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APPENDICES

APPENDIX A

DETIAL OF SOME SUBSTANCES

1. **Amphotericin B** (Lund, 1994; Merck, 2001)

1.1 Chemical name

Amphotericin B is a mixture of antifungal polyenes produced by the growth of certain strains of Streptomyces nodosus or by any other means. It consists largely of amphotericin B which is (3*R*,5*R*,8*R*,9*R*,11*S*,13*R*,15*S*, 16*R*,17*S*,19*R*,34*S*, 35*R*,36*R*,37*S*)-19-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy) -16-carboxy-3,5,8, 9,11,13,15,35-octahydroxy-34,36-dimethyl-13,17-epoxyoctatriaconta-20,22,24,26,28, 30,32-heptaen-37-olide.

1.2 Empirical formula

$C_{47}H_{73}NO_{17}$
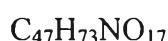
1.3 Structural formula
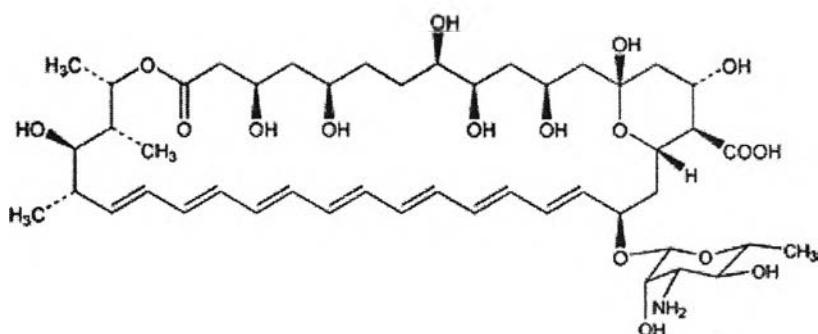
1.4 Appearance

Yellow to orange, odourless or almost odourless powder

1.5 Typical properties

Melting point   : begins to decompose above 200°C

Solubility : insoluble in water at pH 6 to 7. Soluble at pH 2 or pH 11 in water about 0.1 mg/ml. Water soluble increased by sodium desoxycholate. Soluble in DMF 2 to 4 mg/ml; in DMF+HCl : 60 to 80 mg/ml; in DMSO; 30 to 40 mg/ml.

1.6 Stability

Amphotericin B in the solid state appears to be stable for long periods of time when stored at moderate temperature and protected from light and air. The major route of degradation of amphotericin, in aqueous solution, is thought to be epoxidation and *trans-cis* isomerism, although degradation products have not been identified. Dilute solutions are light-sensitive. Amphotericin B is inactivated at low pH values.

2. Glyceryl behenate (The Council of Europe, 2001; Kibbe, A.H., 2000)

2.1 Chemical name

Glycerobehenate

Glycerol dibehenate

2.2 Empirical formula

$C_{69}H_{134}O_6$

2.3 Structural formula

$$
\begin{array}{l}
\quad\quad\quad\quad O \\
\quad\quad\quad\quad \| \\
CH_2O\!-\!\!-\!C\!-\!\!-CH_2(CH_2)_{19}CH_3 \\
|\quad\quad\quad O \\
|\quad\quad\quad \| \\
CHO\!-\!\!-C\!-\!\!-CH_2(CH_2)_{19}CH_3 \\
|\quad\quad\quad O \\
|\quad\quad\quad \| \\
CH_2O\!-\!\!-\!C\!-\!\!-CH_2(CH_2)_{19}CH_3
\end{array}
$$
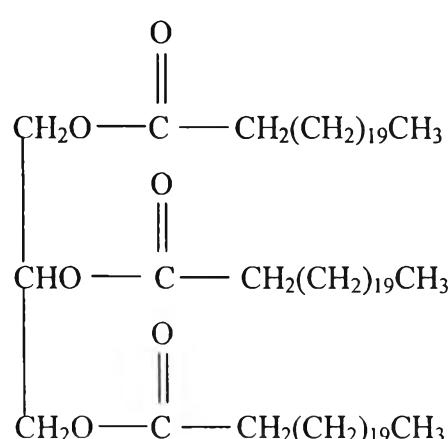
2.4 Appearance

Glycerol behenate is fine powder or white or almost white with a faint odor.

2.5 Typical properties

Melting point   : 65-77°C

Saponification value : 145-164

Solubility   : glyceryl behenate is insoluble in water, soluble in methylene chloride and partly soluble in alcohol.

2.6 Stability

Glyceryl behenate should be stored in an airtight container, protected from light and moisture.

2.7 Safety

Glyceryl behenate is generally regarded as an essential nontoxic and nonirritant material.

3. Glyceryl palmitostearate (Kibbe, A.H., 2000)

3.1 Chemical name

Octadecanoic acid, 2,3-dihydroxypropyl ester mixed with 3-hydroxy-2-[(1-oxohexadecyl)-oxy] propyl octadecanoate, 2-[(1-oxohexadecyl)-oxy]-1,3-propanediyl dioctadecanoate and 1,2,3-propane triol

3.2 Empirical formula

Glyceryl palmitostearate is a mixture of mono-, di-, and tri-glycerides of C_{16} and C_{18} fatty acids.

3.3 Structural formula

See section 3.1 and 3.2

3.4 Appearance

Glyceryl palmitostearate is fine powder with a faint odor.

3.5 Typical properties

Melting point   : 52-55°C

Saponification value : 175-195

Solubility   : freely soluble in chloroform and dichloromethane; practically insoluble in ethanol (95%), mineral oil, and water.

3.6 Stability

Glyceryl palmitostearate should not be stored at temperatures above 35°C in an airtight container, protected from light and moisture.

3.7 Safety

Glyceryl palmitostearate is generally regarded as an essential nontoxic and nonirritant material.

4. Medium Chain Triglyceride Oil (Kibbe, A.H., 2000)

4.1 Chemical name

Medium-chain triglycerides; glyceryl tricaprylate/caprate; Miglyol 812; MCT oil; thin vegetable oil

4.2 Empirical formula

Medium chain triglyceride is the fixed oil extracted from the hard, dried fraction of the endosperm of Cocos nucifera L. by hydrolysis, fractionation of the fatty acids obtained, and re-esterification. 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.

4.3 Structural formula

$$
\begin{array}{l}
\text{H}_2\text{C} \diagup \text{O} \!\!-\!\! \underset{\underset{\text{O}}{\|}}{\text{C}} \!\!-\!\! (\text{CH}_2)_x\text{CH}_3 \\[2em]
\text{HC} \!-\! \text{O} \!\!-\!\! \underset{\underset{\text{O}}{\|}}{\text{C}} \!\!-\!\! (\text{CH}_2)_x\text{CH}_3 \\[2em]
\text{H}_2\text{C} \diagdown \text{O} \!\!-\!\! \underset{\underset{\text{O}}{\|}}{\text{C}} \!\!-\!\! (\text{CH}_2)_x\text{CH}_3
\end{array}
$$
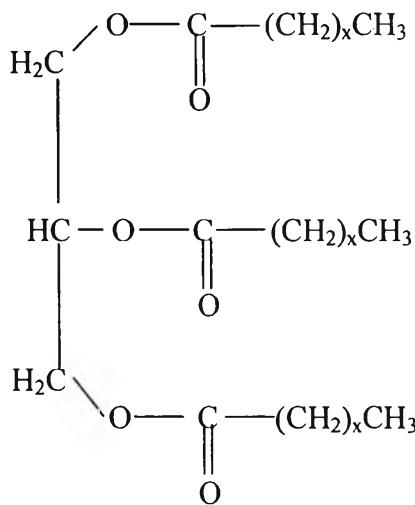
Where x = 6,8

4.4 Appearance

A colorless to slightly yellowish oily liquid which is practically odorless and tasteless. It solidifies at about 0°C and has a low viscosity even at temperatures near its solidification point.

4.5 Typical properties

Viscosity at 20°C   : 28-32 mPa s

Saponification value   : 325-345

Solubility   : soluble, in all proportions at 20°C, in acetone, benzene, 2-butanone, carbon tetrachloride, chloroform, dichloromethane, ethanol (95%), ether, ethyl acetate, petroleum ether; practically insoluble in water.

4.6 Stability

Medium chain triglycerides are stable over the wide range of storage temperatures. They should be stored protected from light in a well-filled and well-closed container. When stored dry, in sealed containers, medium-chain triglycerides remain stable for many years.

4.7 Safety

Medium chain triglycerides are used in a variety of pharmaceutical formulations including oral, parenteral, and topical products and are generally regarded as essentially nontoxic and nonirritant materials.

5. Propylene glycol (Kibbe, A.H., 2000)

5.1 Chemical name

1,2-Propanediol

5.2 Empirical formula

$C_3H_8O_2$

5.3 Structural formula
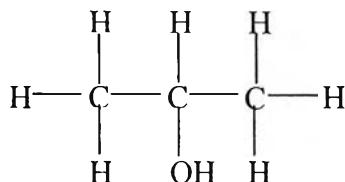
5.4 Appearance

Propylene glycol is a clear, colorless, viscous, practically odorless liquid with slightly acrid taste resembling glycerin.

5.5 Typical properties

Boiling point : 188°C

Melting point : -59°C

Solubility : miscible with acetone, chloroform, ethanol (95%), glycerin and water; soluble 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils but will dissolve some essential oils.

5.6 Stability

Propylene glycol is stable in well closed container at cool temperature, but at high temperatures, in the open, it tends to oxidize, giving rise to products such as propinaldehyde, lactic acid, pyruvic acid and acetic acid.

5.7 Safety

Propylene glycol is widely used in a variety of pharmaceutical formulations and is generally regarded as a nontoxic material.

6. Glycerin (Kibbe, A.H., 2000)

6.1 Chemical name

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

6.2 Empirical formula

$C_3H_8O_3$

6.3 Structural formula

```
        H
        |
  H —— C —— OH
        |
  H —— C —— OH
        |
  H —— C —— OH
        |
        H
```
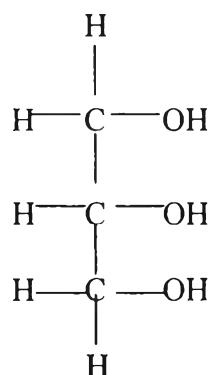
6.4 Appearance

Glycerin is a clear colorless. Odorless, syrupy and hygroscopic liquid.

6.5 Typical properties

Melting point : 17.9°C

Solubility   : Glycerin is miscible with water, alcohol and methanol. One part of glycerin dissolves in 11 parts of ethyl acetate and in 500 parts of ethylether. It is insoluble in benzene, chloroform, ether, mineral oil, fixed and volatile oils, hydrogenated hydrocarbons and aromatic hydrocarbons.

6.6 Stability

Glycerin may crystallize if stored at low temperatures; the crystals do not melt until raised to 20°C. Glycerin should be stored in an airtight container, in a cool, dry, place.

6.7 Safety

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

7   Polyethylene glycol 400 (Kibbe, A.H., 2000)

7.1 Chemical name

α-Hydro-ω-hydroxy-poly(oxy-1,2-ethanediyl)

7.2 Empirical formula

$HOCH_2(CH_2OCH_2)mCH_2OH$

7.3 Structural formula

$$HO-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-(CH_2OCH_2)m-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-OH$$
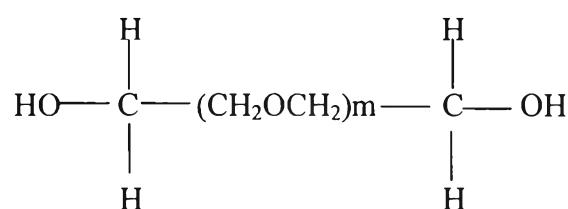
Where m represents the average number of oxyethylene groups. In case of polyethylene glycol 400 (PEG 400), m is 8.7. Its average molecular weight is 380-420.

7.4 Appearance

PEG 400 is clear, colorless, viscous liquids. It has a slight, but characteristic odor and a bitter, slightly burning taste.

7.5 Typical properties

Viscosity : 90.0 mm^2/s at 25°C

Solubility : PEG 400 is soluble in water and miscible in all proportions with other polyethylene glycols (after melting, if necessary). It soluble in acetone, alcohols, benzene, glycerin, and glycols.

7.6 Stability

PEG 400 is chemically stable in air and in solution. It can be sterilized by autoclaving, filtration, or gamma irradiation. It should be stored in well-closed containers in a cool, dry, place. Stainless steel, aluminium, glass, or lined steel containers are preferred.

7.7 Safety

Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials. However, adverse reactions to polyethylene glycols have been reported and although of the relatively low toxicity, any toxicity appears to be greatest with propylene glycols of low molecular weight.

8   Tween® 80 (Kibbe, A.H., 2000)

8.1 Chemical name

Polyoxyethylene 20 sorbitan monooleate

8.2 Empirical formula

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

8.3 Structural formula

$$CH_2$$

$$HCO(C_2H_4O)_wH$$

$$H(OC_2H_4)_xOCH$$

$$HC$$

$$O$$

$$HCO(C_2H_4O)_yH$$

$$CH_2O(C_2H_4O)_zOCR$$
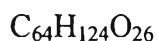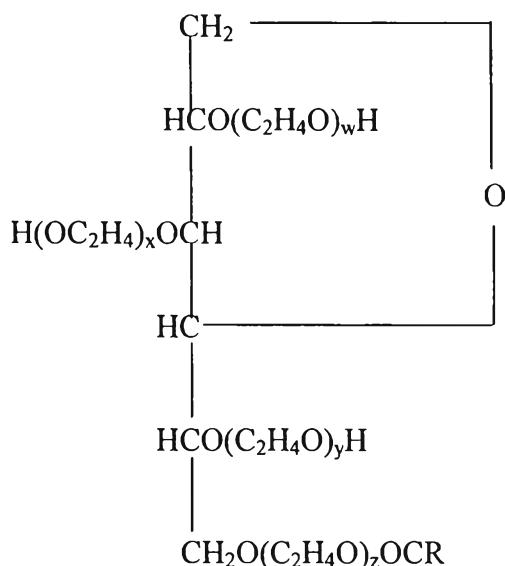
Polyoxyethylene sorbitan monoester

w+x+y+z = 20

R = oleic acid

8.4 Appearance

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

8.5 Typical properties

HLB value	: 15.0
Specific gravity at 25°C	: 1.08
Viscosity	: 425 mPa s
Solubility	: Tween® 80 is miscible with water,

alcohol, dehydrate alcohol, ethylacetate, and methyl alcohol; practically insoluble in liquid paraffin and fixed oils.

8.6 Stability

Tween® 80 is stable to electrolytes and weak acids and bases. It should be stored in a well-closed container, protected from light, in a cool, dry, place.

8.7 Safety

Tween® 80 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 Tween® 80, calculated as total polysorbate esters, at up to 25 mg/kg.

9. Tween® 20 (Kibbe, A.H., 2000)

9.1 Chemical name

Polyoxyethylene sorbitan monolaurate

9.2 Empirical formula

$C_{58}H_{114}O_{26}$

9.3 Structural formula

$$CH_2$$

$$HCO(C_2H_4O)_wH$$

$$H(OC_2H_4)_xOCH$$

$$HC$$

$$O$$

$$HCO(C_2H_4O)_yH$$

$$CH_2O(C_2H_4O)_zOCR$$
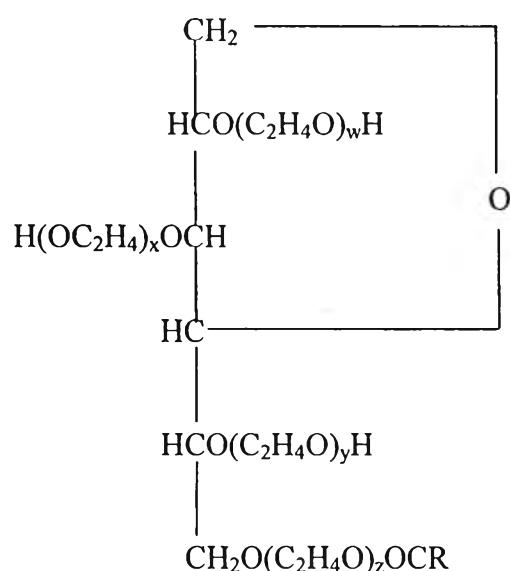
Polyoxyethylene sorbitan monoester

$$w+x+y+z = 20$$

R = lauric acid

9.4 Appearance

Tween® 20 is yellow oily liquid at 25°C

9.5 Typical properties

HLB value	: 16.7
Specific gravity at 25°C	: 1.1
Viscosity	: 400 mPa s
Solubility	: Tween® 20 is soluble in ethanol and

water, insoluble in mineral oil and vegetable oil.

9.6 Stability

Tween® 20 is stable to electrolytes and weak acids and bases.

9.7 Safety

Tween® 20 is widely used in cosmetics, food products, parenteral, oral and topical pharmaceutical formulations and generally regarded as nontoxic and nonirritant material.

10. Polyoxyl 35 castor oil (Cremophor® EL) (Kibbe, A.H., 2000; Strickley, R.G., 2004)

10.1 Chemical name

Polyoxyl 35 castor oil; Polyoxyethylene glycerol triricinoleat 35

10.2 Empirical formula

Polyoxyl 35 castor oil has hydrophobic constituents comprised of about 83% of the total mixture. The main component is 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.

10.3 Structural formula

$$H_2C \diagup \overset{O-C-R_1}{\underset{O}{\|}}$$

$$HC-O-\overset{C}{\underset{O}{\|}}-R_2$$

$$H_2C \diagdown \overset{O-C-R_3}{\underset{O}{\|}}$$

$R_{1,2,3} = (CH_2)_6 \diagdown \diagup = \diagdown \diagup (CH_2)_6\text{-}CH_3$

$(OCH_2CH_2)_{x,y,z}OH$
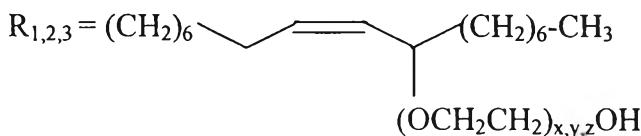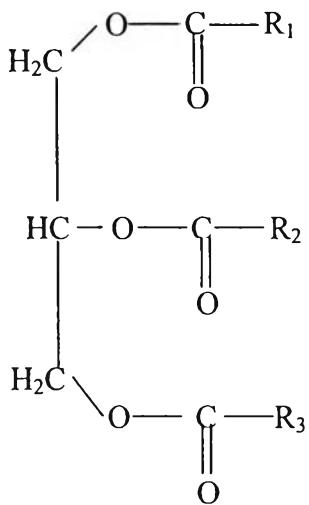
R = Polyethylene glycol ricinoleate

$x+y+z = 35$

10.4 Appearance

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

10.5 Typical properties

Melting point : 19-20°C

HLB value : 12-14

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 and xylene.

10.6 Stability

Cremophor® EL is stable in many organic solvents such as chloroform, ethanol, and propan-2-ol; it also forms clear, stable, aqueous solutions. Aqueous solutions of Cremophor® EL are stable in the presence of low concentrations of electrolyte such as acids or salts, with the exception of mercuric chloride.

10.7 Safety

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

11. Polyoxyl 40 hydrogenated castor oil (Cremophor® RH40) (Kibbe, A.H., 2000; Strickley, R.G., 2004)

11.1 Chemical name

Polyoxyl 40 hydrogenated castor oil; glycerol polyethyleneglycol oxystearate; hydrogenated castor oil POE-40; PEG-40 hydrogenated castor oil.

11.2 Empirical formula

Approximately 75% of the components of the mixture are hydrophobic. These comprise mainly fatty acid esters of glycerol polyethylene glycol and fatty acid esters of polyethylene glycol. The hydrophilic portion consists of polyethylene glycols and glycerol ethoxylates.

11.3 Structural formula

$$H_2C \diagup O-\underset{\underset{O}{\|}}{C}-R_1$$

$$HC-O-\underset{\underset{O}{\|}}{C}-R_2$$

$$H_2C \diagdown O-\underset{\underset{O}{\|}}{C}-R_3$$

$$R_{1,2,3} = (CH_2)_9 \diagdown \diagup (CH_2)_6\text{-}CH_3$$
$$(OCH_2CH_2)_{x,y,z}OH$$
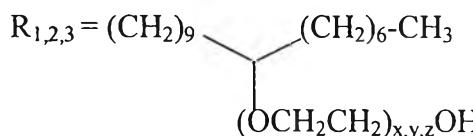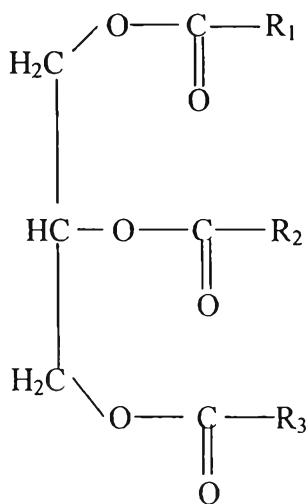
R = Polyethylene glycol 12-oxystearate

$x+y+z = 40$

11.4 Appearance

Cremophor® RH40 occurs as a white, semisolid paste which liquefies at 30°C. It has a very faint characteristic odor and a slight taste in aqueous solution.

11.5 Typical properties

Melting point : ≈ 30°C

HLB value : 14-16

Solubility : Cremophor® RH40 is soluble in ethyl alcohol, fatty acid, fatty alcohol, olive oil, chloroform, and water.

11.6 Stability

Aqueous of Cremophor® RH40 heated for polonged periods may separated into solid and liquid phases on cooling. However, the product can be

restored to its original form. It should be stored in well-filled, airtight container, protected from light, in a cool, dry, place.

11.7 Safety

Cremophor® RH40 is used a variety of oral, topical, and parenteral pharmaceutical formulations. Acute and chronic toxicity tests in animals have shown it to be essentially nontoxic and nonirritant material. However, several serious anaphylactic reactions have been observed in humans and animals following parenteral. The precise mechanism of the reaction is not known.

12. Poloxamer (Kibbe, A.H., 2000)

12.1 Chemical name

α-Hydro-ω-hydroxypropoly-(oxyethylene)-poly-(oxypropylene)-poly-(oxyethylene) block copolymer

12.2 Empirical formula

$HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$

12.3 Structural formula

$$HO(CH_2CH_2O)_a(CH_2CHO)_b(CH_2CH_2O)_aH$$
$$|$$
$$CH_3$$
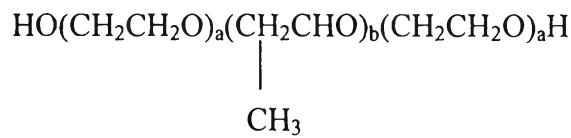
The poloxamer polyols are series of closely related block copolymers of ethylene oxide and propylene oxide. Two grades are shown as following.

Poloxamer	a	b	Average molecular weight
188	80	27	7680-9510
407	101	56	9840-14600

12.4 Appearance

Both poloxamer 118 and 407 generally occur as white-colored, waxy, free flowing prilled granules or as cast solids. It is practically odorless and tasteless.

12.5 Typical properties

Melting point and HLB of both poloxamers are shown below

Poloxamer	Melting point (°C)	HLB
188	52	29
407	56	22

Solubility : Poloxamers are freely soluble in water, ethanol, and isopropyl alcohol.

12.6 Stability

Poloxamers are stable materials, Aqueous solution are stable in the presence of acids, alkalis, and metal ions. However, aqueous solutions do support mold growth. The bulk material should be stored in a well-closed container in a cool, dry place.

12.7 Safety

Poloxamers are used in variety of oral, parenteral and topical pharmaceutical formulations and is generally regarded as nontoxic and nonirritant material. Poloxamers are not metabolized in the body.

13. Polyoxyethylene Stearates (Kibbe, A.H., 2000)

13.1 Chemical name

Polyoxyl 40 stearate : PEG-40 stearate; polyoxyethyleneglycol 2000 monostearate; polyoxyethylene (40) monostearate; Myrj 52.

Polyoxyl 100 stearate : PEG-100 stearate; polyoxyethyleneglycol 4400 monostearate; polyoxyethylene (100) monostearate; Myrj 59.

13.2 Empirical formula

Polyoxyl 40 stearate : $C_{98}H_{196}O_{42}$

Polyoxyl 100 stearate : $C_{218}H_{436}O_{102}$

13.3 Structural formula

$$R \underline{\quad\quad} \overset{\overset{\textstyle O}{\|}}{C} \underline{\quad\quad} (OCH_2CH_2)_{\overline{n}} \underline{\quad\quad} OH$$
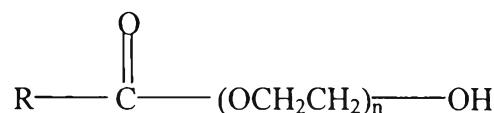
Where, the average value of n is 40 for Polyoxyl 40 stearate, 100 for Polyoxyl 100 stearate. In the structure, R represents the alkyl group of the parent fatty acid. With stearic acid, R is $CH_3(CH_2)_{16}$.

13.4 Appearance

Polyoxyl 40 stearate is waxy solid, with a faint, bland, fat-like odor, off-white to light tan in color. Polyoxyl 100 stearate is solid material.

13.5 Typical properties

The listed of polyoxyethylene stearates properties are shown as follow

Name	HLB	Melting point	saponification value
Myrj 52	16.9	≈ 38	25-35
Myrj 59	18.8	≈ 46	9-20

Solubility : polyoxyethylene stearates are soluble in ethanol (95%) and water but insoluble in mineral oil.

13.6 Stability

polyoxyethylene stearates are generally stable in the presence of electrolytes and weak acids or bases. Strong acids and bases can cause gradual hydrolysis and sponification. The bulk material should be stored in a well-closed container, in a dry place, at room temperature.

13.7 Safety

Although polyoxyethylene stearates are primarily used as emulsifying agents in topical pharmaceutical formulations certain materials, particularly polyoxyl 40 stearate, have also been used in intravenous injections and oral preparations. Polyoxyethylene stearates have been extensively tested for toxicity in animals and are widely used in pharmaceutical formulations and cosmetics. They are generally regarded as essentially nontoxic and nonirritant materials.

14. Soy lecithin (Kibbe, A.H., 2000)

14.1 Chemical name

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

14.2 Empirical 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 as separated from a crude vegetable oil source. The composition of lecithin and hence its physical properties varies enormously depending upon the source of the lecithin and the degree of purification.

14.3 Structural formula

$$H_2C \diagup O - \underset{\underset{O}{\|}}{C} - R_1$$

$$HC - O - \underset{\underset{O}{\|}}{C} - R_2$$

$$H_2C \diagdown O - \underset{\underset{O}{\|}}{\overset{\overset{O^-}{|}}{P}} - OCH_2CH_2N^+(CH_3)_3$$
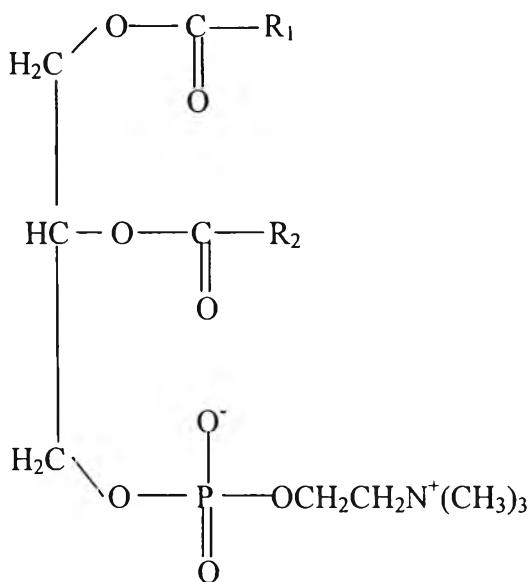
α-phosphatidylcholine

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

The structure shows phosphatidylcholine, in its α-form. In the β-form, the phosphorus containing group and the R_2 group exchange the positions.

Two commercially available soy lecithins used in this study are shown below.

Components	Phospholipon®90	Epikuron®200
Phosphatidylcholine	90 (H)	96.0
Lysophosphatidylcholine	4 (H)	2.1

H = hydrogenated

14.4 Appearance

Lecithin is brown to light yellow, depending on whether it is bleached or unbleached or degree of purity. It has practically no odor.

14.5 Typical properties

Saponification value : 196

Solubility : Lecithin is soluble in aliphatic and aromatic hydrocarbon, hydrogenated hydrocarbons, mineral oil and fatty acids. It is practically insoluble in cold vegetable and animal oils, polar solvents and water. When mixed with water however, lecithin hydrates to form emulsions.

14.6 Stability

Lecithin decomposes at extreme pH. They are also hygroscopic and subject to microbial degradation. When heated, lecithin oxidizes, darken, and decompose. Temperatures of 160-180°C will cause degradation within 24 hours.

14.7 Safety

Lecithin is a component of cell membranes and is therefore consumed as a normal part of the diet. Although excessive consumption may be harmful, it is highly biocompatible and oral doses of up to 80 g daily have been used therapeutically in the treatment of tardive dyskinesia.

APPENDIX B

PHYSICOCHEMICAL PROPERTIES OF DRUG-FREE SLN

Table b1 Particle size of GP-SLN containing various type of surfactants and co-surfactants on the ratio of lipid: (surfactant:co-surfactant):water = 10:(20:20):50

Formulation	Mean particle size by PCS (nm)	
	Z value $\pm$ SD	PI
GB:(Tw80+Gly):Water	236.7 $\pm$ 1.1	0.321
GB:(CreEL+Gly):Water	26.3 $\pm$ 0.3	0.162
GB:(CreRH+Gly) :Water	229.1 $\pm$ 3.2	0.284
GB:(Tw80+PG) :Water	381.3 $\pm$ 11.2	0.347
GB:(CreEL+PG) :Water	22.2 $\pm$ 0.2	0.081
GB:(CreRH+PG) :Water	222.6 $\pm$ 13.4	0.352
GB:(Tw80+PEG) :Water	170.1 $\pm$ 4.0	0.330
GB:(CreEL+PEG) :Water	46.5 $\pm$ 1.0	0.244
GB:(CreRH+PEG) :Water	113.9 $\pm$ 1.4	0.317

Table b2 Particle size of GP-SLN containing various type of surfactants and co-surfactants on the ratio of lipid: (surfactant:co-surfactant):water = 10:(25:25):50

Formulation	Mean particle size by PCS (nm)	
	Z value $\pm$ SD	PI
GP:(Tw80+Gly):Water	41.3 $\pm$ 0.9	0.300
GP:(CreEL+Gly):Water	26.8 $\pm$ 0.4	0.200
GP:(CreRH+Gly) :Water	45.0 $\pm$ 0.4	0.274
GP:(Tw80+PG) :Water	171.4 $\pm$ 7.7	0.313
GP:(CreEL+PG) :Water	21.7 $\pm$ 0.6	0.147
GP:(CreRH+PG) :Water	222.5 $\pm$ 4.4	0.332
GP:(Tw80+PEG) :Water	129.2 $\pm$ 1.0	0.277
GP:(CreEL+PEG) :Water	81.9 $\pm$ 0.2	0.257
GP:(CreRH+PEG) :Water	166.0 $\pm$ 1.5	0.152

Table b3 The equation and coefficients of determination of osmolality of GB-SLN dispersions prepared by WME method

Formula	Equation	Coefficient of determination (R^2)
GB:(Tw80+Gly):Water	y = 6.15x – 24.83	0.9994
GB:(CreEL+Gly):Water	y = 4.45x + 19.08	0.9949
GB:(CreRH+Gly):Water	y = 4.55x + 15.80	0.9983
GB:(Tw80+ PG):Water	y = 5.77x + 13.45	0.9959
GB:(CreEL+ PG):Water	y = 6.51x – 6.75	0.9913
GB:(CreRH+ PG):Water	y = 5.60x + 17.40	0.9986
GB:(Tw80+ PEG):Water	y = 1.23x + 5.30	0.9996
GB:(CreEL+ PEG):Water	y = 1.39x – 1.85	0.9965
GB:(CreRH+ PEG):Water	y = 1.28x + 2.80	0.9983

Table b4 The equation and coefficients of determination of osmolality of GP-SLN dispersions prepared by WME method

Formula	Equation	Coefficient of determination (R^2)
GP:(Tw80+Gly):Water	y = 4.89x + 2.85	0.9986
GP:(CreEL+Gly):Water	y = 5.23x - 2.25	0.9918
GP:(CreRH+Gly):Water	y = 4.56x + 15.40	0.9997
GP:(Tw80+ PG):Water	y = 5.68x + 7.40	0.9884
GP:(CreEL+ PG):Water	y = 5.75x + 9.75	0.9836
GP:(CreRH+ PG):Water	y = 5.25x + 18.35	0.9906
GP:(Tw80+ PEG):Water	y = 1.48x - 1.50	0.9902
GP:(CreEL+ PEG):Water	y = 1.38x - 0.40	0.9956
GP:(CreRH+ PEG):Water	y = 1.51x - 1.65	0.9945

Table b5 pH and osmolality of drug free SLN containing 3% GB or GP and various amount of Tw80 or Tw20

Formulation	pH	Osmolality (mosmol/kg)	Formulation	pH	Osmolality (mosmol/kg)
3GB+1Tw20	5.63 ± 0.01	8.67 ± 0.58	3GB+1Tw80	5.79 ± 0.02	6.33 ± 0.58
3GB+2Tw20	5.25 ± 0.01	17.67 ± 0.58	3GB+2Tw80	5.89 ± 0.02	9.67 ± 0.58
3GB+3Tw20	5.05 ± 0.01	28.00 ± 1.00	3GB+3Tw80	5.89 ± 0.02	12.33 ± 0.58
3GB+4Tw20	4.91 ± 0.01	33.33 ± 1.15	3GB+4Tw80	5.94 ± 0.01	17.00 ± 1.00
3GB+5Tw20	4.87 ± 0.02	40.67 ± 0.58	3GB+5Tw80	6.05 ± 0.02	19.00 ± 1.58
3GP+1Tw20	5.77 ± 0.03	8.67 ± 0.58	3GP+1Tw80	5.32 ± 0.03	8.00 ± 0.00
3GP+2Tw20	5.92 ± 0.08	18.33 ± 1.53	3GP+2Tw80	6.14 ± 0.02	9.00 ± 0.00
3GP+3Tw20	5.74 ± 0.01	21.67 ± 0.58	3GP+3Tw80	5.63 ± 0.06	10.67 ± 0.58
3GP+4Tw20	5.37 ± 0.04	24.67 ± 0.58	3GP+4Tw80	6.09 ± 0.02	12.33 ± 0.58
3GP+5Tw20	5.39 ± 0.06	30.00 ± 0.00	3GP+5Tw80	5.70 ± 0.04	17.33 ± 1.15

Table b6 pH and osmolality of drug free SLN containing 3% GB or GP and various amount of CreEL or CreRH

Formulation	pH	Osmolality (mosmol/kg)	Formulation	pH	Osmolality (mosmol/kg)
3GB+1CreEL	5.24 ± 0.02	5.67 ± 0.58	3GB+1CreRH	5.73 ± 0.01	13.00 ± 0.00
3GB+2CreEL	4.87 ± 0.02	8.33 ± 0.58	3GB+2CreRH	5.59 ± 0.02	15.00 ± 0.00
3GB+3CreEL	5.05 ± 0.02	11.67 ± 0.58	3GB+3CreRH	5.55 ± 0.01	19.00 ± 1.00
3GB+4CreEL	4.93 ± 0.01	14.00 ± 0.00	3GB+4CreRH	5.18 ± 0.03	20.67 ± 0.58
3GB+5CreEL	5.03 ± 0.03	17.67 ± 0.58	3GB+5CreRH	4.67 ± 0.02	25.33 ± 0.58
3GP+1CreEL	5.38 ± 0.03	6.33 ± 0.58	3GP+1CreRH	5.74 ± 0.03	6.33 ± 0.58
3GP+2CreEL	5.17 ± 0.02	9.00 ± 0.00	3GP+2CreRH	5.52 ± 0.03	9.00 ± 0.00
3GP+3CreEL	5.10 ± 0.04	11.67 ± 0.58	3GP+3CreRH	5.41 ± 0.04	12.00 ± 0.00
3GP+4CreEL	4.96 ± 0.01	16.67 ± 0.58	3GP+4CreRH	5.33 ± 0.02	16.67 ± 0.58
3GP+5CreEL	4.92 ± 0.02	20.33 ± 0.58	3GP+5CreRH	5.29 ± 0.02	20.00 ± 0.00

Table b7 pH and osmolality of drug free SLN containing 3% GB or GP and various amount of P118 or P407

Formulation	pH	Osmolality (mosmol/kg)	Formulation	pH	Osmolality (mosmol/kg)
3GB+1P118	6.03 ± 0.03	6.00 ± 0.00	3GB+1P407	5.81 ± 0.02	8.33 ± 0.58
3GB+2P118	5.93 ± 0.03	8.33 ± 0.58	3GB+2P407	5.93 ± 0.02	11.33 ± 2.08
3GB+3P118	5.99 ± 0.01	13.00 ± 0.00	3GB+3P407	6.03 ± 0.03	16.00 ± 2.00
3GB+4P118	6.08 ± 0.07	22.33 ± 0.58	3GB+4P407	5.97 ± 0.01	21.33 ± 0.58
3GB+5P118	6.16 ± 0.02	29.00 ± 0.00	3GB+5P407	6.14 ± 0.02	33.00 ± 1.00
3GP+1P118	5.21 ± 0.02	11.33 ± 0.58	3GP+1P407	5.58 ± 0.03	10.67 ± 0.58
3GP+2P118	5.39 ± 0.01	14.00 ± 0.00	3GP+2P407	5.48 ± 0.01	14.00 ± 0.00
3GP+3P118	5.57 ± 0.04	18.00 ± 0.00	3GP+3P407	5.70 ± 0.01	19.67 ± 0.58
3GP+4P118	5.67 ± 0.01	22.00 ± 1.00	3GP+4P407	6.04 ± 0.02	24.00 ± 0.00
3GP+5P118	5.70 ± 0.04	28.33 ± 1.15	3GP+5P407	5.94 ± 0.03	30.67 ± 1.53

Table b8 pH and osmolality of drug free SLN containing 3% GB or GP and various amount of M52 or M59

Formulation	pH	Osmolality (mosmol/kg)	Formulation	pH	Osmolality (mosmol/kg)
3GB+1M52	5.80 ± 0.02	8.67 ± 0.58	3GB+1M59	5.07 ± 0.03	5.00 ± 0.00
3GB+2M52	5.57 ± 0.02	17.67 ± 0.58	3GB+2M59	4.29 ± 0.01	8.00 ± 0.00
3GB+3M52	5.40 ± 0.02	28.00 ± 1.00	3GB+3M59	4.18 ± 0.02	11.67 ± 0.58
3GB+4M52	5.44 ± 0.01	33.33 ± 1.15	3GB+4M59	4.12 ± 0.02	13.67 ± 0.58
3GB+5M52	5.46 ± 0.02	40.67 ± 0.58	3GB+5M59	4.09 ± 0.02	18.00 ± 0.00
3GP+1M52	5.23 ± 0.02	8.67 ± 0.58	3GP+1M59	4.86 ± 0.02	12.67 ± 0.58
3GP+2M52	5.68 ± 0.03	18.33 ± 1.53	3GP+2M59	4.26 ± 0.03	15.00 ± 0.00
3GP+3M52	5.49 ± 0.03	21.67 ± 0.58	3GP+3M59	4.20 ± 0.02	18.00 ± 0.00
3GP+4M52	5.67 ± 0.05	24.67 ± 0.58	3GP+4M59	4.13 ± 0.03	22.33 ± 0.58
3GP+5M52	5.65 ± 0.03	30.00 ± 0.00	3GP+5M59	3.93 ± 0.08	26.00 ± 1.00

APPENDIX C

HPLC Validation

The content of AmB in the various formations and the drug release testing could be determined by HPLC assay with UV detection. In this study, The wavelength used to analyze the content of AmB in preparations after extraction was 403 nm which was the λ_{max} of AmB in DMSO: MeOH (1:999 %v/v) as shown in Figure c1. The validation of the HPLC condition is presented as follows.
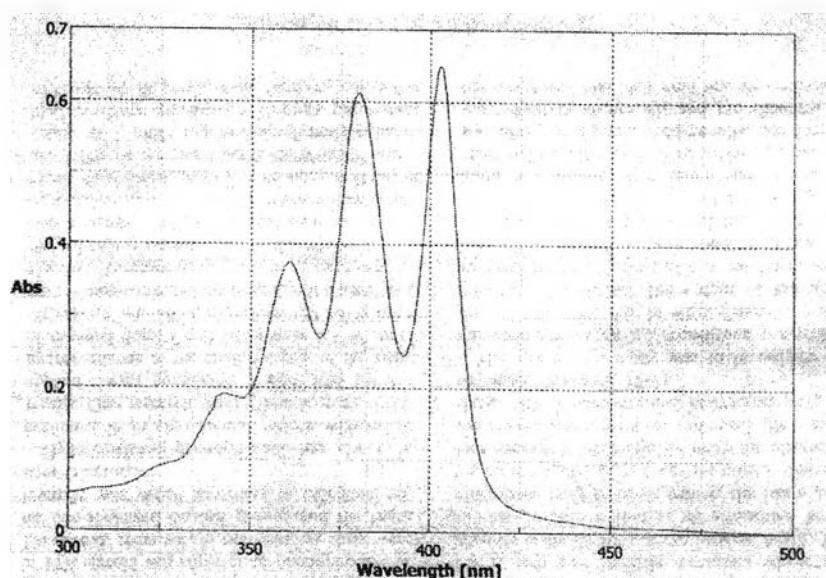
Figure c1 The UV spectrum of AmB dissolved in DMSO: MeOH (1:999 %v/v)

1. Specificity

Figure c2-c4 showed the chromatograms of water, PBS, DMF, DMSO, the extraction of drug-free SLN prepared by WME method; the chromatograms of drug-free SLN, NLC, SLN-L and NLC-L prepared by HPH method and various concentration of standard solution AmB, respectively. AmB was eluted as a distinct peak with the retention time of 4.20-4.40 minutes and the peaks of solvent which had a retention time of 1.90-2.40 minutes. All of the extraction of drug-free preparations had no peak which interfere the AmB peak when were injected in the same condition. This resulted indicated that it was specific to detect AmB content without interferences.
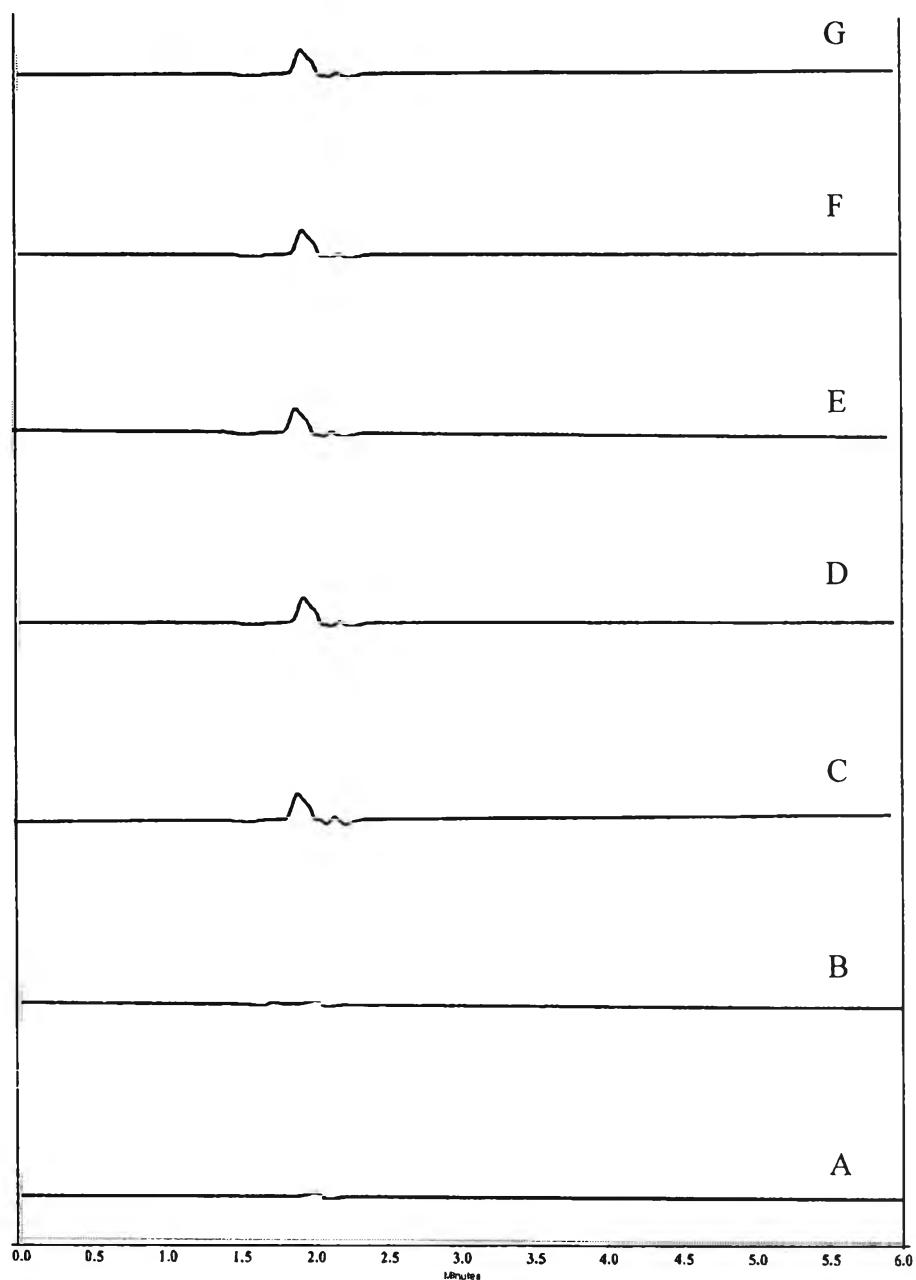
Figure c2 The HPLC chromatograms of water (A); PBS pH 7.4 (B); DMF (C); DMSO (D); and the extraction of drug-free prepared by WME method (WME1 (E); WME2 (F); WME3 (G)).
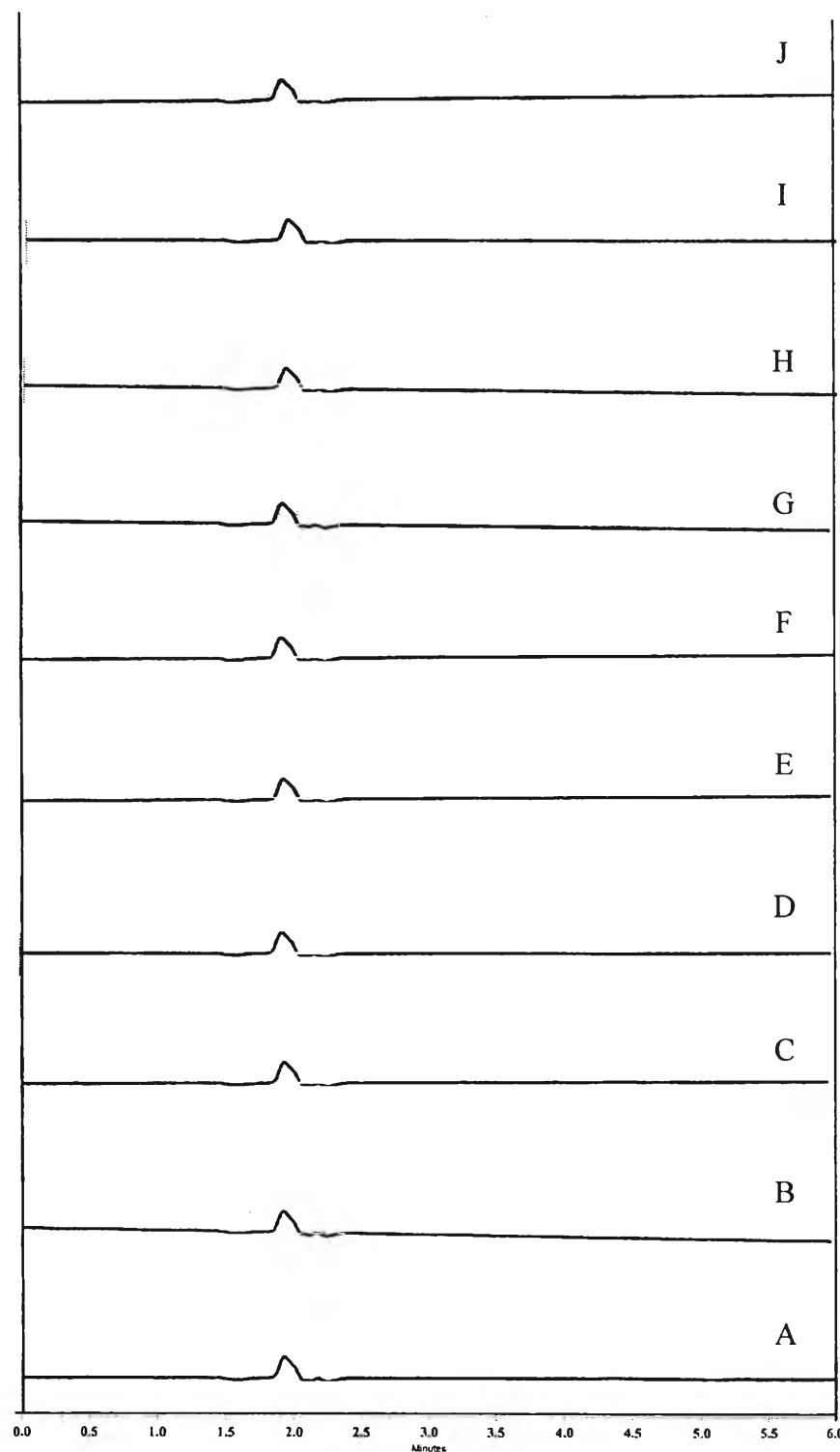
Figure c3 The HPLC chromatograms of the extraction of drug-free prepared by HPH method; SLN1 (A), SLN2 (B), SLN3 (C), SLN4(D), NLC1 (E), NLC2 (F), NLC3 (G), NLC4 (H), SLN-L (I) and NLC-L (J).
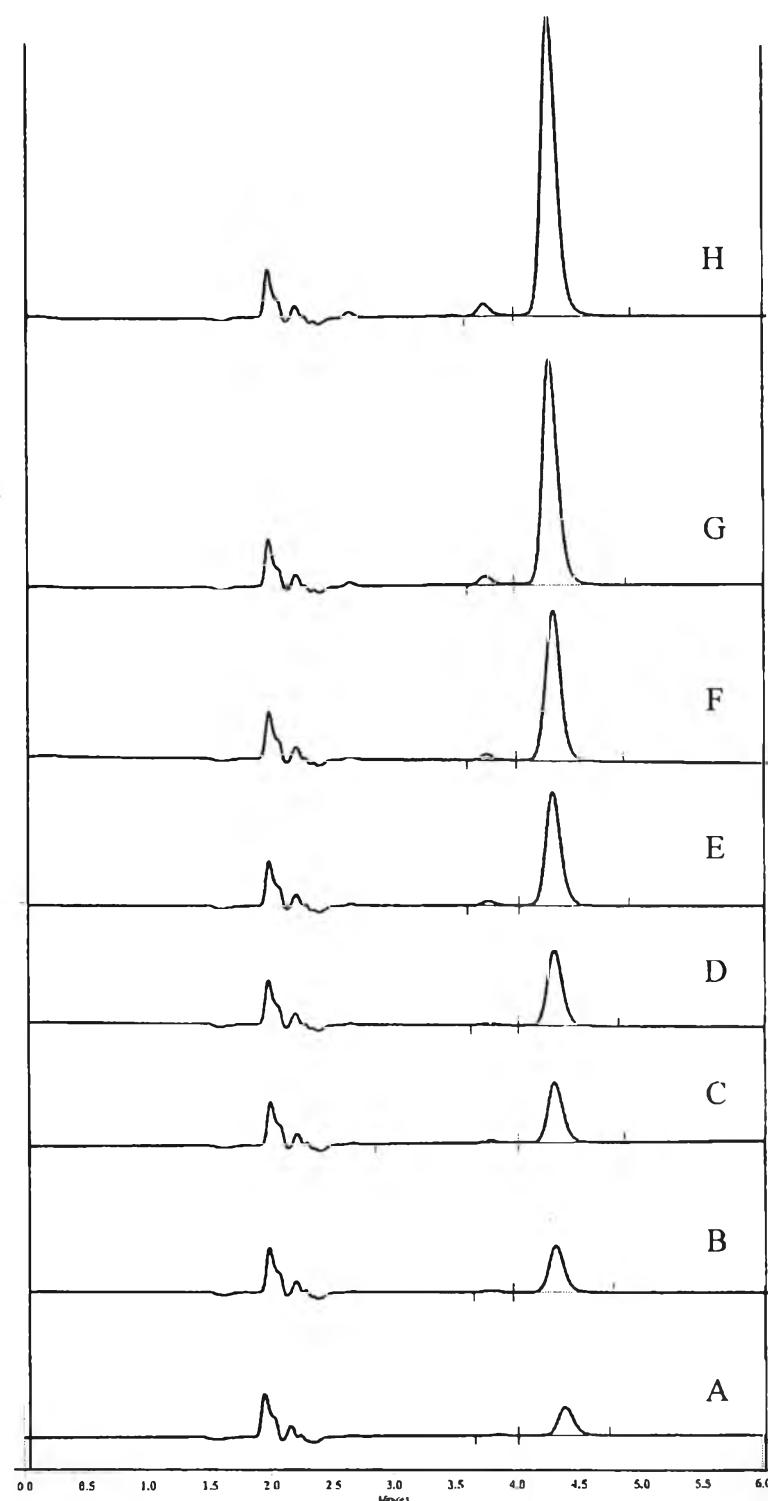
Figure c4 The HPLC chromatograms of the standard solutions of AmB; 0.8 µg/ml (A); 1.2 µg/ml (B); 1.6 µg/ml (C); 2.0 µg/ml (D); 3.0 µg/ml (E); 4.0 µg/ml (F); 6.0 µg/ml (G); and 8.0 µg/ml (H).

2. Precision

Table c1-c2 showed data of within run precision and between run precision of AmB assayed by the HPLC method, respectively. The percentage of coefficient of variation (%CV) values of peak area in both within run and between run precisions were low (0.08-0.4% and 0.36-1.42%, respectively) Therefore, The HPLC condition could be used to determine the amount of AmB over a period of time studied.

Table c1 Data of within run precision of AmB assayed by the HPLC method

AmB concentration (μg/ml)	Peak areas of AmB					
	n1	n2	n3	mean	SD	% CV
0.8128	79850	79870	79484	79734.67	217.31	0.27
1.2192	121621	120926	121378	121308.33	352.70	0.29
1.6256	161864	161200	161889	161651.00	390.78	0.24
2.0320	202311	201778	202094	202061.00	268.03	0.13
3.0480	299455	299458	299888	299600.33	249.13	0.08
4.0640	402825	402210	405235	403423.33	1598.80	0.40
6.0960	613627	613412	612602	613213.67	540.52	0.09
8.1280	813946	814872	815300	814706.00	692.10	0.08

Table c2 Data of between run precision of AmB assayed by the HPLC method

AmB concentration (μg/ml)	Peak areas of AmB					
	day1	day2	day3	mean	SD	% CV
0.7904	77195.67	77537.25	75723.87	76818.93	963.61	1.25
1.1856	116388.00	117965.19	118883.55	117745.58	1262.19	1.07
1.5808	155078.33	157196.05	156563.30	156279.23	1087.06	0.70
1.9760	195061.00	196492.39	198227.02	196593.47	1585.43	0.81
2.9640	292214.33	291343.63	293532.28	292363.41	1101.92	0.38
3.9520	393502.33	392305.36	390712.15	392173.28	1399.77	0.36
5.9280	598320.67	596314.08	592588.72	595741.16	2908.61	0.49
7.9040	810146.67	792253.47	789216.03	797205.39	11309.91	1.42

3. Accuracy

Table c3A-c3C showed the percentage of analytical recovery in each concentration of AmB for three determinations. The mean percent recoveries were 100.19%, 100.58% and 99.98% with the %CV values of percent recovery were very low 0.84%, 2.03% and 1.15%, respectively. That indicated the HPLC method could be used to accurately determine AmB within the concentration range of 0.8-8.0 µg/ml.

4. Linearity

The chromatograms of AmB dissolved in DMSO: MeOH (1:999 %v/v) are shown in Figure c4. The retention time of AmB was about 4.24-4.35. The calibration curve was plotted between the peak area and the concentrations of AmB in µg/ml. The results are shown in Table c4A-c4C and Figure c5A-c5C. Linear regression analysis was performed with the coefficient of determination (R^2) of 0.9998-0.9999. These results concluded that the HPLC condition was acceptable for determining the content of AmB in preparations.

Table c3A Data of accuracy of AmB assayed by the HPLC method (No. 1)

Actual concentration (µg/ml)	Mean Peak area	Mean Analytical concentration (µg/ml)	% Mean Recovery
0.8128	79734.67	0.8207	100.98
1.2192	121308.33	1.2341	101.22
1.6256	161651.00	1.6352	100.59
2.0320	202061.00	2.0370	100.24
3.0480	299600.33	3.0068	98.65
4.0640	403423.33	4.0390	99.39
6.0960	613213.67	6.1249	100.47
8.1280	814706.00	8.1282	100.00
		Mean	100.19
		SD	0.84
		% CV	0.84

Table c3B Data of accuracy of AmB assayed by the HPLC method (No. 2)

Actual concentration (μg/ml)	Mean Peak area	Mean Analytical concentration (μg/ml)	% Mean Recovery
0.7904	77195.67	0.8285	104.82
1.1856	116388.00	1.2097	102.04
1.5808	155078.33	1.5861	100.34
1.9760	195061.00	1.9750	99.95
2.9640	292214.33	2.9201	98.52
3.9520	393502.33	3.9054	98.82
5.9280	598320.67	5.8977	99.49
7.9040	810146.67	7.9582	100.69
		Mean	100.58
		SD	2.04
		% CV	2.03

Table c3C Data of accuracy of AmB assayed by the HPLC method (No. 3)

Actual concentration (μg/ml)	Mean Peak area	Mean Analytical concentration (μg/ml)	% Mean Recovery
0.7744	74191.00	0.7581	97.89
1.1616	116477.00	1.1808	101.65
1.5488	153394.00	1.5499	100.07
1.9360	194214.33	1.9580	101.13
2.9040	287590.33	2.8915	99.57
3.8720	382803.00	3.8433	99.26
5.8080	580593.00	5.8206	100.22
7.7440	773240.00	7.7465	100.03
		Mean	99.98
		SD	1.15
		% CV	1.15

Table c4A Data of calibration curve of standard AmB solutions (No. 1)

AmB concentration (μg/ml)	Peak areas of AmB					
	n1	n2	n3	mean	SD	% CV
0.8128	79850	79870	79484	79734.67	217.31	0.27
1.2192	121621	120926	121378	121308.33	352.70	0.29
1.6256	161864	161200	161889	161651.00	390.78	0.24
2.0320	202311	201778	202094	202061.00	268.03	0.13
3.0480	299455	299458	299888	299600.33	249.13	0.08
4.0640	402825	402210	405235	403423.33	1598.80	0.40
6.0960	613627	613412	612602	613213.67	540.52	0.09
8.1280	813946	814872	815300	814706.00	692.10	0.08

Figure c5A Calibration curve of AmB assay by HPLC method (No.1)

Table c4B Data of calibration curve of standard AmB solutions (No. 2)

| AmB concentration (μg/ml)	Peak areas of AmB					
	n1	n2	n3	mean	SD	% CV
0.7904	77263	77121	77203	77195.67	71.28	0.09
1.1856	115711	116415	117038	116388.00	663.91	0.57
1.5808	154956	154839	155440	155078.33	318.63	0.21
1.9760	194808	195253	195122	195061.00	228.69	0.12
2.9640	292142	292375	292126	292214.33	139.37	0.05
3.9520	393485	393024	393998	393502.33	487.23	0.12
5.9280	598133	597998	598831	598320.67	447.09	0.07
7.9040	973958	974015	976038	810146.67	203.44	0.03
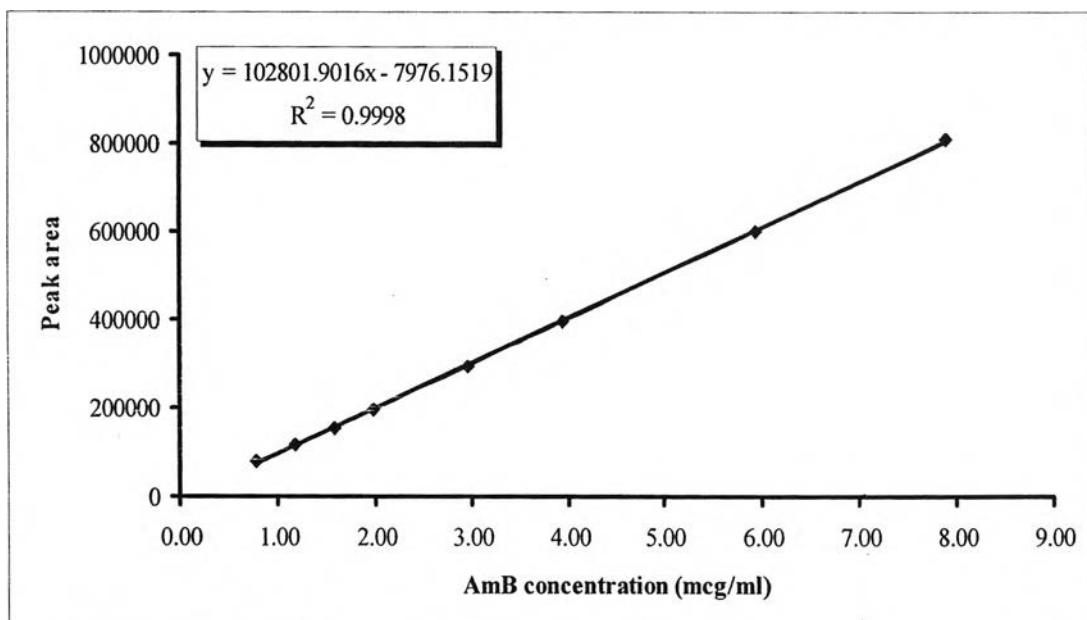
Figure c5B Calibration curve of AmB assay by HPLC method (No.2)

Table c4C Data of calibration curve of standard AmB solutions (No. 3)

AmB concentration (μg/ml)	Peak areas of AmB					
	n1	n2	n3	mean	SD	% CV
0.7744	74236	74449	73888	74191.00	283.19	0.38
1.1616	116674	116021	116736	116477.00	396.12	0.34
1.5488	153832	153164	153186	153394.00	379.48	0.25
1.9360	193934	194448	194261	194214.33	260.16	0.13
2.9040	287430	287153	288188	287590.33	535.80	0.19
3.8720	381652	383421	383336	382803.00	997.71	0.26
5.8080	576025	576687	589067	580593.00	7346.16	1.27
7.7440	768458	768452	782810	773240.00	8287.86	1.07
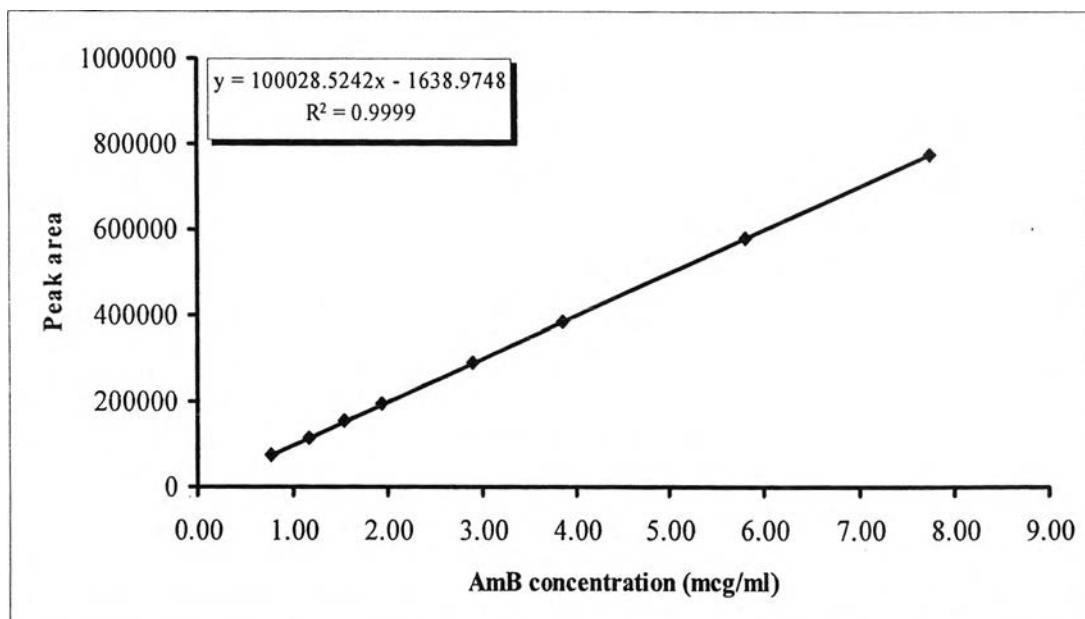
Figure c5C Calibration curve of AmB assay by HPLC method (No.3)

Accuracy of the extraction of the drug

Due to the poor solubility characteristics of AmB and complexity of the formulation of AmB-SLN, a novel extraction procedure has been modified from Wilkinson, et al (1998) by using two organic solvents. The use of dimethylformamide (DMF) and cyclohexane enabled the AmB to be solubilized into the DMF, with the solid lipid being extracted into the upper cyclohexane layer. Because of the complexity of the extractive procedure, no suitable internal standard could be found with similar extractive characteristics to that of AmB. The method was validated by submitting to the extraction procedure, followed by HPLC analysis.

The recoveries of AmB from placebo were assessed by spiking placebo (SLN containing all the components except the drug) with AmB and following the extraction procedure used for the dosage form. Placebo was spiked in triplicate at five levels spanning 50-150% of the amount of AmB in the preparations. The average recovery for AmB five levels was 96.33% with a coefficient of variation of 1.42% as shown in Table c5. Linear regression analysis of the dependence of the average amount recovered (y) on the average amount added (x) gave the equation $y=0.9536x + 0.0344$, with a correlation coefficient of 0.9999 as shown in Figure c6.

It was concluded that the AmB was quantitatively extracted into the DMF layer, free of all other interfering substances and that there were no losses into either the cyclohexane layer or by adsorption to the precipitated components. The solid lipid was extracted into the cyclohexane layer and discarded, preventing contamination and damage of the HPLC.

Table c5 Recovery of AmB from spiked placebo

Amount of AmB added (mcg)	Amount of AmB recovered	Recovery (%)
2.03	1.90	93.26
	2.01	98.82
	2.01	99.01
3.05	2.91	95.59
	2.94	96.30
	2.95	96.78
4.06	3.89	95.82
	3.93	96.69
	3.91	96.15
5.08	4.91	96.58
	4.87	95.91
	4.93	97.03
6.10	5.82	95.47
	5.83	95.58
	5.85	95.91
	Mean	96.33
	SD	1.37
	%CV	1.42

Figure c6 The linear regression analysis between the average amount recovered and the average amount added.

APPENDIX D

SPECTROSCOPIC DATA OF AMB

Table d1 The characteristic bands of various AmB-WME spectra at the drug concentration range of 2.0 – 12.0 µg/ml.

Formulations	Absorption spectrum wavelength (nm)			
	Peak I	Peak II	Peak III	Peak IV
AmB-WME1	341.5	363.5	386.0	408.0
AmB-WME2	343.5	364.0	386.0	408.0
AmB-WME3	320.5	363.5	385.5	408.0

Table d2 The characteristic bands of various AmB-SLN spectra at the drug concentration range of 2.0 – 12.0 µg/ml.

Formulations	Absorption spectrum wavelength (nm)			
	Peak I	Peak II	Peak III	Peak IV
AmB-SLN1	346.0	365.0	387.5	414.0
AmB-SLN2	348.0	366.5	388.0	414.0
AmB-SLN3	354.0	367.5	388.5	414.0
AmB-SLN4	352.0	370.0	389.5	414.5

Table d3 The characteristic bands of various AmB-NLC spectra at the drug concentration range of 2.0 – 12.0 µg/ml.

Formulations	Absorption spectrum wavelength (nm)			
	Peak I	Peak II	Peak III	Peak IV
AmB-NLC1	344.0	361.5	386.0	414.5
AmB-NLC2	348.0	366.5	388.5	414.5
AmB-NLC3	351.0	367.0	388.5	414.5
AmB-NLC4	352.5	369.5	389.5	414.5

Table d4 The characteristic bands of various AmB-SLN-L spectra at the drug concentration range of 2.0 – 12.0 µg/ml.

Formulations	Absorption spectrum wavelength (nm)			
	Peak I	Peak II	Peak III	Peak IV
AmB-SLN-L1	327.0	360.0	385.0	407.5
AmB-SLN-L2	334.0	362.0	386.0	408-414*
AmB-SLN-L3	347.0	364.5	387.0	408-414*
AmB-SLN-L4	347.5	366.0	387.5	409-414*

* : wavelength shift according to AmB concentration

Table d5 The characteristic bands of various AmB-NLC-L spectra at the drug concentration range of 2.0 – 12.0 µg/ml.

Formulations	Absorption spectrum wavelength (nm)			
	Peak I	Peak II	Peak III	Peak IV
AmB-NLC-L1	327.5	362.5	385.5	408.0
AmB-NLC-L2	323.0	363.0	385.5	408.0
AmB-NLC-L3	346.0	362.0	386.0	408.0
AmB-NLC-L4	345.5	364.5	386.5	409.0

APPENDIX E

RELEASE DATA OF AMB

Calibration curve for the validated HPLC assays of AmB was performed when dissolved AmB in the aid of DMSO:MeOH (1:999 v/v) and followed diluted to PBS solutions in the concentration range 0.4-2.2 µg/ml. Correlation coefficient was 0.9995. Each point represents the average of three measurements and the error was calculated as standard deviation (±SD)


The calibration curve shows:

$$y = 55334x - 494.83$$
$$R^2 = 0.9995$$
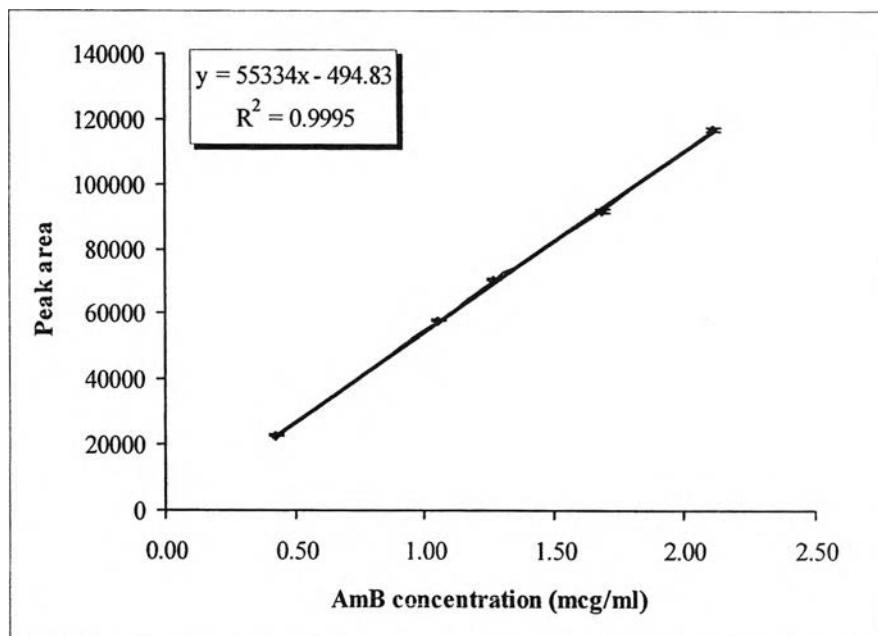
Figure e1 The calibration curve of AmB dissolved in PBS, pH 7.4

Table e1 Release of AmB from 1% AmB loaded SLN

Time (hr)	AmB amount (μg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	12.80	7.54	10.10	5.57	3.28	4.39	4.41	1.14
4	29.75	14.12	15.93	12.93	6.14	6.92	8.66	3.72
6	47.74	23.35	35.60	20.75	10.15	15.47	15.46	5.30
8	65.13	32.90	43.90	28.31	14.30	19.08	20.57	7.12
12	90.75	48.14	60.86	39.45	20.93	26.46	28.95	9.51
16	110.40	60.55	74.03	48.00	26.32	32.18	35.50	11.21
24	136.21	77.38	97.14	59.21	33.64	42.23	45.03	13.01

Table e2 Release of AmB from 1% AmB loaded NLC

Time (hr)	AmB amount (μg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	20.70	33.50	8.00	0.80	15.10	3.60	6.50	7.57
4	36.74	39.31	15.90	5.46	17.72	7.16	10.11	6.64
6	46.55	46.40	39.61	11.27	20.91	17.85	16.67	4.93
8	91.18	61.41	51.36	17.50	27.67	23.14	22.77	5.10
12	66.67	60.42	64.35	25.67	27.23	29.00	27.30	1.66
16	74.47	57.82	70.93	24.96	26.05	31.96	27.66	3.77
24	54.03	61.91	74.25	24.13	27.90	33.46	28.50	4.69

Table e3 Release of AmB from 1% AmB loaded SLN-L

Time (hr)	AmB amount (µg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.10	1.27	1.13	0.64	0.73	0.66	0.68	0.05
4	2.59	4.08	2.69	1.50	2.36	1.56	1.81	0.48
6	4.44	7.91	6.79	2.58	4.58	3.93	3.70	1.02
8	6.51	12.94	10.26	3.77	7.50	5.95	5.74	1.87
12	10.30	21.57	18.05	5.97	12.51	10.47	9.65	3.34
16	13.75	27.09	24.49	7.97	15.70	14.20	12.63	4.10
24	18.50	36.41	32.74	10.73	21.11	18.98	16.94	5.48

Table e4 Release of AmB from 2.5% AmB loaded SLN

Time (hr)	AmB amount (µg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.21	0.00	0.46	0.18	0.00	0.38	0.19	0.19
4	0.79	0.96	5.89	0.65	0.80	4.87	2.11	2.39
6	2.77	2.95	12.28	2.29	2.44	10.15	4.96	4.49
8	5.95	5.96	16.50	4.92	4.92	13.63	7.82	5.03
12	10.63	11.73	22.69	8.78	9.69	18.74	12.41	5.51
16	17.55	16.34	28.94	14.50	13.50	23.92	17.31	5.75
24	27.30	25.82	35.79	22.55	21.33	29.57	24.49	4.45

Table e5 Release of AmB from 2.5% AmB loaded NLC

Time (hr)	AmB amount (µg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.14	0.20	0.00	0.12	0.17	0.00	0.10	0.09
4	1.11	1.48	0.69	0.94	1.25	0.58	0.92	0.34
6	2.92	2.94	1.99	2.46	2.48	1.68	2.21	0.46
8	5.42	6.58	3.81	4.57	5.55	3.21	4.44	1.17
12	9.04	12.92	9.07	7.62	10.90	7.65	8.72	1.88
16	12.19	16.35	14.64	10.28	13.79	12.35	12.14	1.77
24	16.91	24.16	23.64	14.27	20.37	19.93	18.19	3.41

Table e6 Release of AmB from 2.5% AmB loaded SLN-L

Time (hr)	AmB amount (µg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.31	0.30	0.00	0.30	0.28	0.19	0.17
4	1.54	0.85	0.98	1.47	0.81	0.94	1.07	0.35
6	8.20	2.57	2.92	7.81	2.45	2.78	4.35	3.00
8	11.53	4.52	5.77	10.99	4.30	5.49	6.93	3.56
12	12.34	7.23	9.62	11.76	6.89	9.17	9.27	2.44
16	14.39	10.47	15.82	13.71	9.97	15.07	12.92	2.64
24	18.27	14.20	19.07	17.40	13.52	18.16	16.36	2.49

Table e7 Release of AmB from 5% AmB loaded SLN

Time (hr)	AmB amount (μg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	14.55	16.44	12.61	6.24	7.05	5.41	6.23	0.82
4	59.73	60.40	42.09	25.63	25.92	18.06	23.20	4.46
6	96.66	92.64	78.80	41.48	39.75	33.81	38.35	4.02
8	108.08	109.24	105.88	46.37	46.87	45.43	46.23	0.73
12	107.73	115.67	122.42	46.23	49.63	52.53	49.46	3.15
16	106.51	115.17	123.70	45.70	49.42	53.08	49.40	3.69
24	96.88	107.81	119.64	41.57	46.26	51.34	46.39	4.89

Table e8 Release of AmB from 5% AmB loaded NLC

Time (hr)	AmB amount (μg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	6.35	6.39	30.05	2.96	2.98	14.00	6.65	6.37
4	33.69	34.54	56.68	15.70	16.10	26.42	19.41	6.08
6	56.66	52.12	65.81	26.41	24.30	30.67	27.13	3.25
8	62.23	64.29	71.62	29.00	29.97	33.38	30.78	2.30
12	69.23	73.27	72.02	32.27	34.15	33.57	33.33	0.97
16	68.95	71.24	70.77	32.14	33.21	32.99	32.78	0.57
24	64.34	65.88	65.29	29.99	30.71	30.43	30.37	0.36

Table e9 Release of AmB from 5% AmB loaded SLN-L

Time (hr)	AmB amount (μg)			% Release			Mean	SD
	No.1	No.2	No.3	No.1	No.2	No.3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	7.62	9.15	2.50	3.25	3.90	1.07	2.74	1.48
4	22.14	33.77	9.22	9.44	14.40	3.93	9.26	5.23
6	45.60	53.73	22.56	19.44	22.91	9.62	17.32	6.89
8	60.30	65.78	37.16	25.70	28.04	15.84	23.20	6.47
12	76.91	75.76	54.91	32.79	32.30	23.41	29.50	5.28
16	81.18	81.24	75.41	34.61	34.64	32.15	33.80	1.43
24	81.61	90.97	101.73	34.79	38.78	43.37	38.98	4.29

APPENDIX F

RAW DATA OF ^{1}H-NMR SPECTRA
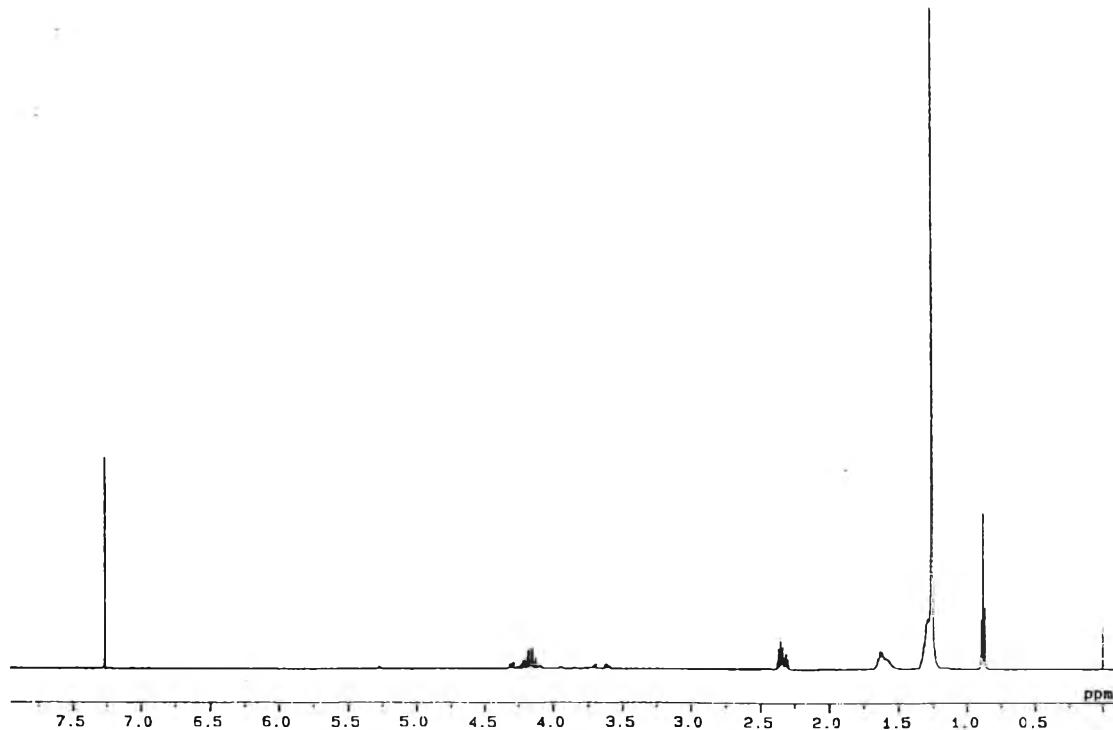
Figure f1A The 500 MHz ^{1}H-NMR spectrum of GP in CDCL$_3$
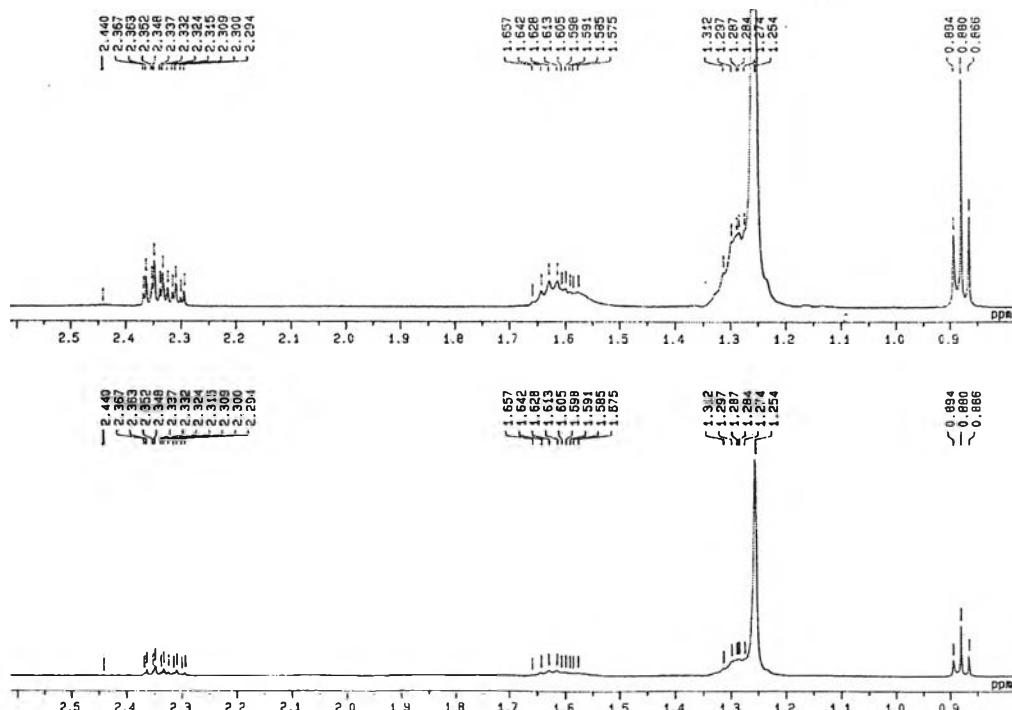
Figure f1B The 500 MHz ^{1}H-NMR spectrum of GP in CDCL$_3$
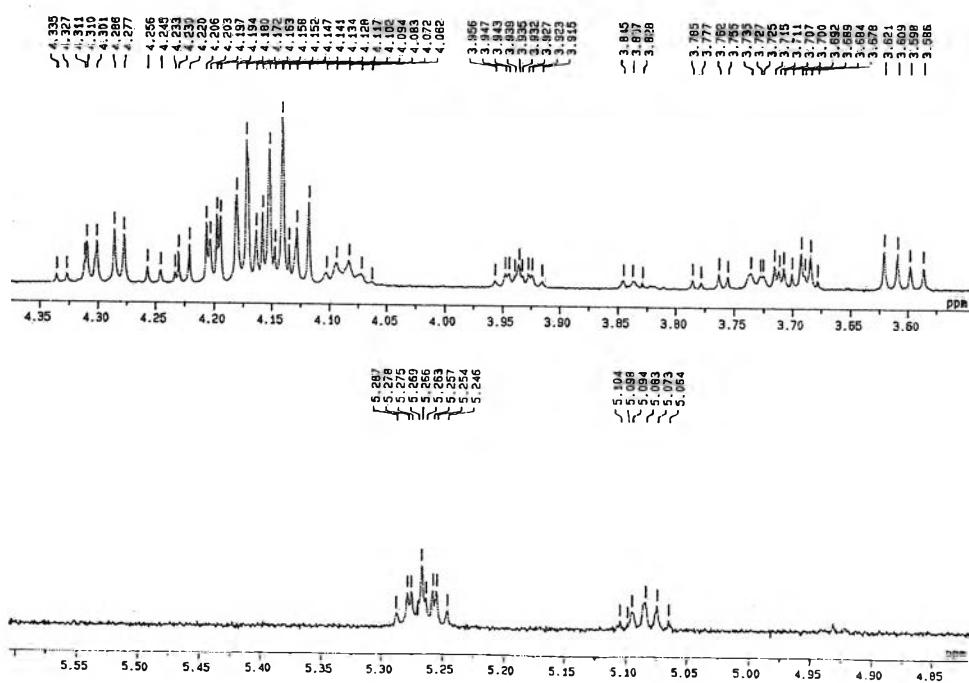
Figure f1C The 500 MHz ^{1}H-NMR spectrum of GP in CDCL$_3$
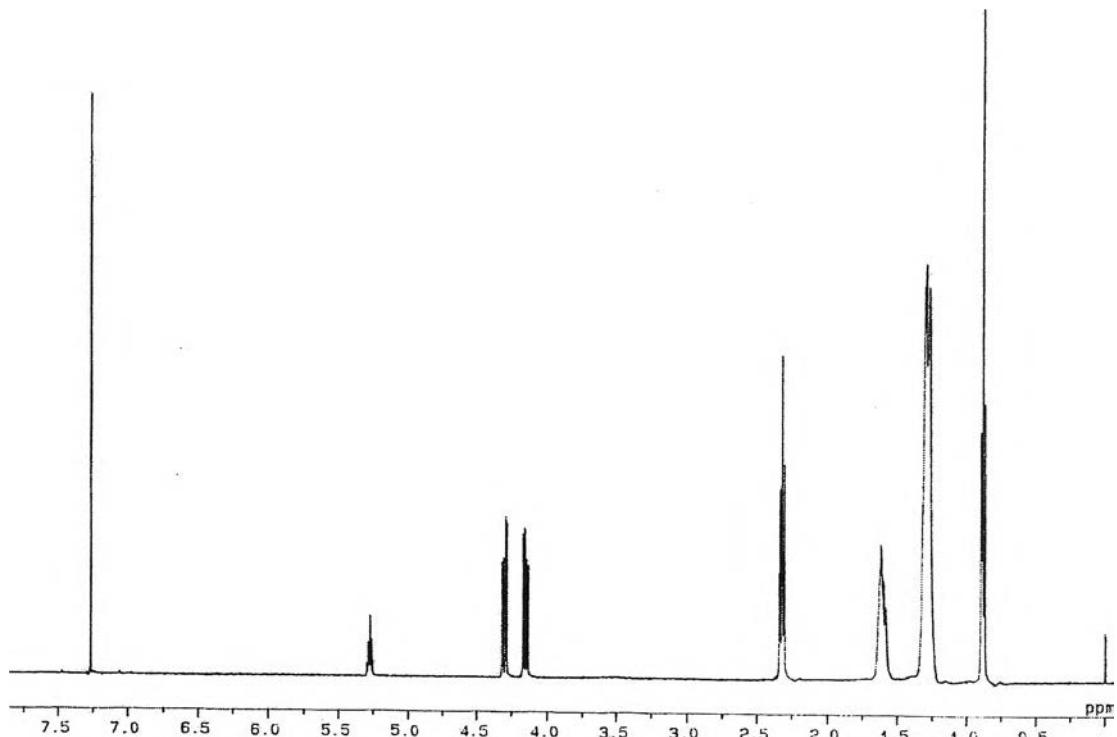
Figure f2A The 500 MHz ^{1}H-NMR spectrum of MCT oil in CDCL$_3$
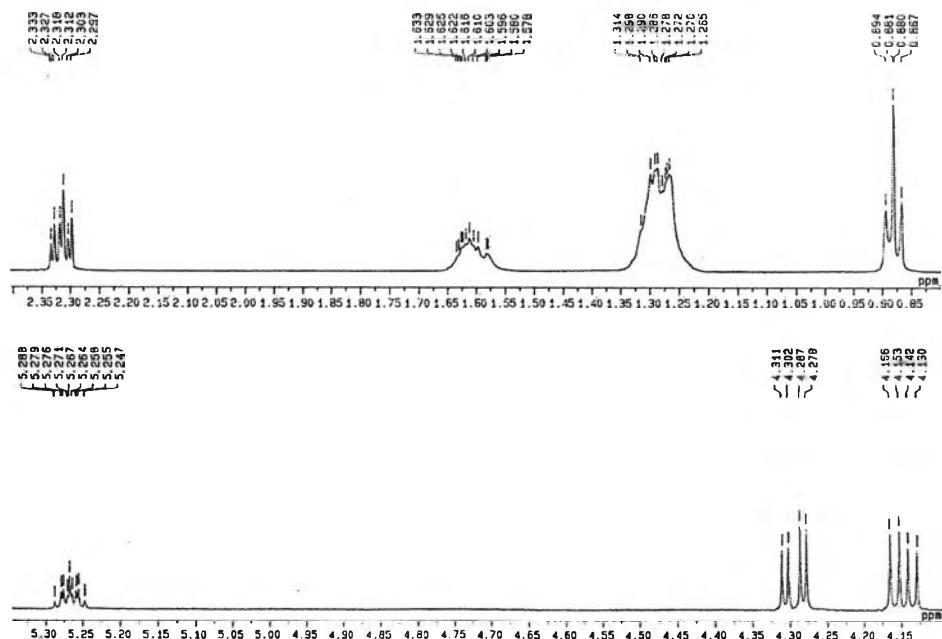
Figure f2B The 500 MHz ^{1}H-NMR spectrum of MCT oil in CDCL$_3$
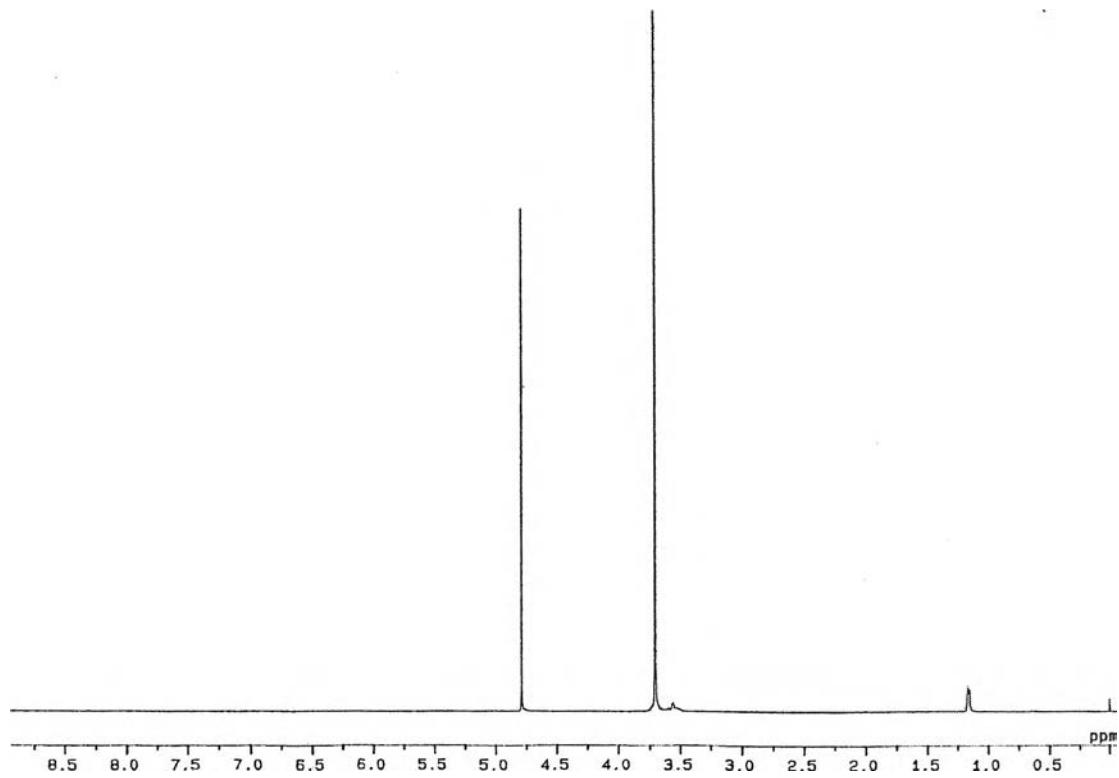
Figure f3A The 500 MHz ^{1}H-NMR spectrum of P407 in D$_2$O
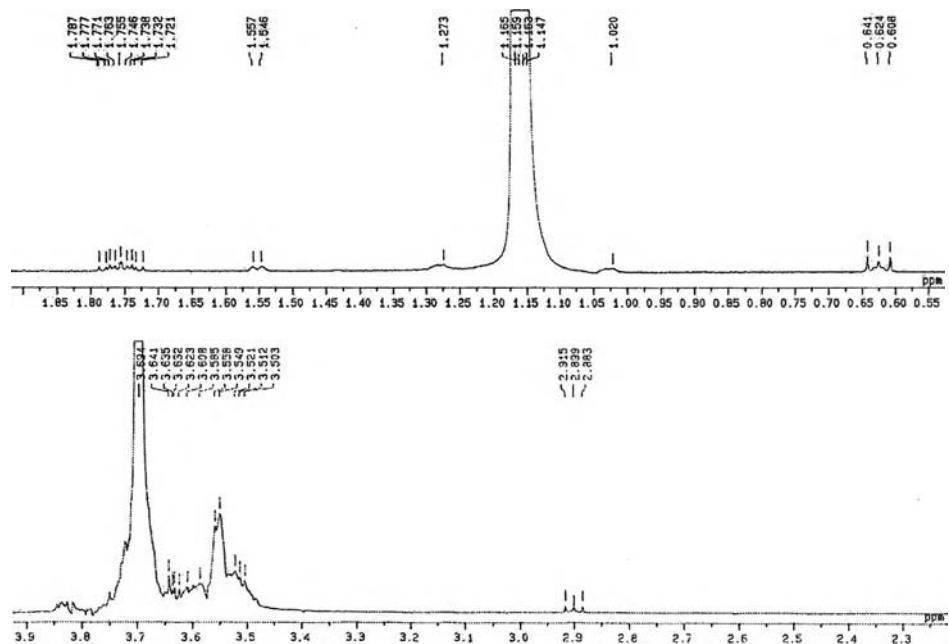
Figure f3B The 500 MHz ^{1}H-NMR spectrum of P407 in D$_2$O
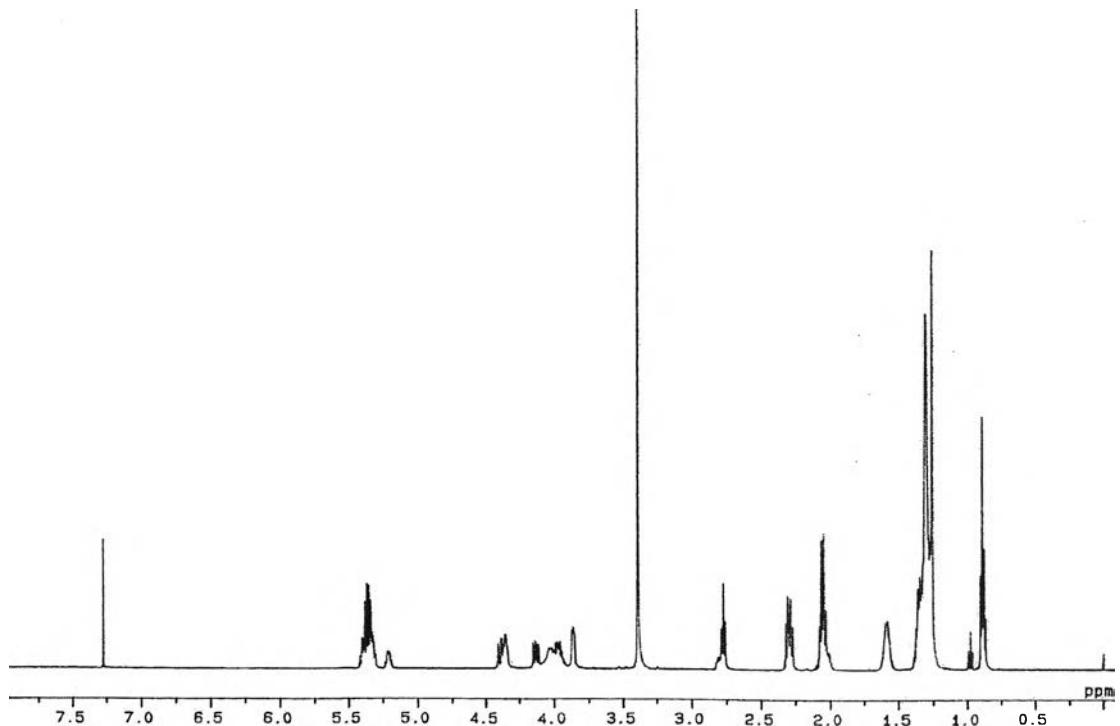
Figure f4A The 500 MHz ^{1}H-NMR spectrum of PL in CDCl$_3$
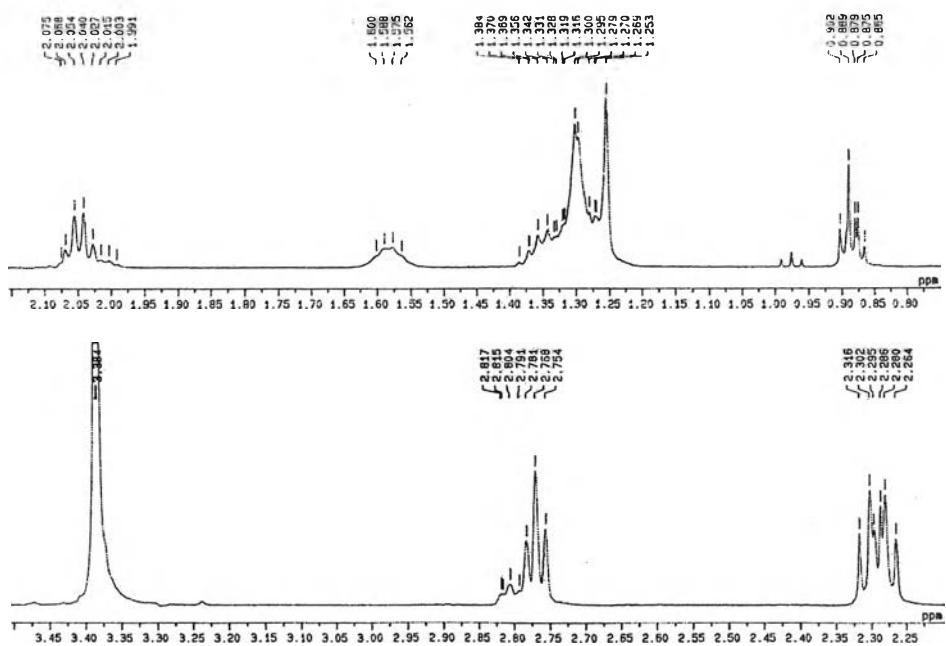
Figure f4B The 500 MHz ^{1}H-NMR spectrum of PL in CDCl$_3$
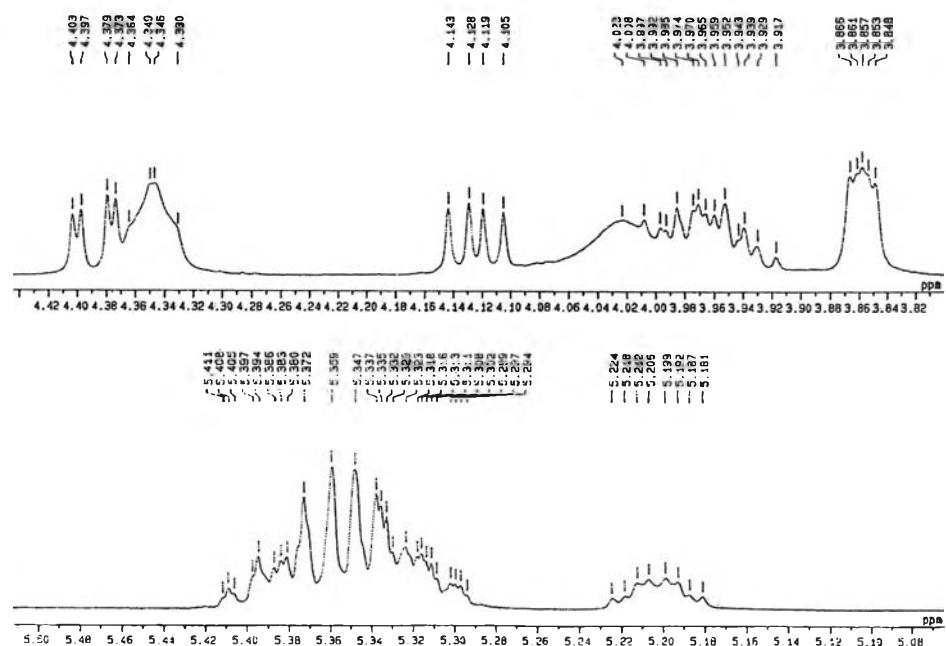
Figure f4C The 500 MHz ^{1}H-NMR spectrum of PL in CDCl$_3$
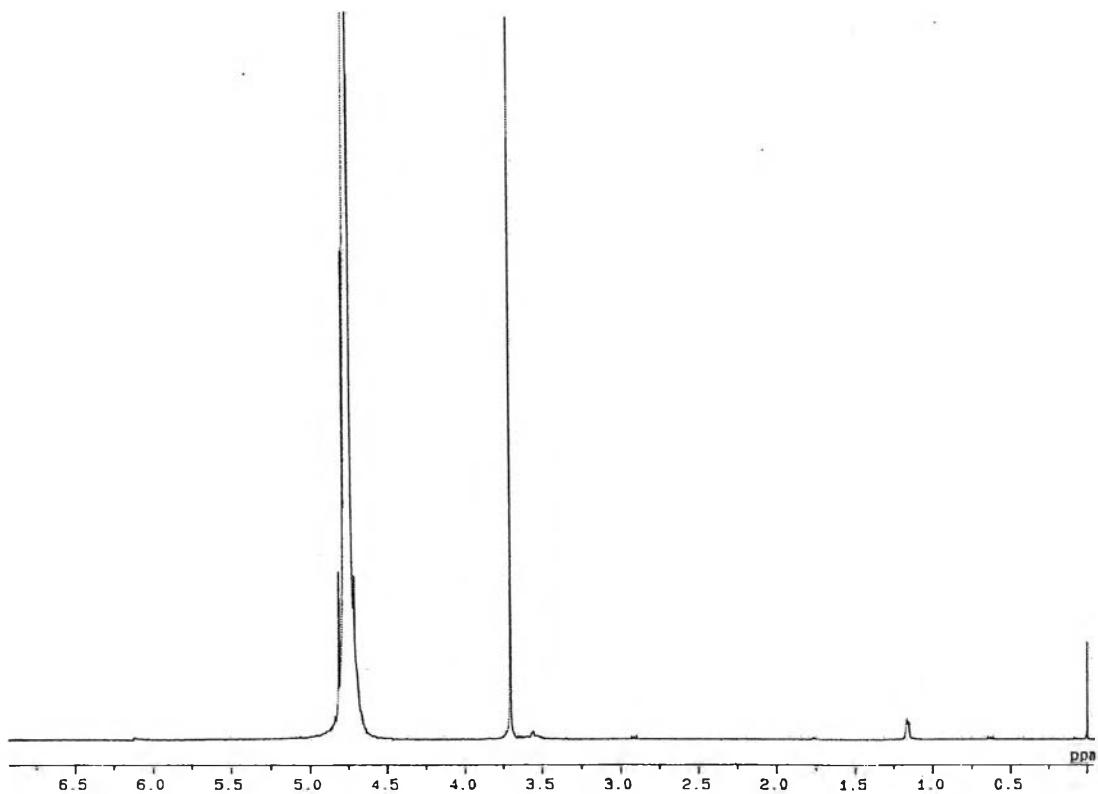
Figure f5A The 500 MHz ^{1}H-NMR spectrum of AmB-SLN in D_2O
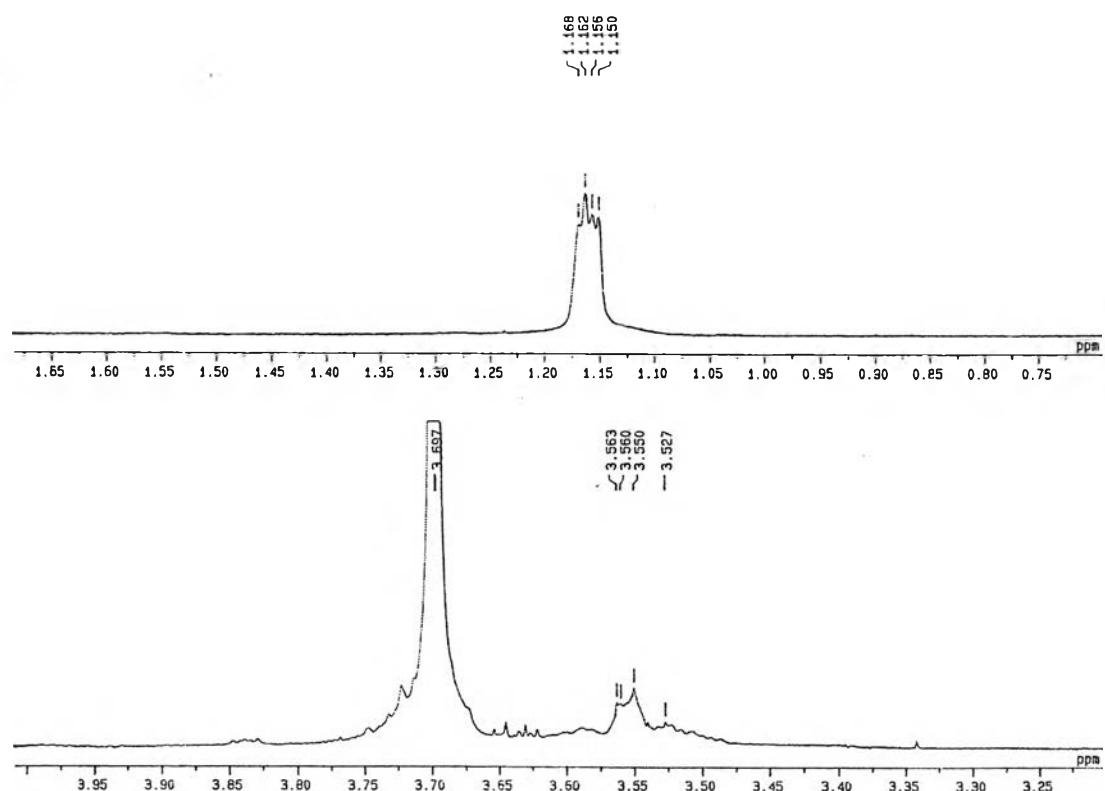
Figure f5B The 500 MHz ^{1}H-NMR spectrum of AmB-SLN in D_2O
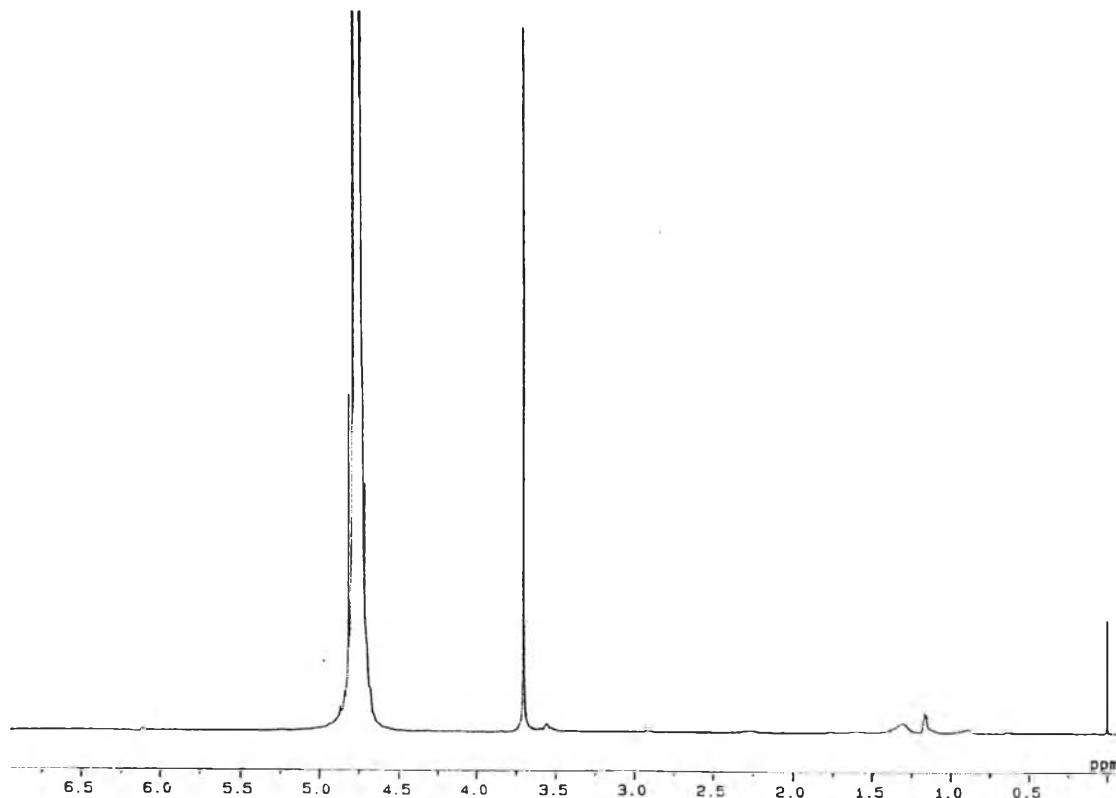
Figure f6A The 500 MHz ^{1}H-NMR spectrum of AmB-NLC in D$_2$O
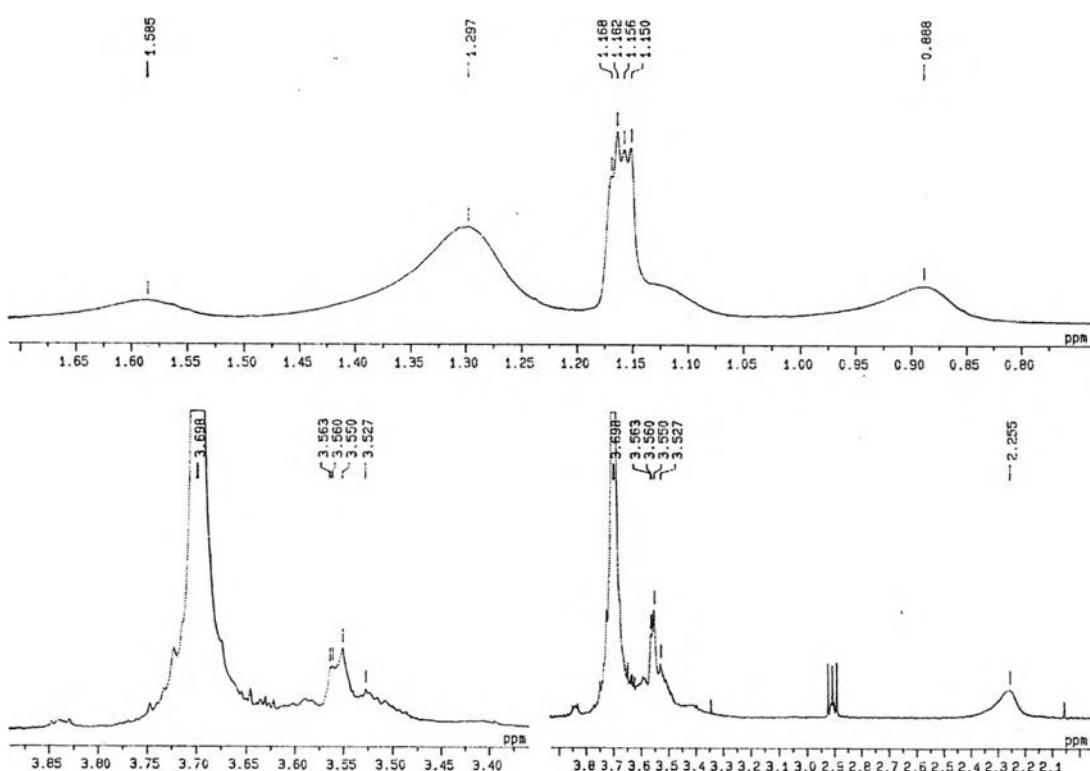
Figure f6B The 500 MHz ^{1}H-NMR spectrum of AmB-NLC in D$_2$O
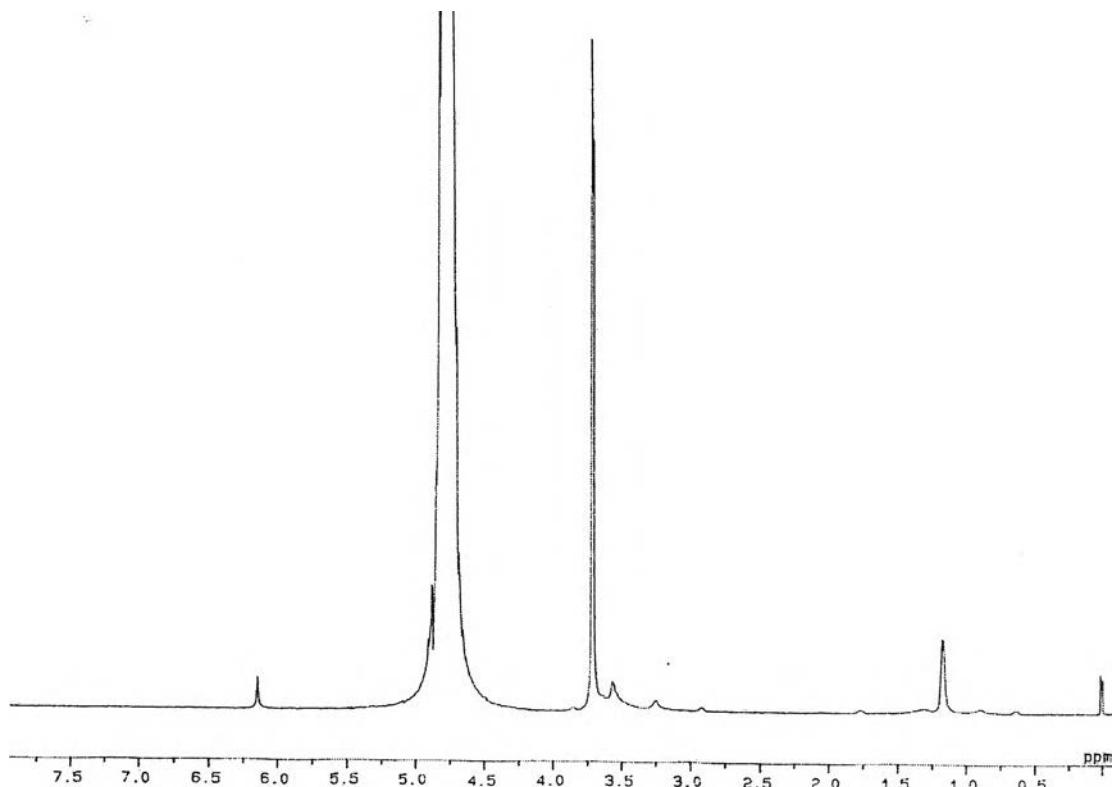
Figure f7A The 500 MHz ^{1}H-NMR spectrum of AmB-SLN-L in D_2O
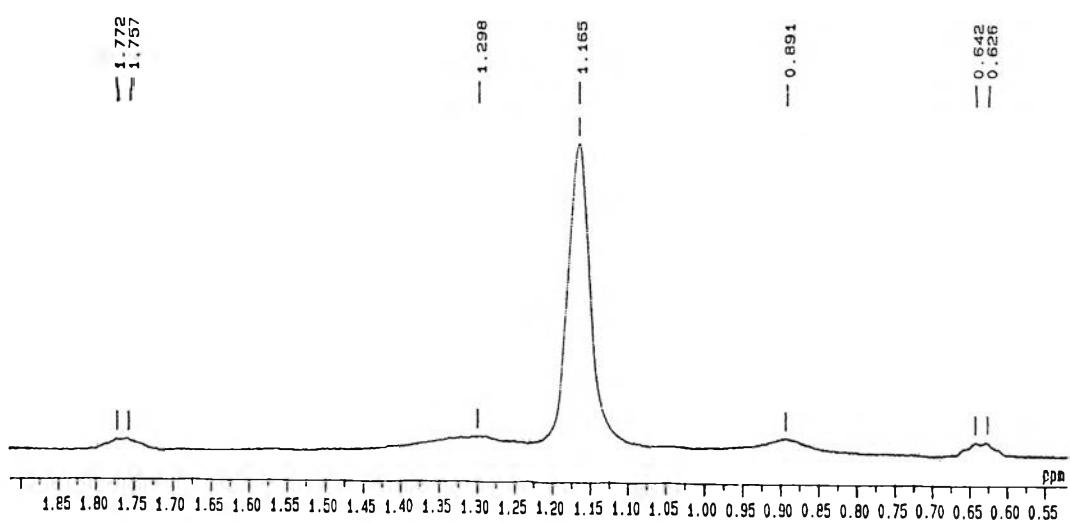
Figure f7B The 500 MHz ^{1}H-NMR spectrum of AmB-SLN-L in D_2O
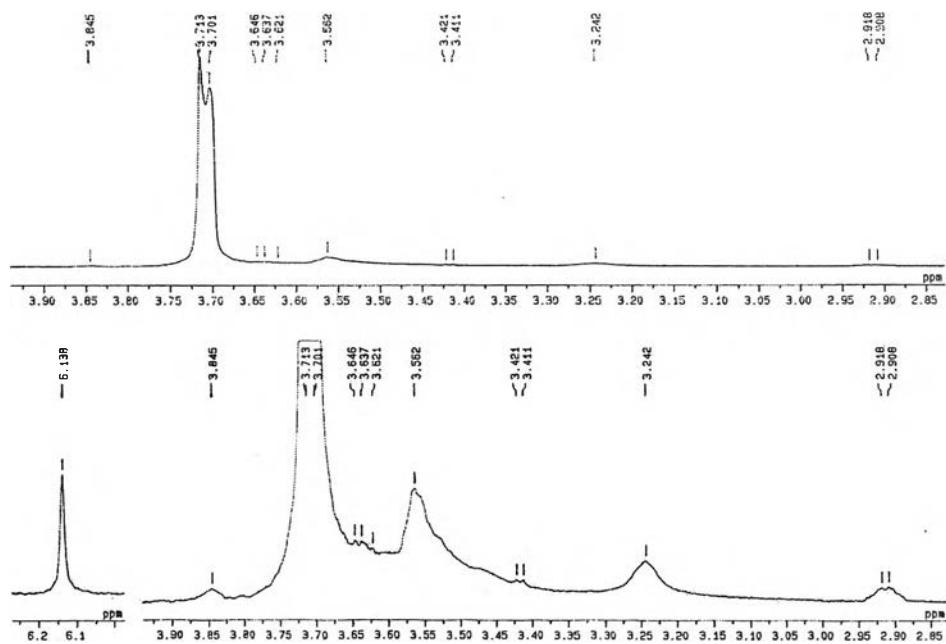
Figure f7C The 500 MHz ^{1}H-NMR spectrum of AmB-SLN-L in D_2O
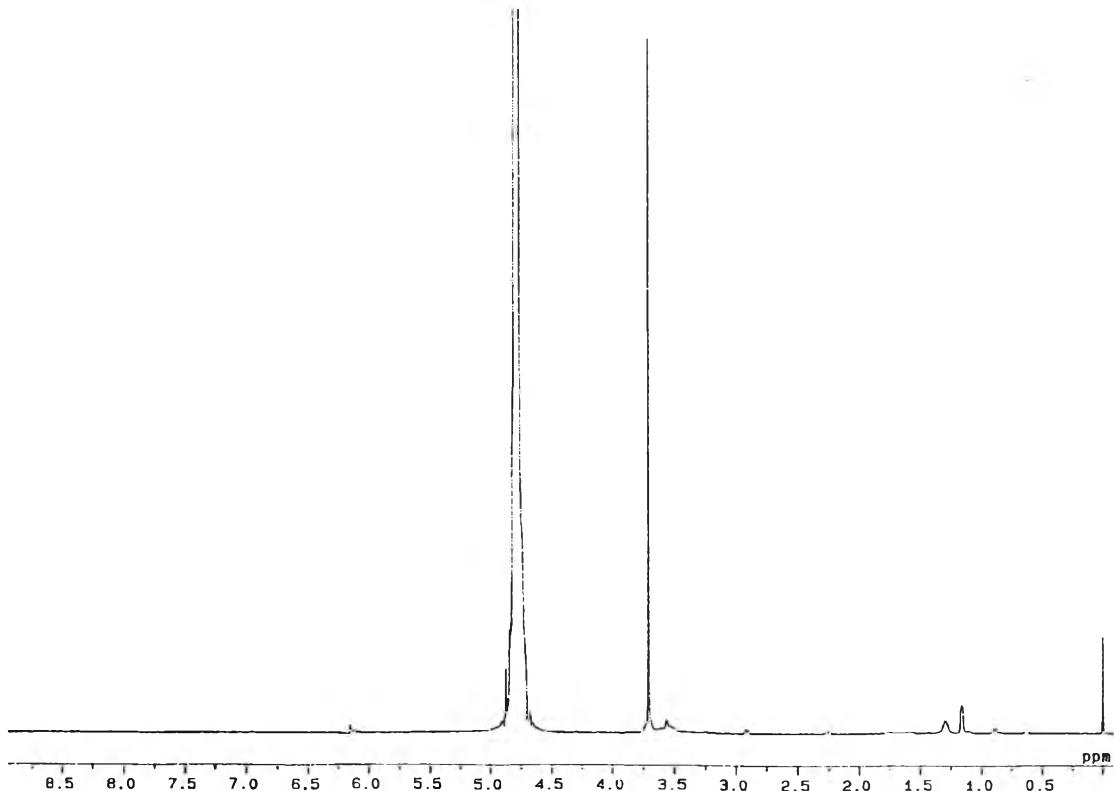
Figure f8A The 500 MHz ^{1}H-NMR spectrum of AmB-NLC-L in D_2O
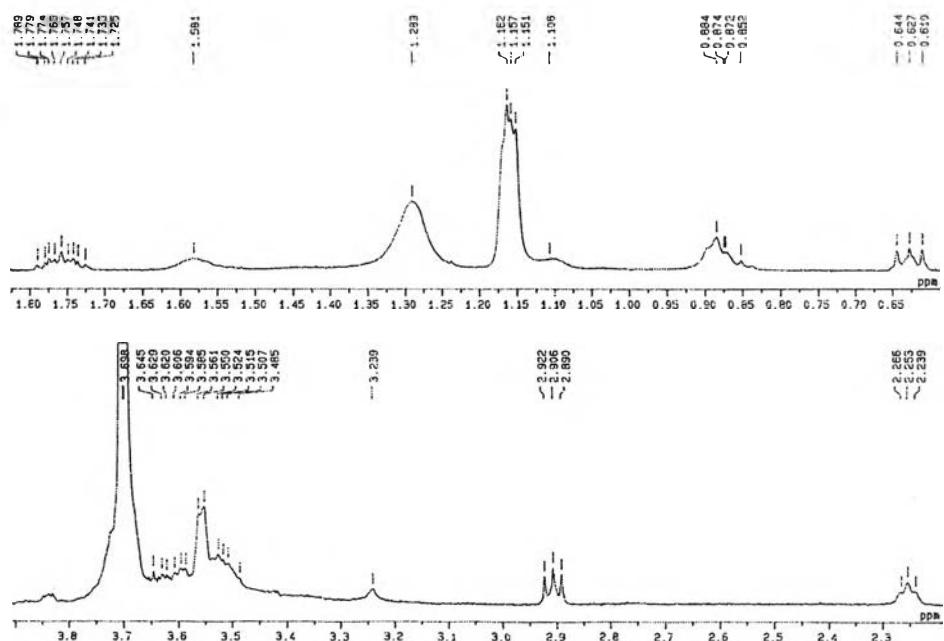
Figure f8B The 500 MHz ^{1}H-NMR spectrum of AmB-NLC-L in D$_2$O

APPENDIX G

HEMOLYSIS ACTIVITY DATA

Table g1 Hemolysis of sheep RBC at the varied levels of AmB as fungizone[®]

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	7.65	5.30	5.21	6.05	1.38
2.00	21.71	15.99	5.76	14.48	8.08
3.00	24.50	13.48	17.57	18.52	5.57
4.00	14.97	22.02	25.71	20.90	5.46
5.00	25.48	31.14	20.41	25.68	5.37
6.00	31.85	40.96	25.36	32.72	7.83
8.00	44.57	36.88	30.63	37.36	6.98
10.00	56.95	45.14	56.35	52.81	6.65
20.00	82.21	89.75	69.38	80.44	10.30
30.00	98.39	97.74	99.26	98.46	0.76
40.00	98.81	99.82	99.31	99.31	0.50

Table g2 Hemolysis of sheep RBC at the varied levels of AmB as AmB-WME3

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.66	-0.06	-0.57	0.01	0.62
2.00	0.30	-0.19	0.84	0.32	0.51
3.00	1.17	1.47	1.00	1.21	0.24
4.00	1.17	2.64	0.91	1.57	0.93
5.00	1.92	0.99	1.95	1.62	0.55
6.00	1.68	2.31	1.31	1.77	0.51
8.00	2.04	1.39	1.77	1.73	0.32
10.00	1.88	1.81	1.55	1.75	0.18
20.00	1.87	1.76	1.67	1.77	0.10
30.00	1.56	1.66	2.34	1.85	0.43
40.00	2.54	2.04	1.85	2.14	0.36

Table g3 Hemolysis of sheep RBC at the varied levels of AmB as AmB-WME9

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	1.29	1.58	0.98	1.28	0.30
2.00	1.44	2.35	1.18	1.66	0.62
3.00	3.45	2.03	1.56	2.35	0.98
4.00	2.53	2.71	3.29	2.84	0.40
5.00	3.01	2.62	2.97	2.87	0.22
6.00	1.76	3.06	3.82	2.88	1.04
8.00	3.70	3.25	3.78	3.58	0.28
10.00	1.57	4.41	4.82	3.60	1.77
20.00	3.02	3.49	4.36	3.62	0.68
30.00	3.71	3.94	3.48	3.71	0.23
40.00	4.54	4.64	4.98	4.72	0.23

Table g4 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN1

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.56	0.74	1.14	0.81	0.30
2.00	1.41	3.47	1.51	2.13	1.16
3.00	42.09	14.90	1.74	19.58	20.58
4.00	78.04	34.95	14.51	42.50	32.43
5.00	81.62	41.83	28.39	50.61	27.68
6.00	69.44	86.22	40.47	65.38	23.15
8.00	94.27	89.62	70.22	84.70	12.75
10.00	93.86	94.96	96.27	95.03	1.21
20.00	99.88	93.67	90.61	94.72	4.72
30.00	100.84	95.28	92.23	96.12	4.37
40.00	97.74	95.85	94.21	95.93	1.76

Table g5 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN2

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.34	0.94	1.48	0.92	0.57
2.00	1.22	0.75	0.60	0.85	0.32
3.00	1.48	1.21	1.76	1.48	0.28
4.00	2.15	2.08	1.53	1.92	0.34
5.00	2.07	1.65	2.13	1.95	0.26
6.00	1.57	1.57	3.18	2.11	0.93
8.00	2.12	2.91	2.59	2.54	0.40
10.00	2.79	3.13	2.74	2.89	0.21
20.00	4.81	3.88	3.64	4.11	0.62
30.00	5.68	5.94	6.23	5.95	0.28
40.00	8.17	7.68	7.65	7.83	0.29

Table g6 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN3

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	-0.03	-0.09	0.14	0.01	0.12
2.00	0.68	0.40	0.64	0.57	0.15
3.00	0.83	0.97	1.15	0.98	0.16
4.00	0.91	1.37	0.85	1.04	0.29
5.00	1.35	1.24	1.17	1.26	0.09
6.00	1.86	1.51	1.86	1.74	0.20
8.00	2.56	2.37	3.24	2.72	0.45
10.00	2.43	2.96	3.04	2.81	0.33
20.00	6.49	6.18	6.31	6.33	0.16
30.00	8.77	9.30	9.16	9.07	0.27
40.00	12.36	12.17	12.96	12.50	0.41

Table g7 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN4

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.35	1.60	1.26	1.07	0.65
2.00	1.08	0.70	0.98	0.92	0.20
3.00	1.00	0.93	1.19	1.04	0.14
4.00	1.09	1.10	1.33	1.17	0.14
5.00	1.11	1.83	1.91	1.62	0.44
6.00	2.37	1.81	1.52	1.90	0.43
8.00	2.97	2.93	1.55	2.48	0.81
10.00	2.28	2.26	3.55	2.70	0.74
20.00	4.89	4.79	3.68	4.45	0.67
30.00	7.18	6.98	8.79	7.65	0.99
40.00	7.33	8.63	9.09	8.35	0.92

Table g8 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC1

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	1.28	1.56	1.60	1.48	0.17
2.00	1.66	1.93	4.29	2.63	1.45
3.00	10.53	2.89	3.70	5.71	4.20
4.00	8.72	6.52	6.84	7.36	1.18
5.00	8.29	10.03	16.70	11.67	4.44
6.00	23.68	10.99	13.41	16.03	6.74
8.00	46.52	21.15	19.02	28.90	15.30
10.00	51.63	52.38	51.29	51.77	0.56
20.00	60.71	72.14	91.74	74.86	15.69
30.00	89.52	90.54	85.92	88.66	2.43
40.00	97.06	98.92	102.08	99.35	2.54

Table g9 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC2

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.09	0.20	0.35	0.21	0.13
2.00	0.57	0.54	0.57	0.56	0.02
3.00	0.82	0.72	0.86	0.80	0.07
4.00	1.21	1.31	1.10	1.21	0.10
5.00	1.41	1.43	1.42	1.42	0.01
6.00	1.88	1.77	1.81	1.82	0.06
8.00	2.16	2.19	2.31	2.22	0.08
10.00	2.72	2.92	2.95	2.86	0.12
20.00	5.81	6.58	6.06	6.15	0.39
30.00	8.45	8.55	8.60	8.53	0.08
40.00	11.32	11.84	11.79	11.65	0.29

Table g10 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC3

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.66	0.93	0.83	0.81	0.14
2.00	1.65	4.26	3.49	3.13	1.34
3.00	4.05	3.27	2.63	3.32	0.71
4.00	4.09	4.31	4.12	4.18	0.12
5.00	4.93	5.39	5.09	5.14	0.23
6.00	6.17	6.16	6.21	6.18	0.03
8.00	8.04	8.89	8.30	8.41	0.43
10.00	10.62	11.98	10.38	10.99	0.86
20.00	22.34	23.60	23.20	23.05	0.64
30.00	32.49	32.69	32.53	32.57	0.10
40.00	40.64	43.37	41.49	41.83	1.40

Table g11 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC4

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	1.23	0.11	0.16	0.50	0.63
2.00	0.58	0.51	0.60	0.56	0.05
3.00	0.99	1.23	1.04	1.09	0.13
4.00	1.48	1.65	0.92	1.35	0.38
5.00	1.60	2.16	2.06	1.94	0.30
6.00	2.37	2.35	2.47	2.40	0.07
8.00	3.22	3.19	3.30	3.24	0.06
10.00	4.26	4.23	3.92	4.14	0.19
20.00	8.51	9.03	8.74	8.76	0.26
30.00	13.10	12.82	13.51	13.14	0.35
40.00	15.64	15.77	16.23	15.88	0.31

Table g12 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L1

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.70	0.89	0.90	0.83	0.11
2.00	3.42	4.19	5.11	4.24	0.85
3.00	7.40	6.57	7.69	7.22	0.58
4.00	9.20	12.20	9.19	10.20	1.74
5.00	20.77	18.61	13.10	17.49	3.95
6.00	39.13	55.14	20.07	38.12	17.56
8.00	49.63	58.61	68.65	58.96	9.52
10.00	-	-	-	-	-
20.00	-	-	-	-	-
30.00	-	-	-	-	-
40.00	-	-	-	-	-

Table g13 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L2

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	1.61	-0.80	0.45	0.42	1.20
2.00	-0.80	1.76	1.10	0.69	1.33
3.00	1.10	2.40	1.71	1.74	0.65
4.00	1.36	1.91	2.35	1.87	0.50
5.00	1.94	2.48	2.23	2.22	0.27
6.00	2.10	2.53	2.52	2.38	0.25
8.00	2.21	2.96	2.01	2.40	0.50
10.00	4.01	4.23	4.10	4.11	0.11
20.00	5.84	6.10	6.23	6.06	0.20
30.00	8.66	9.32	9.56	9.18	0.46
40.00	11.63	11.40	12.37	11.80	0.50

Table g14 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L3

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	-0.15	0.03	0.23	0.04	0.19
2.00	0.34	0.33	0.59	0.42	0.14
3.00	1.34	1.03	1.16	1.17	0.16
4.00	1.86	1.72	1.64	1.74	0.11
5.00	2.05	1.97	2.80	2.27	0.46
6.00	2.51	2.76	2.51	2.59	0.14
8.00	3.38	3.05	2.96	3.13	0.22
10.00	3.73	3.81	3.96	3.83	0.12
20.00	8.74	8.17	8.37	8.43	0.29
30.00	11.32	11.69	11.70	11.57	0.22
40.00	14.46	14.49	14.98	14.65	0.29

Table g15 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L4

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.10	0.19	0.00	0.09	0.10
2.00	0.48	0.40	0.27	0.38	0.10
3.00	0.85	0.70	0.53	0.70	0.16
4.00	0.93	1.16	0.88	0.99	0.15
5.00	1.33	1.28	1.63	1.42	0.19
6.00	2.36	1.97	1.66	2.00	0.35
8.00	2.66	2.49	2.44	2.53	0.12
10.00	2.80	2.77	3.05	2.87	0.15
20.00	9.46	4.61	5.32	6.46	2.62
30.00	7.22	7.97	7.31	7.50	0.41
40.00	9.99	9.94	11.42	10.45	0.84

Table g16 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L1

AmB concentration (μg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	25.42	24.92	24.51	24.95	0.46
2.00	63.67	55.44	21.86	46.99	22.15
3.00	66.21	56.20	61.51	61.30	5.01
4.00	59.77	64.91	64.50	63.06	2.86
5.00	64.94	64.24	66.39	65.19	1.10
6.00	-	-	-	-	-
8.00	-	-	-	-	-
10.00	-	-	-	-	-
20.00	-	-	-	-	-
30.00	-	-	-	-	-
40.00	-	-	-	-	-

Table g17 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L2

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.17	0.57	0.05	0.27	0.27
2.00	0.25	0.59	0.53	0.46	0.18
3.00	0.85	1.08	0.67	0.87	0.21
4.00	0.96	0.94	1.20	1.03	0.14
5.00	0.96	1.91	1.42	1.43	0.48
6.00	1.38	1.66	1.55	1.53	0.14
8.00	1.72	1.80	1.91	1.81	0.09
10.00	2.26	2.41	2.55	2.41	0.14
20.00	5.88	5.80	5.91	5.87	0.06
30.00	6.99	8.06	8.31	7.79	0.70
40.00	9.31	9.95	10.09	9.78	0.42

Table g18 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L3

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.28	-0.07	-0.09	0.04	0.20
2.00	0.38	0.38	0.51	0.42	0.07
3.00	0.92	0.78	0.35	0.68	0.30
4.00	1.14	0.87	0.83	0.95	0.17
5.00	1.83	1.96	1.99	1.93	0.09
6.00	2.34	2.22	2.20	2.25	0.07
8.00	2.82	3.07	3.30	3.07	0.24
10.00	3.85	3.86	3.83	3.85	0.01
20.00	7.06	6.57	7.01	6.88	0.27
30.00	17.61	20.20	21.37	19.73	1.93
40.00	32.24	30.80	28.12	30.38	2.09

Table g19 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L4

AmB concentration (µg/ml)	% hemolysis			Mean	SD
	No.1	No.2	No.3		
1.00	0.05	-0.01	0.09	0.05	0.05
2.00	0.12	0.08	0.18	0.12	0.05
3.00	0.44	0.53	0.59	0.52	0.07
4.00	0.75	0.81	0.85	0.81	0.05
5.00	1.05	1.02	0.90	0.99	0.08
6.00	1.38	1.23	1.21	1.27	0.09
8.00	2.21	1.73	1.65	1.86	0.30
10.00	2.46	2.35	2.30	2.37	0.08
20.00	6.01	5.87	5.71	5.86	0.15
30.00	8.08	8.19	8.58	8.29	0.26
40.00	17.01	17.46	17.95	17.47	0.47

VITA

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