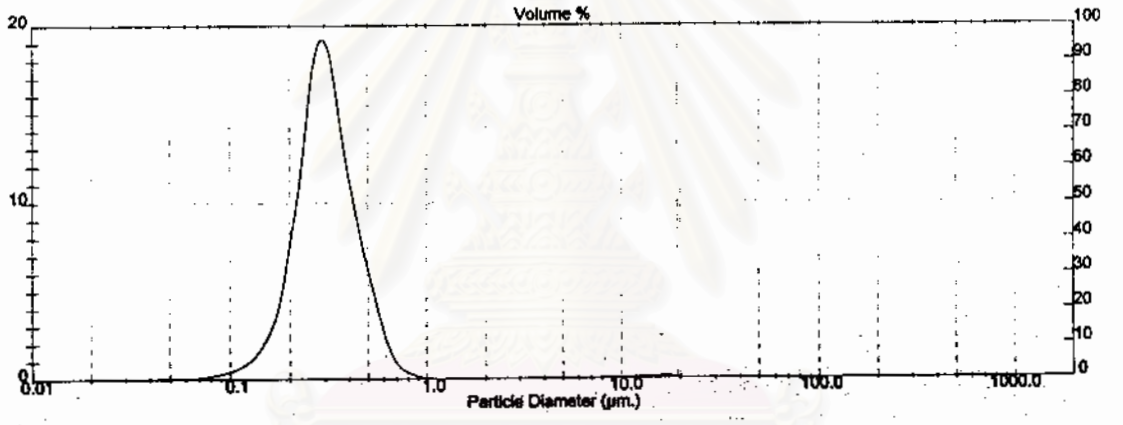


Figure c29. Particle size distribution of formulation 10SB+2LE+1.5T80 after autoclaving and storage for 1 month at room temperature

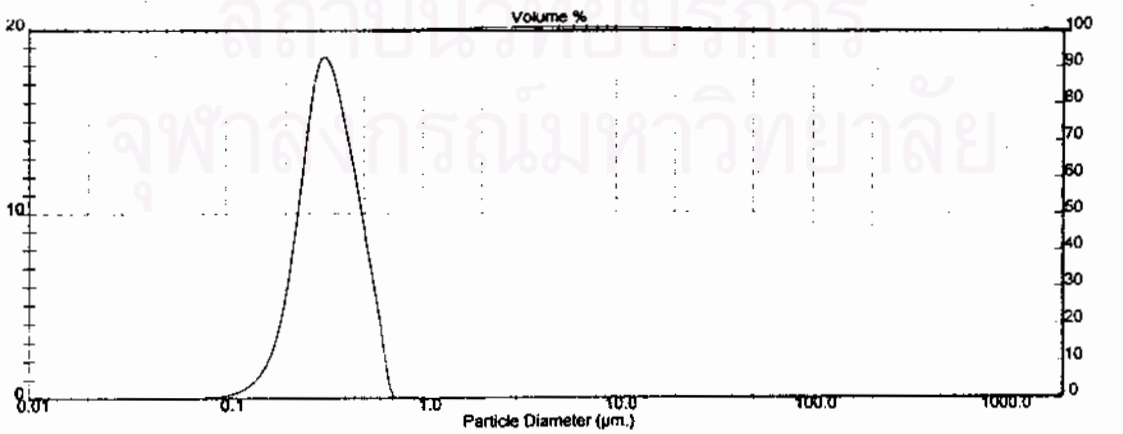


Figure c30. Particle size distribution of formulation 10SB+2LE+2T80 before autoclaving

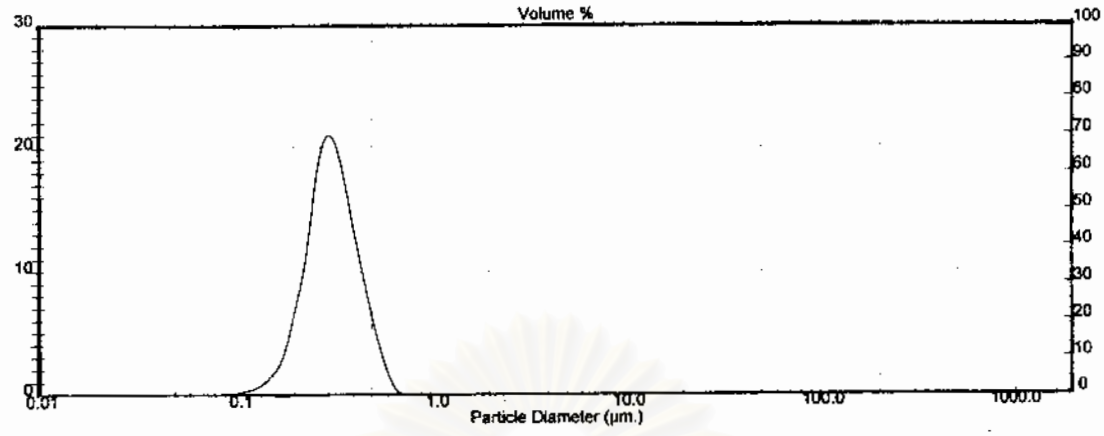


Figure c31. Particle size distribution of formulation 10SB+2LE+2T80 after autoclaving and storage for 24 hours at room temperature

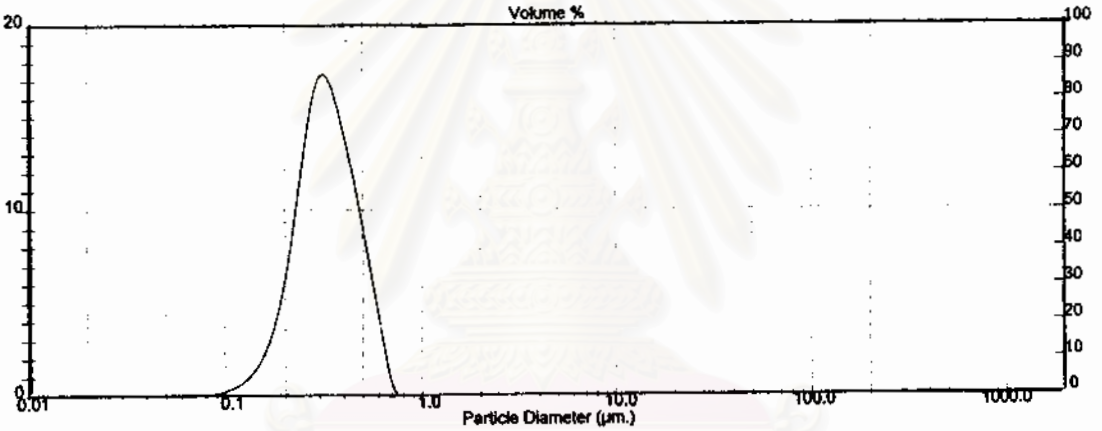


Figure c32. Particle size distribution of formulation 10SB+2LE+2T80 after autoclaving and storage for 7 days at room temperature

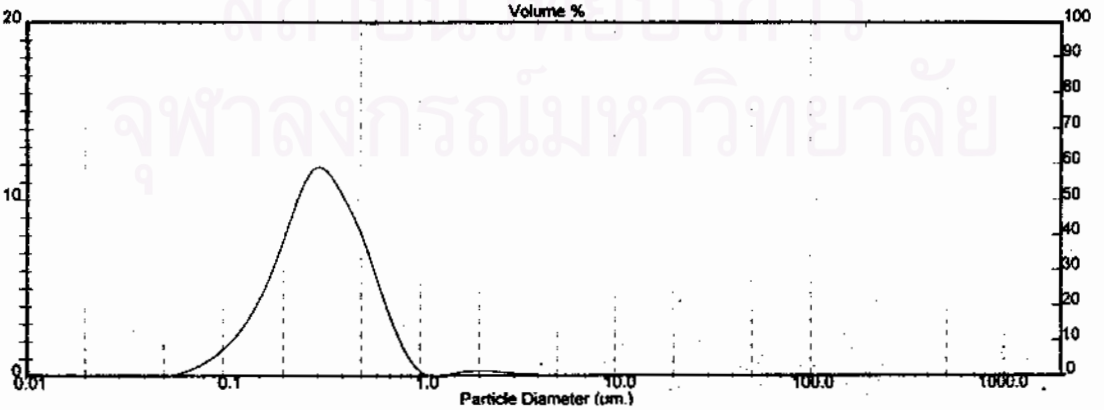


Figure c33. Particle size distribution of formulation 10SB+1.71LE+1.29T80 before autoclaving.

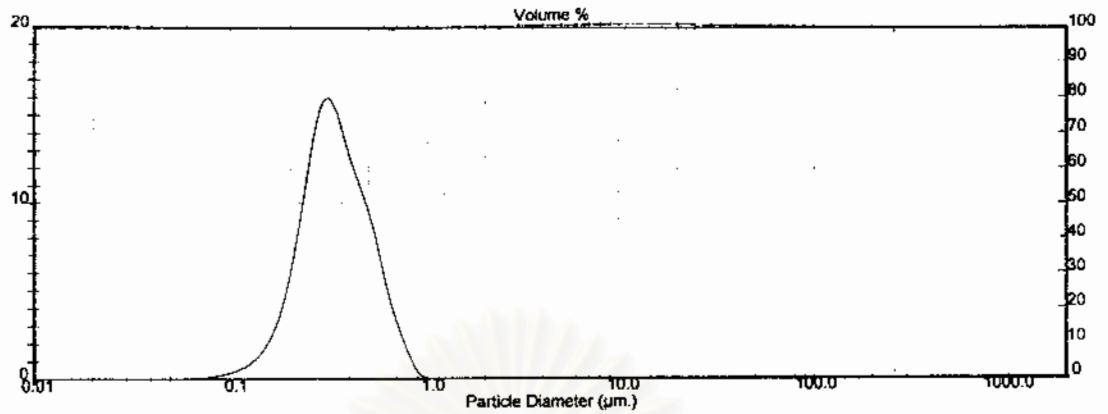


Figure c34. Particle size distribution of formulation 10SB+1.71LE+1.29T80 after autoclaving and storage for 24 hours at room temperature

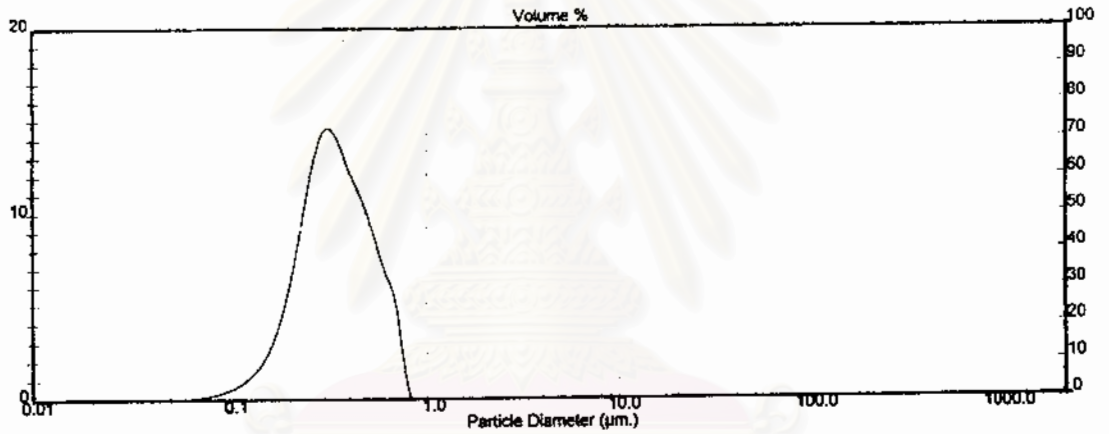


Figure c35. Particle size distribution of formulation 10SB+1.71LE+1.29T80 after autoclaving and storage for 7 days at room temperature

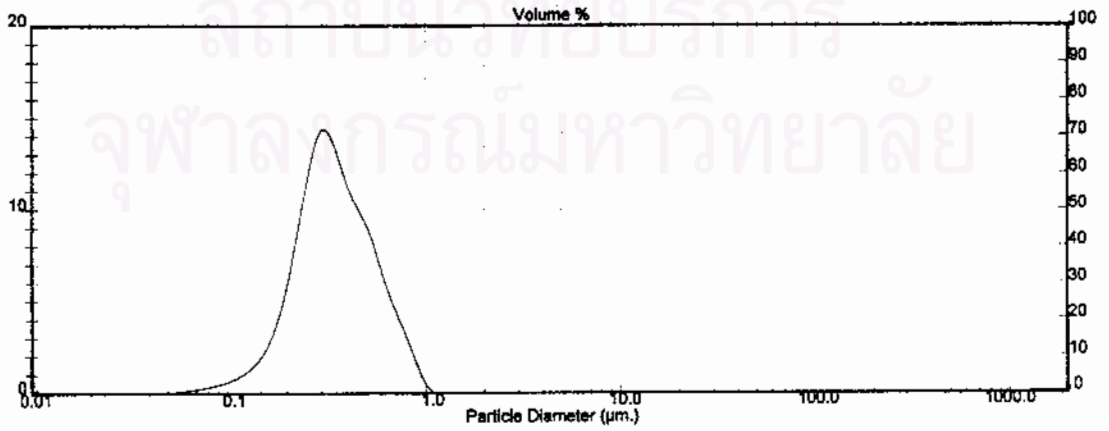


Figure c36. Particle size distribution of formulation 10SB+1.71LE+1.29T80 after autoclaving and storage for 1 month at room temperature.

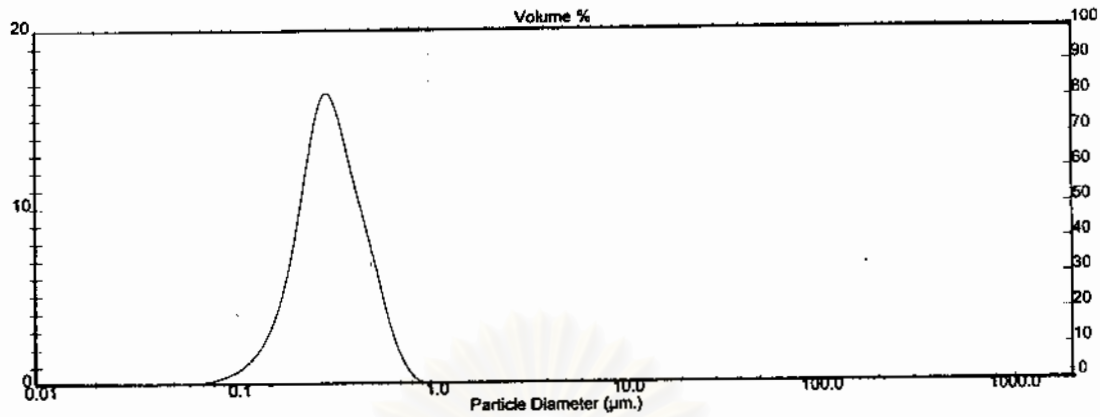


Figure c37. Particle size distribution of formulation 10SB+2.29LE+1.71T80 before autoclaving

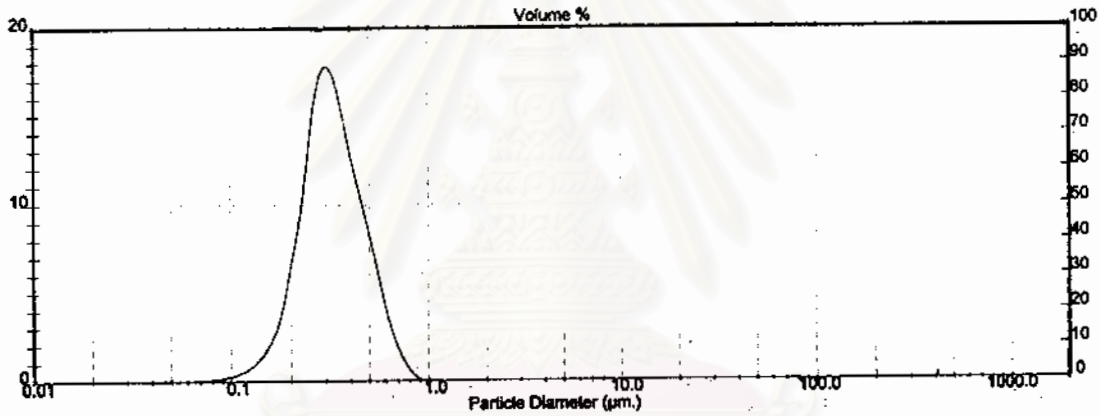


Figure c38. Particle size distribution of formulation 10SB+2.29LE+1.71T80 after autoclaving and storage for 24 hours at room temperature

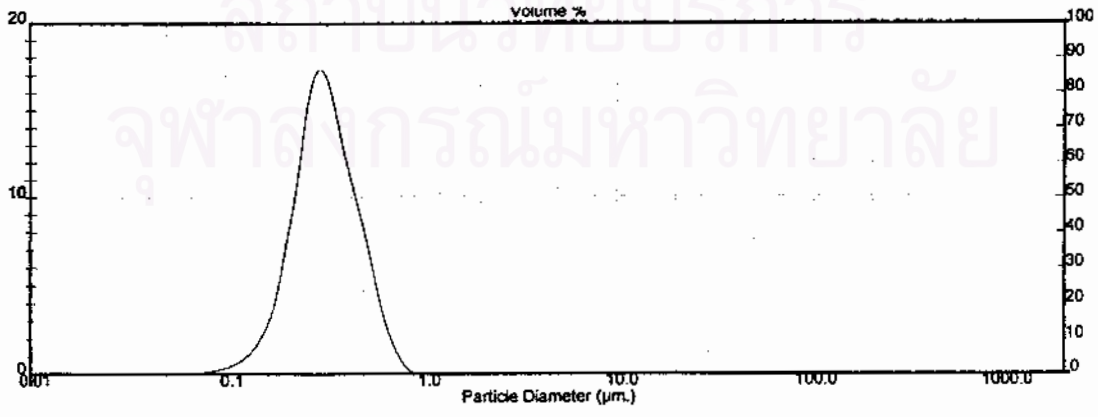


Figure c39. Particle size distribution of formulation 10SB+2.29LE+1.71T80 after autoclaving and storage for 7 days at room temperature

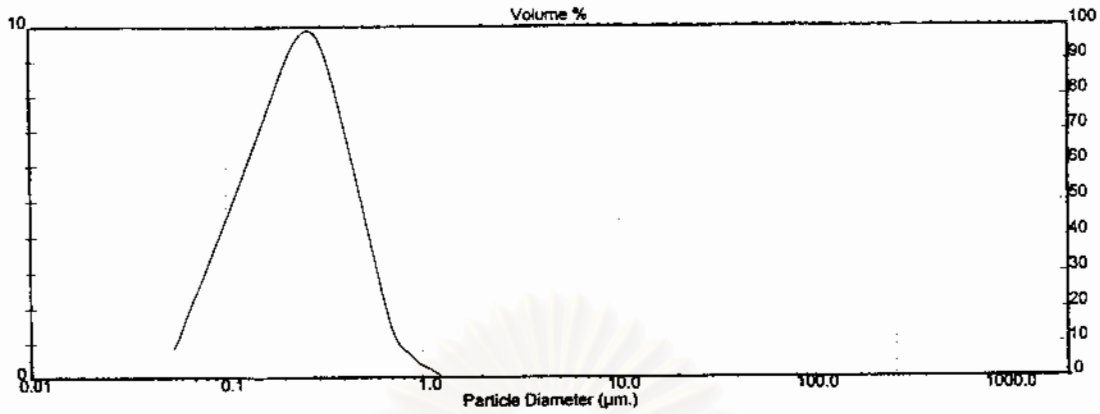


Figure c40. Particle size distribution of formulation 10SB+2.86LE+2.14T80 before autoclaving

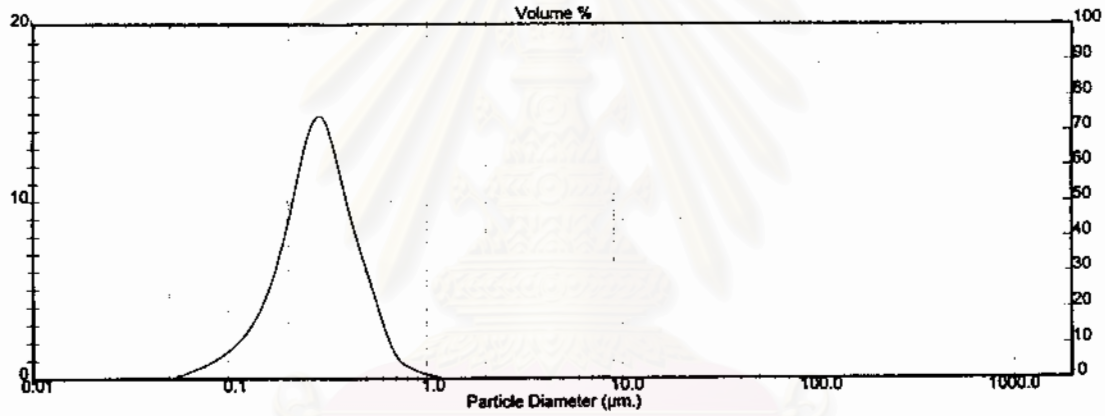


Figure c41. Particle size distribution of formulation 10SB+2.86LE+2.14T80 after autoclaving and storage for 24 hours at room temperature

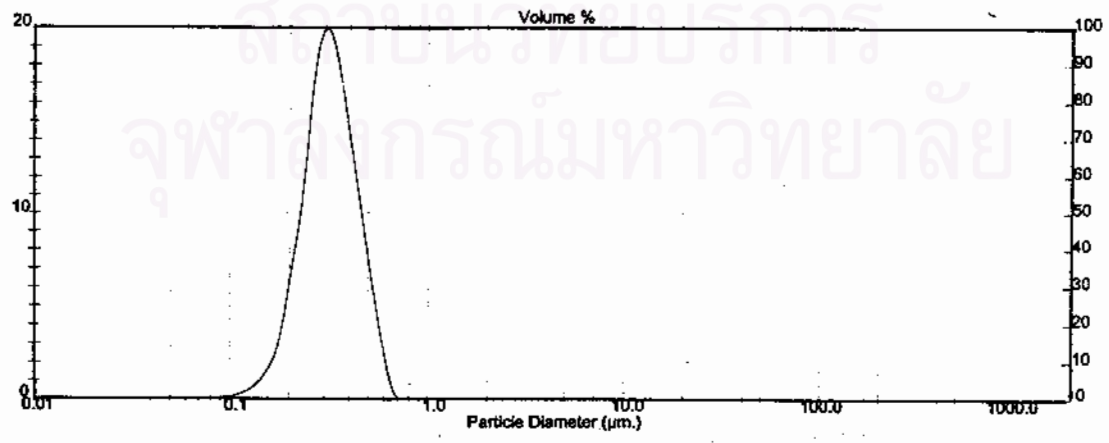


Figure c42. Particle size distribution of formulation 10SB+2.86LE+2.14T80 after autoclaving and storage for 7 days at room temperature

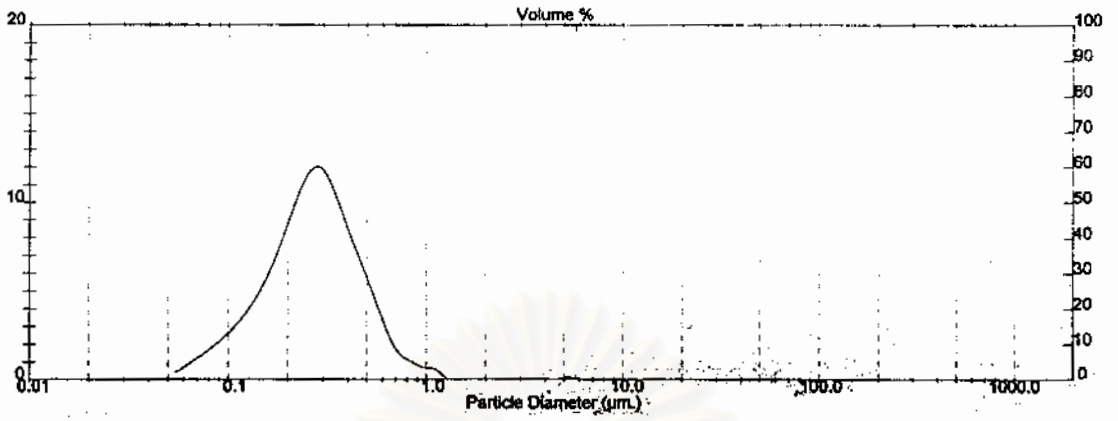


Figure c43. Particle size distribution of formulation 5MCT+1LE+1T80 before autoclaving

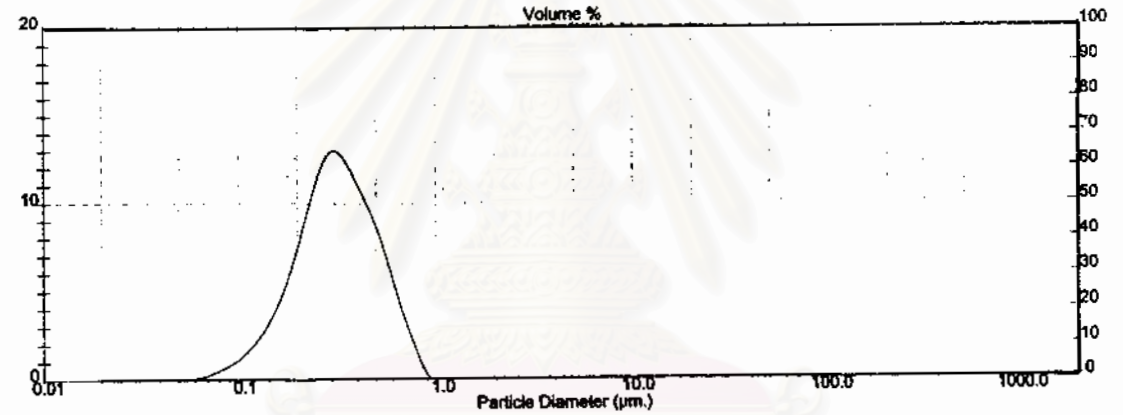


Figure c44. Particle size distribution of formulation 5MCT+1LE+1T80 after autoclaving and storage for 24 hours at room temperature

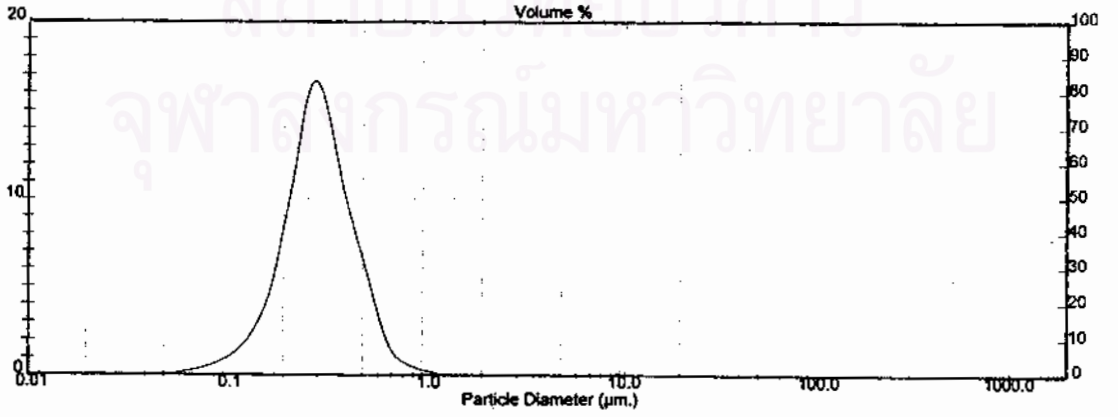


Figure c45. Particle size distribution of formulation 5MCT+1LE+1T80 after autoclaving and storage for 7 days at room temperature

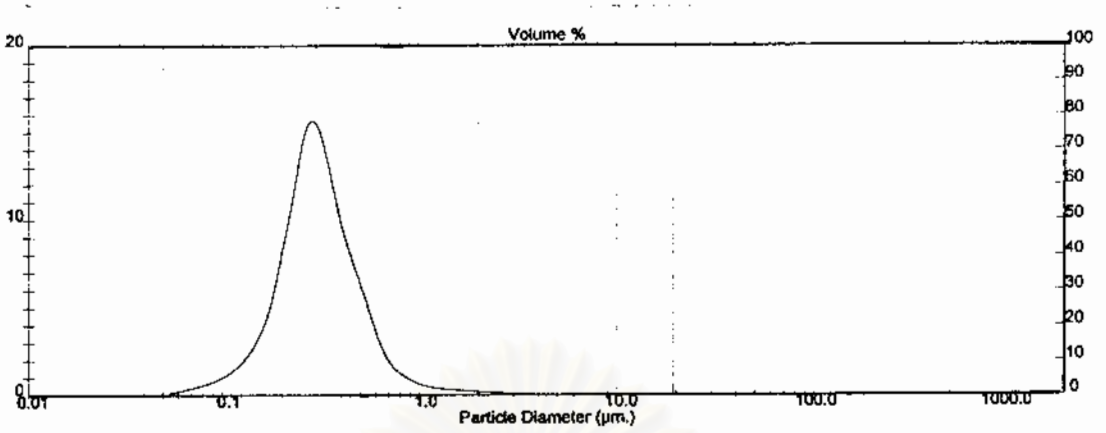


Figure c46. Particle size distribution of formulation 5MCT+1LE+1T80 after autoclaving and storage for 1 month at room temperature

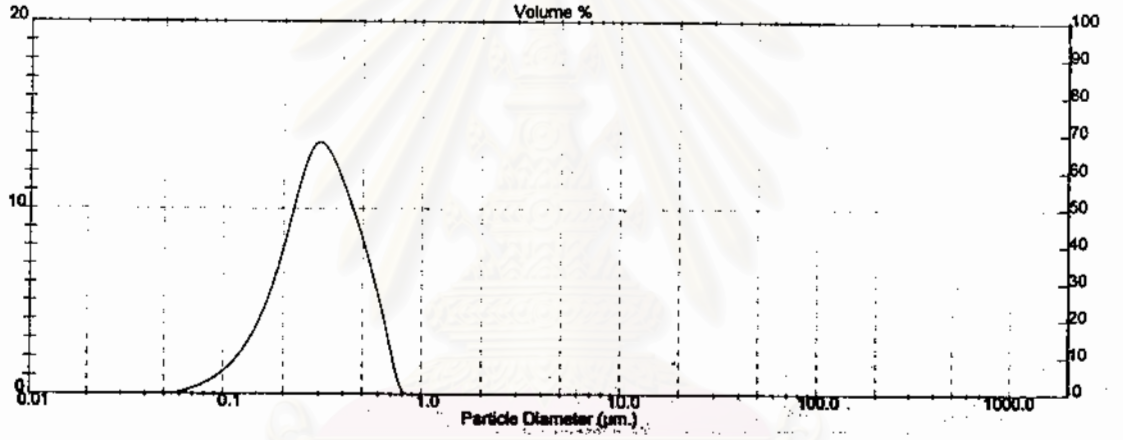


Figure c47. Particle size distribution of formulation 5MCT+1LE+1T80 after autoclaving and storage for 2 months at room temperature

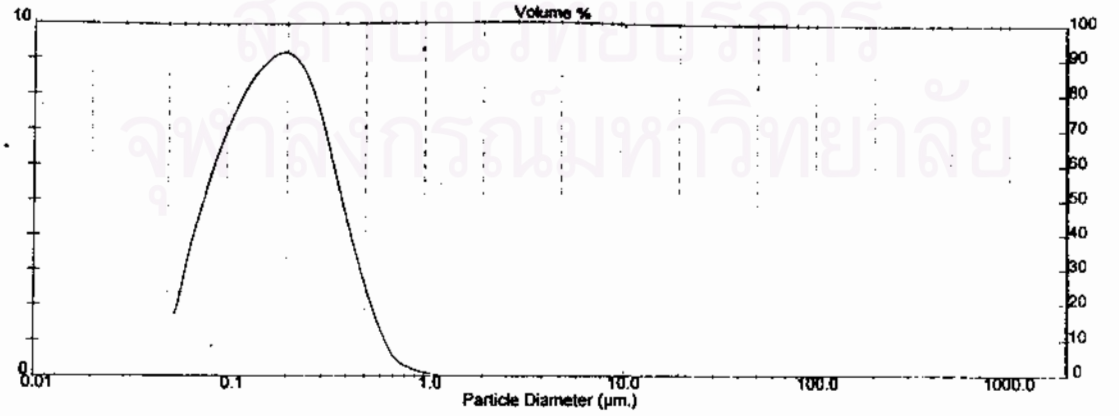


Figure c48. Particle size distribution of formulation 5MCT+1LE+1T80 after autoclaving and storage for 3 months at room temperature

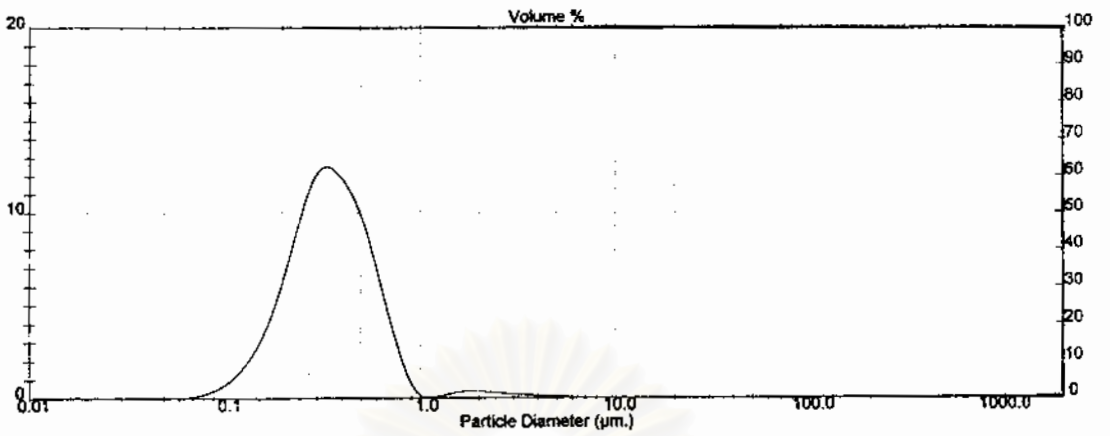


Figure c49. Particle size distribution of autoclaved formulation 5MCT+1LE+1T80 after accelerated stability test

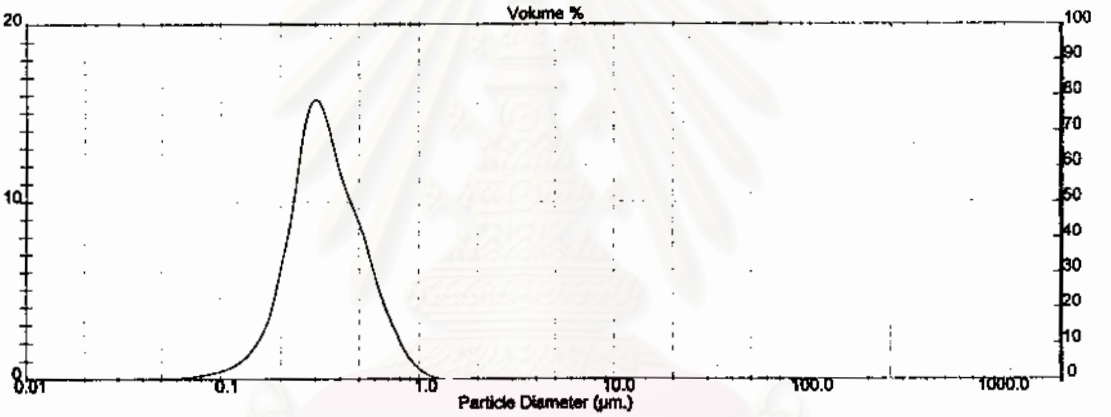


Figure c50. Particle size distribution of formulation 5MCT+1LE+1P188 before autoclaving

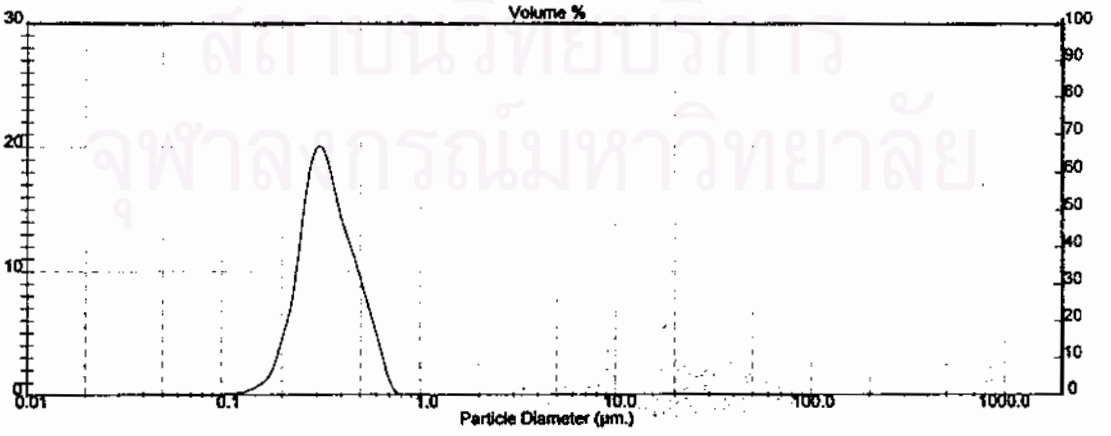


Figure c51. Particle size distribution of formulation 5MCT+1LE+1P188 after autoclaving and storage for 24 hours at room temperature

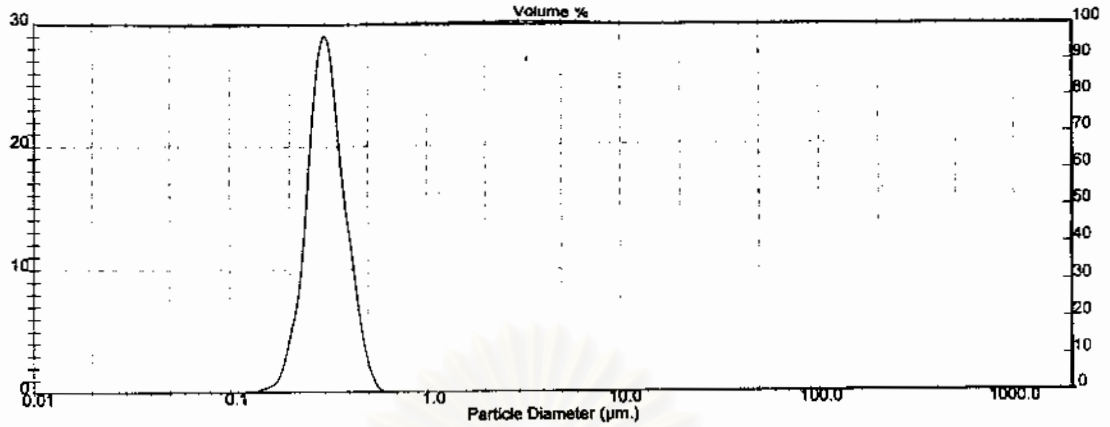


Figure c52. Particle size distribution of formulation 5MCT+1LE+1P188 after autoclaving and storage for 7 days at room temperature

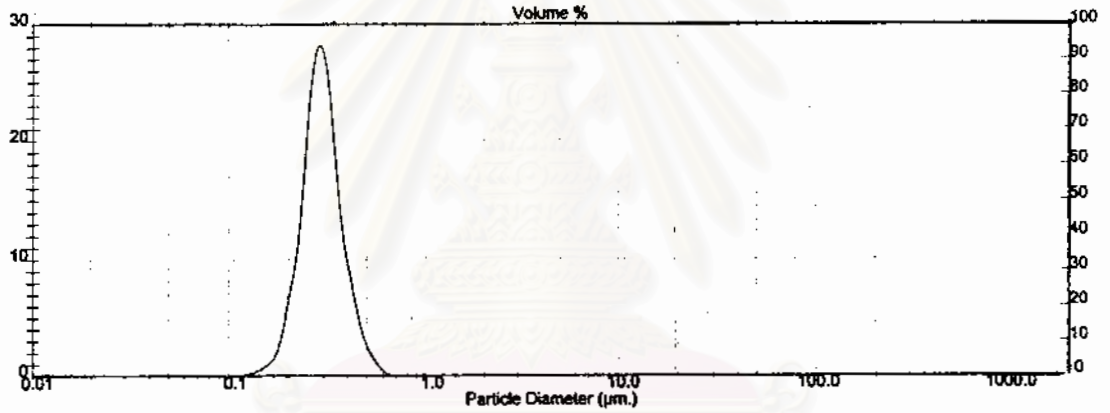


Figure c53. Particle size distribution of formulation 5MCT+1LE+1P188 after autoclaving and storage for 1 month at room temperature

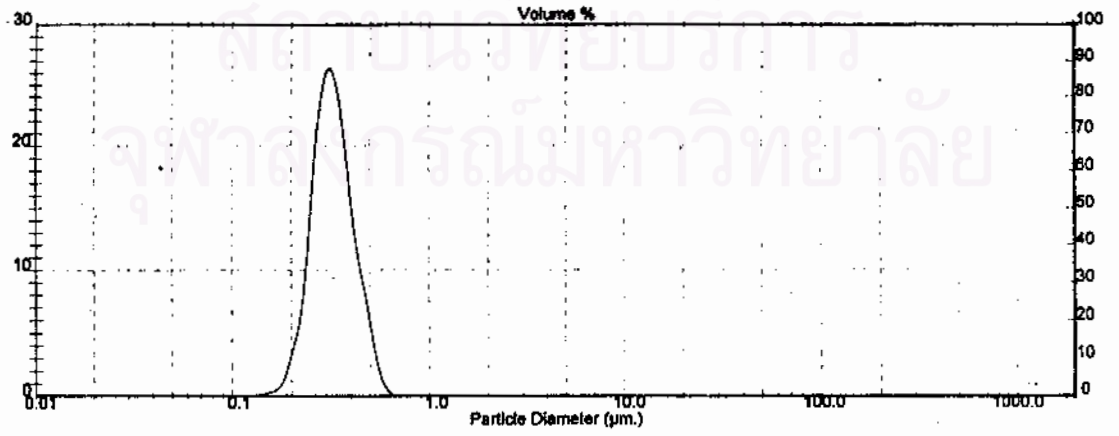


Figure c54. Particle size distribution of 10% Intralipid®

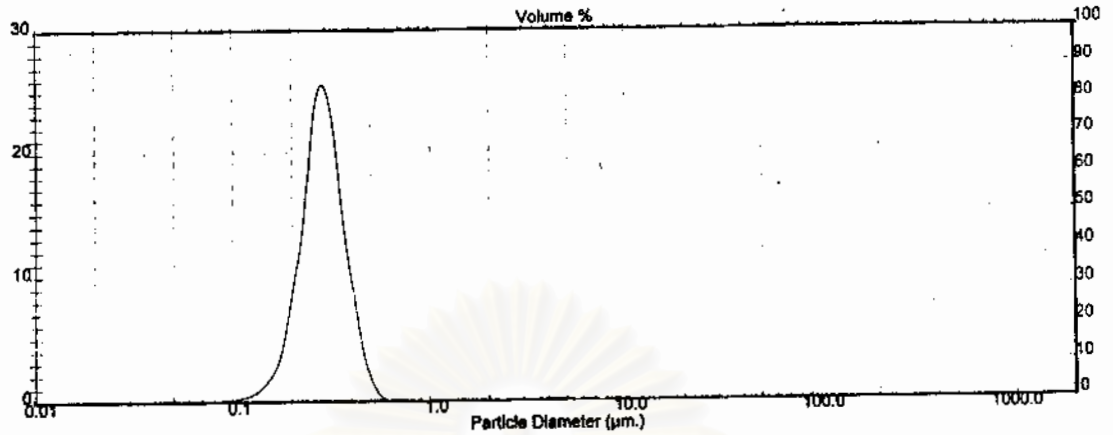


Figure c55. Particle size distribution of 10% Lipofundin®MCT/LCT

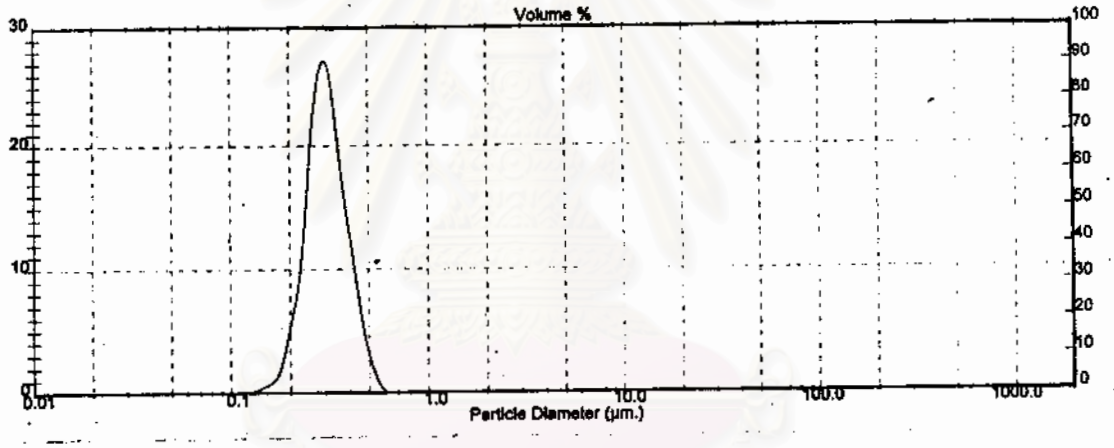


Figure c56. Particle size distribution of 10% Lipofundin-S®

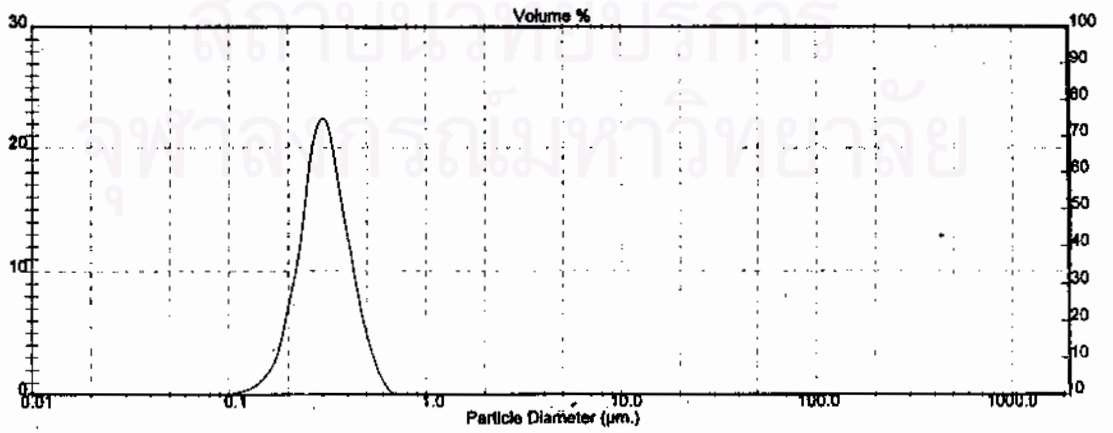


Figure c57. Particle size distribution of 10% Pharmalipid

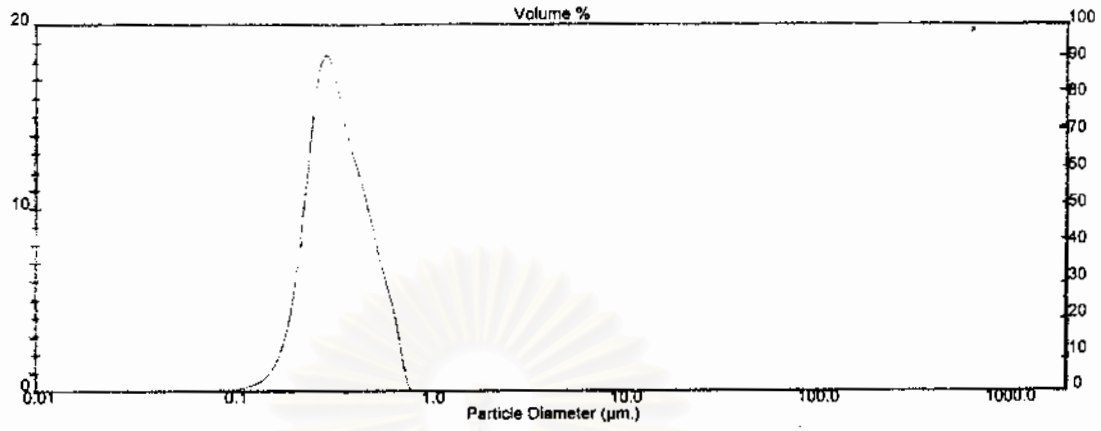


Figure c58. Particle size distribution of 20% Intralipid®

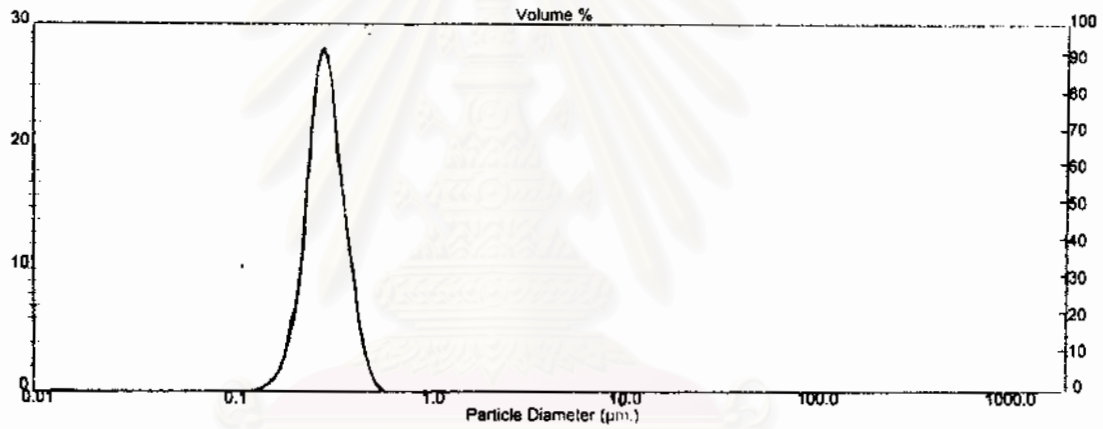


Figure c59. Particle size distribution of 20% Lipofundin®MCT/LCT

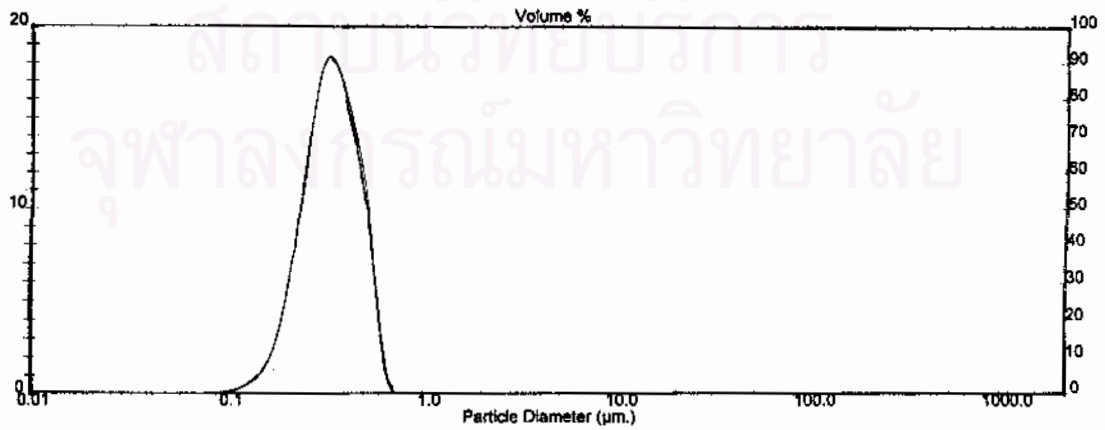


Figure c60. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Intralipid at 0 hour.

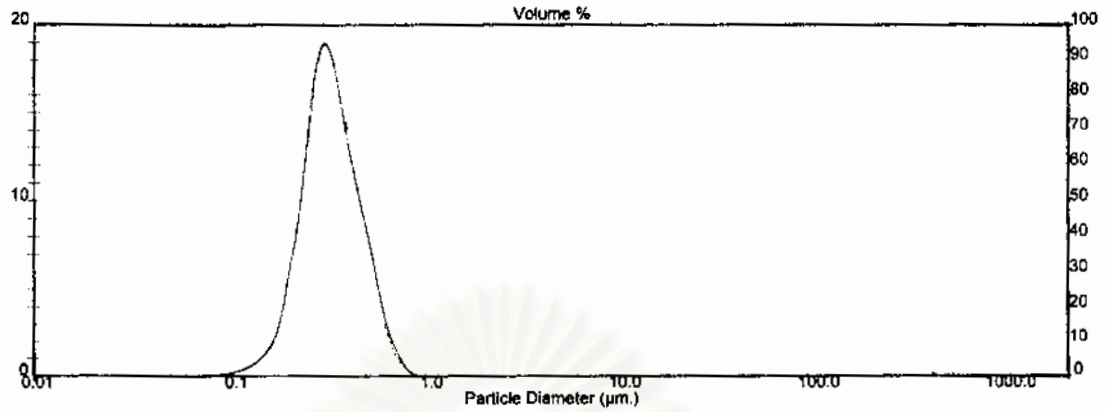


Figure c61. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Intralipid® at 24 hours.

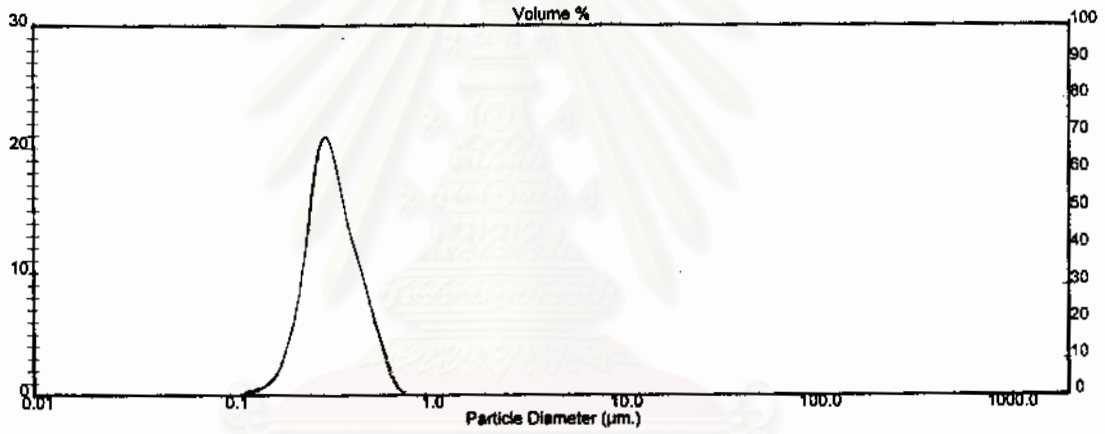


Figure c62. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Intralipid® at 0 hour.

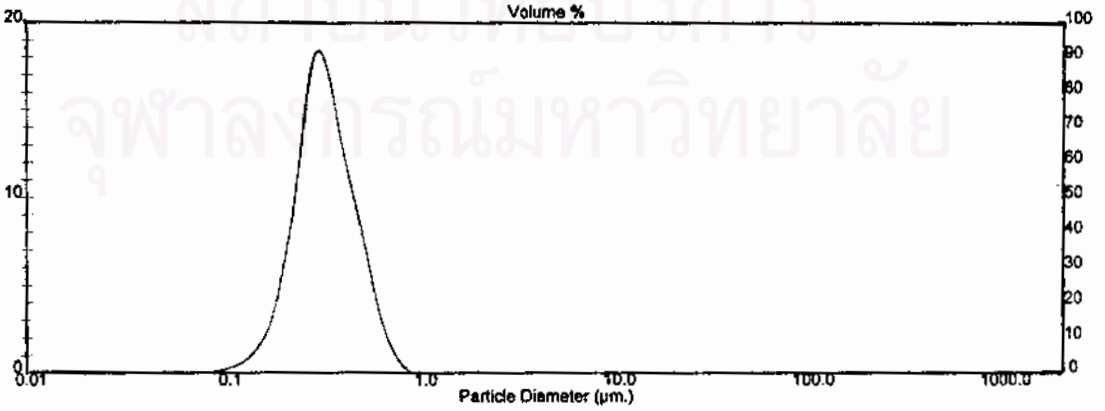


Figure c63. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Intralipid® at 24 hours.

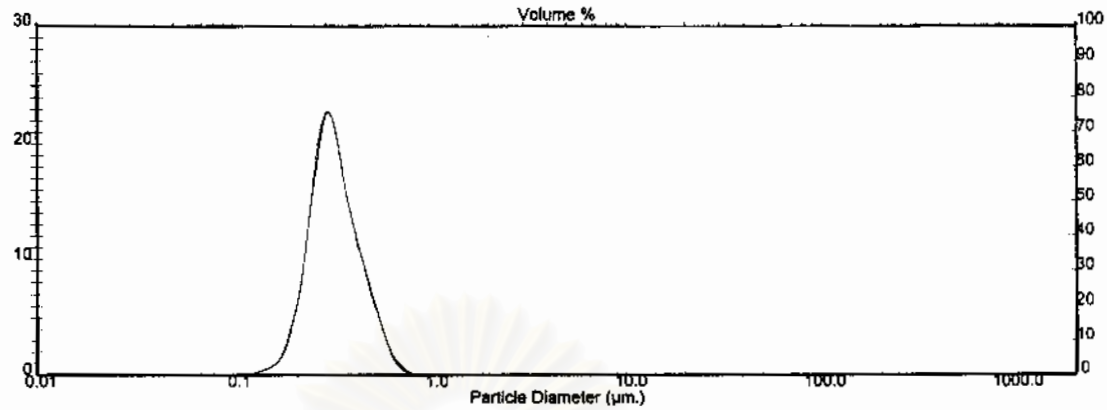


Figure c64. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Intralipid® at 0 hour.

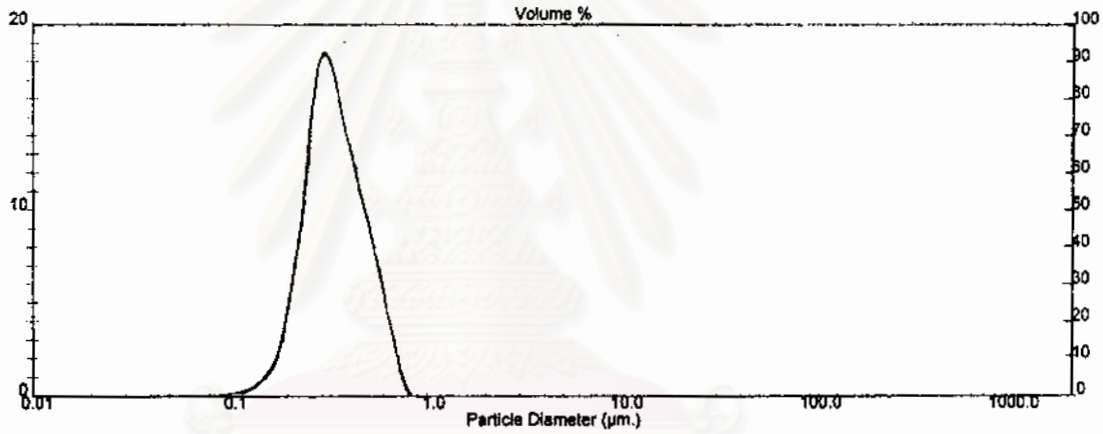


Figure c65. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Intralipid® at 24 hours.

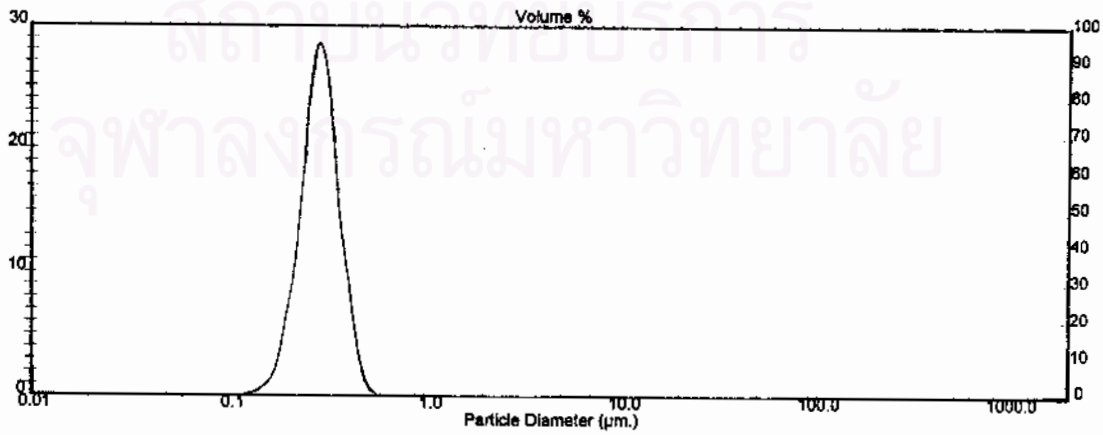


Figure c66. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 0 hour.

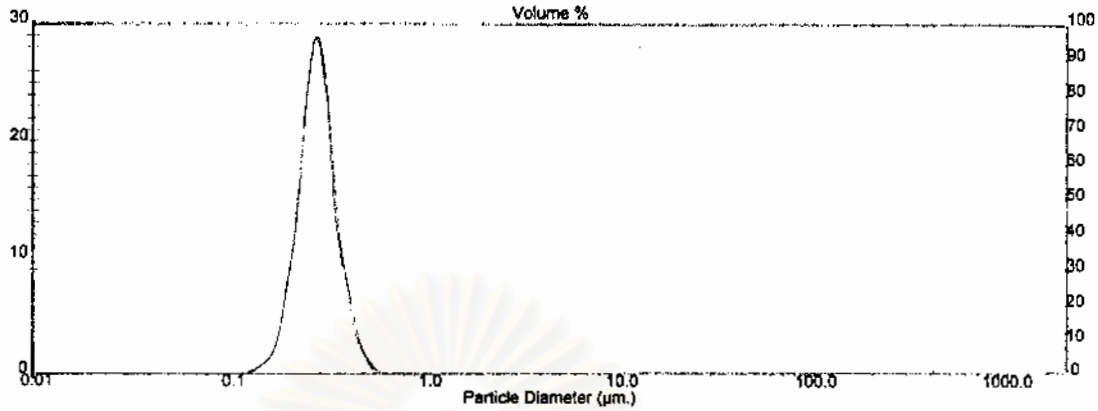


Figure c67. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 24 hours.

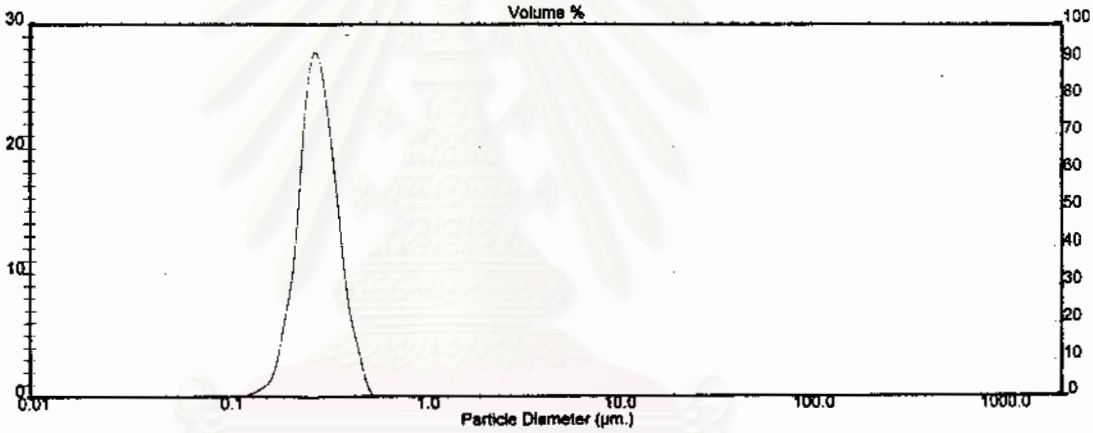


Figure c68. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 0 hour.

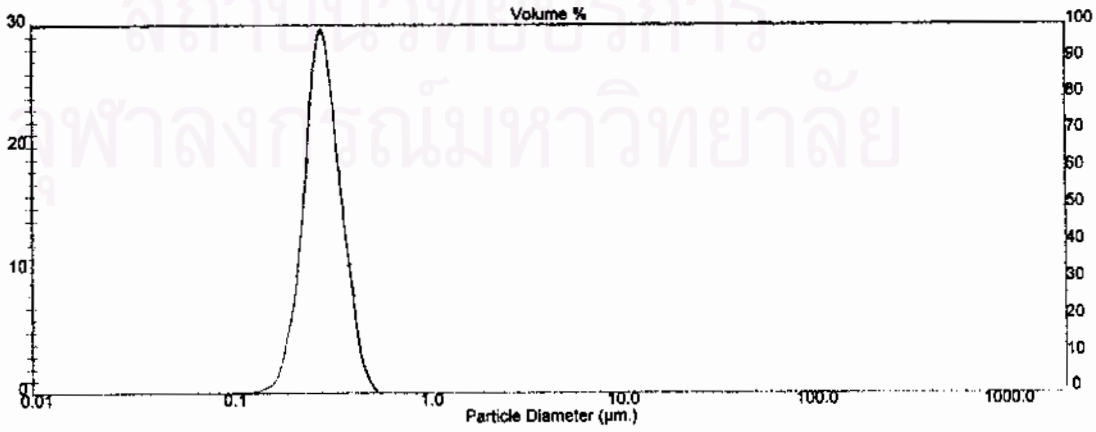


Figure c69. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 24 hours.

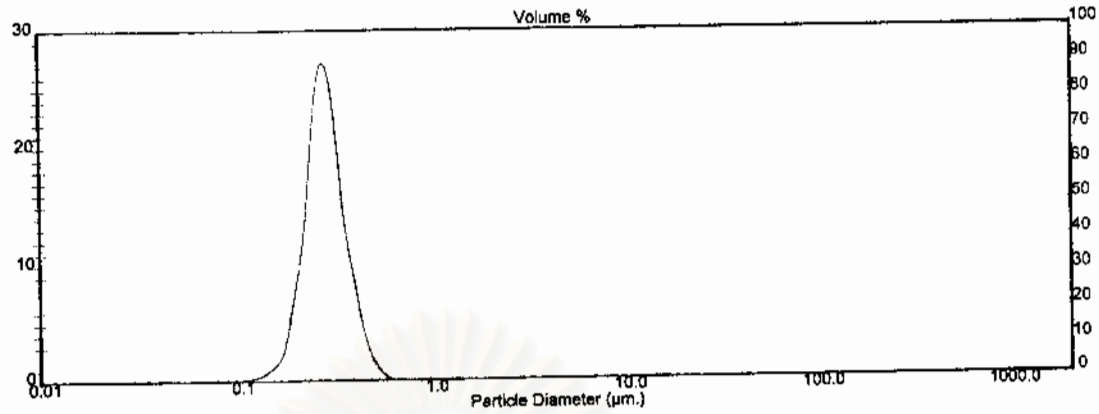


Figure c70. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 0 hour.

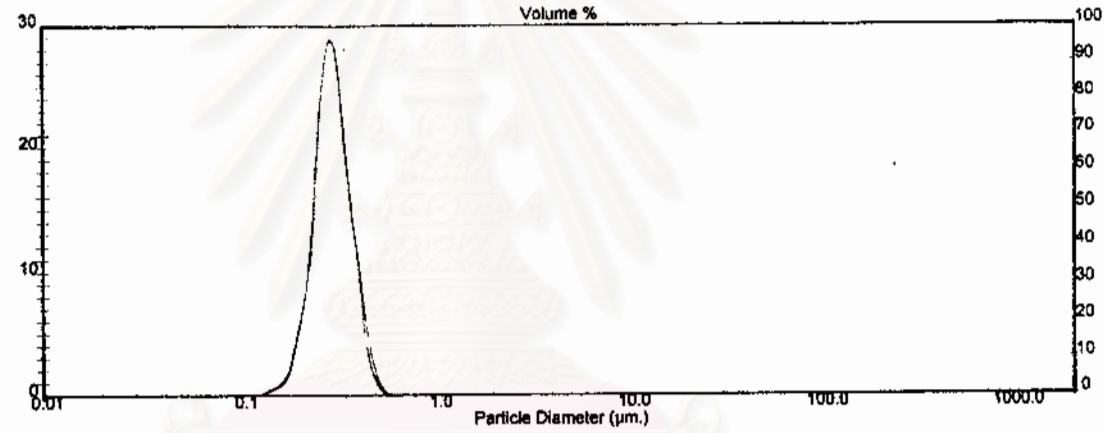


Figure c71. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Lipofundin®MCT/LCT at 24 hours.

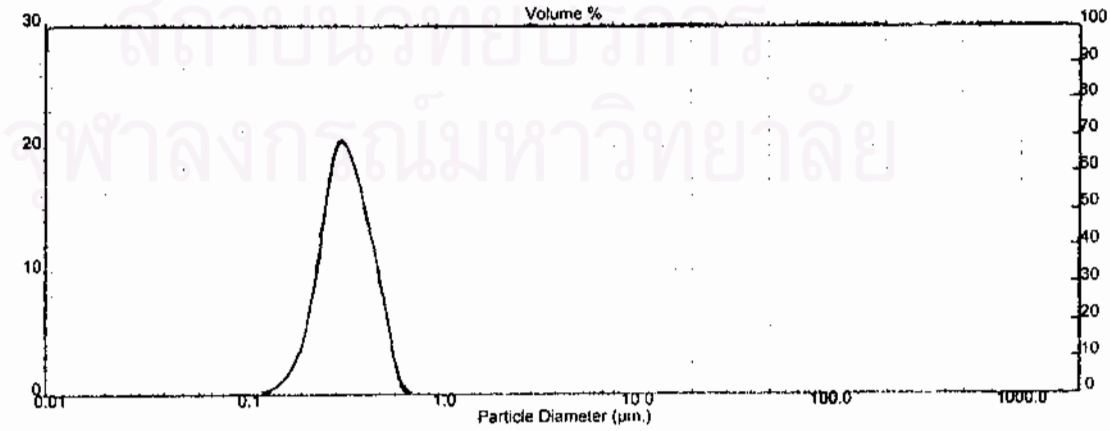


Figure c72. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 0 hour.

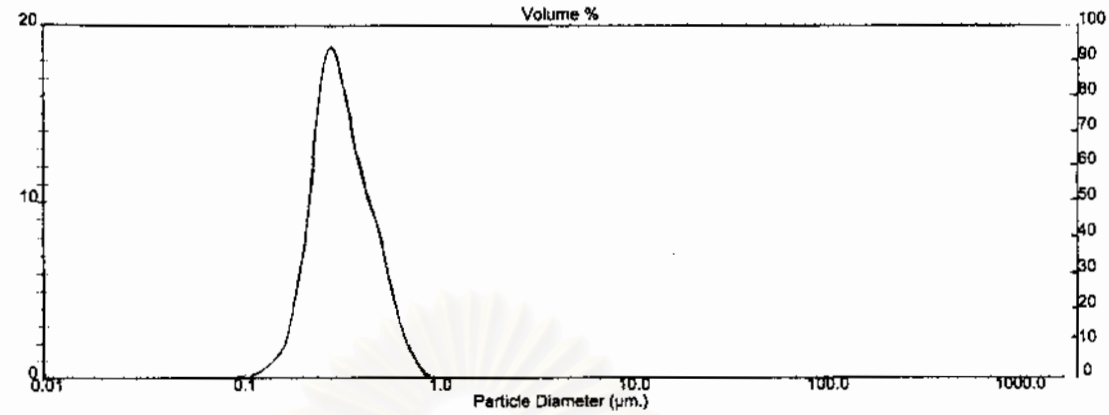


Figure c73. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 24 hours.

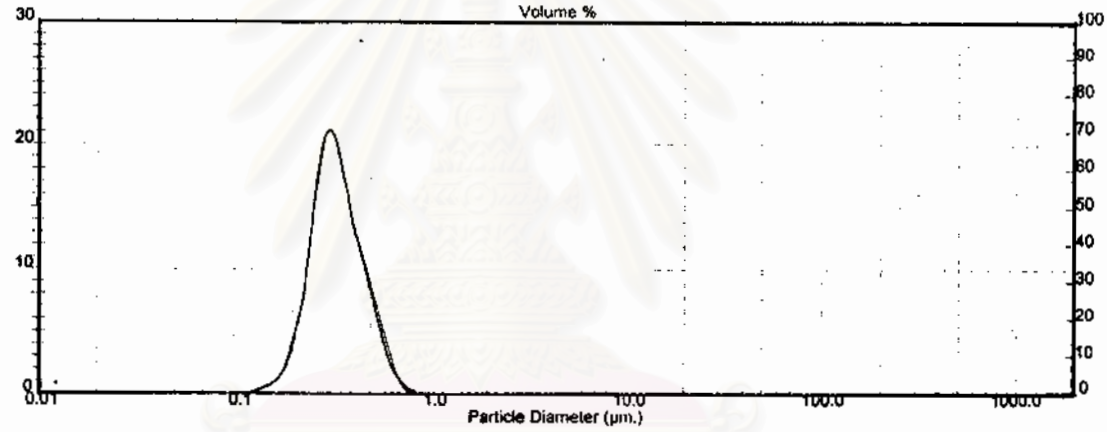


Figure c74. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 0 hour.

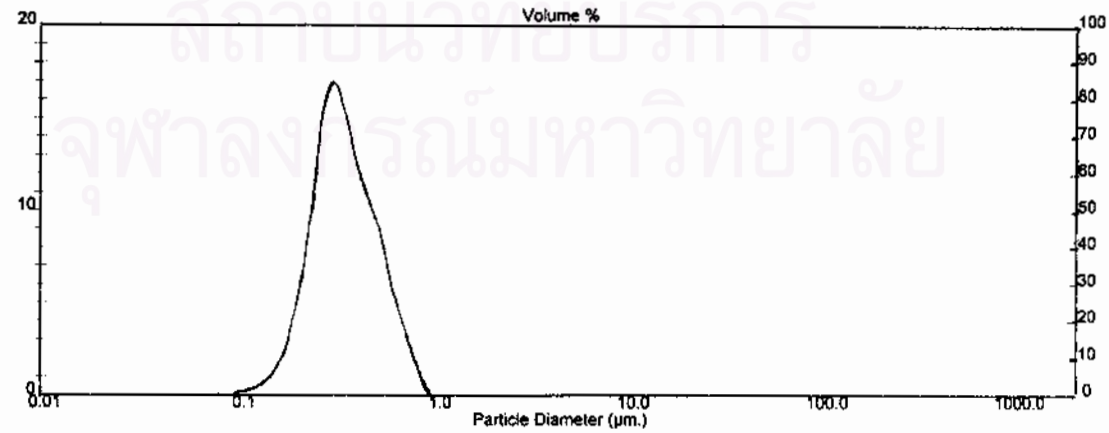


Figure c75. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 24 hours.

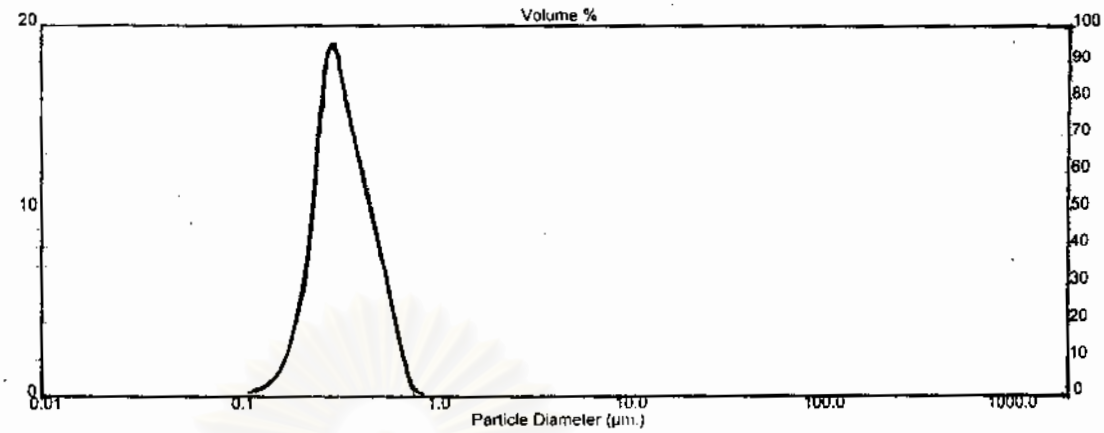


Figure c76. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 0 hour.

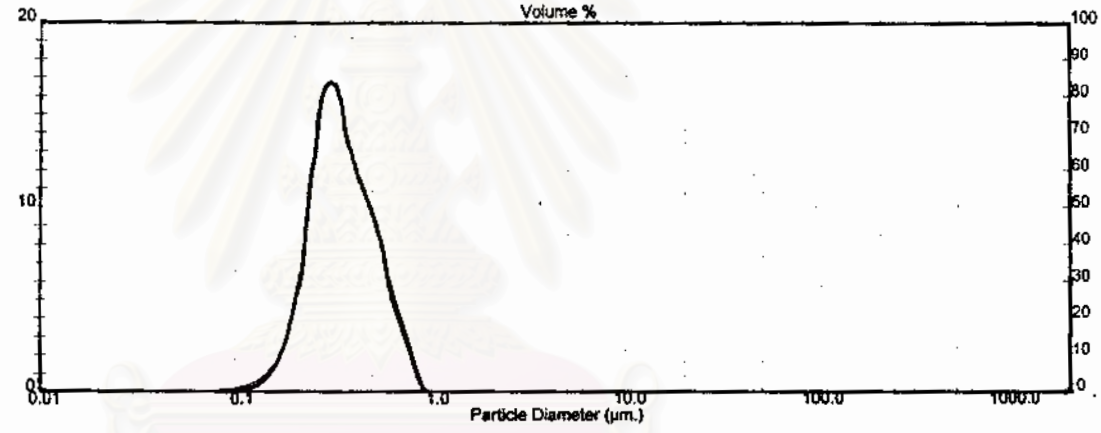


Figure c77. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Lipofundin-S® at 24 hours.

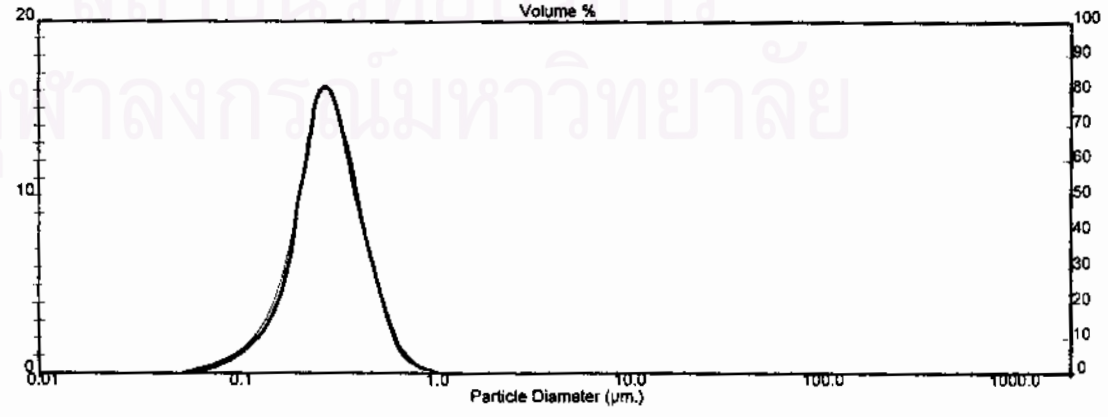


Figure c78. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 0 hour.

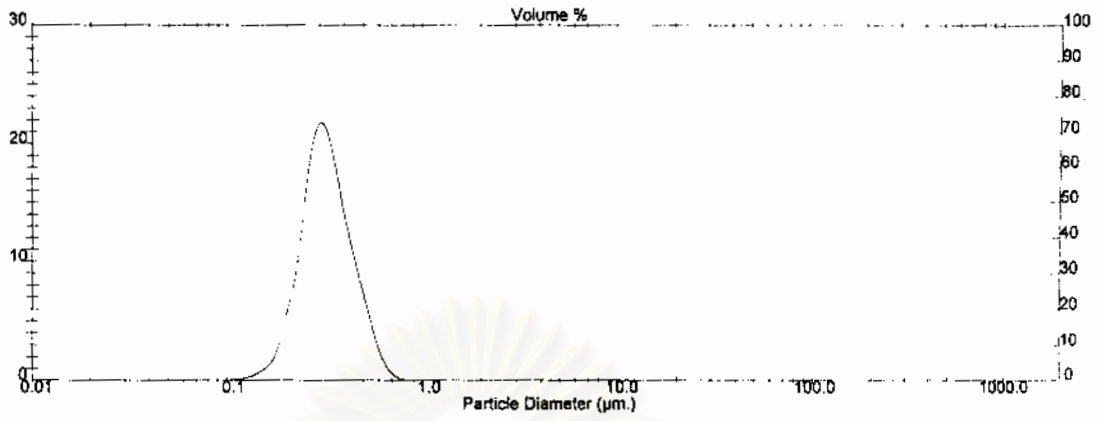


Figure c79. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 24 hours.

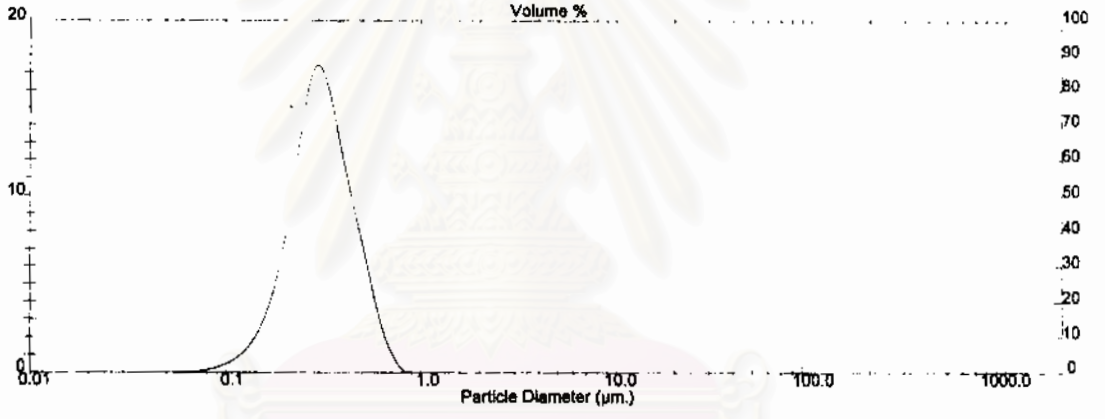


Figure c80. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 0 hour.

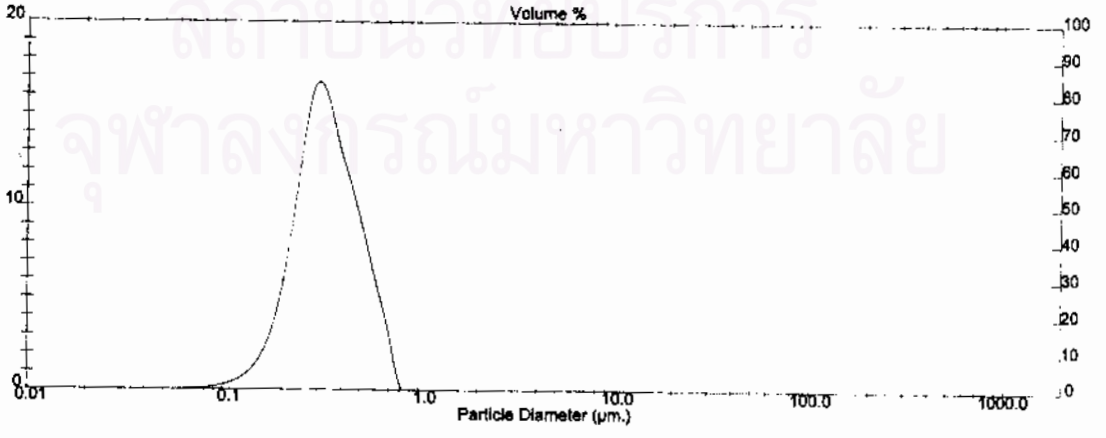


Figure c81. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 24 hours.

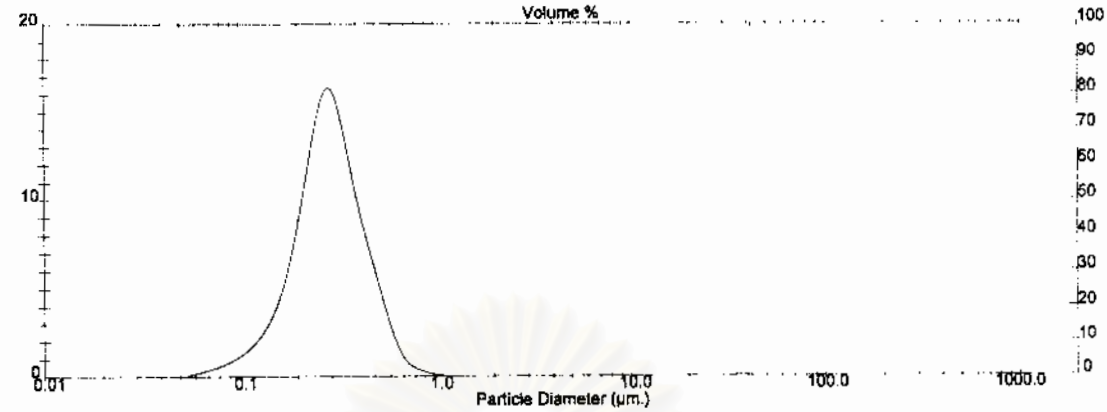


Figure c82. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Pharmalipid in the presence of Addamel-N® and OMVI® at 0 hour.

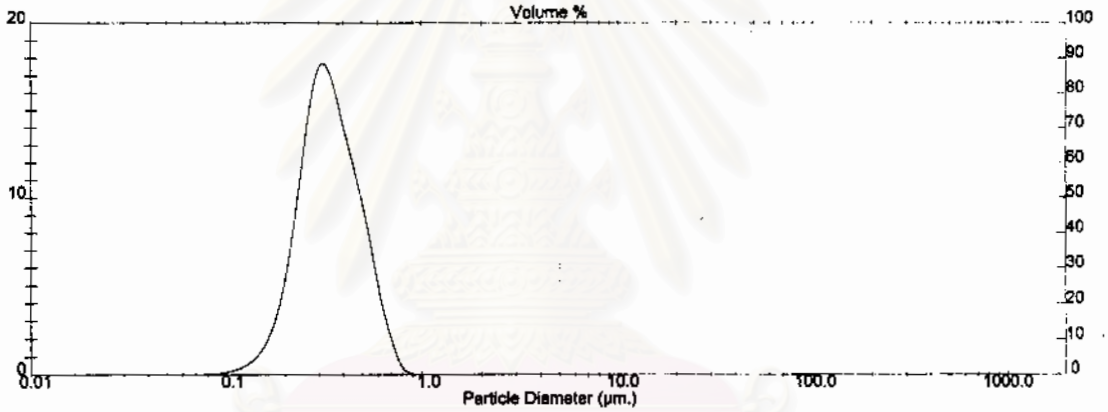


Figure c83. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:10% Pharmalipid in the presence of Addamel-N® and OMVI® at 24 hours.

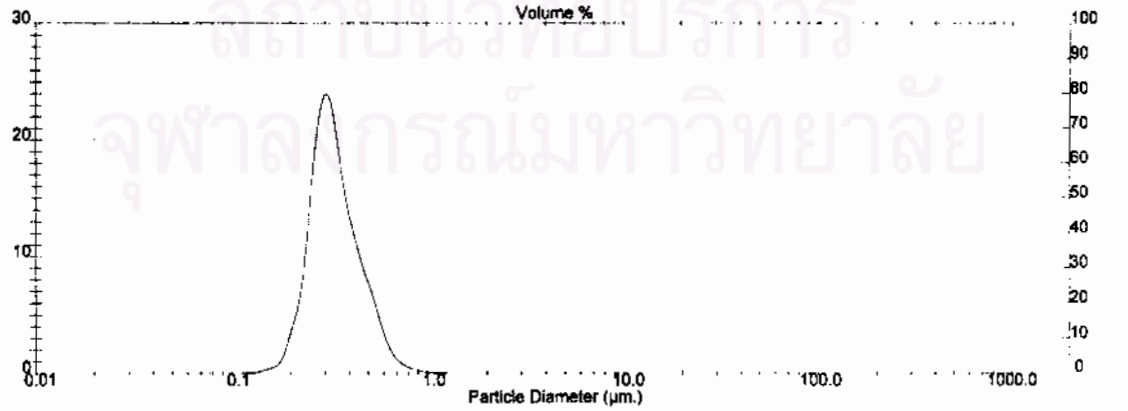


Figure c84. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 0 hour.

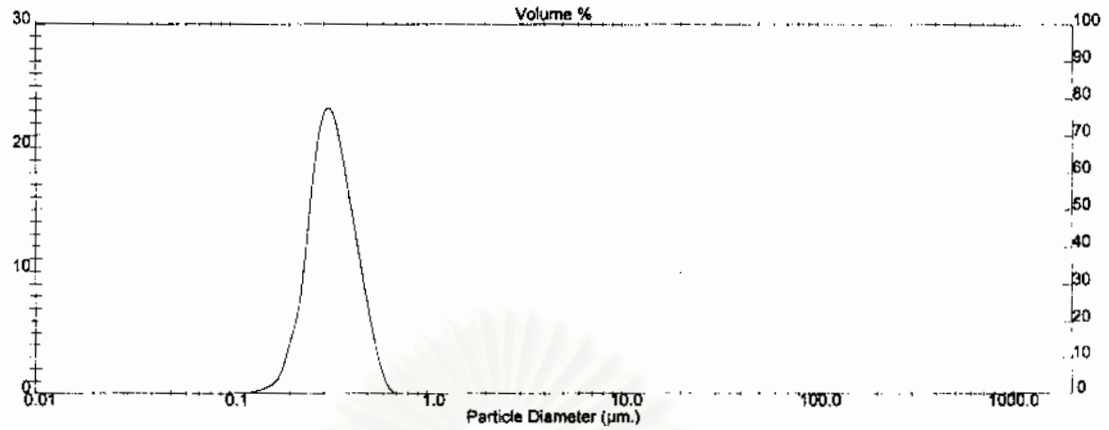


Figure c85. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:10% Pharmalipid at 24 hours.

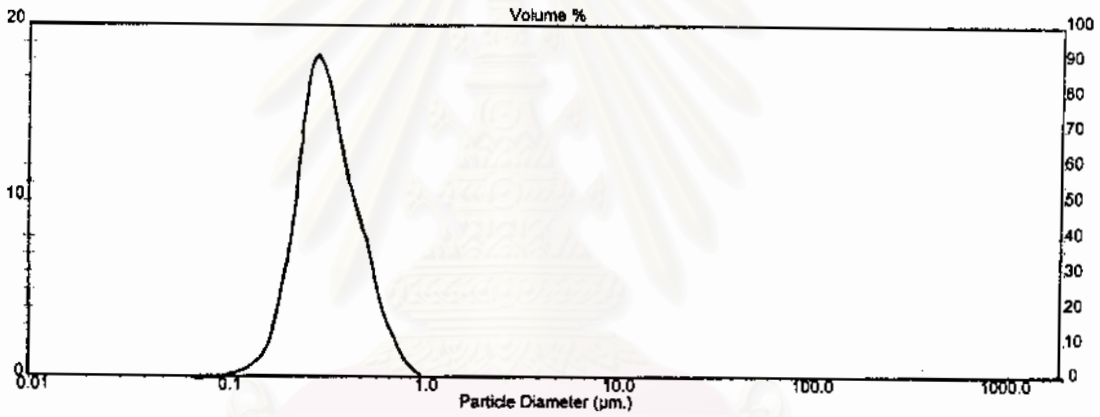


Figure c86. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:20% Intralipid® at 0 hour.

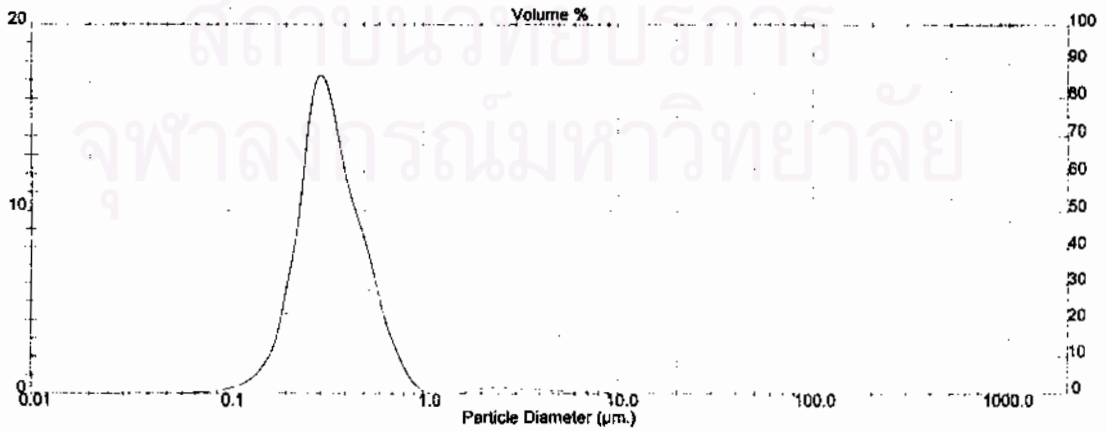


Figure c87. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

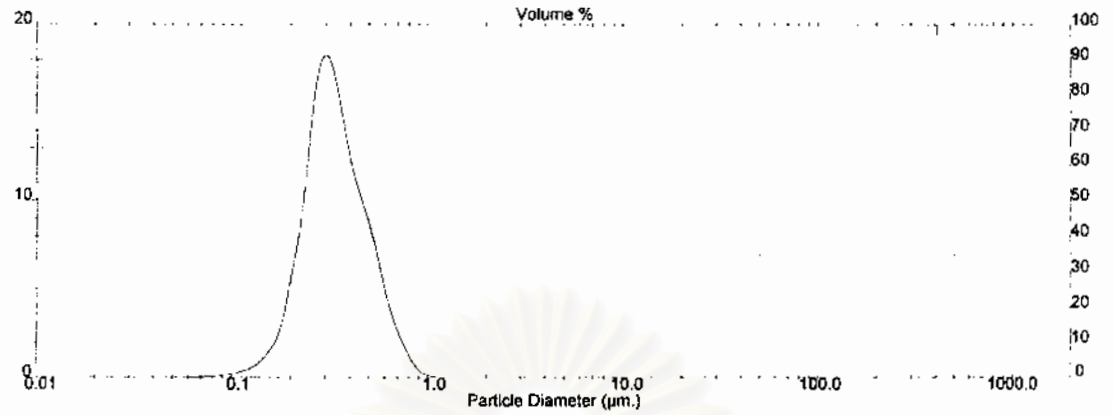


Figure c88. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Intralipid® at 0 hour.

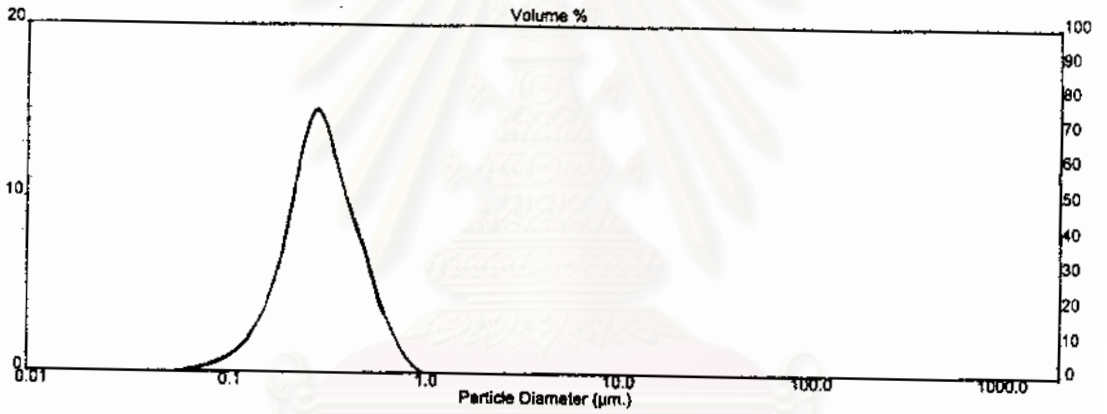


Figure c89. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

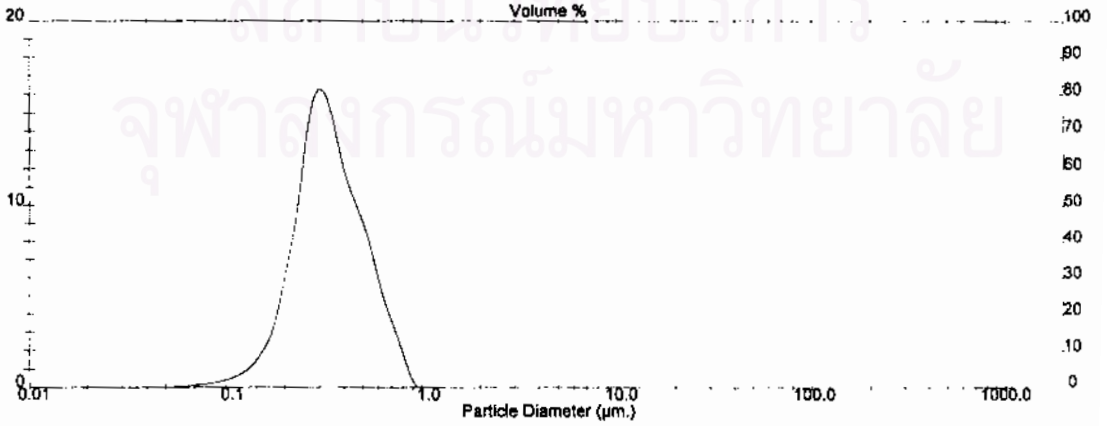


Figure c90. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Intralipid® in the presence of Addamel-N® and OMVI® at 0 hour.

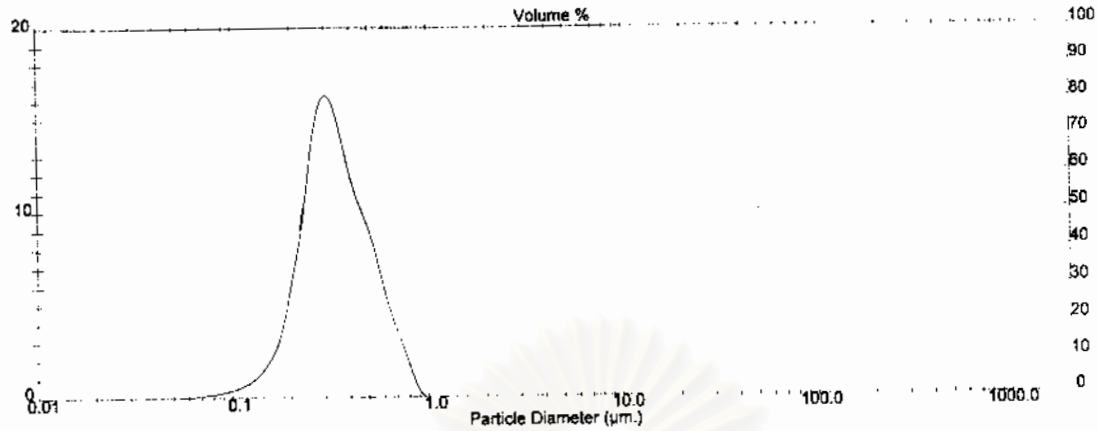


Figure c91. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Intralipid® in the presence of Addamel-N® and OMVI®at 24 hours.

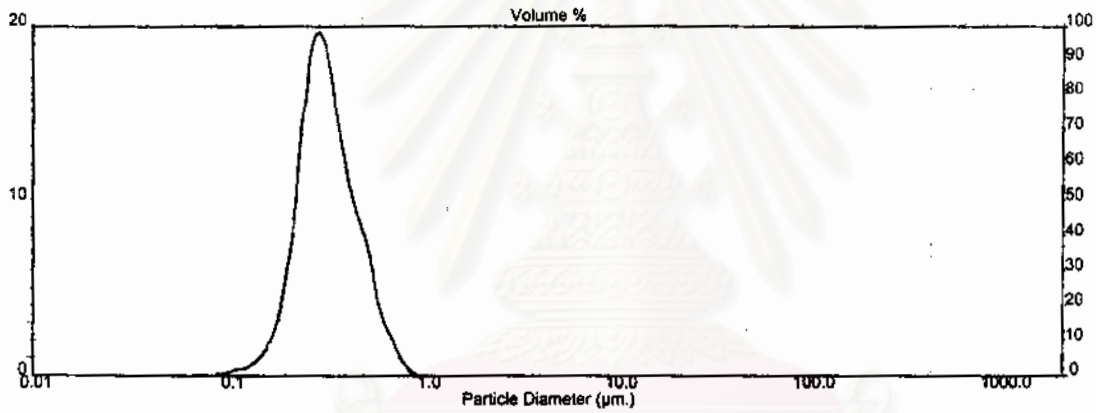


Figure c92. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:20% Intralipid® at 0 hour.

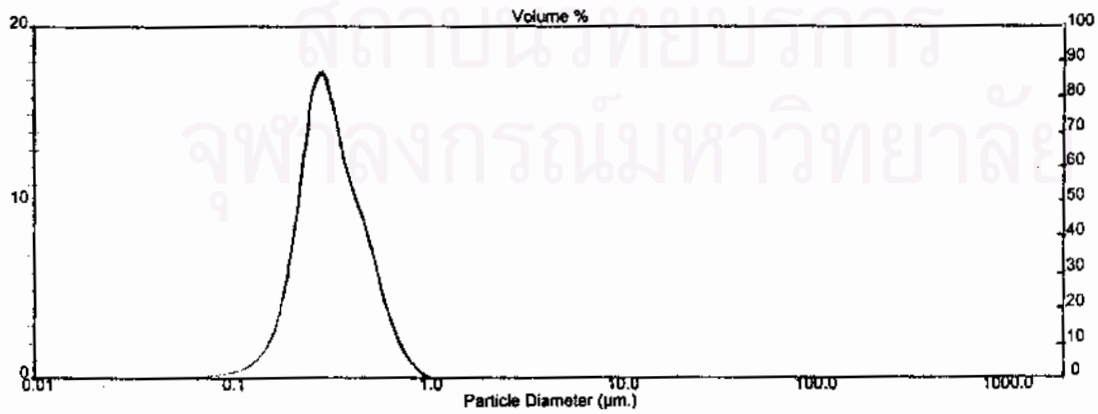


Figure c93. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

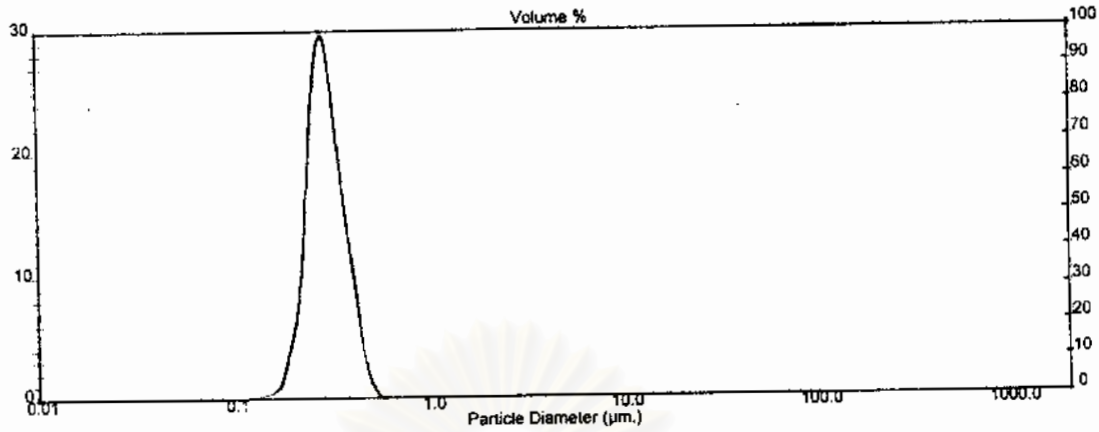


Figure c94. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 0 hour.

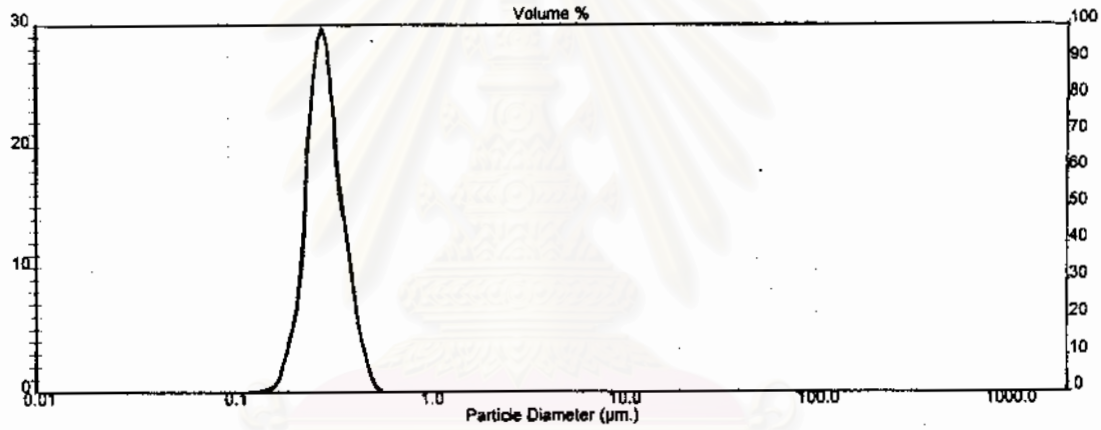


Figure c95. Particle size distribution of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 24 hours.

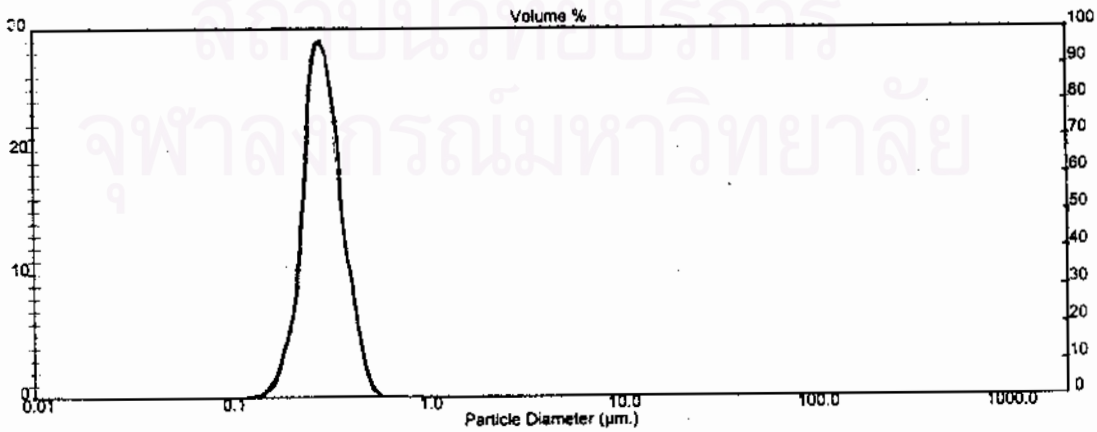


Figure c96. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 0 hour.

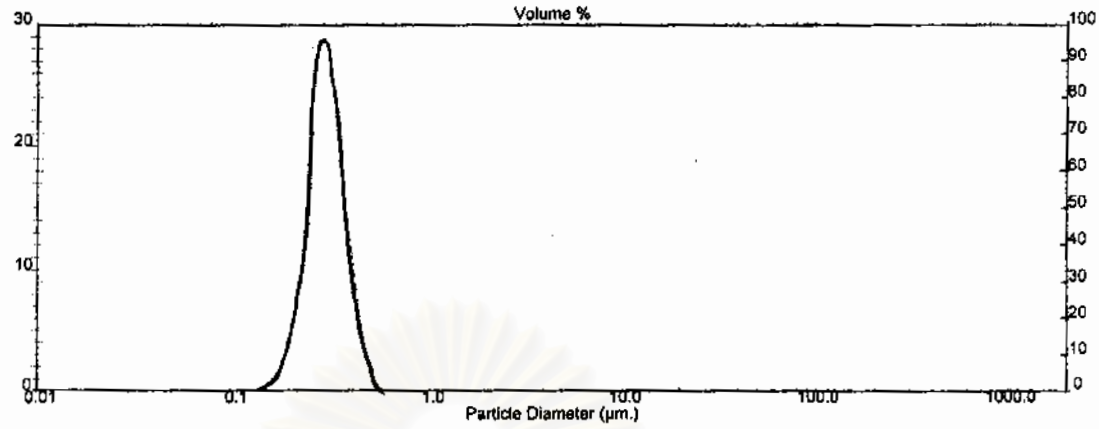


Figure c97. Particle size distribution of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 24 hours.

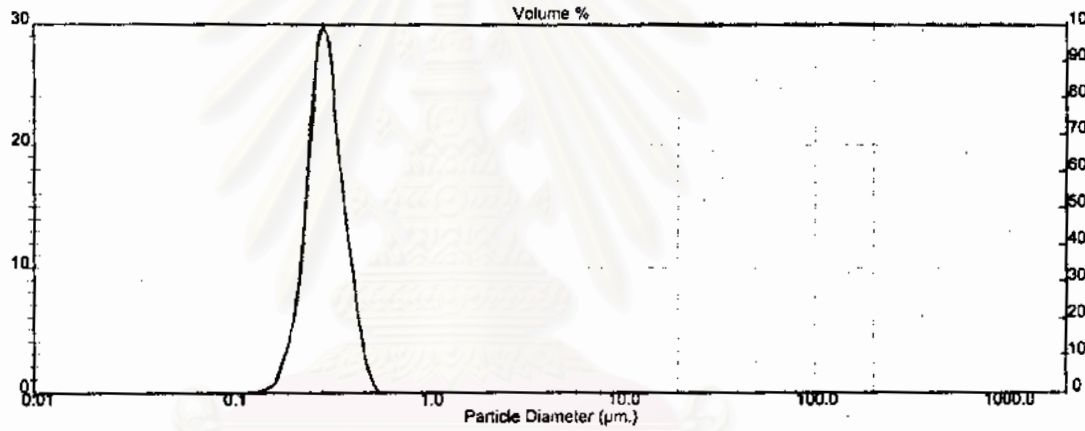


Figure c98. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 0 hour.

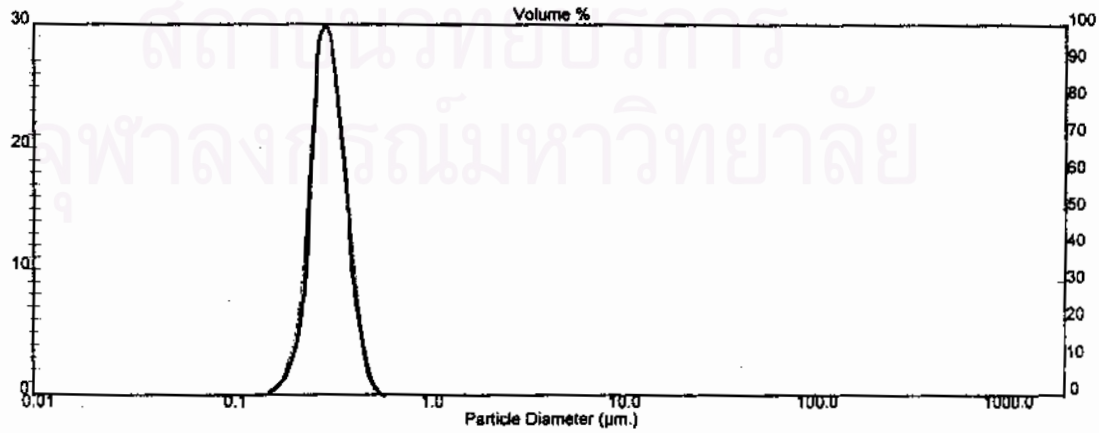


Figure c99. Particle size distribution of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:20% Lipofundin®MCT/LCT at 24 hours.

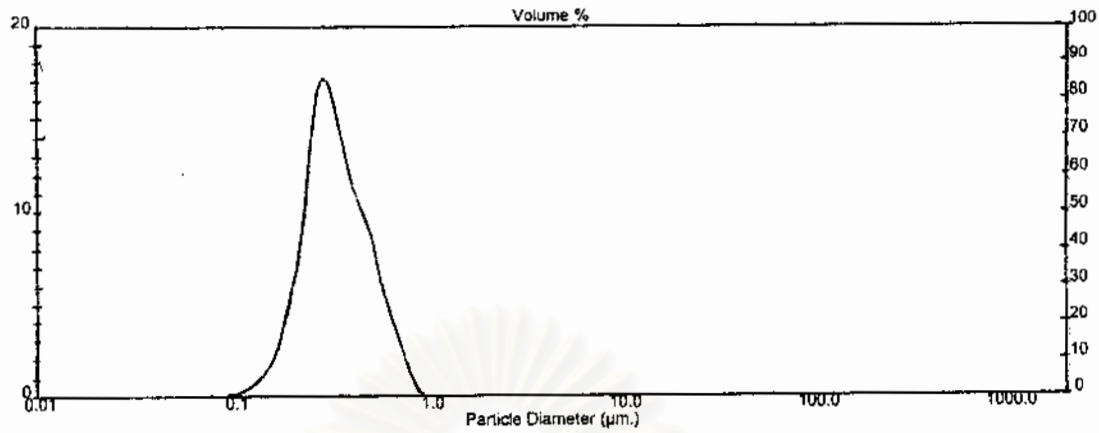


Figure c100. Particle size distribution of cream layer of TNA formulation containing 2:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

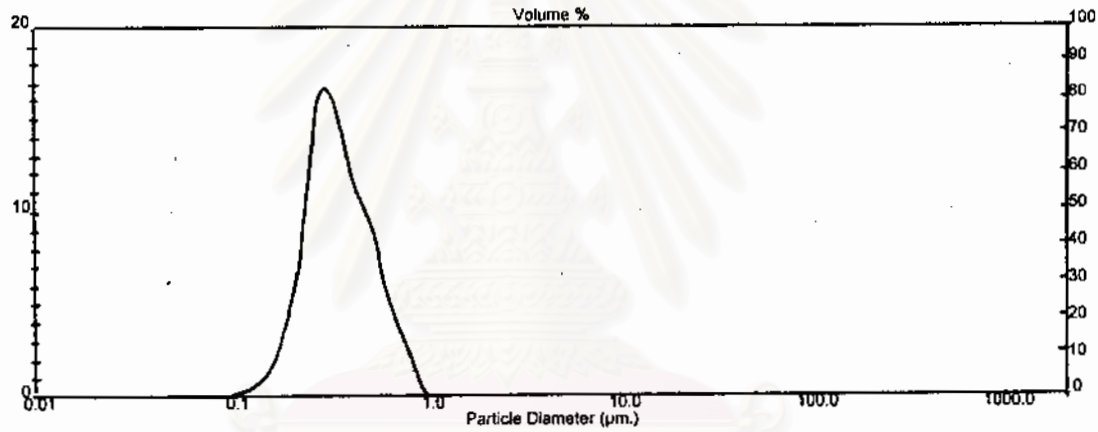


Figure c101. Particle size distribution of cream layer of TNA formulation containing 3:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

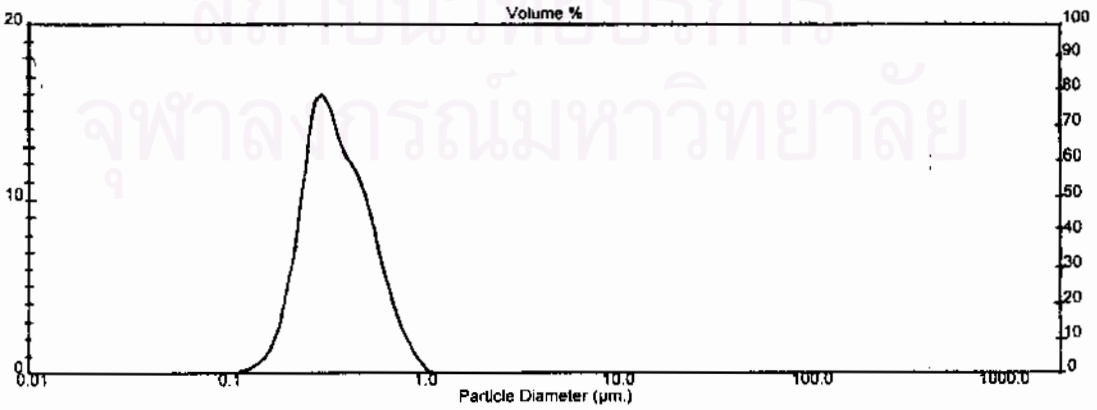


Figure c102. Particle size distribution of cream layer of TNA formulation containing 4:1 volume ratio of Vamin®Glucose:20% Intralipid® at 24 hours.

APPENDIX D

Zeta potential of lipid emulsion

The surface forces effects in the controlling the behavior of colloidal systems. This force is much more important than the gravity effects. The Zeta meter system 3.0 can measure the effect of electrostatic charge of the particle that causes the electrical repulsion between adjacent particles and the attractive force (called the van der Waals force). The net result depends on the relative magnitude of both forces. Numberous encounters occur between particles as they are moved by mechanical agitation, convection currents and Brownian motions. The outcome of these collisions will depend in part on the net attractive or repulsive force between the colloids. This charge produces a difference in electrical potential, in millivolts, between the surface of each colloid and the bulk of the suspending liquid. This difference is called zeta potential.

The Zeta meter system 3.0 is an instrument for zeta potential measurement. The concept of this instrument is that the charged colloid will move when the suspension is placed between two electrodes that have a DC voltage across them, and its velocity (called electrophoresis mobility) will be proportional to the zeta potential. This phenomena is known as electrophoresis.

The Helmholtz-Smoluchwski equation is the most elementary expression for zeta potential. The basic formula shows a direct relation between zeta potential and electrophoresis mobility as follows: (Muslin Limpanasitthikul, 1991)

$$\zeta = \frac{v}{E} \times \frac{4\pi\eta}{\in} \times \left(9 \times 10^4\right)$$

Where: ζ = Zeta potential of the suspened particle in volts

v = The velocity of migration in cm/sec

E = The potential gradient in volts/cm

η = The velocity of the medium in poise (dyne sec/cm^2) at temperature (millivolts) , t

$\in$ = The dielectric constant of the medium

The term *v/E* means the electrophoresis mobility (*EM*). This is determined by the Zeta meter system 3.0 in microns/sec per volts/cm. So it is preferable to calculate the zeta potential in practical millivolts, the formula then becomes: (Zeta meter system 3.0, Instrumental manual).

$$\zeta = 113{,}000 \times \frac{\eta}{\in} \times EM$$

Where: ζ = Zeta potential of the suspened particle in millivolts

EM = The electrophoresis mobility at actual temperature (millivolts) in micons/sec per volts/cm

Table d1 shown the interaction between zeta potential determinations and the probable response of the suspension being tested. Table d2 shown the zeta potential values of standard Minusil® solution. Table d3-d6 shown the zeta potential of lipid emulsions and TNA preparations.

Table d1. The interaction between zeta potential determinations and the probable response of the suspension being tested (from the Zeta meter system 3.0 manual)

Stability characteristics	Average ZP in millivolts
Maximum agglomeration and precipitation	+3 to 0
Excellent agglomeration and precipitation	-1 to -4
Fair agglomeration and precipitation	-5 to -10
Threshold of agglomeration (agglomerates of 2 to 10 colloids)	-11 to -20
Plateau of slight stability (few agglomerates)	-21 to -30
Moderate stability (no agglomerates)	-31 to -40
Good stability	-41 to -50
Very good stability	-51 to -60
Excellent stability	-61 to -80
Maximum stability	-81 to -125

Table d2. Zeta potential of standard Minusil® suspension (millivolts)

Sample 1

-51.312	-47.039	-47.937	-52.000	-47.515	-52.367	-48.515	-51.601	-46.359	-49.257
-45.671	-47.593	-48.257	-53.445	-52.078	-50.835	-48.257	-46.749	-47.515	-49.523
-47.937	-52.578	-45.460	-52.921	-46.273	-49.968	-48.359	-51.312	-46.148	-50.601
-50.734	-50.835	-46.515	-50.835	-45.460	-47.117	-52.367	-45.828	-50.523	-45.460
-48.359	-49.070	-51.023	-46.062	-46.749	-50.734	-47.437	-48.804	-45.828	-49.781
count	50			mean	-48.898			SD	2.347

Sample 2

-50.390	-51.604	-47.382	-50.101	-45.882	-48.624	-44.617	-42.984	-44.828	-44.039
-45.437	-53.234	-48.281	-43.242	-41.765	-47.437	-50.289	-41.296	-44.039	-44.109
-50.632	-53.234	-46.062	-48.706	-46.828	-47.914	-45.593	-50.945	-49.179	-47.567
-47.859	-44.671	-44.671	-52.367	-52.929	-50.820	-46.960	-47.437	-43.984	-49.992
-48.179	-45.304	-44.984	-47.593	-46.617	-45.304	-51.531	-43.242	-44.828	-46.746
count	50			mean	-47.245			SD	3.073

Sample 3

-48.359	-49.812	-51.343	-44.749	-44.906	-51.976	-48.992	-46.148	-46.178	-44.249
-55.984	-48.835	-50.414	-48.093	-46.328	-53.773	-49.437	-43.195	-45.195	-44.039
-54.085	-52.640	-55.539	-53.867	-48.257	-48.968	-48.015	-48.093	-50.335	-48.515
-55.164	-48.093	-49.703	-45.671	-45.117	-50.523	-46.148	-54.195	-53.109	-46.148
-56.570	-53.828	-46.593	-53.195	-49.492	-51.398	-50.632	-52.078	-51.312	-50.234
count	50			mean	-49.590			SD	3.478

Table d3. Zeta potential of lipid emulsions containing soybean oil and various type and amount of emulsifiers before and after autoclaving (millivolts)

Formulation		Zeta potential (millivolts)[1]			Formulation		Zeta potential (millivolts)[1]		
		Sample 1	Sample 2	Sample 3			Sample 1	Sample 2	Sample 3
5SB+1LE+1T80	a)	-28.025	-27.364	-28.423	10SB+2LE+1P188	a)	-28.380	-28.003	-28.914
	b)	-29.169	-28.170	-28.809		b)	-28.474	-29.456	-28.931
	c)	-28.647	-27.271	-29.460		c)	-27.429	-28.890	-28.849
5SB+1LE+1P188	a)	-31.996	-31.312	-30.357	20SB+2LE+1T80	a)	-36.354	-34.456	-34.467
	b)	-31.377	-31.646	-30.570		b)	-35.500	-35.381	-34.543
	c)	-32.933	-32.942	-32.338	10SB+2LE+1.5T80	a)	-32.030	-31.773	-31.219
	d)	-30.428	-28.157	-27.636		b)	-30.259	-33.578	-31.533
5SB+2LE+1T80	a)	-28.032	-28.334	-28.182		c)	-32.385	-32.493	-33.263
	b)	-28.444	-28.238	-27.207		d)	-30.774	-31.778	-32.373
	c)	-31.371	-31.474	-31.708	10SB+2LE+2T80	a)	-31.578	-33.378	-31.389
	d)	-23.523	-25.520	-24.546		b)	-31.772	-30.350	-30.822
						c)	-31.385	-31.618	-30.288
5SB+2LE+1P188	a)	-25.911	-24.330	-25.409	10SB+1.71LE+1.29T80	a)	-32.499	-32.014	-31.841
	b)	-29.840	-29.881	-29.111		b)	-33.469	-33.588	-33.512
	c)	-29.388	-30.077	-30.242		c)	-31.269	-31.931	-32.125
	d)	-25.748	-23.594	-25.213		d)	-31.607	-31.757	-31.680
10SB+1LE+1T80	a)	-32.296	-33.107	-33.033	10SB+2.28LE+1.72T80	a)	-33.731	-32.741	-32.885
	b)	-31.650	-30.533	-31.345		b)	-32.626	-34.330	-32.043
10SB+2LE+1T80	a)	-28.697	-27.502	-28.357		c)	-32.150	-32.970	-32.122
	b)	-30.732	-30.151	-29.071	10SB+2.86LE+2.14T80	a)	-31.845	-31.956	-32.048
	c)	-30.377	-28.816	-29.246		b)	-30.759	-31.944	-32.185
						c)	-32.461	-31.175	-32.791

[1] = show mean of 50 particles of each sample

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature

Table d4. Zeta potential of lipid emulsions containing MCT oil and various type and amount of emulsifiers before and after autoclaving (millivolts)

Formulation		Zeta potential (millivolts)[1]			Formulation		Zeta potential (millivolts)[1]		
		Sample 1	Sample 2	Sample 3			Sample 1	Sample 2	Sample 3
5MCT+1LE+1T80	a)	-29.785	-29.602	-29.492	5MCT+1LE+1P188	a)	-30.067	-30.142	-30.919
	b)	-30.874	-29.866	-29.095		b)	-31.309	-30.061	-31.195
	c)	-28.828	-27.669	-28.109		c)	-30.508	-29.714	-30.436
	d)	-30.512	-30.044	-29.071		d)	-30.750	-29.034	-31.382
	e)	-26.671	-29.161	-30.504					
	f)	-29.879	-29.933	-29.298					
	g)	-22.701	-20.817	-23.623					

[1] = show mean of 50 particles of each sample

a): before autoclaving, b): after autoclaving and storage for 24 hours at room temperature, c): after autoclaving and storage for 1 week at room temperature, d): after autoclaving and storage for 1 month at room temperature, e): after autoclaving and storage for 2 months at room temperature, f): after autoclaving and storage for 3 month at room temperature, g): after autoclaving and storage in accelerated stability test

Table d5. Zeta potential of 10% and 20% commercial lipid emulsion and 10% Pharmalipid

Formulation	Zeta potential (millivolts)[1]		
	Sample 1	Sample 2	Sample 3
10% Intralipid®	-44.700	-44.726	-45.333
10% Lipofundin®MCT/LCT	-38.480	-39.968	-39.250
10% Lipofundin-S®	-42.235	-41.314	-41.079
10% Pharmalipid	-36.360	-35.355	-36.711
20% Intralipid®	-45.086	-43.453	-43.581
20% Lipofundin®MCT/LCT	-39.052	-38.836	-39.042

[1] = show mean of 50 particles of each sample

Table d6. Zeta potential of TNA system after mixing at0 hour and 24 hours at room temperature

Formulation at volume of VG to emulsion		Zeta potential (millivolts)[1]			Formulation at volume of VG to emulsion		Zeta potential (millivolts)[1]		
		Sample 1	Sample 2	Sample 3			Sample 1	Sample 2	Sample 3
VG:10% Intralipid 2:1	h)	-44.364	-44.594	-45.168	VG:10%Pharmalipid 3:1	h)	-26.449	-25.933	-26.570
	i)	-42.548	-44.584	-44.669		i)	-26.473	-24.919	-25.778
VG:10% Intralipid 3:1	h)	-43.078	-44.684	-44.764	VG:10%Pharmalipid 3:1 (++)	h)	-24.991	-25.531	-24.722
	i)	-41.701	-42.892	-41.563		i)	-30.634	-28.992	-30.115
VG:10% Intralipid 4:1	h)	-45.663	-44.745	-44.544	VG:10%Pharmalipid 4:1	h)	-28.393	-27.293	-25.310
	i)	-41.611	-42.589	-43.024		i)	-29.630	-28.498	-28.276
VG:10% Lipofundin					VG:20% Intralipid 2:1	h)	-44.659	-44.193	-44.589
MCT/LCT 2:1	h)	-39.093	-39.273	-39.039		i)	-44.339	-43.551	-44.378
	i)	-35.866	-37.366	-36.717	VG:20% Intralipid 3:1	h)	-44.258	-43.900	-43.750
VG:10% Lipofundin						i)	-41.675	-43.348	-43.660
MCT/LCT 3:1	h)	-38.950	-37.896	-38.918	VG:20% Intralipid 3:1 (++)	h)	-43.544	-43.212	-42.912
	i)	-35.445	-36.031	-36.103		i)	-41.896	-40.009	-40.330
VG:10% Lipofundin					VG:20% Intralipid 4:1	h)	-45.534	-43.962	-44.556
MCT/LCT 4:1	h)	-39.114	-39.069	-39.514		i)	-41.720	-42.920	-42.861
	i)	-39.170	-38.886	-38.545	VG:20% Lipofundin MCT/LCT				
VG:10%Lipofundin-S 2:1	h)	-42.061	-42.227	-41.038	2:1	h)	-40.029	-39.868	-40.746
	i)	-40.119	-40.432	-40.576		i)	-37.762	-37.423	-38.565
VG:10%Lipofundin-S 3:1	h)	-42.558	-41.511	-41.024	VG:20% Lipofundin MCT/LCT				
	i)	-40.791	-41.359	-40.913	3:1	h)	-39.030	-39.169	-39.396
VG:10%Lipofundin-S 4:1	h)	-41.763	-41.240	-41.667		i)	-37.508	-38.215	-38.783
	i)	-39.293	-40.483	-40.313	VG:20% Lipofundin MCT/LCT				
VG:10%Pharmalipid 2:1	h)	-35.812	-34.971	-34.416	4:1	h)	-40.473	-39.492	-39.376
	i)	-29.016	-26.166	-25.650		i)	-39.403	-39.577	-39.958

[1] = show mean of 50 particles of each sample
VG = Vamin®Glucose
(++) = added Addamel-N and OMVI
h): after mixing at 0 hour, I): after mixing for 24 hours

BIOGRAPHY

Miss Supinda Ruangthurakit was born on January 16, 1971 in Rayong province, Thailand. She received her Bachelor of Science in Pharmacy Degree from the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand in 1995. After graduation, she works at Queen Sawangwattana (Somdejpraboromrajthevi na Sriracha) Memorial Hospital, Chonburi province.