# References


Anthony, C., and Tsang, S. 1981. α-Tocopherol retards autoxidation and prolong the shelf-life of liposomes. ***Int. J. Pharm.*** 8: 101-110.

Artmann, C., Roding, J., Ghyczy, M., and Pratzel, H. G. 1990a. Liposomes from soya phospholipids as: percutaneous drug carriers 1st communication: qualitative in vivo investigations with antibody-loads liposomes. ***Drug Res.*** 40(II),12,: 1363-1365.

Artmann, C., Roding, J., Ghyczy, M., and Pratzel, H. G. 1990b. Liposomes from soya phospholipids as percutaneous drug carriers: 2nd communication: qualitative in vivo investigations with radioactively labeled liposomes. ***Drug Res.*** 40(II),12,: 1365-1368.

Bangham, A.D., Standish, M.M., and Watkins, J.C. 1965. Diffusion of univalent ions across the lamellae of swollen phospholipid. ***J. Mol. Biol.*** 13: 238-252.

Barenholz, Y., Gibbes, D., Litman, B. J., Thompson, T. E., and Carlson, F. D. 1977. A simple method for the preparation of homogeneous phospholipid. ***Biochemistry.*** 16(12): 2806-2810.

Barenholzt, Y., Amselem, S., and Lichtenberg, D. 1979. A new method for preparation of phospholipid vesicles (liposomes)-french press. ***FEBS. Lett.*** 99(1): 210-214.

Batzri, S., and Korn, E. 1973. Single bilayer liposomes prepared without sonication. ***Biochim. Biophys. Acta.*** 298: 1015-1019.

Blume, A. 1992. Phospholipids as basic ingredients. In: Braun-Falco, O., Korting, H. C., and Maibach, H. I. (eds.), ***Liposome Dermatics***, Griesbach

Conference. Spring - Verlag Berlin. 30-37.

Bouwstra, J.A., Hofland, H.E.J., Spies, F., Fooris, G.S., and Junginger, H.E. 1992. Change in the structure of human stratum corneum induced by liposomes. In: Braun-Falco, O., Korting, H. C., and Maibach, H. I. (eds.), ***Liposome Dermatics***, Griesbach Conference. Spring - Verlag Berlin. 121-136.

Brandl, M., Bachmann, D., Drechsler, M., and Bauer, K. H. 1990. Liposome preparation by a new high pressure homogenizer gaulin micron lab 40. ***Drug Del. Ind. Pharm.*** 16(14): 2167-2191.

Brown, K. A., and Collins, A. 1977. Action of nonsteroidal anti-inflammatory drugs on human and rat peripheral leukocyte migration in vitro. ***Ann. Rheum. Dis.*** 36: 239-249.

Brunner, J., Skrabal, P., and Hauser, H. 1976. Single bilayer vesicles prepared without sonication physico-chemical properties. ***Biochim. Biophys. Acta.*** 455: 322-331.

Cevc, G. 1992. Rational for the production and dermal application of lipid vesicles. In: Braun-Falco, O., Korting, H. C., and Maibach, H. I. (eds.), ***Liposome Dermatics***, Griesbach Conference. Spring - Verlag Berlin. 82-85.

Cevc, G., and Blume, G. 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. ***Biochim. Biophys. Acta.*** 1104: 226-232.

Cevc, G., Schatzlein, A., and Blume, G. 1995. Transdermal drug carriers basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. ***J. Control. Release.*** 36: 3-16.

Chien, Y. W. 1992. Transdermal drug delivery and delivery systems. In: ***Novel drug delivery systems.*** 2nd, Revised and expanded. pp. 301-304. New York: Marcel Dekker Inc.

Citernesi, U., and Sciacchitano, M. 1995. Phospholipid/active ingredient complexes. ***Cosmet. Toiletries.*** 110(November): 57-68.

Deamer, D. W., and Bangham, A. D. 1973. Large volumes by an ether vaporization method. ***Biochim. Biophys. Acta.*** 433: 629-634.

De Gier, J., Mandersloot, J. G., and Van Deenen, L. L. M. 1968. Lipid composition and permeability of liposomes. ***Biochim. Biophys. Acta.*** 150: 666-675.

Du Plessis, J., Ramachandran, C., Weiner, N., and Muller, D.G. 1994. The influence of particle size of liposomes on the deposition of drug into the skin. ***Int. J. Pharm.*** 103: 277-282.

Du Plessis, J., Weiner, N., and Muller, D. G. 1994. The influence of in vivo treatment of skin with liposomes on the topical absorption of a hydrophilic and a hydrophobic drug in vitro. ***Int. J. Pharm.*** 103: R1-R5.

Elvers, K. T., and Wright, S. J. L. 1995. Antibacterial activity of the anti-inflammatory compound ibuprofen. ***Letters in Applied Microbiology.*** 20: 82-84.

Evoy, M. G. eds. 1995. ***AHFS Drug information.*** The American Society of Health-System Pharmacists: New York: 1311-1315.

Foldvari, M., Gesztes, A., and Mezei, M. 1990. Dermal drug delivery by liposome encapsulation: clinical and electron microscopic studies. ***J. microencapsulation*** 79: 479-789.

George, C. F. 1996. ***British National Formulary.*** Number 31 (March 1996). The British Medical Association and the Royal Pharmaceutical Society of Great Britain. London: The Pharmaceutical Press: 424.

Gesztes, A., and Mezei, M. 1988. Topical anesthesia of the skin by liposome-encapsulated tetracaine. ***Anesth. Analg.*** 67: 1079-1081.

Ghyczy, M., and Gareiss, J. 1994. Liposomes from vegetable phosphatidylcholine.

*Cosmet. Toiletries.* 109(July): 75-80.

Gruner, S. M. 1985. Novel-multilayered lipid vesicles: comparison of physical characteristics of multilamellar liposomes and stable plurilamellar vesicles. ***Biochemistry.*** 24: 2833-2842.

Hauser, H., and Gains, N. 1982. Spontaneous vesiculation of phospholipids: A simple and quick method of forming unilamellar vesicles. ***Proc. Natl. Acad. Sci.*** 79: 1683-1687.

Inoue, K., and Kitagawa, T. 1974. Effect of exogenous lysolecithin on liposomal membranes its relative to membrane fluidity. ***Biochim. Biophys. Acta.*** 363: 361-372.

Johnson, S. M. 1973. The effect of charge and cholesterol on the size and thickness of sonicated phospholipid vesicles. ***Biochim. Biophys. Acta.*** 307: 27-41.

Katare, O.P., Vyas, S. P., and Dixit, V. K. 1990. Effervescent granule based proliposomes of ibuprofen. ***J. microencapsulation*** 7(4): 455-462.

Katz, A., Ryan, P., Lalonde, C., West, K., and Demiling, R. 1985. Topical ibuprofen decreases thromboxane release from the endotoxin-stimulated burn wound. ***J. Trauma.*** 26(2): 157-162.

Kim, S., Turker, M. S., Chi, E. Y., Sela, S., and Martin, G. M. 1983. Preparation of multivesicular liposomes. ***Biochim. Biophys. Acta.*** 728: 339-348.

Kirby, C., Clarke, J., and Gregoriadis, G. 1980. Cholesterol content of small unilamellar liposomes controls phospholipid loss to high density lipoproteins in the presence of serum. ***FEBS Lett.*** 11(2): 324-328.

Kulkarni, S. B., Betageri, G. V., and Singh, M. 1995. Factors affecting microencapsulation of drug in liposomes. ***J. Microencapsulation*** 12: 229-246.

Lasch, J., Laub, R., and Wohlrab, W. 1991. How deep do intact liposomes penetrate

into human skin? ***J. Control. Release.*** 18: 55-58.

Lasic, D. D., Belic, A., and Valentincic, T. 1988. A new method for the instant preparation of large unilamellar vesicles. ***J. Am Chem Soc.*** 110: 970-971.

Lund, W. eds. 1994. ***The pharmaceutical codex. 12 th ed. Principles and Practice of Pharmaceutics.*** pp. 908-911. London: The pharmaceutical press.

Mayer, L. D., Hope, M. J., Cullis, P. R., and Janoff, A. S. 1985. Solute distributions and trapping efficiencies observed in freeze-thawed multilamellar vesicles. ***Biochim. Biophys. Acta.*** 817: 193-196.

Memoli, A., Palermiti, L.G., Travagli, V., and Alhaique, F. 1993. Liposomes in cosmetics: Which kind of phospholipid? Which loading method? ***J. Soc. Cosmet. Chem.*** 44(March/April): 123-128.

Mezei, M., and Gulasekharam, V. 1980. Liposomes - a selective drug delivery system for the topical route of administration. I. lotion dosage form. ***Life. Sci.*** 26: 1473-1477.

Mezei, M., and Gulasekharam, V. 1982. Liposomes - a selective drug delivery system for the topical route of administration: gel dosage form. ***J. Pharm. Pharmacol.*** 34: 473-477.

Michel, C., Purmann, T., Mentrup, E., Seiller, E., and Kreuter, J. 1992. Effect of liposomes on percutaneous penetration of lipophillic materials. ***Int. J. Pharm.*** 84: 93-105.

Montenegro, L., Panico, A.M., Ventimiglia, A., and Bonina, F.P. 1996. In vitro retinoic acid and skin permeation from different liposome formulations. ***Int. Pharm.*** 133: 89-96.

Nattermann Phospholipid GmbH. 1995a. Soybean lecithin and phospholipid fractions-A brief overview. In: Nattermann Phospholipid GmbH (eds.), ***Nattermann Phospholipid Substance description***: 8.

Nattermann Phospholipid GmbH. 1995b. In: Nattermann Phospholipid GmbH (eds.), ***Nattermann Phospholipid Substance description.***

Nattermann Phospholipid GmbH. 1996. Possible relevance of fluid or crystalline-phase liposomes as vectors. 1-6.

Neidleman, S. L. 1993. Occurrence and response to environmental stresses in nonmammalian organisms. In: Ceve, G. (ed.), ***Phospholipid handbook.*** New York: Marcel Dekker, Inc. 23-38.

New, R R. C. 1990a. Preparation of liposomes. In: ***Liposomes a practical approach.*** pp. 33-38. Oxford: IRL Press.

New, R R. C. 1990b. Introduction. In: ***Liposomes a practical approach.*** pp. 23. Oxford: IRL Press.

New, R R. C. 1990c. Introduction. In: ***Liposomes a practical approach.*** pp. 4. Oxford: IRL Press.

New, R R. C. 1990d. Introduction. In: ***Liposomes a practical approach.*** pp. 19-21. Oxford: IRL Press.

New, R R. C. 1990e. Preparation of liposomes. In: ***Liposomes a practical approach.*** pp. 56-58. Oxford: IRL Press.

New, R R. C. 1990f. Preparation of liposomes. In: ***Liposomes a practical approach.*** pp. 68-69. Oxford: IRL Press.

New, R R. C. 1990g. Preparation of liposomes. In: ***Liposomes a practical approach.*** pp. 99-100. Oxford: IRL Press.

Niki, E., Takahashi, M., and Komuro, E. 1986. Antioxidant activity of vitamin E in liposomal membranes. ***Chem. Lett.*** 1573-1576.

Oku, N., and MacDonald, R. C. 1983. Differential effects of alkali metal chloride on formation of giant liposomes by freezing and thawing and dialysis. ***Biochemistry.*** 22(4): 855-863.

Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J., and Papahadjopoulos, D. 1979. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membrane. ***Biochim. Biophys. Acta.*** 557: 9-23.

Papahadjopoulos, D., Vail, W. J., Jacobson, K., and Poste, G. 1975. Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles. ***Biochim. Biophys. Acta.*** 394: 483-491.

Payne, N. I., Timmins, P., Ambrose, C. V. , Ward, M. D., and Ridgway, F. 1986. Proliposomes: A Novel solution to an old problem. ***J. Pharm Sci.*** 75(4): 325-329.

Perrett, S., Golding, M., and Williams, P. 1991. A simple method for the preparation of liposomes for pharmaceutical applications: characterization of the liposomes. ***J. Pharm Sci.*** 43: 154-161.

Rao, C. S., Schoenwald, R. D., Barfknecht, C. F., and Laban, S. L. 1992. Biopharmaceutical evaluation of ibufenac, ibuprofen and their hydroxyethoxy analogs in the rabbit eye. ***J. Pharmacokinetics and Biopharmaceutics.*** 20(4): 357-389.

Reeves, J. P., and Dowben, R. M. 1969. Formation and properties of thin walled phospholipid vesicles. ***J. Cell Physiol.*** 73: 49-60.

Rhoden, V., and Goldin, S. M. 1979. Formation of unilamellar lipid vesicles of controlled dimensions by detergent dialysis. ***Biochemistry.*** 18(9): 4173-4176.

Robert, L., Hamilton, Jr., Goerke, J., Luke, S. S. G., and Williams, M. C. 1980. Unilamellar liposomes made with the French pressure cell: a simple preparative and semiquantitative technique. ***J. Lip. Res.*** 21: 981-992.

Sanderson, N. M., and Jones, M. 1995. Interaction of cationic liposomes with skin bacteria. ***Biochem. Soc. Trans.*** 23: 561S.

Sanyal, A. K., Roy, D., Chowdhury, B., and Banerjee, A. B. 1993. Ibuprofen a unique anti-inflammatory compound with antifungal activity against dermatophytes. ***Letters in Applied Microbiology.*** 17: 109-111.

Sharma, A., and Sharma, U. S. 1997. Liposomes in drug delivery: progress and limitations. ***Int. J. Pharm.*** 154: 123-140.

Sivakamai, P. A., Mythily, S., Alamelu, S. and Panduranga Ras, K. 1994. Liposome-ibuprofen conjugate and the release characteristics of ibuprofen. ***Indian Drugs.*** 31(12): 568-573.

Stamp, D., and Juljano, R. L. 1979. Factor affecting the encapsulation of drugs within liposomes. ***Can. J. Physiol. Pharmacol.*** 57: 535-539.

Strauss, G. 1989. Liposomes : From theoretical model to cosmetic tool. ***J. Soc. Cosmet. Chem.*** 40 (January/February): 54-60.

Szoka, F., and Papahadjopoulous, D. 1978. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. ***Proc. Natl. Acad. Sci. USA*** 75(9): 4194-4198.

Szoka, F., Olson, F., Heath, T., Vail, J., Mayhew, E., and Papahadjopoulous, D. 1980. Preparation of unilamellar liposomes of intermediate size (0.1-0.2 $\mu$m) by a combination of reverse phase evaporation and extrusion through polycarbonate membranes. ***Biochim. Biophys. Acta.*** 601: 559-571.

Talsma, H., Ozer, A. Y., Bloois, L. V., and Crommelin, D. J. A. 1989. The size reduction of liposomes with a high pressure homogenizer (microfluidizer$^{TM}$). Characterization of prepared dispersions and comparison with conventional methods. ***Drug Del. Ind. Pharm.*** 15(2): 197-207.

Vemuri, S., and Rhodes, C.T. 1995. Preparation and characterization of liposomes as therapeutic delivery systems: a review. ***Pharmaceutica. Acta. Helv.*** 70: 95-111.

Vemuri, S., Yu, C. D., Wangsatorntanakun, V., and Roosdorp, N. 1990. Large-scale production of liposomes by a microfluidizer. ***Drug Del. Ind. Pharm.*** 16(15): 2243-2256.

Wade, A., and Weller, P. J. (eds.) 1994a. ***Handbook of pharmceutical exipients.*** 2nd ed. Washington: American Pharmaceutical Association. London: The Pharmaceutical Press, 12-13.

Wade, A., and Weller, P. J. (eds.) 1994b. ***Handbook of pharmaceutical exipients.*** 2nd ed. Washington: American Pharmaceutical Association. London: The Pharmaceutical Press, 121-122.

Weiner, N., Martin, F., and Riaz, M. 1989. Liposomes as a drug delivery system. ***Drug Del. Ind. Pharm.*** 15: 1523-1554.

Wohlrab, W., and Lasch, J. 1987. Penetration kinetics of liposomal hydrocortisone in human skin. ***Dermatologica*** 174: 18-22.

Yamauchi, H., Kiyoto, Y., Suzuki, N., and Kikuchi, H. 1994. Application of newly developed technology to liposomes. ***JSPS-NRCT Core University Exchanges System on pharmaceutical sciences second joint seminar:*** recent advances on pharmaceutics and pharmaceutical technology. Bangkok, Thailand. OP6.

Yotsuyanagi, T., Nonomura, M., and Ikeda, K. 1981. Ultrasonic size reduction of liposomes and volume estimation of internal aqueous phase. ***J. Paren. Sci. Techno.*** 35(6): 271-275.

Zuidam, N. J., Gouw, H. K. M. E., Barenholz, Y., and Crommelin, D. J. A. 1995. Physical (in)stability of liposomes upon chemical hydrolysis : the role of lysophosphatidylcholine and fatty acid. ***Biochim. Biophys. Acta.*** 1240: 101-110.

Zumbuehl, O., and Weder, H. G. 1981. Liposomes of controllable size the range of

40 to 80 nm by defined dialysis of lipid/detergent mixed micelles. ***Biochim. Biophys. Acta.*** 640: 252-262.

# APPENDICES

# Appendix I

## Details of Some Substances

**1. Soybean lecithin** (Phospholipon® 90) (Nattermann Phospholipid GmbH,1995)

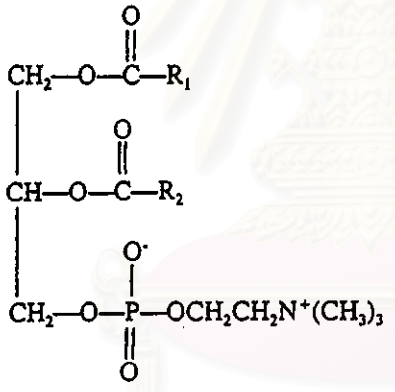
**1. Scientific names:**

(3-sn-Phosphatidyl)-choline, soya

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

Cholinephospholic acid diglyceride ester

**2. Chemical formula:**



$R_1$ and $R_2$ = fatty acid residues

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

mean molecular weight : 775 g/mol

**3. Composition:**

| | |
|---|---|
| Phosphatidylcholine | 93 ± 3% |
| Lysophosphatidylcholine | 3 ± 3% |
| d,l-α-Tocopherol | min. 0.1% |

Typical fatty acid composition:

| | |
|---|---|
| palmitic acid | $12 \pm 2\%$ |
| stearic acid | $3 \pm 1\%$ |
| oleic acid | $10 \pm 3\%$ |
| linoleic acid | $66 \pm 5\%$ |
| linolenic acid | $5 \pm 2\%$ |

**4. General properties :**

| | |
|---|---|
| Form | granular or waxy |
| Color | yellow |
| Melting point | ca. 180 °C with decomposition |
| Density | ca. 1 g/cm$^3$ |
| pH-value | 5.5 - 7.5 |
| (c=1 % in 1 % NaCl-solution) | |
| Specific rotation | ca. 7 ° |
| (c=10% in $CHCl_3$ : $CH_3OH$ : $H_2O$ = 65:25:4=v:v:v) | |
| Solubilities: | |
| Soluble in | ethanol |
| | propylene glycol |
| | toluene |
| | hexane |
| | ether |
| | chloroform |
| | petroleum ether |
| Sparingly soluble in | acetone |
| | methyl acetate |

Dispersible in water

**5. Stability data:**

Phospholipon® 90 is chemically and physically stable for at least 3 years in closed original containers and the specified storage temperature.

**6. Applications:**

- Preparation of mixed micelles for drugs
- Phosphatidylcholine source for drugs and dietetics
- Production of liposomes for pharmacy, dermatology and cosmetics for the improvement of skin humidity and skin penetration
- Suggested concentration for topical application:

cosmetic: 0.5-3%

pharma: 2-10%

**7. Safety data :**

Phospholipon® 90 is not dangerous goods according to the currently valid transport regulations of the working materials regulation of February 11, 1982 or the chemicals law. Not names in the list of water-polluting substances. The product can be regarded as nontoxic for both humans and animals. Toxicity and skin toleration data are available on request.

## 2. Cholesterol (Wade and Weller, 1994b)

### 1. Nonproprietary names

USPNF : Cholesterol

### 2. Synonyms

Cholesterin

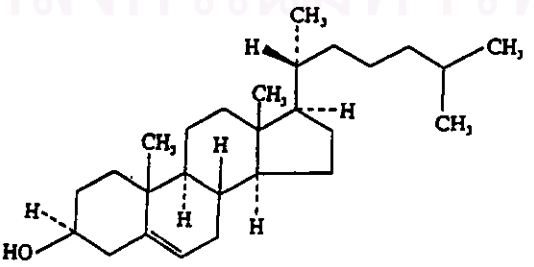
### 3. Chemical name and CAS registry number

Cholest-5-en-3$\beta$ - ol [57-88-5]

### 4. Empirical formal Molecular weight

$C_{27}H_{46}O$ 386.67

### 5. Structural formula



**6. Function category**

Emollient; emulsifying agent

**7. Application in pharmaceutical formulation or technology**

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations between 0.3-5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity. Cholesterol additionally has a physiological role.

**8. Description**

White or faintly yellow, almost odorless, pearly leaflets, needles, powder or granules. On prolonged exposure to light and air cholesterol acquires a yellow to tan color.

**9. Pharmacopeial specifications**

| Test | USP NF XVII |
|---|---|
| Identification | + |
| Melting range | 147-150°C |
| Specific rotation | -34° to -38° |
| Acidity | + |
| Loss on drying | ≤ 0.3% |
| Residue on ignition | ≤ 0.1% |

**10. typical properties**

Boiling point: 360°C

Density: 1.052 g/cm$^3$ for anhydrous form

Dielectric constant (pK$_a$): 5.41

Melting point: 147-150°C

Solubility

| Solvent | Solubility at 20°C unless otherwise stated |
|---|---|
| Acetone | soluble |
| Benzene | 1 in 7 |
| Chloroform | 1 in 4.5 |
| Ethanol | 1 in 147 at 0°C |
| | 1 in 78 at 20°C |
| | 1 in 29 at 40°C |
| | 1 in 19 at 50°C |
| | 1 in 13 at 60°C |
| Ethanol (95%) | 1 in 78 (slowly) |
| | 1 in 3.6 at 80°C |
| Ether | 1 in 2.8 |
| Hexane | 1 in 52 |
| Isopropyl myristate | 1 in 19 |
| Methanol | 1 in 294 at 0°C |
| | 1 in 153 at 20°C |
| | 1 in 53 at 40°C |
| | 1 in 34 at 50°C |
| | 1 in 23 at 60°C |

| Solvent | Solubility at 20°C unless otherwise stated |
|---|---|
| Vegetable oils | soluble |
| Water | practically insoluble |

Specific rotation $[\alpha]_D^{20}$: -39.5° (2% w/v solution in chloroform)
-31.5° (2% w/v solution in ether)

**11. Stability and storage conditions**

Cholesterol is stable and should be stored in a well-closed container, protected from light.

**12. Incompatibilities**

Precipitated by digitonin.

**13. Safety**

Cholesterol is generally regarded as an essentially nontoxic and nonirritant material at levels employed as an excipient.

## 3. Alpha tocopherol (Wade and Weller, 1994a)

**1. Nonproprietary names**

BP : Alpha tocopherol

PhEur : α-Tocopherolum

USP : Vitamin E

**2. Synonyms**

(±)-3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-ol; E307; synthetic alpha tocopherol; *all-rac*-α-tocopherol; *dl*-α-tocopherol; 5,7,8-trimethyltocol.

**3. Chemical name and CAS registry number**

(±)-(2*RS*,4′*RS*,8′*RS*)-2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol [10191-41-0]

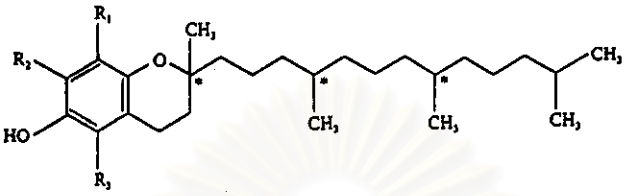
Note that alpha tocopherol has three chiral centers giving rise to eight isomeric forms. The naturally occurring form is known as *d*-alpha tocopherol or (2*R*,4′*R*,8′*R*)-alpha-tocopherol. The synthetic form, *dl*-alpha tocopherol or simply alpha tocopherol, occurs as a racemic mixture containing equimolar quantities of all the isomers.

Similar considerations apply to beta, delta and gamma tocopherol and tocopherol esters.

**4. Empirical formula** **Molecular weight**

$C_{29}H_{50}O_2$ 430.69

**5. Structural formula**



$R_1 = R_2 = R_3 = CH_3$

* Indicates chiral centers

**6. Function category**

Antioxidant ; therapeutic agent.

**7. Applications in pharmaceutical formulation or technology**

Alpha tocopherol is primarily recognized as a source of vitamin E and the commercially available materials and specifications reflect this purpose. Whilst alpha tocopherol also exhibits antioxidant properties, the beta, delta and gamma tocopherol are considered to be more effective as antioxidant.

Of widespread regulatory acceptability, tocopherol are of value in oil or fat-based pharmaceutical products and are normally used in the concentration of 0.001-0.05 %.

There is frequently an optimum concentration; thus the autoxidation of linoleic acid and methyl linolenate is reduced at low concentrations of alpha

tocopherol but accelerated by higher concentrations. Antioxidant effectiveness can be increased by the addition of oil soluble synergists such as lecithin and ascorbyl palmitate.

**8. Description**

Alpha tocopherol is a practically odorless, clear, colorless, yellow, yellowish-brown or greenish-yellow colored viscous oil.

**9. Pharmacopeial specifications**

| Test | PhEur 1990 | USP XXIII |
|---|---|---|
| Identification | + | + |
| Acidity | - | + |
| Acid value | ≤ 2 | - |
| Heavy metals | ≤ 20 ppm | - |
| Sulfated ash | ≤ 0.1% | - |
| Assay | 96.0-102.0% | 96.0 - 102.0% |

Note that the USP XXII describes vitamin E as comprising *d*-or *dl*-alpha tocopherol; *d*-or *dl*-alpha tocopheryl acetate; *d*-or *dl*-alpha tocopheryl acid succinate. However, the PhEur 1990 and the BP 1993 describe alpha tocopherol and alpha tocopheryl acetate in separate monographs.

The diversity of the tocoperols described in the various pharmacopeial monographs makes a comparison of specifications difficult.

## 10. Typical properties

Solubility:

| | |
|---|---|
| practically insoluble in | water |
| freely soluble in | acetone |
| | ethanol |
| | ether |
| | vegetable oils |

## 11. Stability and storage conditions

Tocopherols are slowly oxidized by atmospheric oxygen and rapidly by ferric and silver salts. Oxidation products include rapidly tocopherooxide, tocopherylhydroquinone and tocopherylhydroquinone, as well as dimers and trimers. Tocopherol esters are more stable to oxidation than the free tocopherols but are in consequence less effective antioxidants. Tocopherols should be stored under an inert gas, in an airtight container in a cool, dry, place and protected from light.

## 12. Incompatibilities

Tocopherols are incompatible with peroxides and metal ions especially iron, copper and silver. Tocopherols may be absorbed into plastic.

## 13. Safety

Tocopherols (vitamin E) occur in many food substances that are consumed as part of the normal diet. The daily nutritional requirement has been

clearly defined but is estimated to be 3-20 mg. Absorption from the gastrointestinal tract is dependent upon normal pancreatic function and the presence of bile. Tocopherols are widely distributed throughout the body with some ingested tocopherol metabolized in the liver; excretion of metabolites is via the urine or bile. Individuals with vitamin E deficiency are usually treated by oral administration of tocopherols although intramuscular and intravenous administration may sometimes be used.

Tocopherols are well tolerated although large oral doses may cause diarrhea or other gastrointestinal disturbances. Topical application of tocopherols may cause contact dermatitis.

The use of tocopherols as antioxidants in pharmaceuticals and food products is unlikely to pose any hazard to human heath since the daily intake from such uses is small compared to the intake of naturally occurring tocopherols in the diet. The WHO has set an acceptable daily intake of tocopherol used as an antioxidant at 0.15-2 mg/kg body-weight.

# Appendix II

## Quantitative Analysis Data of Ibuprofen

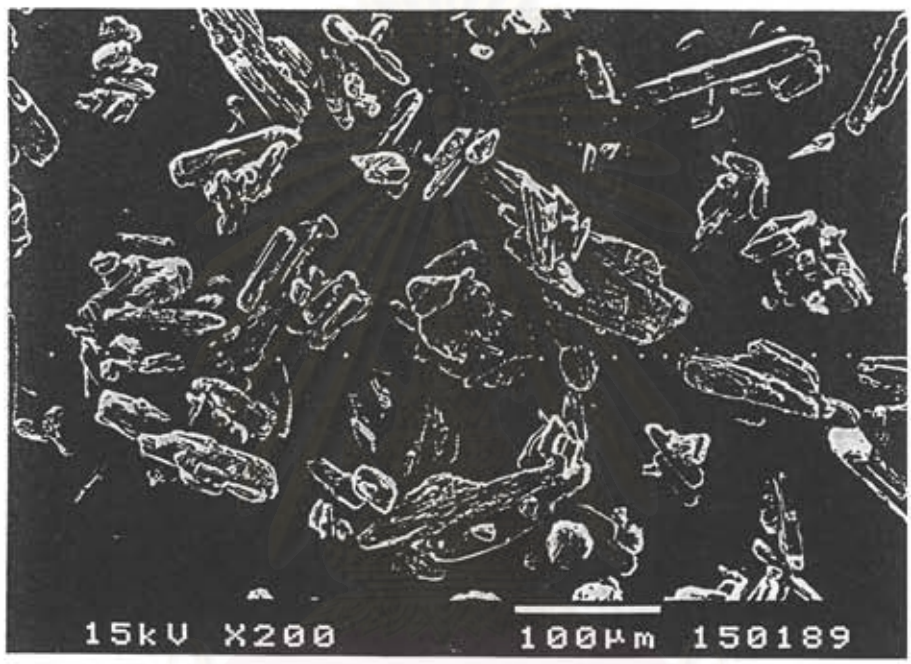



**Figure 24** Scanning electron photomicrograph of ibuprofen powder, magnification : 200x

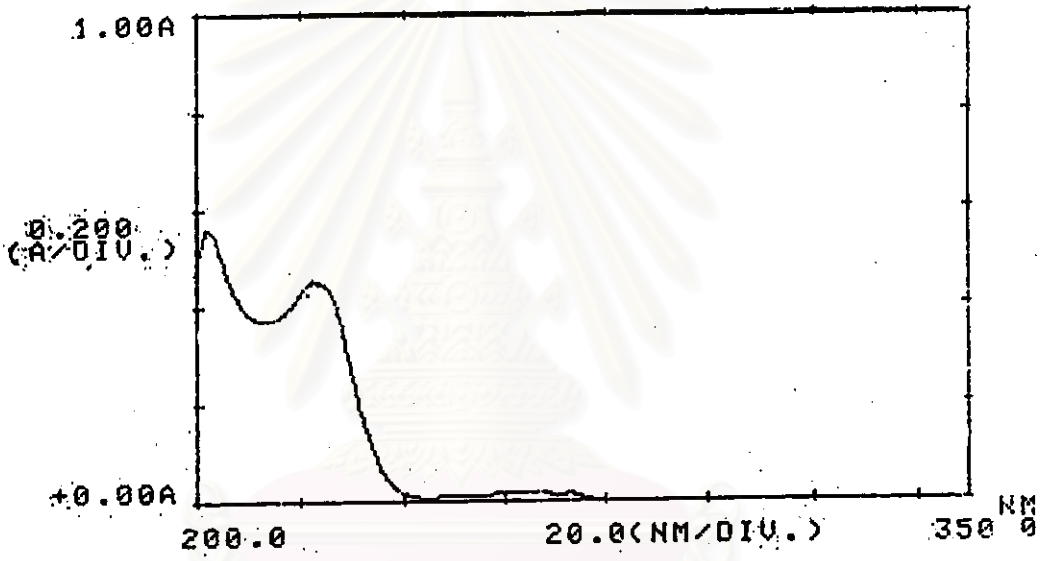



**Figure 25** Spectrum of ibuprofen in phosphate buffer saline pH 7.4

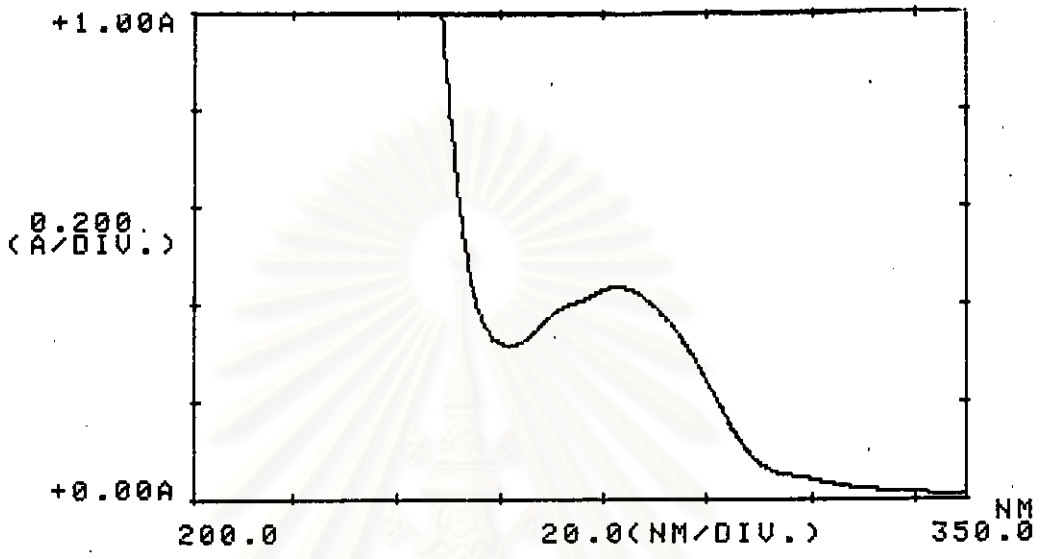



**Figure 26** Spectrum of empty liposomes prepared with 90.9 μmol/ml of soybean lecithin in isopropyl alcohol

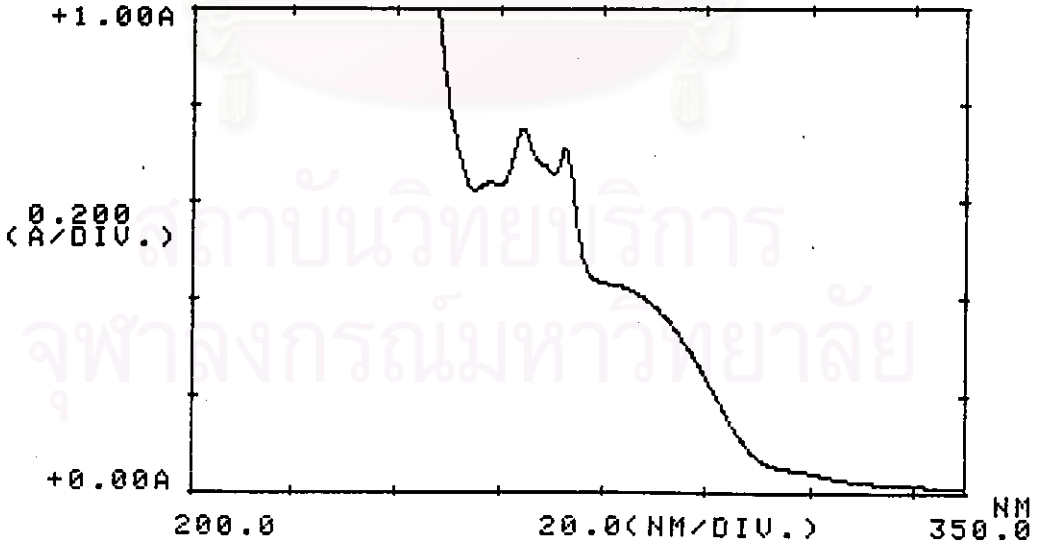



**Figure 27** Spectrum of ibuprofen in ibuprofen liposomes (formula 3) prepared with 90.9 μmol/ml of constant soybean lecithin concentration and 2.7 mg/ml of ibuprofen in isopropyl alcohol

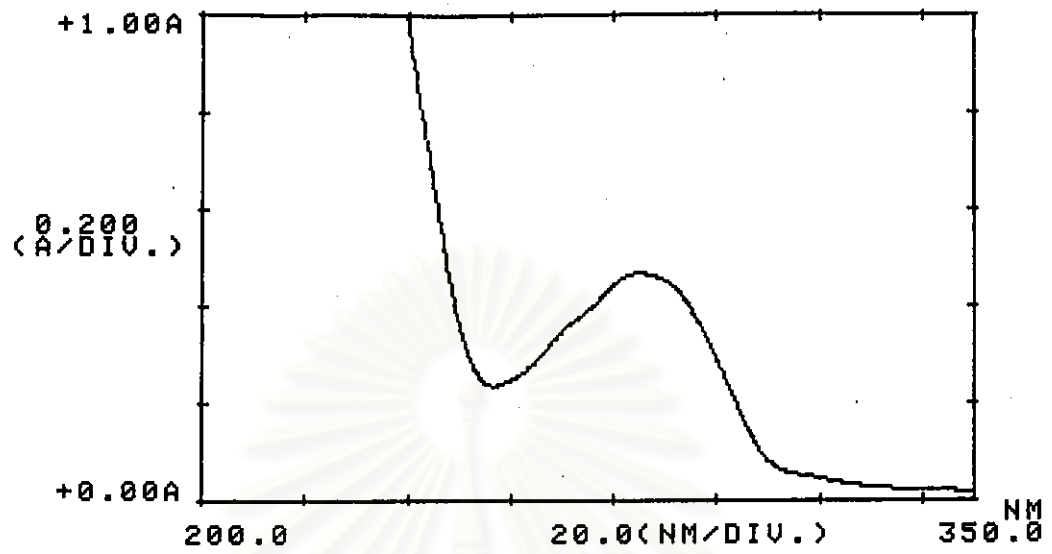



**Figure 28** Spectrum of empty liposomes prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.025% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid in isopropyl alcohol

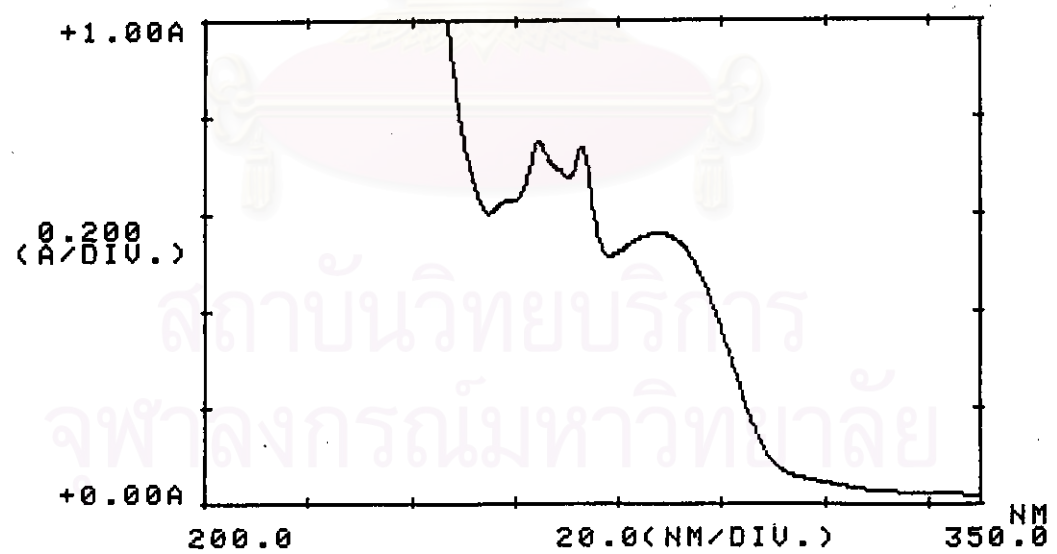



**Figure 29** Spectrum of ibuprofen in ibuprofen liposomes (formula 21) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.025% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen in isopropyl alcohol

**Table 25** Calibration curve data of ibuprofen in phosphate buffer saline pH 7.4 at wavelength 222.6 nm

| Std. No. | Conc. (μg/ml) | Abs. | Inversely estimated conc.[a] | % theory[b] |
|---|---|---|---|---|
| 1 | 4.000 | 0.179 | 3.969 | 99.22 |
| 2 | 8.000 | 0.358 | 7.969 | 99.61 |
| 3 | 12.000 | 0.543 | 12.103 | 100.86 |
| 4 | 16.000 | 0.718 | 16.013 | 100.08 |
| 5 | 20.000 | 0.894 | 19.946 | 99.73 |
| | | | | Mean 99.90 |
| | | | | SD. 0.62 |
| | | | | %CV.[c] 0.62 |

[a] Obtained from the fitted curve

$$\text{Abs.} = 0.0014 + (0.04475 \times \text{conc.}) \quad : r^2 = 0.999$$

$$\text{Inversely estimated concentration} = \frac{(\text{absorbance} - 0.0014)}{0.04475}$$

[b] $$\% \text{ Theory} = \frac{\text{Inversely estimated concentration} \times 100}{\text{known concentration}}$$

[c] $$\text{Coefficient of variation} = \frac{\text{SD.} \times 100}{\text{Mean}}$$

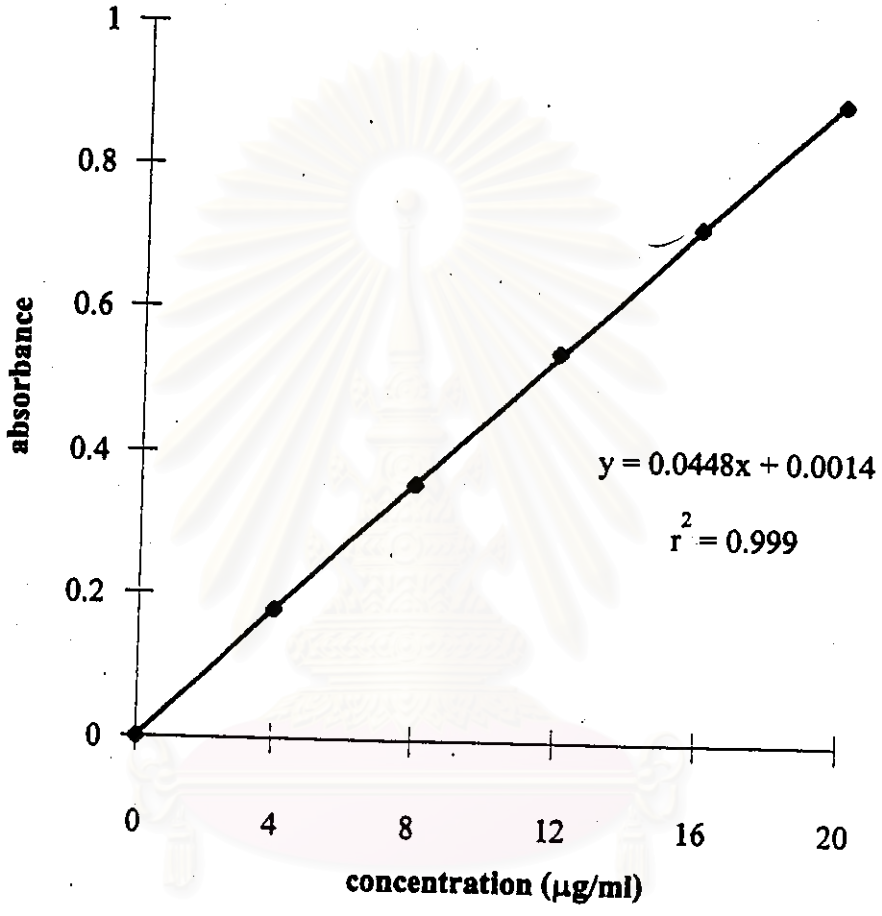



**Figure 30** Calibration curve of ibuprofen in phosphate buffer saline pH 7.4 at wavelength 222.6 nm

**Table 26** The percentage drug entrapment of ibuprofen liposomes (formula 3) prepared with 90.9 μmol/ml of constant soybean lecithin concentration and 2.7 mg/ml of ibuprofen (1:0.144 molar ratio of soybean lecithin to drug) at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | % drug entrapment (SD) | conc. of ibuprofen entrapped (μmol/ml) (SD) |
|---|---|---|
| 1 | 99.21 | 13.11 |
| 2 | 99.22 | 13.11 |
| 3 | 99.01 | 13.09 |
| 4 | 99.21 | 13.11 |
| average (SD) | 99.16 (0.10) | 13.10 (0.01) |

**Table 27** Particle size of ibuprofen liposomes (formula 3) prepared with 90.9 μmol/ml of 90.9 μmol/ml of constant soybean lecithin concentration and 2.7 mg/ml of ibuprofen (1:0.144 molar ratio of soybean lecithin to drug) at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | size (μm) | | | average size (μm) (SD) |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| 1 | 7.76 | 7.74 | 7.75 | 7.75 (0.01) |
| 2 | 6.12 | 6.10 | 6.08 | 6.10 (0.02) |
| 3 | 6.48 | 6.28 | 6.26 | 6.34 (0.12) |
| average size (μm) (SD) | | | | 6.73 (0.77) |

**Table 28** The percentage drug entrapment of ibuprofen liposomes (formula 6) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | % drug entrapment (SD) | conc. of ibuprofen entrapped (μmol/ml) (SD) |
|---|---|---|
| 1 | 99.16 | 13.11 |
| 2 | 99.09 | 13.10 |
| 3 | 99.14 | 13.10 |
| 4 | 99.12 | 13.10 |
| average (SD) | 99.13 (0.03) | 13.10 (0) |

**Table 29** Particle size of ibuprofen liposomes (formula 6) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | size (μm) | | | average size (μm) (SD) |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| 1 | 5.89 | 5.85 | 5.83 | 5.86 (0.03) |
| 2 | 5.65 | 5.60 | 5.58 | 5.61 (0.04) |
| 3 | 5.68 | 5.66 | 5.65 | 5.66 (0.02) |
| average size (μm) (SD) | | | | 5.71 (0.11) |

**Table 30** The percentage drug entrapment of ibuprofen liposomes (formula 15) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | % drug entrapment (SD) | conc. of ibuprofen entrapped (μmol/ml) (SD) |
|---|---|---|
| 1 | 98.24 | 12.99 |
| 2 | 98.00 | 12.96 |
| 3 | 98.00 | 12.96 |
| 4 | 98.56 | 13.03 |
| average (SD) | 98.20 (0.26) | 12.98 (0.03) |

**Table 31** Particle size of ibuprofen liposomes (formula 15) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | size (μm) | | | average size (μm) (SD) |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| 1 | 5.59 | 5.56 | 5.52 | 5.56 (0.04) |
| 2 | 4.79 | 4.66 | 4.62 | 4.69 (0.09) |
| 3 | 4.87 | 4.75 | 4.72 | 4.78 (0.08) |
| average size (μm) (SD) | | | | 5.01 (0.42) |

**Table 32** The percentage drug entrapment of ibuprofen liposomes (formula 21-24) prepared with 0.025, 0.0125, 0.00625 and 0.001 % of (±)-α-tocopherol, 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole % of stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen (n = 4) at $t_1$ (after storage at 4°C for 1 month)

| Formula | amount of (±)-α-tocopherol (%) | % drug entrapment | | | | average % drug entrapment (SD) | average conc. of ibuprofen entrapped (μmol/ml) (SD) |
|---|---|---|---|---|---|---|---|
| | | $n_1$ | $n_2$ | $n_3$ | $n_4$ | | |
| 21 | 0.025 | 98.42 | 98.38 | 98.42 | 98.34 | 98.39 (0.04) | 13.01 (0.00) |
| 22 | 0.0125 | 98.40 | 98.53 | 98.49 | 98.42 | 98.46 (0.06) | 13.02 (0.00) |
| 23 | 0.00625 | 98.11 | 98.08 | 98.00 | 98.15 | 98.09 (0.06) | 12.97 (0.01) |
| 24 | 0.001 | 98.42 | 98.26 | 97.89 | 98.45 | 98.26 (0.26) | 12.99 (0.03) |

**Table 33** Particle size of ibuprofen liposomes (formula 21-24) prepared with 0.025, 0.0125, 0.00625 and 0.001 % of (±)-α-tocopherol, 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole % of stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Formula | Batch no. | size (μm) | | | average size (μm) (SD) |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| 21 | 1 | 12.04 | 10.30 | 8.69 | |
| | 2 | 12.89 | 10.61 | 8.74 | |
| | 3 | 7.81 | 7.22 | 6.88 | 9.46 (2.12) |
| 22 | 1 | 5.66 | 5.67 | 5.67 | |
| | 2 | 5.81 | 5.25 | 5.03 | |
| | 3 | 5.54 | 5.28 | 5.18 | 5.45 (0.27) |
| 23 | 1 | 5.97 | 5.95 | 5.93 | |
| | 2 | 6.66 | 6.50 | 6.41 | |
| | 3 | 6.71 | 6.50 | 6.36 | 6.33 (0.31) |
| 24 | 1 | 5.46 | 5.45 | 5.45 | |
| | 2 | 6.02 | 5.91 | 5.84 | |
| | 3 | 6.83 | 6.58 | 6.54 | 6.01 (0.53) |

**Table 34** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 3) prepared with 90.9 μmol/ml of constant soybean lecithin concentration and 2.7 mg/ml of ibuprofen (1:0.144 molar ratio of soybean lecithin to drug) at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.138 | 2.988 | 74.700 | 99.09 |
| 2 | 0.139 | 3.010 | 75.250 | 99.08 |
| 3 | 0.134 | 2.900 | 72.500 | 99.11 |
| 4 | 0.145 | 3.143 | 78.575 | 99.04 |
| average (SD) | | 3.010 (0.100) | 75.256 (2.511) | 99.08 (0.03) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0025)}{0.045343625}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 35** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 6) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs. [a] | Conc. [b] (μg/ml) (SD) | Amount of unencapsulated drug [c] (μg) (SD) | % drug entrapment [d] (SD) |
|---|---|---|---|---|
| 1 | 0.177 | 3.848 | 96.200 | 98.82 |
| 2 | 0.215 | 4.686 | 117.150 | 98.57 |
| 3 | 0.215 | 4.686 | 117.150 | 98.57 |
| 4 | 0.205 | 4.466 | 111.650 | 98.64 |
| average (SD) | | 4.422 (0.396) | 110.538 (9.904) | 98.65 (0.12) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0025)}{0.045343625}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 36** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 15) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.284 | 6.315 | 157.875 | 98.07 |
| 2 | 0.281 | 6.248 | 156.200 | 98.09 |
| 3 | 0.290 | 6.449 | 161.225 | 98.03 |
| 4 | 0.292 | 6.494 | 162.350 | 98.02 |
| average (SD) | | 6.376 (0.114) | 159.412 (2.863) | 98.05 (0.03) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0014)}{0.04475}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 37** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 21) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.025% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.247 | 5.476 | 136.900 | 98.33 |
| 2 | 0.231 | 5.115 | 127.875 | 98.44 |
| 3 | 0.267 | 5.928 | 148.200 | 98.19 |
| 4 | 0.241 | 5.341 | 133.525 | 98.37 |
| average (SD) | | 5.465 (0.343) | 136.625 (8.568) | 98.33 (0.10) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0044)}{0.0443}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 38** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 22) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.0125% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.252 | 5.589 | 139.725 | 98.29 |
| 2 | 0.256 | 5.679 | 141.975 | 98.26 |
| 3 | 0.321 | 7.147 | 178.675 | 97.82 |
| 4 | 0.203 | 4.483 | 112.075 | 98.63 |
| average (SD) | | 5.724 (1.093) | 143.112 (27.330) | 98.25 (0.33) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0044)}{0.0443}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 39** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 23) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.00625% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.239 | 5.296 | 132.400 | 98.38 |
| 2 | 0.243 | 5.386 | 134.650 | 98.35 |
| 3 | 0.297 | 6.605 | 165.125 | 97.98 |
| 4 | 0.231 | 5.115 | 127.875 | 98.44 |
| average (SD) | | 5.60 (0.679) | 140.012 (16.977) | 98.29 (0.21) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0044)}{0.0443} : r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 40** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 24) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.001% of (±)- α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_0$ (freshly prepared)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.310 | 6.898 | 172.450 | 97.89 |
| 2 | 0.273 | 6.063 | 151.575 | 98.15 |
| 3 | 0.234 | 5.183 | 129.575 | 98.42 |
| 4 | 0.209 | 4.618 | 115.450 | 98.59 |
| average (SD) | | 5.690 (1.001) | 142.262 (25.019) | 98.26 (0.31) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0044)}{0.0443}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 41** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 3) prepared with 90.9 μmol/ml of constant soybean lecithin concentration and 2.7 mg/ml of ibuprofen (1:0.144 molar ratio of soybean lecithin to drug) at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.116 | 2.592 | 64.800 | 99.21 |
| 2 | 0.114 | 2.547 | 63.675 | 99.22 |
| 3 | 0.144 | 3.224 | 80.600 | 99.01 |
| 4 | 0.116 | 2.592 | 64.800 | 99.21 |
| average (SD) | | 2.739 (0.324) | 68.469 (8.105) | 99.16 (0.10) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.001)}{0.044361277}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 42** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 6) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.129 | 2.754 | 64.850 | 99.16 |
| 2 | 0.139 | 2.980 | 74.500 | 99.09 |
| 3 | 0.132 | 2.822 | 70.550 | 99.14 |
| 4 | 0.134 | 2.867 | 71.675 | 99.12 |
| average (SD) | | 2.856 (0.095) | 71.394 (4.052) | 99.13 (0.03) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0075)}{0.044125} : r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 43** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 15) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs. [a] | Conc. [b] (μg/ml) (SD) | Amount of unencapsulated drug [c] (μg) (SD) | % drug entrapment [d] (SD) |
|---|---|---|---|---|
| 1 | 0.252 | 5.753 | 143.825 | 98.24 |
| 2 | 0.287 | 6.558 | 163.950 | 98.00 |
| 3 | 0.286 | 6.535 | 163.375 | 98.00 |
| 4 | 0.207 | 4.718 | 117.950 | 98.56 |
| average (SD) | | 5.891 (0.867) | 147.275 (21.673) | 98.20 (0.26) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0019)}{0.043475}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 44** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 21) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.025% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.234 | 5.183 | 129.575 | 98.42 |
| 2 | 0.239 | 5.296 | 132.400 | 98.38 |
| 3 | 0.234 | 5.183 | 129.575 | 98.42 |
| 4 | 0.245 | 5.431 | 135.775 | 98.34 |
| average (SD) | | 5.273 (0.118) | 131.831 (2.947) | 98.39 (0.04) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0044)}{0.0443}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 45** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 22) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.0125% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.234 | 5.248 | 131.200 | 98.40 |
| 2 | 0.215 | 4.819 | 120.475 | 98.53 |
| 3 | 0.221 | 4.954 | 123.850 | 98.49 |
| 4 | 0.230 | 5.158 | 128.950 | 98.42 |
| average (SD) | | 5.045 (0.194) | 126.119 (4.859) | 98.46 (0.06) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0019)}{0.044225}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 46** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 23) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.00625% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.275 | 6.156 | 153.900 | 98.12 |
| 2 | 0.281 | 6.291 | 157.275 | 98.08 |
| 3 | 0.292 | 6.538 | 163.450 | 98.00 |
| 4 | 0.270 | 6.044 | 151.100 | 98.15 |
| average (SD) | | 6.257 (0.213) | 156.431 (5.317) | 98.09 (0.06) |

[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0009)}{0.044525}$ : $r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen

**Table 47** Calculation of the percentage drug entrapment of ibuprofen liposomes (formula 24) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.001% of (±)-α-tocopherol, 90.9 μmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_1$ (after storage at 4°C for 1 month)

| Batch no. | Abs.[a] | Conc.[b] (μg/ml) (SD) | Amount of unencapsulated drug[c] (μg) (SD) | % drug entrapment[d] (SD) |
|---|---|---|---|---|
| 1 | 0.231 | 5.168 | 129.200 | 98.42 |
| 2 | 0.254 | 5.684 | 142.100 | 98.26 |
| 3 | 0.308 | 6.897 | 172.425 | 97.89 |
| 4 | 0.227 | 5.078 | 126.950 | 98.45 |
| average (SD) | | 5.707 (0.837) | 142.669 (20.930) | 98.26 (0.26) |

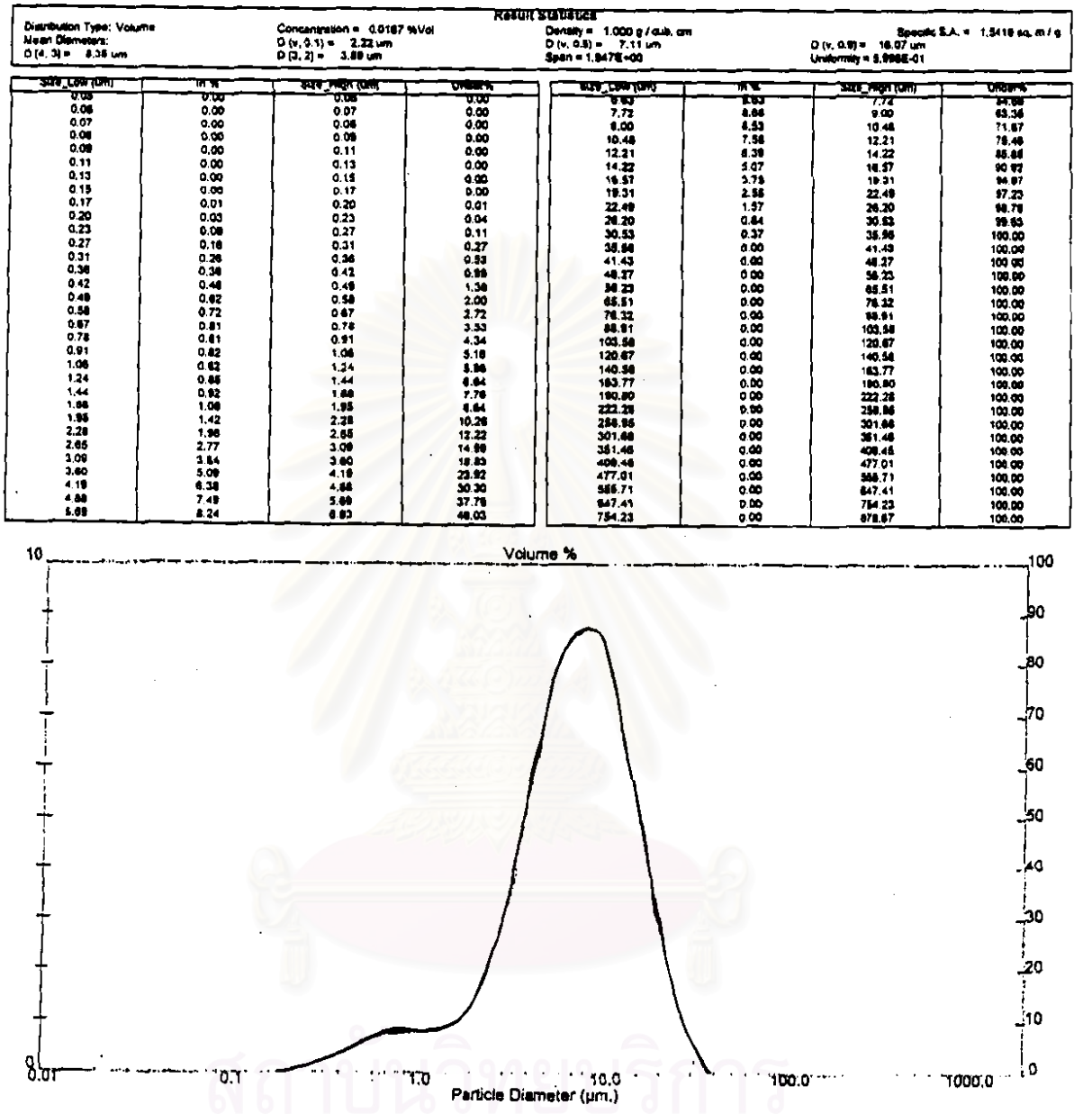
[a] Absorbance of unencapsulated ibuprofen in 25 ml of supernatant liquid after subtraction by average absorbance of supernatant liquid of corresponding empty liposomes

[b] $\text{Conc.} = \frac{(\text{abs.}^{a} - 0.0009)}{0.044525} \quad : r^2 = 0.999$

[c] Amount of unencapsulated ibuprofen in 3 ml of ibuprofen liposomes

[d] $\%\ \text{drug entrapment} = \frac{(\text{Amount of total drug}^{e} - \text{Amount of unencapsulated drug})}{\text{Amount of total drug}} \times 100$

[e] total amount of added ibuprofen




| Size_Low (um) | In % | Size_High (um) | Under% |
|---|---|---|---|
| 0.05 | 0.00 | 0.06 | 0.00 |
| 0.06 | 0.00 | 0.07 | 0.00 |
| 0.07 | 0.00 | 0.08 | 0.00 |
| 0.08 | 0.00 | 0.09 | 0.00 |
| 0.09 | 0.00 | 0.11 | 0.00 |
| 0.11 | 0.00 | 0.13 | 0.00 |
| 0.13 | 0.00 | 0.15 | 0.00 |
| 0.15 | 0.00 | 0.17 | 0.00 |
| 0.17 | 0.01 | 0.20 | 0.01 |
| 0.20 | 0.03 | 0.23 | 0.04 |
| 0.23 | 0.08 | 0.27 | 0.11 |
| 0.27 | 0.16 | 0.31 | 0.27 |
| 0.31 | 0.26 | 0.36 | 0.53 |
| 0.36 | 0.38 | 0.42 | 0.99 |
| 0.42 | 0.48 | 0.49 | 1.38 |
| 0.49 | 0.62 | 0.58 | 2.00 |
| 0.58 | 0.72 | 0.67 | 2.72 |
| 0.67 | 0.81 | 0.78 | 3.53 |
| 0.78 | 0.81 | 0.91 | 4.34 |
| 0.91 | 0.82 | 1.06 | 5.16 |
| 1.06 | 0.82 | 1.24 | 5.98 |
| 1.24 | 0.86 | 1.44 | 6.84 |
| 1.44 | 0.92 | 1.68 | 7.76 |
| 1.68 | 1.06 | 1.95 | 8.84 |
| 1.95 | 1.42 | 2.28 | 10.26 |
| 2.28 | 1.96 | 2.65 | 12.22 |
| 2.65 | 2.77 | 3.09 | 14.99 |
| 3.09 | 3.84 | 3.60 | 18.83 |
| 3.60 | 5.09 | 4.19 | 23.92 |
| 4.19 | 6.38 | 4.88 | 30.30 |
| 4.88 | 7.49 | 5.69 | 37.78 |
| 5.69 | 8.24 | 6.63 | 46.03 |

| Size_Low (um) | In % | Size_High (um) | Under% |
|---|---|---|---|
| 6.63 | 8.63 | 7.72 | 54.66 |
| 7.72 | 8.86 | 9.00 | 63.36 |
| 9.00 | 8.53 | 10.48 | 71.87 |
| 10.48 | 7.58 | 12.21 | 79.46 |
| 12.21 | 6.39 | 14.22 | 85.86 |
| 14.22 | 5.07 | 16.57 | 90.92 |
| 16.57 | 3.75 | 19.31 | 94.67 |
| 19.31 | 2.58 | 22.49 | 97.25 |
| 22.49 | 1.57 | 26.20 | 98.78 |
| 26.20 | 0.84 | 30.53 | 99.63 |
| 30.53 | 0.37 | 35.56 | 100.00 |
| 35.56 | 0.00 | 41.43 | 100.00 |
| 41.43 | 0.00 | 48.27 | 100.00 |
| 48.27 | 0.00 | 56.23 | 100.00 |
| 56.23 | 0.00 | 65.51 | 100.00 |
| 65.51 | 0.00 | 76.32 | 100.00 |
| 76.32 | 0.00 | 88.91 | 100.00 |
| 88.91 | 0.00 | 103.58 | 100.00 |
| 103.58 | 0.00 | 120.67 | 100.00 |
| 120.67 | 0.00 | 140.56 | 100.00 |
| 140.56 | 0.00 | 163.77 | 100.00 |
| 163.77 | 0.00 | 190.80 | 100.00 |
| 190.80 | 0.00 | 222.28 | 100.00 |
| 222.28 | 0.00 | 258.86 | 100.00 |
| 258.86 | 0.00 | 301.66 | 100.00 |
| 301.66 | 0.00 | 351.46 | 100.00 |
| 351.46 | 0.00 | 409.45 | 100.00 |
| 409.45 | 0.00 | 477.01 | 100.00 |
| 477.01 | 0.00 | 555.71 | 100.00 |
| 555.71 | 0.00 | 647.41 | 100.00 |
| 647.41 | 0.00 | 754.23 | 100.00 |
| 754.23 | 0.00 | 878.67 | 100.00 |

**Figure 31** An example of size distribution diagram of ibuprofen liposomes (formula 3) prepared with 90.9 µmol/ml of soybean lecithin concentration and 2.7 mg/ml of ibuprofen (1:0.144 molar ratio of soybean lecithin to drug) at $t_0$ (freshly prepared)

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 17.32 um

Concentration = 0.0265 %Vol
D (v, 0.1) = 2.77 um
D [3, 2] = 5.32 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 12.01 um
Span = 3.023E+00

Specific S.A. = 1.1277 sq. m / g
D (v, 0.9) = 38.09 um
Uniformity = 1.007E+00

| Size_Low (um) | In % | Size_High (um) | Undersize |
|---|---|---|---|
| 0.05 | 0.00 | 0.06 | 0.00 |
| 0.06 | 0.00 | 0.07 | 0.00 |
| 0.07 | 0.00 | 0.08 | 0.00 |
| 0.08 | 0.00 | 0.09 | 0.00 |
| 0.09 | 0.00 | 0.11 | 0.00 |
| 0.11 | 0.00 | 0.13 | 0.00 |
| 0.13 | 0.00 | 0.15 | 0.00 |
| 0.15 | 0.00 | 0.17 | 0.00 |
| 0.17 | 0.00 | 0.20 | 0.00 |
| 0.20 | 0.00 | 0.23 | 0.00 |
| 0.23 | 0.02 | 0.27 | 0.03 |
| 0.27 | 0.07 | 0.31 | 0.09 |
| 0.31 | 0.14 | 0.36 | 0.24 |
| 0.36 | 0.23 | 0.42 | 0.47 |
| 0.42 | 0.34 | 0.49 | 0.80 |
| 0.49 | 0.45 | 0.58 | 1.25 |
| 0.58 | 0.52 | 0.67 | 1.78 |
| 0.67 | 0.58 | 0.78 | 2.36 |
| 0.78 | 0.56 | 0.91 | 2.91 |
| 0.91 | 0.55 | 1.06 | 3.46 |
| 1.06 | 0.56 | 1.24 | 4.02 |
| 1.24 | 0.61 | 1.44 | 4.64 |
| 1.44 | 0.72 | 1.68 | 5.36 |
| 1.68 | 0.91 | 1.95 | 6.27 |
| 1.95 | 1.26 | 2.28 | 7.53 |
| 2.28 | 1.79 | 2.65 | 9.32 |
| 2.65 | 2.56 | 3.09 | 11.88 |
| 3.09 | 3.50 | 3.60 | 15.38 |
| 3.60 | 4.44 | 4.19 | 19.82 |
| 4.19 | 5.14 | 4.88 | 24.96 |
| 4.88 | 5.39 | 5.69 | 30.34 |
| 5.69 | 5.12 | 6.63 | 35.46 |

| Size_Low (um) | In % | Size_High (um) | Undersize |
|---|---|---|---|
| 6.63 | 4.57 | 7.72 | 40.03 |
| 7.72 | 3.91 | 9.00 | 43.94 |
| 9.00 | 3.34 | 10.48 | 47.28 |
| 10.48 | 3.04 | 12.21 | 50.32 |
| 12.21 | 3.05 | 14.22 | 53.37 |
| 14.22 | 3.40 | 16.57 | 56.77 |
| 16.57 | 4.10 | 19.31 | 60.87 |
| 19.31 | 5.09 | 22.49 | 65.96 |
| 22.49 | 6.27 | 26.20 | 72.23 |
| 26.20 | 7.03 | 30.53 | 79.25 |
| 30.53 | 6.93 | 35.56 | 86.18 |
| 35.56 | 5.90 | 41.43 | 92.08 |
| 41.43 | 4.27 | 48.27 | 96.36 |
| 48.27 | 2.64 | 56.23 | 98.99 |
| 56.23 | 1.01 | 65.51 | 100.00 |
| 65.51 | 0.00 | 76.32 | 100.00 |
| 76.32 | 0.00 | 88.91 | 100.00 |
| 88.91 | 0.00 | 103.58 | 100.00 |
| 103.58 | 0.00 | 120.67 | 100.00 |
| 120.67 | 0.00 | 140.58 | 100.00 |
| 140.58 | 0.00 | 163.77 | 100.00 |
| 163.77 | 0.00 | 190.80 | 100.00 |
| 190.80 | 0.00 | 222.28 | 100.00 |
| 222.28 | 0.00 | 258.95 | 100.00 |
| 258.95 | 0.00 | 301.68 | 100.00 |
| 301.68 | 0.00 | 351.46 | 100.00 |
| 351.46 | 0.00 | 409.45 | 100.00 |
| 409.45 | 0.00 | 477.01 | 100.00 |
| 477.01 | 0.00 | 555.71 | 100.00 |
| 555.71 | 0.00 | 647.41 | 100.00 |
| 647.41 | 0.00 | 754.23 | 100.00 |
| 754.23 | 0.00 | 878.67 | 100.00 |

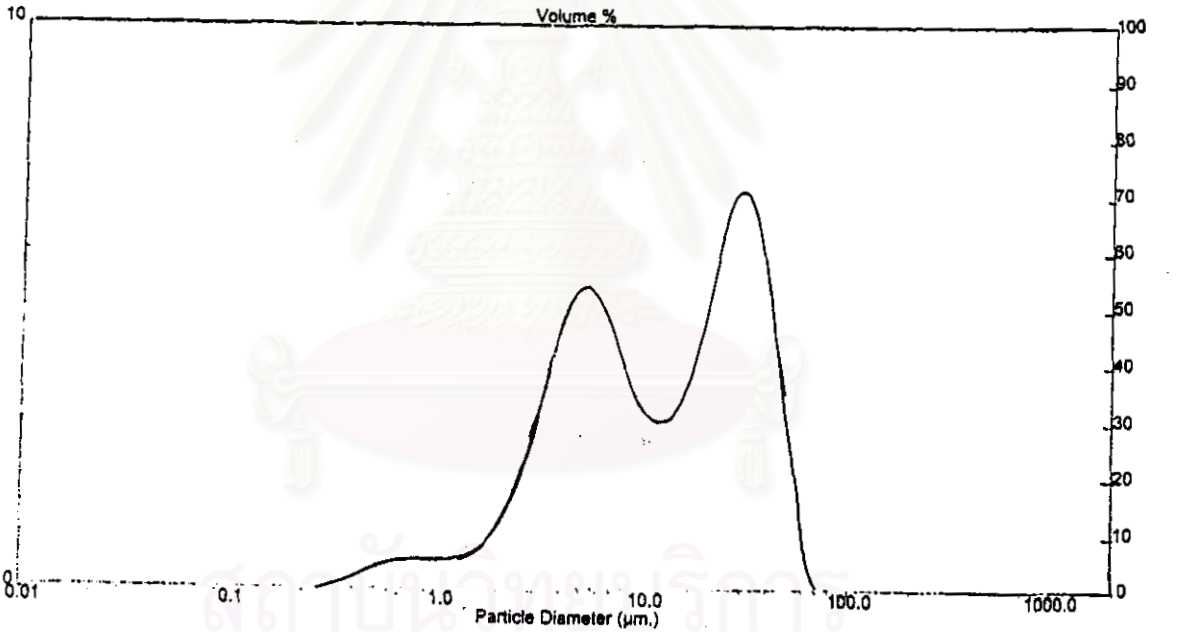



**Figure 32** An example of size distribution diagram of ibuprofen liposomes (formula 21) prepared with 9:1 molar ratio of soybean lecithin to cholesterol, 2.50 mole% of stearylamine, 0.025% of (±)- α-tocopherol, 90.9 µmol/ml of constant whole lipid and 2.7 mg/ml of ibuprofen at $t_i$ (after storage at 4°C for 1 month)

# VITAE

Miss Narumol Vangvithya was born on August 9, 1965 in Saraburi, Thailand. She graduated her Bachelor Degree of Sciences in Pharmacy from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 1989. After graduation, she had been worked as a pharmacist at Banmoh Hospital, Saraburi province from 1989 to 1995. After that, she has moved to work in pharmacy department in Praphutthabat Hospital, Saraburi before entering the Master's Degree program in Pharmacy at Chulalongkorn University.