REFERENCES

Ahn, B.-N., Kim, S.-K. and Shim, C.-K. 1995. Proniosomes as an intranasal dosage form for the sustained delivery of propranolol. J. Control. Release 34: 203-210.

Alkan-Onyuksel, H. and Son, K. 1992. Mixed micelles as proliposomes for the solubilization of teniposide. Pharm. Res. 9(12): 1556-1562.

Anssen, M. J. H., Crommelin, D. J. A., Strom, G. and hulshoff, A. 1985. Doxorubicin decomposition in storage, effect of pH, type of buffer and liposome encapsulation. Int. J. Pharm. 28: 1-11.

Armbruster, C., Vorbach, H., Steindl, F. and El Menyawi, I. 2001. Intracellular concentration of the HIV protease inhibitors indinavir and saquinavir in human endothelial cells. J. Antimicrob. Chemother. 47: 487-490.

Arunothayanun, P., Uchegbu, I. F., Craig, D. Q. M., Turton, J. A. and Florence A. T. 1999a. Preparation and in vitro/ in vivo evaluation of luteinizing hormone releasing hormone (LHRH)-loaded polyhedral and spherical/ tubular niosomes. J. Pharm. Sci. 88(1): 34-38.

Arunothayanun, P., Uchegbu, I. F., Craig, J. A. and Florence, A. T. 1999b. In vitro/ in vivo characterization of polyhedral niosomes. Int. J. Pharm. 183: 57-60.

Azmin, M. N., Florence, A. T., Handjani-Vila, R. M., Stuart, J. F. B. and Vanlerberghe, G. 1984. The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J. Pharm. Pharmacol. 37: 237-242.

Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T. and Rogerson, A. 1985. The preparation and properties of niosomes-non-ionic surfactant vesicles. J. Pharm. Pharmacol. 37: 863-868.

Baillie, A.J., Coombs, G. H., Dolan, T. F. and Laurie, J. 1986. Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate. J. Pharm. Pharmacol. 37: 863-868.

Balasubramaniam, A., Kumar, V.A. and Pillai, K.S. 2002. Formulation and in vivo evaluation of niosome-encapsulated daunorubicin hydrochloride. Drug Dev. Ind. Pharm. 28(10): 1181-1193.

Barber, R. and Shek, P. 1993. Liposomes as a topical occular drug delivery system. In A. Rolland (ed.), Pharmaceutical particulate carriers, pp. 1-20. New York: Marcel Dekker.

Blazek-Welsh, A. I. and Rhodes, D. G. 2001. Maltodextrin-based proniosomes. AAPS Pharmsci. 2001: 1-8.

Booser, D. and Hortobagyi, G. 1994. Anthracycline antibiotics in cancer therapy: focus on drug resistance. Drugs 47: 223-258.

Brittain, H. G. 1999. Methods for characterization of polymorphs and solvates. In H. G. Brittain (ed.), Polymorphism in pharmaceutial solids, pp. 227-278. New York: Marcel Dekker.

Cable, C. 1989. An examination of the effects of surface modifications on the physicochemical and biological properties of non-ionic surfactant vesicles. Doctoral dissertation. University of Strathclyde.

Chen, Y., Ping, Q., Gou, J., Lv, W. and Gao, J. 2003. The absorption behavior of cyclosporine A lecithin vesicles in rat intestinal tissue. Int. J. Pharm. 261: 21-26.

Chung, S.-J. 1999. Future drug delivery research in South Korea. J. Control. Release 62: 73-79.

Couvreur, P., Fattal, E. and Andremont, A. 1991. Liposomes and nanoparticles in the treatment of intracellular bacterial infections. Pharm. Res. 8: 1079–1086.

Davies, P. 2001. Oral dosage forms. In M. Gibson (ed.), Pharmaceutical preformulation and formulation: A practical guide from candidate drug selection to commercial dosage form, pp. 379-458. Englewood: IHS Health Group.

Duncan, R., Florence, A., Uchegbu, I. and Cociacinch, F. 1997. Drug polymer conjugates encapsulated within niosomes. International Patent Application. PCT/GB97/00072.

Echeogyen, L. E., Hernandez, J. C., Kaifer, A. E. Gokel, G. W. and Ehegoyen, L. 1988. Aggregation of steroidal ethers: The first example of nonionic liposomes (niosomes) formed from neutral crown ether compounds. J. Chem. Soc. Chem. Comm. 12: 836-837.

Eldridge, J. H., Hammond, C. J., Meulbroek, J. A., Staas, J. K., Gilley, R. M and Tice, T. R. 1990. Controlled vaccine release in the gut-associated lymphoid tissues, I. Orally administred biodegradable microspheres target the peyer's patches. J. Control. Release 11: 205-214.

Erdogan, S., Özer, A.Y., Ercan, M. T., Eryilmaz, M. and Hincal, A. A. 1996. In vivo studies on iopromide radiopaque niosomes. J. Pharm. Sci. 6: 87-93.

Fang, J.-Y., Hong, C.-T., Chiu, W.-T. and Wang, Y.-Y. 2001. Effect of liposomes and niosomes on skin permeation of enoxacin. Int. J. Pharm. 219: 61-72.

Florence, A. T. 1993. Nonionic surfactant vesicles: preparation and characterization. In G. Gregoriadis (ed.), Liposome Technology, pp. 157-176. Boca Raton: CRC.

Francis, M. F., Cristea, M. and Winnik, M. F. 2004. Polymeric micelles for oral drug delivery: Why and how*. Pure Appl. Chem. 76(7–8): 1321–1335.

François, G., Christine, F. and Aloïse, M. 2004. Transport of HIV protease inhibitors through the blood-brain barrier and interactions with the efflux proteins, p-glycoprotein and multidrug resistance proteins. J. Acquir. Immune Defic. Syndr. 35(2): 649-658.

Frfkjaer, S., Hjorth, E. and Wfrtis, O. 1984. Stability testing of liposomes during storage. In G. Gregoriadis (ed.), Liposome technology, vol. 1. 2nd ed., pp. 235-245. Boca Raton: CRC.

Gebicki, J. M., Du, J., Collins, J. and Tweeddale, H. 2000. Peroxidation of proteins and lipids in suspensions of liposome, in blood serum, and in mouse myeloma cell. Acta Biochemica. Polonica. 47: 901-911.

Gennaro, A. R. 2000. Remington: The science and practice of pharmacy. 20th ed. Baltimore: Lippincott Williams and Wilkins.

Gianasi, E., Cociancich, F., Uchegbu, I. F., Florence, A. T. and Duncan, R. 1997. Pharmaceutical and biological characterisation of a doxorubicin-polymer conjugate (PK1) entrapped in sorbitan monostearate Span 60 niosomes. Int. J. Pharm. 148(2): 139-148.

Gregoriadis, G. 1976. The carrier potential of liposomes in biology and medicine. N. Engl. J. Med. 295: 704-710.

Gregoriadis, G., Florence, A. and Patel, Harish M. 1993. Liposome in drug delivery. Langhorne: Harwood Academic.

Handjani-Vila, R. M., Ribier, A., Rondot, B. A. and Vanlerberghe, G. 1979. Dispersion of lamellar pharse of nonionic lipids in cosmetic products. Int. J. Cosmet. Sci. 1: 303-314.

Hanson W. A. 1991. Handbook of dissolution testing. 2nd ed. Revised, Oregon: Aster.

Hao, Y., Zhao, F., Li, N., Yang, Y. and Li, K. 2002. Studies on a high encapsulation of colchicine by a niosome system. Int. J. Pharm. 244: 73-80.

Hayat, A.-O., and Son, K. 1992. Mixed micelles as proliposomes for the solubilization of teniposide. Pharm. Res. 9(12): 1556-1562.

Higuchi, T. 1963. Mechanism of sustained action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 52: 1145–1149.

Hu, C. and Rhodes, D.G. 1996. "Proniosomes," A promising novel formulation for delivery of hydrophobic and amphiphilic drugs. AAPS Regional meeting, New Brunswick, NJ.

Hu, C. and Rhodes, D.G. 1999. Proniosomes: A novel drug carrier preparation. Int. J. Pharm. 185(1): 23-35.

Hunter, C. A. Dolan, T. E., Coombs, G. H. and Baillie, A. J. 1988. Vesicular system (niosomes and liposomes) for delivery of sodium stibogluconate in experimental murine visceral leishmaniasis. J. Pharm. Pharmacol. 40: 161-165.

Israelachvili, J. N. 1992. Intermolecular and surface forces. 2nd ed. London: Academic Press.

Iwanaga, K., Ono, S., Narioka, K., Kakemi, M., Morimoto, K., Yamashita, S., Namba, Y. and Oku, N. 1999. Application of surface-coated liposomes for oral delivery of peptide: effects of coating the liposome's surface on GI transit of insulin. J. Pharm. Sci. 88(2): 248-252.

Jenke, D.R. 1996. Chromatographic method validation: A review of current practices and procedures II. Guidelines for primary validation parameters. J. Liq. Chrom. & Rel. Technol. 19(5): 737-757.

Jonsson, B., Lindman, B., Holmberg, K. and Kronberg, B. 1998. Surfactants and polymers in aqueous solution. New York: Wiley.

Jung, B. H., Chung, B. C., Chung, S.-J., Lee, M.-H. and Shim, C.-K. 2000. Prolonged delivery of nicotine in rats via nasal administration of proliposomes. J. Control. Release 66: 73-79.

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

Katare, O. P., Vyas, S. P. and Dixit, V. K. 1991. Proliposomes of indomethacin for oral administration. J. Microencapsul. 8: 1-7.

Kibbe, H. 2000. Handbook of pharmaceutical excipients. 3rd ed. Washington, D. C.: American Pharmaceutical Association and Pharmaceutical.

Kiwada, H., Nimura, H. and Kato, Y. 1985. Tissue distribution and pharmacokinetic evaluation of targeting efficiency of synthesis alkyl glucoside vesicles. Chem. Pharm. Bull. 33: 2475-2482.

Lasic, D. D. 1990. On the thermodynamic stability of liposomes. J. Colloid Interface Sci. 140: 302-304.

Lu, B., Zhang, J. Q. and Yang, H. 2003. Nonphospholipid vesicles of carboplatin for lung targeting. Drug Deli. 10: 87-94.

Ludewig, B., Barchiesi, F., Perricin, M., Zinkernagel, R. M., Hengartner, H. and Schwendener, R. A. 2001. In vivo antigen loading and activation of dendritic cells via a liposomal peptide vaccine mediates protective antiviral and anti-tumour immunity. Vaccine 19: 23-32.

Malmsten, M. 2002. Surfactants and polymers in drug delivery, pp. 1-3, 51-86. New York: Marcel Dekker.

Manosroi, A., Wongtrakul, P., Manosroi, J., Sakai, H., Saguwara, F., Yuasa, M. and Abe, M. 2003. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloid Surf. B-Biointerfaces 30: 129-138.

Mayer, L.D., Bally, M.B., Loughrey, H., Masin, D. and Cullis, P.R. 1990. Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumours. Cancar Res. 50: 575-579.

McEvoy, G. K. 2004. AHSF Drug Information American Society of Health-System Pharmacists, pp. 659-668. Bethesda: the American Society of Health-System Pharmacists.

Medda, S., Mukhopadhyay, S., Basu, M. K. 1999. Evaluation of the in-vivo activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms. J. Antimicrob. Chemo. 44: 791-794.

Namdeo, A. and Jain N. K. 1999. Niosomal delivery of 5-fluorouracil. J. Microencapsul. 16(6): 731-740.

Olivier, J.-C. 2005. Drug transport to brain with targeted nanoparticles. NeuroRx. 2(1): 108–119.

Oommen, E., Tiwari, S. B., Udupa, N., Kamath, R. and Devi, P. U. 1999. Niosome entrapped B-cyclodextrin methotrexate complex as a drug delivery system. Ind. J. Pharmacol. 31: 279-284.

Payne, N. I., Timmins, P., Ambrose, C. V., Ward, M. D. and Ridgeway, F. 1986. Liposomes: A novel solution to an old problem. J. Pharm. Sci., 75: 325-329.

Pillai, G. K. and Salim, M. L. D. 1999. Enhanced inhibition of platelet aggregation in-vitro by niosome-encapsulated indomethacin. Int. J. Pharm. 193: 123-127.

Plessis, J., Ramchandran, C., Weiner, N. and Muller, D. G. 1996. The influence of lipid composition and lamellarity of liposomes on the physical stability of liposomes upon storage. Int. J. Pharm. 127: 273–278.

Rajanaresh, R. A., Singh, U. V., Udupa, N. and Pillai, G. K. 1993. Anti-inflammatory activity of niosomes encapsulated diclofenac sodium in rats. Indian Drugs 30: 275-278.

Reddy, D.N. and Udupa, N. 1993. Formulation and evaluation of oral and transdermal preparations of flurbiprofen and piroxicam incoperated with different carriers. Drug Dev. Ind. Pharm. 19: 843-852.

Rental, C.-O., Bouwstra, J. A., Naisbett, B. and Junginger, H. E. 1999. Niosomes as a novel peroral vaccine delivery system. Int. J. Pharm. 186: 161-167.

Rieger, M. M. 1988. Pharmaceutical dosage forms dispersed systems. In H. A. Lieberman, M. M. Rieger and G. S. Banker (eds.), Surfactants, pp. 293-300. New York: Marcel Dekker.

Roche Pharmaceuticals. Saquinavir mesylate. [online]. Available from: http://www.rocheusa.com/products /fortovase/pi.pdf. [2004, July 8]

Rogerson, A., Cummings, J. and Florence, A. T. 1987. Adriamycin-loaded niosomes-drug entrapment, stability and release. J. Microencapsul. 4: 321-328.

Ruckmani, K., Jayakar, B., Ghosal, S. K. 2000. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemias: encapsulation, storage, and in vitro release. Drug Dev. Ind. Pharm. 26(2): 217-222.

Saettone, M. F., Perini, G., Carafa, M., Santucci, E. and Alhaique, F. 1996. Non-ionic surfactant vesicles as ophthalmic carriers for cyclopentolate: a preliminary evaluation. STP Pharma Sci. 6(1): 94-98.

Schmid, M. H. and Korting, H. C. 1994. Liposomes: a drug carrier system for topical treatment in dermatology. Crit. Rev. Ther. Drug Carrier Syst. 11(2-3): 97-118.

Schreier, H. and Bouwstra, J. 1994. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J. Control. Release 30: 1–15.

Schwartz, J. B., Silmdnelli, A. P. and Higuchi, W. I. 1968. Drug release from wax matrices II. J. Pharm. Sci. 57: 278-282.

Shahiwala, A. and Misra, A. 2002. Studies in topical application of niosomeally entrapped nimesulide. J. Pharm. Sci. 5: 220-225.

Siegal, T., Horowitz, A. and Gabizon, A. 1995. Doxorubicin encapsulated in sterically stagilised liposomes for the treatment of a brain tumour model: biodistribution and therapeutic efficiency. J. Neurosurg. 83: 1029-1037.

Supersaxo, A., Hein, W. R. and Steffen, H. 1991. Mixed micelles as a proliposomal lymphotropic drug carrier. Pharm. Res. 8: 1286-1291.

Takada, M., Yuzuriha, T., Iwamoto, K. and Sunamoto, J. 1984. Increased lung uptake of liposomes coated with polysaccharides. Biochem. Biophys. Acta 802: 237-243.

Tan, L. K., Thenmozhiyal, J. C. and Ho, P. C. 2003. Stability of extemporaneously prepared saquinavir formulations. J. Clin. Pharm. Theraputic. 28: 457-463.

Tanaka, M. 1990. Properties of aqueous vesicle dispersion formed with poly(oxyethylene) hydrogenated caster oil. J. Am. Oil Chem. Soc. 67: 55-60.

The United States Pharmacopeial Convention, III. 2004. The United States Pharmacopeia 27/The National Formulary 22: USP 27/NF22. Philadelphia: National Publishing.

Tomizawa, H., Aramaki, Y., Fujii, Y., Hara, T., Suzuki, N., Yachi, K., Kikuchi, H. and Tsuchiya, S. 1993. Uptake of phosphatidylserine liposomes by rat Payer's patches following intraluminal administration. Pharm. Res. 10: 549-552.

Torchilin, V. 2005. Recent advances with liposomes as pharmaceutical carriers. Nature Rev. 4: 145-160.

Uchegbu, I. F. and Vyas, S. P. 1998. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int. J. Pharm. 172: 33-70.

Uchegbu, I. F. 1998. The biodistribution of novel 200-nm palmitoyl muramic acid vesicles Int. J. Pharm. 162(1-2): 19-27.

Uchegbu, I. F. and Florence, A. T. 1995. Non-ionic surfactant vesicles (Niosomes): physical and pharmaceutical chemistry. Adv. Colloid Interface Sci. 58: 1-55.

Uchegbu, I. F., Double, J.A., Kelland, L.R., Turton, J.A. and Florence, A.T. 1996. The activity of doxorubicin niosomes against an ovarian-cancer cell-line and 3 in-vivo mouse-tumor models. J. Drug Target. 3: 399-409.

Uchegbu, J. F., Doible, J. A., Turton, J. A. and Florence, A. T. 1995. Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span 60) niosomes in the mouse. Pharm. Res. 12: 1019-1024.

van Boekel, M. A. J. S. 1998. Effect of heating on maillard reactions in milk. Food Chem. 62(4): 403-414.

van den Bergh, B. A. I., Bouwsatra, J. A., Junginger, H. E. and Wertz, P. W. 1999. Elasticity of vesicles affects hairless mouse skin structure and permeability. J. Control. release 62: 367-379.

Varshosaz, J., Pardakhty, A. and Hajhashemi, V.-I. 2003. Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. Drug Deli. 10: 251-262.

Vora, B., Khopade, A. J., Jain, N. K. 1998. Proniosomes based transdermal delivery of levonorgestrel for effective contraception. J. Control. Release 54: 149-165.

Vyas, S. P. and Venkatesan, N. 1999. Poly(phthaloyl-L-lysine)-coated multilamellar vesicles for controlled drug delivery: in vitro and in vivo performance evaluation. Pharm. Acta Helve. 74: 51-58.

Walters, K. A., Dugard, P. H. and Florence, A. T. 1981. Nonionic surfactants and gastric mucosal transport of paraquat. J. Pharm. Pharmacol. 33: 207-213.

Wenninger, J. A. and McEwen, G. N. 1992. CTFA cosmetic ingredient handbook. 2nd ed. Washington, D. C.: The cosmetic, Toiletry, and Fragrance Association.

Wigginton, S. M., Chu, B. C., Weisman, H. and Howell, S. B. 1980. Methotrexate pharmacokinetics after intra-articular injection in patients with rheumatoid arthritis. Arthritis Rheum. 23: 119–122.

Wong, M., Anthony, F. H., Tillack, W. and Thompson, T. E. 1982. Fusion of dipalmitoylphosphatidylcholine vesicles at 4. Biochemistry 21: 4126-4132.

Yoshida, H., Lehr, C. M., Kok, W., Junginger, H. E. and Bouwstra, J. A. 1992. Niosomes for oral Delivery of Peptide Drugs. J. Control. Release 21: 145-153.

Yoshioka, T., Sternberg, B., Florence, A. T. 1994. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span20, 40, 60 and 80) and a sorbitan triester (Span85). Int. J. Pharm. 105: 1-6.

จุไรรัตน์ รักวาทิน. 2547. แนวทางการเสนอรายงานความคงสภาพของตำรับยาเพื่อการประกอบการขอขึ้นทะเบียนตำรับ. หน้า 33. กรุงเทพมหานคร: กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข.

พิมพ์จิต ดามพวรรณ และ วัชรินทร์ รุกขไชยศิริกุล. 2542. สเปกโทรสโกปีของสารอินทรีย์. หน้า 230-290. สงขลา: คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์.

APPENDICES

APPENDIX I

Data of nonionic surfactants

Brij®30

Polyoxyl 4 lauryl ether

Emperical formula	:	$CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_4OH$
Molecular weight	:	362
Chemical class	:	Alkoxylated alcohols
HLB	:	9.7
Melting point	:	-
Functional category	:	Emulsifying agent, solubilizing agent, wetting agent
Structural formula	:	$CH_3(CH_2)_x(OCH_2CH_2)_yOH$ Where $(x+1)$ is the number of carbon atoms in the alkyl chain and y is the number of ethylene oxide groups in the hydrophilic chain.

Brij®52

Polyoxyl 2 cetyl ether

Emperical formula	:	$CH_3(CH_2)_{14}CH_2(OCH_2CH_2)_4OH$
Molecular weight	:	330
Chemical class	:	Alkoxylated alcohols
HLB	:	5.3
Melting point	:	33
Functional category	:	Emulsifying agent, solubilizing agent, wetting agent
Structural formula	:	$CH_3(CH_2)_x(OCH_2CH_2)_yOH$ Where $(x+1)$ is the number of carbon atoms in the alkyl chain and y is the number of ethylene oxide groups in the hydrophilic chain.

Brij®72

Polyoxyl 2 stearyl ether

Emperical formula	:	$CH_3(CH_2)_{16}CH_2(OCH_2CH_2)_4OH$
Molecular weight	:	359
Chemical class	:	Alkoxylated alcohols
HLB	:	4.9
Melting point	:	43
Functional category	:	Emulsifying agent, solubilizing agent, wetting agent
Structural formula	:	$CH_3(CH_2)_x(OCH_2CH_2)_yOH$ Where $(x+1)$ is the number of carbon atoms in the alkyl chain and y is the number of ethylene oxide groups in the hydrophilic chain.

Brij®98

Polyoxyl 20 oleyl ether

Emperical formula	:	$CH_3(CH_2)_7CH{=}CH(CH2)_7CH_2(OCH_2CH_2)_{20}OH$
Molecular weight	:	1150
Chemical class	:	Alkoxylated alcohols
HLB	:	4.9
Melting point	:	43
Functional category	:	Emulsifying agent, solubilizing agent, wetting agent
Structural formula	:	$CH_3(CH_2)_x(OCH_2CH_2)_yOH$ Where $(x+1)$ is the number of carbon atoms in the alkyl chain and y is the number of ethylene oxide groups in the hydrophilic chain.

APPENDIX II

Analysis of saquinavir mesylate

1. The UV-visible spectrophotometric method

1.1 Calibration curve

The amount of saquinavir mesylate (SQV) dissolved in solubility study and dissolution test was determined by the UV-visible spectrophotometric method. The relationship between SQV concentrations versus absorbances in various media i.e. water, 0.1N hydrochloric acid and phosphate buffer pH 6.8 was presented in **Tables 1(II)-3(II)**. The calibration curve of SQV and the linear relationship with the correlation of determination in each medium were also depicted in **Figures 1(II)-3(II)**.

Table 1(II). The absorbance of saquinavir mesylate in water at 240 nm

Concentration (µg/ml)	Absorbance
4	0.2220
6	0.3327
8	0.4434
10	0.5768
12	0.6867
14	0.7982

Table 2(II). The absorbance of saquinavir mesylate in 0.1N hydrochloric acid at 242 nm

Concentration (µg/ml)	Absorbance
4	0.2040
6	0.3063
8	0.4120
10	0.5140
12	0.6330
14	0.7402

Table 3(II). The absorbance of saquinavir mesylate in phosphate buffer pH 6.8 at 240 nm

Concentration (µg/ml)	Absorbance
4	0.2109
6	0.3132
8	0.4163
10	0.5155
12	0.6343
14	0.7172

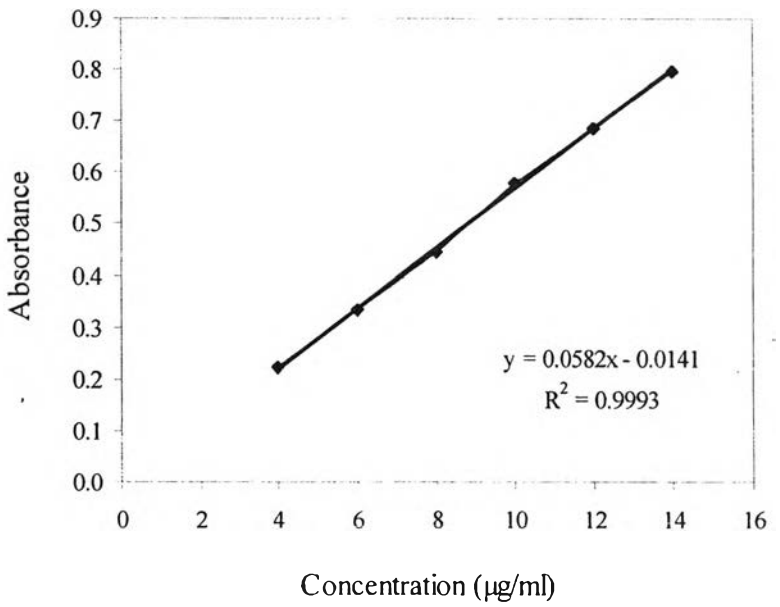


Figure 1(II). Calibration curve of saquinavir mesylate in water at 240 nm

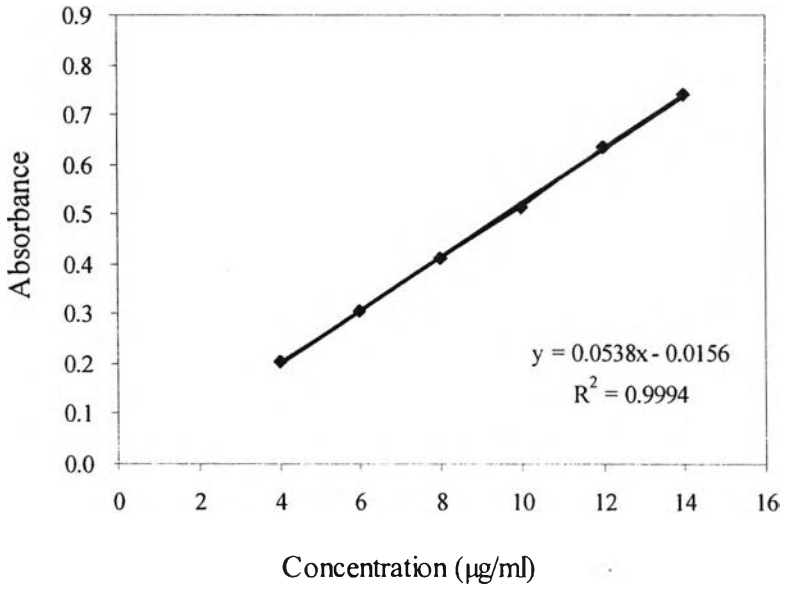


Figure 2(II). Calibration curve of saquinavir mesylate in 0.1N hydrochloric acid at 242 nm

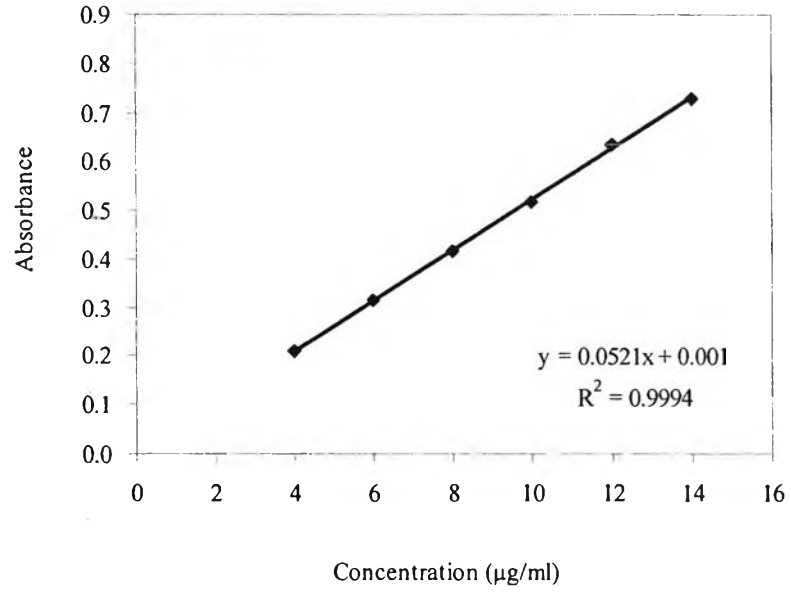


Figure 3(II). Calibration curve of saquinavir mesylate in phosphate buffer pH 6.8 at 240 nm

2. The high performance liquid chromatography method

2.1 Validation of HPLC method

The typical analytical parameters to be considered for assay validation are specificity, linearity (r^2), accuracy (recovery), and precision (%CV).

2.1.1 Specificity

The specificity of the method was determined by comparing the chromatograms between non-active ingredients solution with that of standard solution of saquinavir mesylate. Specificity is established by showing that the active ingredient should have no interference from non-active ingredients.

2.1.2 Accuracy

The accuracy of the proposed method was performed by analyzing placebos spiked with known quantities of active ingredients and evaluated as the percentage of recovery. The final concentration of placebo was 260 µg/ml. This final concentration was equivalent to the concentration of non-active ingredients in the solution assayed for drug content. The final concentration of the saquinavir mesylate was 10, 20 and 30 µg/ml. Three concentrations of the saquinavir mesylate solutions were equivalent to 50%, 100% and 150% of assay concentration within linearity range spiked into the placebo formulation. Each sample was analyzed from triplicate. The percentage of the analytical recovery of each sample was calculated.

2.1.3 Precision

Within run precision

The within run precision was determined by comparing concentration of saquinavir mesylate standard solution prepared and analyzed in the same day. The concentration of standard solution was 20 µg/ml. The percentage of coefficient of variation (%CV) of concentration of saquinavir mesylate from six replicated injections was determined.

Between run precision

The within run precision was determined by comparing concentration of saquinavir mesylate standard solution prepared and analyzed on different six days. The concentration of standard solution was 20 µg/ml. The precision determination was done in six days. In each day, determination of concentration was done in six replicates. The percentage of coefficient of variation (%CV) of concentration of saquinavir mesylate from six groups of standard solution was determined.

2.1.4 Linearity

Triplicate injections of saquinavir mesylate solutions containing drug in various concentrations ranging from 5-50 µg/ml were prepared and analyzed. The linear equation of the curve obtained by plotting the peak areas versus the concentrations was calculated using the least square method.

The results of validation process were presented as following:

Validation of HPLC method

Specificity

The chromatogram of saquinavir standard (SQV) solution was shown in **Figure 5(II)**. SQV was eluted at 7.9 minutes while the solvent peak was detected at 2.8 minutes. The peaks of excipients i.e. Brij®30, Simulsol®M52 and lactose did not appear under this condition of HPLC method. The result indicated that peak of SQV was not interfered with the peaks of other excipients. Therefore, this method would be specific for SQV analysis.

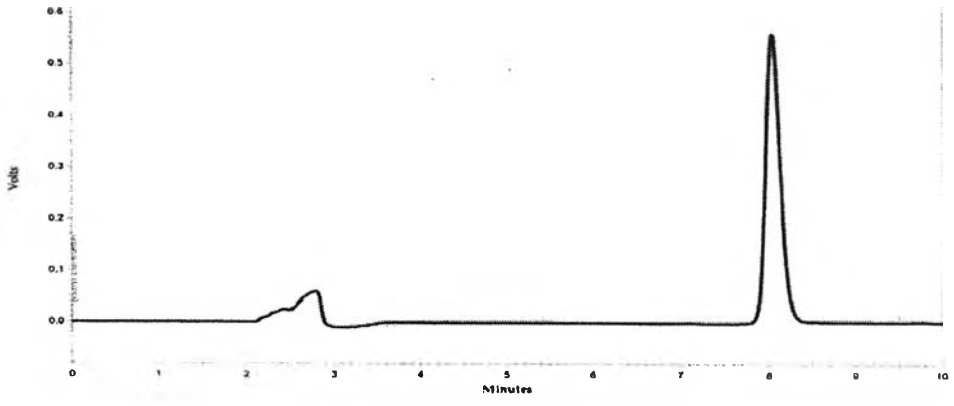


Figure 5(II). HPLC chromatogram of standard solutions of saquinavir mesylate

Accuracy

The percentage of analytical recovery in each concentration of saquinavir mesylate was presented in **Table 4(II)**.

Precision

Table 5(II) and **Table 6(II)** showed data of within and between run precision of saquinavir mesylate assayed by HPLC method, respectively.

Linearity

The linearity of analytical method in **Figure 6(II)** showed the relationship between peak areas and saquinavir mesylate (SQV) concentrations. The coefficient of determination (r^2) of 0.9999 of linear was acceptable using HPLC method for quantitative analysis of SQV in the concentration range studied.

All validation process parameters i.e. specificity, accuracy, precision and linearity were compared to the limited of acceptability in the analytical method validation parameters of HPLC. **Table 8(II)** showed that all parameters were acceptable, indicating that this HPLC method would be suitable for quantitative analysis of saquinavir mesylate.

Table 4(II). Percentage of analytical recovery of saquinavir mesylate (SQV) by HPLC method

Formulation	Actual concentration (µg/ml)	Analytical concentration (µg/ml)			% Recovery					
		n 1	n 2	n 3	n 1	n 2	n 3	Mean	SD	% CV
SQV-C	10	9.9273	10.1417	9.9695	99.2725	101.4175	99.6946	100.1282	1.1363	1.1348
	20	20.2643	20.1432	20.2427	101.3215	100.7158	101.2133	101.0835	0.3231	0.3196
	30	30.3188	30.4057	30.0243	101.0625	101.3524	100.0810	100.8320	0.6663	0.6608

SQV-C = capsule of proniosomes prepared from a mixture of SQV entrapped niosomes and lactose

Table 5(II). Data of within run precision of saquinavir mesylate (SQV) assayed by HPLC method

SQV concentration (µg/ml)	Calculated concentration from calibration curve (µg/ml)								
	n 1	n 2	n 3	n 4	n 5	n 6	Mean	SD	%CV
20	19.6760	19.4812	19.7075	20.0036	19.5280	20.2870	19.7805	0.3085	1.5598

Table 6(II). Data of between run precision assayed by HPLC method

Day	Calculated concentration from calibration curve (µg/ml)								
	n 1	**n 2**	**n 3**	**n 4**	**n 5**	**n 6**	**Mean**	**SD**	**%CV**
1	21.0325	21.3515	21.0318	21.3850	21.4462	21.5449	21.2987	0.2166	1.0172
2	19.9178	19.9762	19.2125	19.5191	19.2237	19.2125	19.5103	0.3585	1.8377
3	20.5453	20.5654	20.6903	20.7054	20.8587	19.7479	20.5188	0.3942	1.9210
4	20.3808	20.4467	19.7080	20.4662	20.6067	20.2876	20.3160	0.3158	1.5546
5	19.9294	19.8697	19.9916	20.0516	20.1090	20.1272	20.0131	0.1017	0.5082
6	20.1189	20.2633	20.2487	20.3381	20.3964	20.7103	20.3459	0.2016	0.9908
						Mean	20.4426	0.3231	1.5920

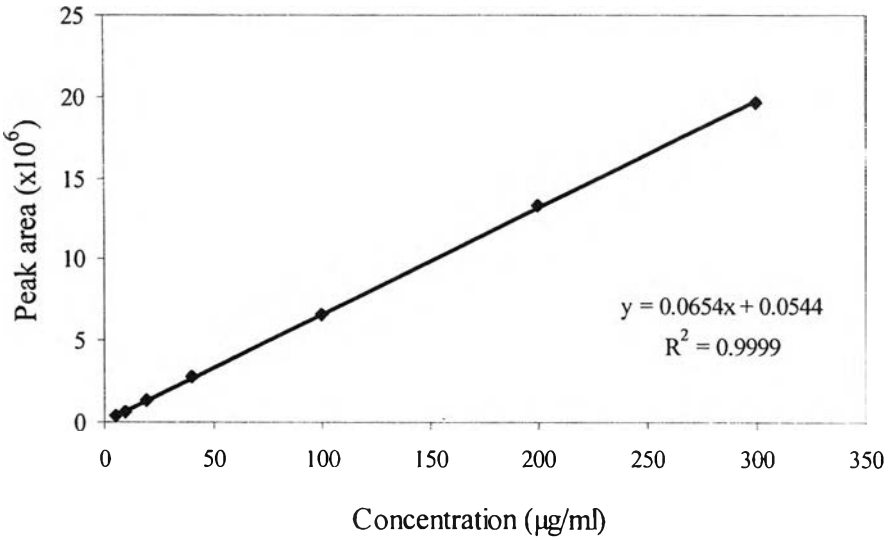


Figure 6(II). Calibration curve of saquinavir mesylate assayed HPLC method

Table 8(II). The analytical method validation parameter of HPLC for saquinavir mesylate

Parameter	Limited of acceptability	Result value
1. Specificity	No other peak interfere major peak [a]	No other peak interfere major peak
2. Accuracy (SD)	95.0%-105% [a]	100.13% - 101.08% (0.32-1.13)
3. Precision (%CV) - Within run precision - Between run precision	≤2 [b]	 1.5598 1.5920
4. Linearity - Correlation coefficient (r^2)	>0.999 [b]	0.9999

[a] (The United States Pharmacopeial Convention, III. (2004))

[b] (Jenke, 1996)

APPENDIX III

Data of drug solubility in three different media

Table 1(III). Concentration of saquinavir mesylate (SQV) dissolved in water at 37°C (n=3)

Time (h)	Concentration of drug (mg/ml)				
	n1	n2	n3	Average	SD
0.5	2.12	2.13	2.15	2.13	0.02
1	2.15	2.29	2.31	2.25	0.08
2	2.28	2.17	2.28	2.24	0.07
4	2.31	2.30	2.32	2.31	0.01
6	2.29	2.40	2.39	2.36	0.06
24	2.38	2.42	2.32	2.37	0.05
48	2.47	2.43	2.45	2.45	0.02

Table 2(III). Concentration of saquinavir mesylate (SQV) dissolved in 0.1N hydrochloric acid at 37°C (n=3)

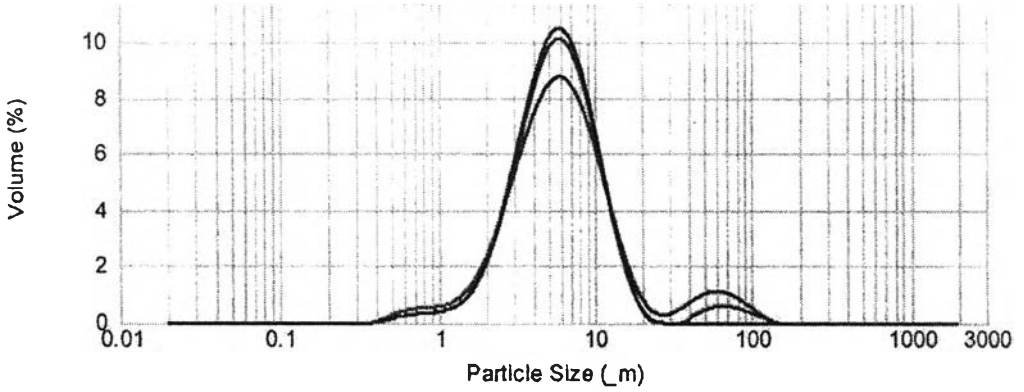
Time (h)	Concentration of drug (mg/ml)				
	n1	n2	n3	Average	SD
0.5	0.06	0.06	0.06	0.06	0.00
1	0.07	0.07	0.07	0.07	0.00
2	0.06	0.06	0.06	0.06	0.00
4	0.06	0.06	0.07	0.06	0.00
6	0.07	0.07	0.07	0.07	0.00
24	0.07	0.07	0.07	0.07	0.00
48	0.07	0.07	0.07	0.07	0.00

Table 3(III). Concentration of saquinavir mesylate (SQV) dissolved in phosphate buffer pH6.8 at 37°C (n=3)

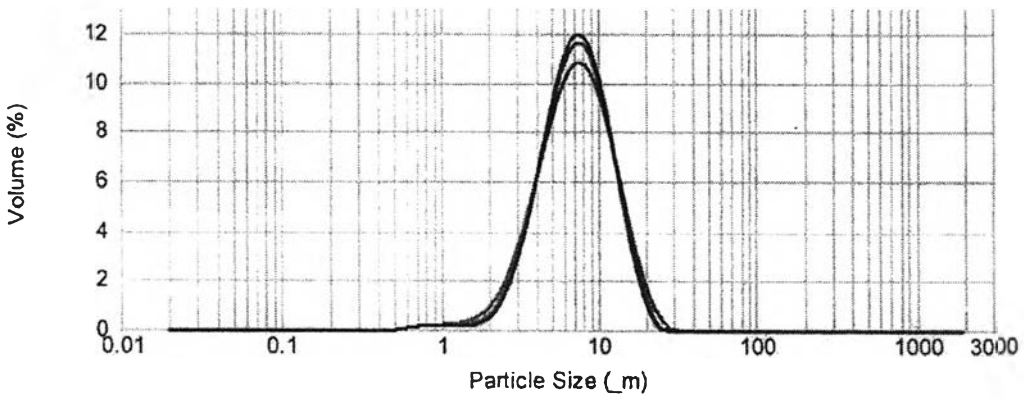
Time (h)	Concentration of drug (mg/ml)				
	n1	n2	n3	Average	SD
0.5	0.04	0.03	0.04	0.04	0.00
1	0.03	0.03	0.03	0.03	0.00
2	0.04	0.05	0.05	0.04	0.00
4	0.05	0.05	0.05	0.05	0.00
6	0.06	0.06	0.05	0.06	0.00
24	0.05	0.05	0.05	0.05	0.00
48	0.06	0.06	0.06	0.06	0.00

APPENDIX IV

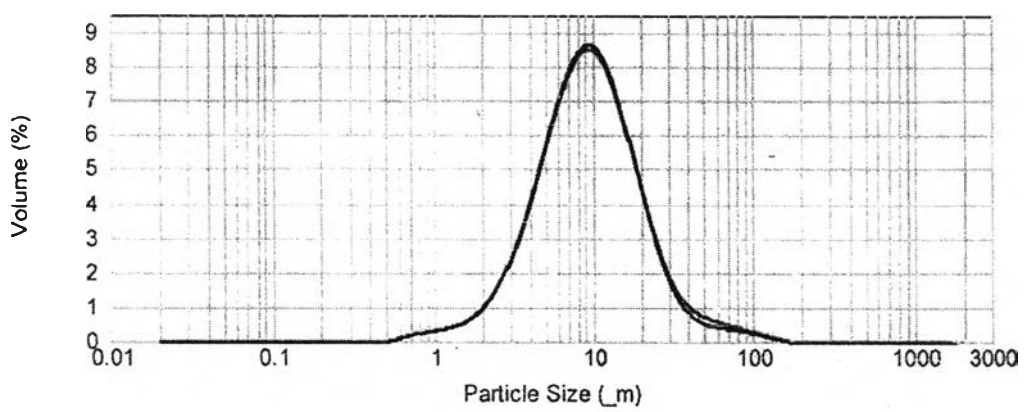
Data of characterization of niosomes



(a) Brij®30 (60:30:10)

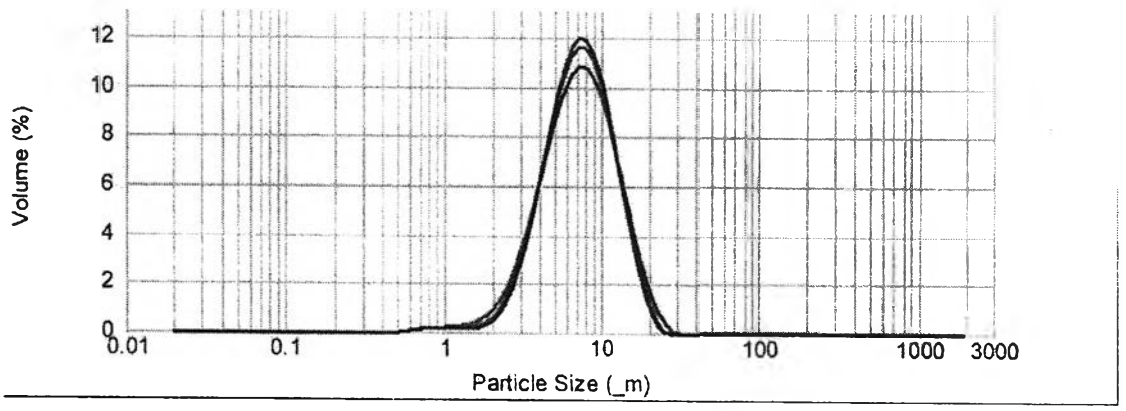


(b) Brij®30 (45:45:10)

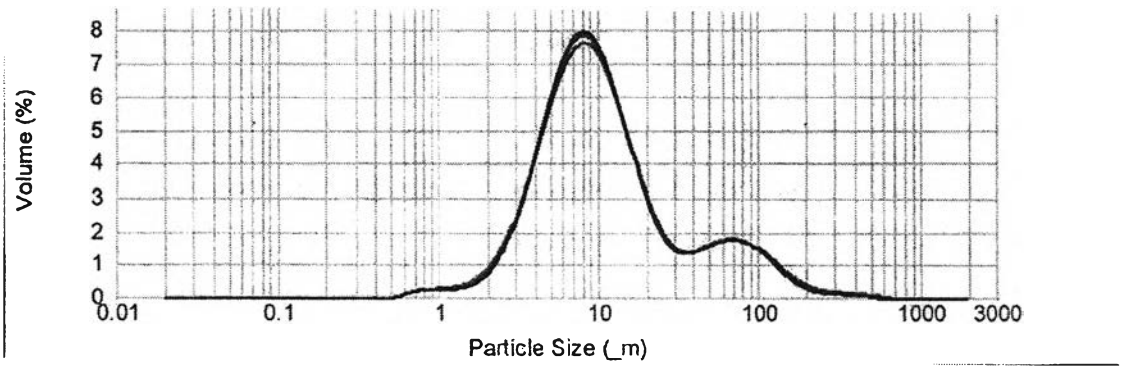


(c) Brij®30 (30:60:10)

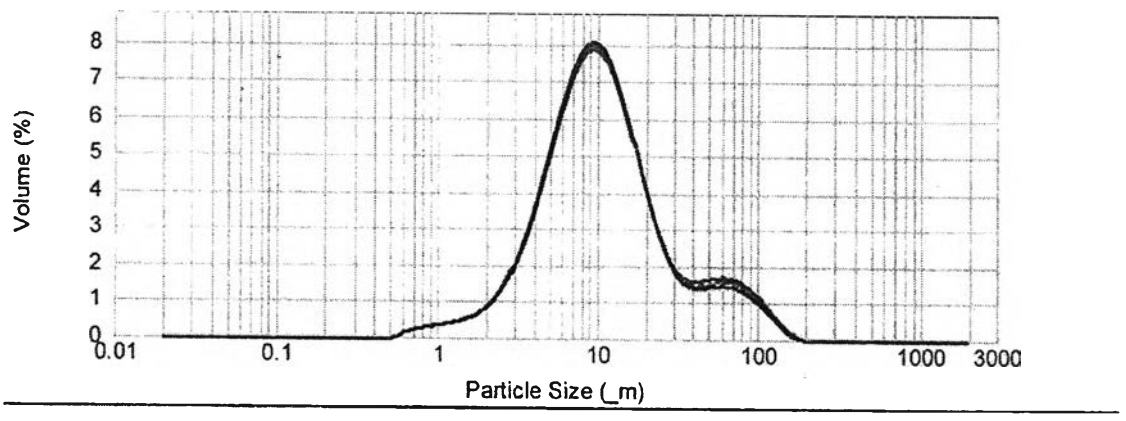
Figure 1(IV) Particle size distribution of Brij®30 niosomes prepared with various ratios of Brij®30: cholesterol: Simulsol®M52 dispersed in water at 70°C



(a) Brij®52 (60:30:10)

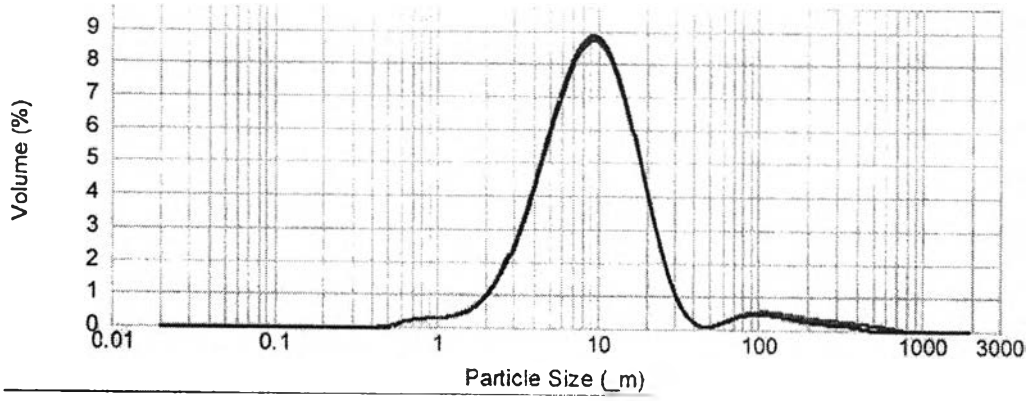


(b) Brij®52 (45:45:10)

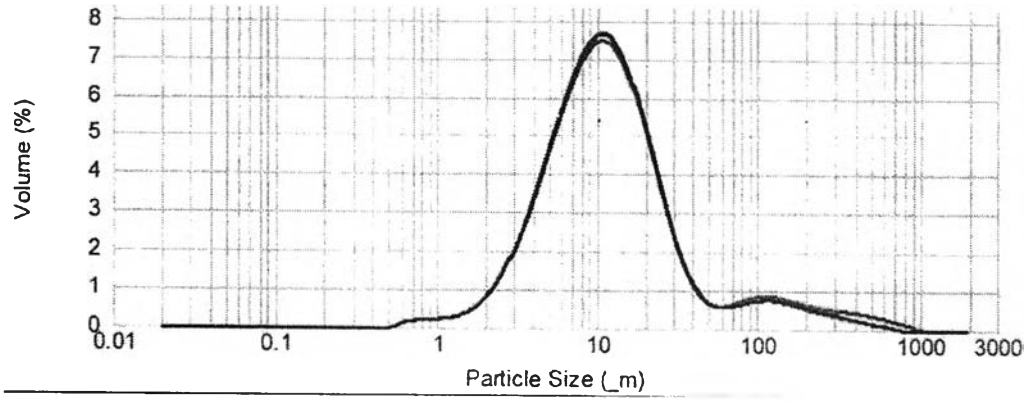


(c) Brij®52 (30:60:10)

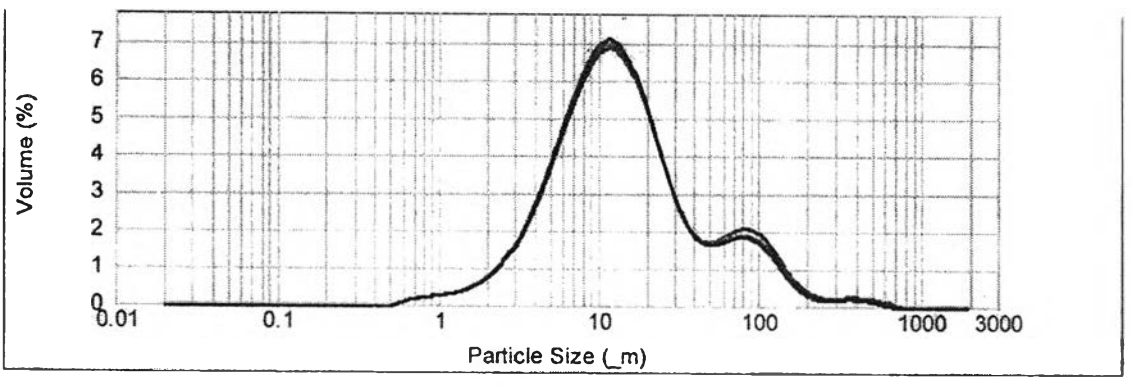
Figure 2(IV) Particle size distribution of Brij®52 niosomes prepared with various ratios of Brij®52: cholesterol: Simulsol®M52 dispersed in water at 70°C



(a) Brij®72 (60:30:10)

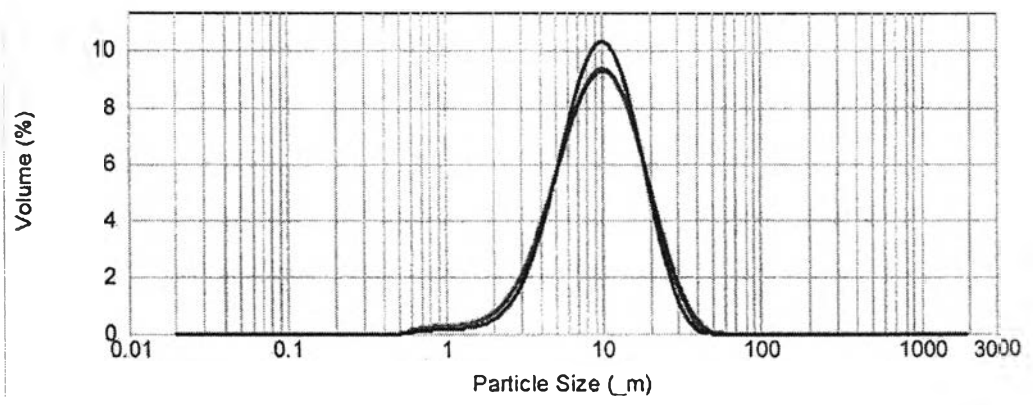


(b) Brij®72 (45:45:10)

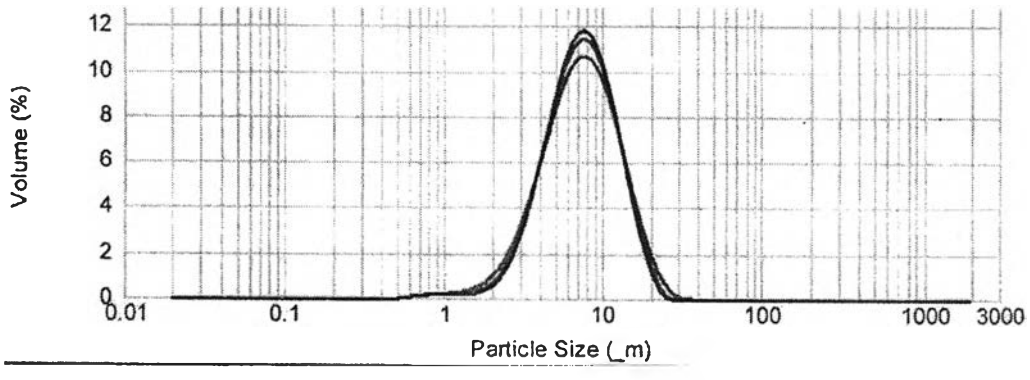


(c) Brij®72 (30:60:10)

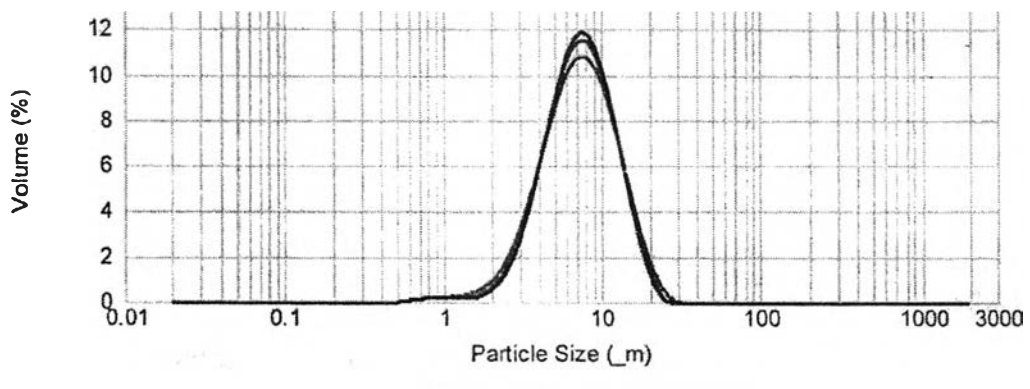
Figure 3(IV) Particle size distribution of Brij®72 niosomes prepared with various ratios of Brij®72: cholesterol: Simulsol®M52 dispersed in water at 70°C



(a) water

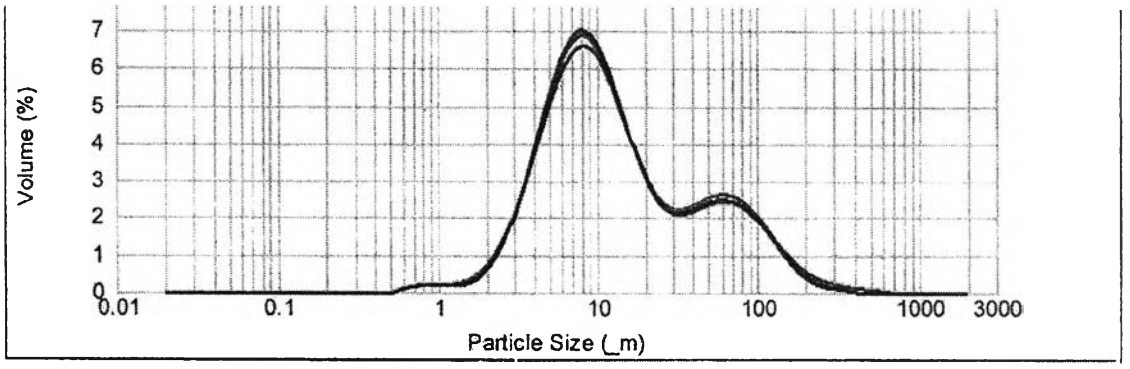


(b) 0.1N Hydrochloric acid

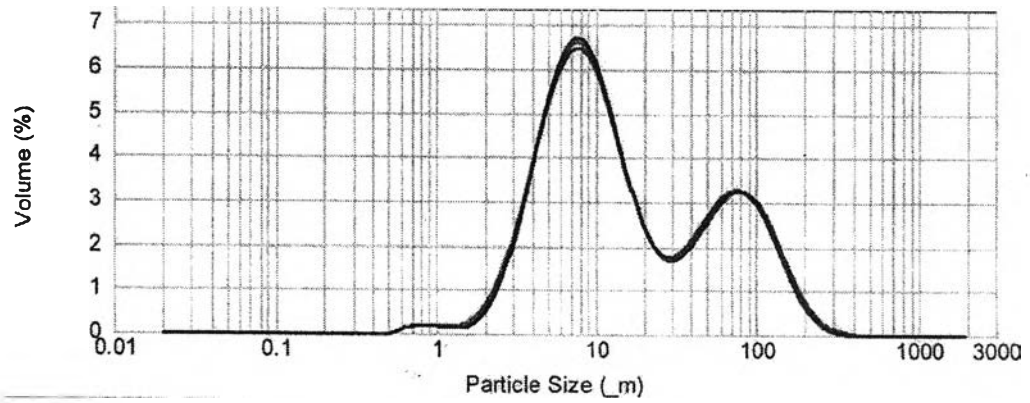


(c) Phosphate buffer pH6.8

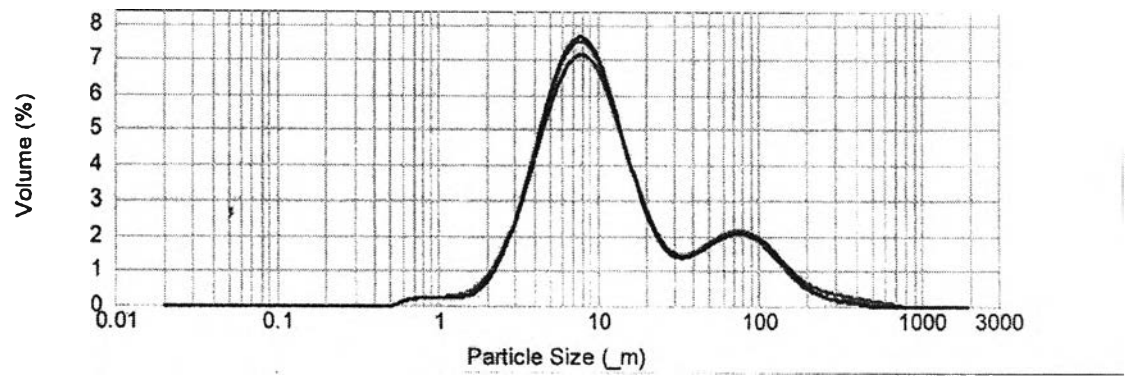
Figure 4(IV) Particle size distribution of Brij®30 niosomes composed of Brij®30: cholesterol: Simulsol®M52 (45:45:10 mole ratio) dispersed in different media at 37°C



(a) water

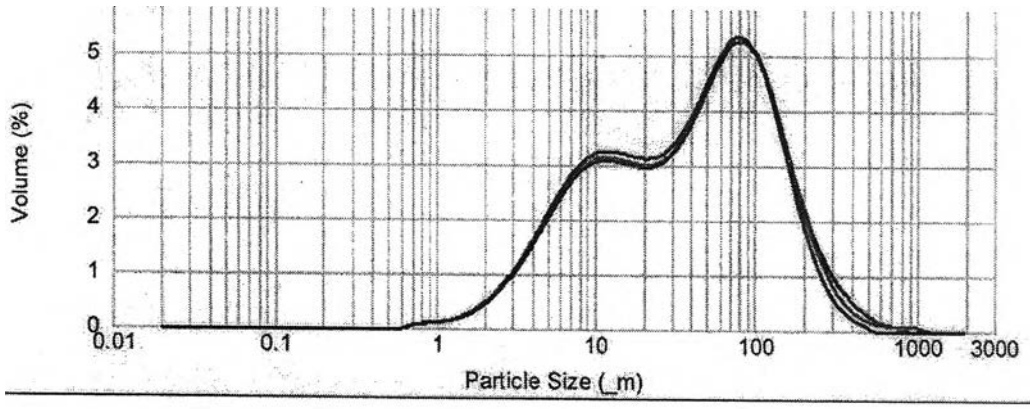


(b) 0.1N Hydrochloric acid

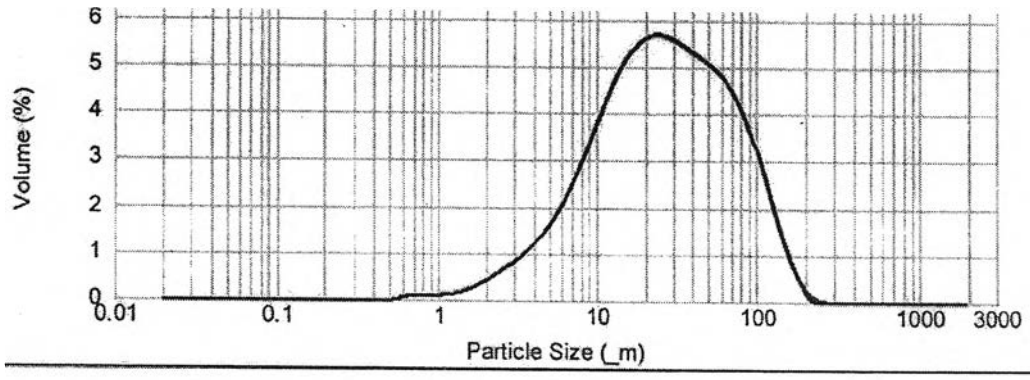


(c) Phosphate buffer pH6.8

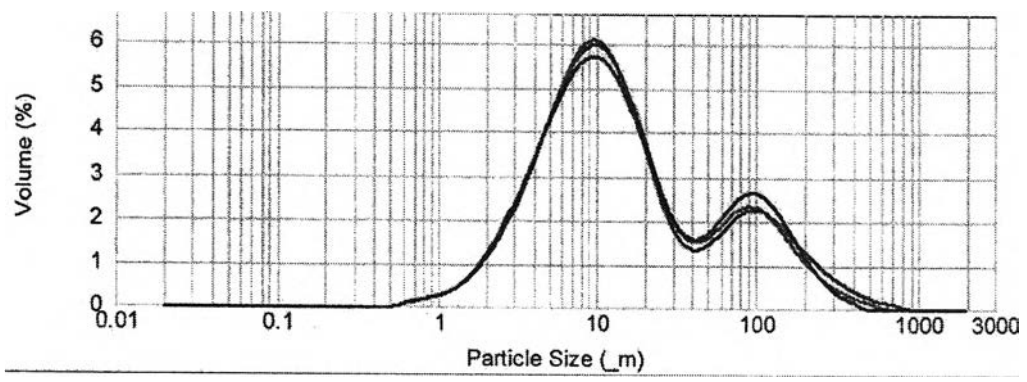
Figure 5(IV) Particle size distribution of Brij®30 niosomes composed of Brij®52: cholesterol: Simulsol®M52 (45:45:10 mole ratio) dispersed in different media at 37°C



(a) water



(b) 0.1N Hydrochloric acid



(c) Phosphate buffer pH6.8

Figure 6(IV) Particle size distribution of Brij®30 niosomes composed of Brij®72: cholesterol: Simulsol®M52 (45:45:10 mole ratio) dispersed in different media at 37°C

Table 1(IV). Mean size of niosomes prepared with various lipid/ surfactant composition in water at 70°C (n=3)

Nonionic surfactant	mole ratio of SF: Chol: SM 52	Size (μm)			Mean (μm)	SD
		n1	n2	n3		
Brij®30	60:30:10	5.696	6.122	5.271	5.70	0.43
	45:45:10	5.732	6.325	5.750	5.94	0.34
	30:60:10	9.177	10.051	8.302	9.18	0.87
Brij®52	60:30:10	8.162	8.197	8.559	8.31	0.22
	45:45:10	8.617	9.373	7.860	8.62	0.76
	30:60:10	10.037	10.587	9.488	10.04	0.55
Brij®72	60:30:10	8.908	9.171	8.335	8.81	0.43
	45:45:10	8.917	9.670	8.159	8.92	0.76
	30:60:10	9.906	11.414	8.398	9.91	1.51
Brij®98	60:30:10	NA	NA	NA	NA	NA
	45:45:10	NA	NA	NA	NA	NA
	30:60:10	NA	NA	NA	NA	NA

SF = nonionic surfactant; Chol = cholesterol; SM52 = Simulsol® M52 and (NA) = not applicable

Table 2(IV). Size of freshly prepared (Day 0) niosomes (45:45:10) dispersed in various media at 37°C (n=3)

Formulation	Medium	Size (μm), Day 0			Mean (μm)	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	10.101	9.742	9.797	9.88	0.19
	0.1 N HCl	7.485	7.692	7.389	7.52	0.15
	PBS pH 6.8	7.157	7.421	8.008	7.53	0.44
Brij®52 (45:45:10)	water	11.851	12.612	9.905	11.46	1.40
	0.1 N HCl	10.884	12.897	10.098	11.29	1.44
	PBS pH 6.8	10.655	9.700	10.923	10.43	0.64
Brij®72 (45:45:10)	water	40.149	41.281	39.020	40.15	1.13
	0.1 N HCl	22.747	24.813	25.613	24.39	1.48
	PBS pH 6.8	12.220	15.144	12.497	13.29	1.61

Table 3(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at room temperature for 1 week (n=3)

Formulation	Medium	Size (μm), 1st week			Mean (μm)	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	10.555	10.123	9.886	10.19	0.34
	0.1 N HCl	7.658	7.961	7.463	7.69	0.25
	PBS pH 6.8	7.207	7.177	7.282	7.22	0.05
Brij®52 (45:45:10)	water	12.861	13.414	12.992	13.09	0.29
	0.1 N HCl	12.271	13.685	13.296	13.08	0.73
	PBS pH 6.8	8.838	8.894	9.070	8.93	0.12
Brij®72 (45:45:10)	water	38.492	40.156	37.689	38.78	1.26
	0.1 N HCl	21.068	22.511	23.744	22.44	1.34
	PBS pH 6.8	11.293	13.012	7.756	10.69	2.68

Table 4(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at room temperature for 2 weeks (n=3)

Formulation	Medium	Size (μm), 2nd week			Mean (μm)	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	10.128	9.642	9.975	9.92	0.25
	0.1 N HCl	11.680	10.714	12.187	11.53	0.75
	PBS pH 6.8	7.421	8.008	7.170	7.533	0.43
Brij®52 (45:45:10)	water	13.595	13.682	13.124	13.47	0.30
	0.1 N HCl	12.987	13.511	13.315	13.27	0.26
	PBS pH 6.8	9.700	10.923	8.780	9.80	1.08
Brij®72 (45:45:10)	water	37.977	40.411	35.522	37.97	2.44
	0.1 N HCl	18.976	20.607	22.103	20.56	1.56
	PBS pH 6.8	10.767	12.812	7.978	10.52	2.43

Table 5(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at 4°C for 1 week (n=3)

Formulation	Medium	Size (µm), 1st week			Mean	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	10.217	9.880	10.467	10.19	0.29
	0.1 N HCl	8.866	9.573	9.368	9.27	0.36
	PBS pH 6.8	7.197	7.346	7.967	7.50	0.41
Brij®52 (45:45:10)	water	12.888	12.643	11.672	12.40	0.64
	0.1 N HCl	10.889	12.643	11.397	11.64	0.90
	PBS pH 6.8	9.447	10.644	10.804	10.30	0.74
Brij®72 (45:45:10)	water	38.414	36.828	34.774	36.67	1.83
	0.1 N HCl	24.990	26.798	28.669	26.82	1.84
	PBS pH 6.8	10.788	11.225	11.962	11.33	0.59

Table 6(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at 4°C for 2 weeks (n=3)

Formulation	Medium	Size (µm), 2nd week			Mean (µm)	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	10.813	11.042	9.819	10.56	0.65
	0.1 N HCl	7.068	8.757	8.211	8.01	0.86
	PBS pH 6.8	7.424	7.982	7.115	7.51	0.44
Brij®52 (45:45:10)	water	12.853	12.388	11.917	12.39	0.47
	0.1 N HCl	9.798	10.787	12.352	10.98	1.29
	PBS pH 6.8	10.511	9.741	9.692	9.98	0.46
Brij®72 (45:45:10)	water	36.551	38.655	38.320	37.84	1.13
	0.1 N HCl	25.101	26.833	28.907	26.95	1.91
	PBS pH 6.8	10.838	11.512	12.150	11.50	0.66

Table 7(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at 45°C for 1 week (n=3)

Formulation	Medium	Size (μm), 1st week			Mean (μm)	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	11.455	10.217	11.037	10.90	0.63
	0.1 N HCl	11.966	12.801	13.396	12.72	0.72
	PBS pH 6.8	7.264	7.028	6.828	7.04	0.22
Brij®52 (45:45:10)	water	10.881	10.615	11.213	10.90	0.30
	0.1 N HCl	13.415	13.678	12.960	13.35	0.36
	PBS pH 6.8	8.437	7.965	8.260	8.22	0.24
Brij®72 (45:45:10)	water	35.247	33.156	34.012	34.14	1.05
	0.1 N HCl	24.101	21.177	22.281	22.52	1.48
	PBS pH 6.8	13.895	12.034	15.369	13.77	1.67

Table 8(IV). Size of niosomes (45:45:10) dispersed at 37°C stored at 45°C for 2 weeks (n=3)

Formulation	Medium	Size (μm), 2nd week			Mean	SD
		n1	n2	n3		
Brij®30 (45:45:10)	water	9.171	7.203	8.187	8.19	0.98
	0.1 N HCl	12.738	13.756	14.720	13.74	0.99
	PBS pH 6.8	7.665	6.822	7.060	7.18	0.43
Brij®52 (45:45:10)	water	10.012	10.721	10.416	10.38	0.36
	0.1 N HCl	12.544	13.121	13.506	13.06	0.48
	PBS pH 6.8	7.680	7.727	8.069	7.83	0.21
Brij®72 (45:45:10)	water	33.681	31.067	33.144	32.63	1.38
	0.1 N HCl	22.044	19.987	23.244	21.76	1.65
	PBS pH 6.8	11.078	12.827	8.023	10.64	2.43

Table 9(IV). The amount of saquinavir mesylate in 60mM Brij®30 niosomes prepared in water 15 ml at 37°C

%mole of Brij30: Chol: SM52	Amount of entrapped drug (mg)					Amount of free drug (mg)					Total solubilized drug (mg)	Entrapment efficiency (EE)	%recovery
	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD			
45: 45: 10	14.3594	14.3594	14.4609	14.3932	0.0586	26.7450	26.7495	26.7265	26.7403	0.0122	41.1335	0.0209	97.04
90: 0: 10	6.2795	6.3469	6.3727	6.3330	0.0481	42.3845	41.9990	41.8745	42.0860	0.2659	48.4190	0.0092	91.24
80: 0: 20	8.3644	8.3129	8.3386	8.3386	0.0258	51.2100	50.8900	51.0500	51.0500	0.1600	59.3886	0.0121	88.63
70: 0: 30	8.5006	8.5263	8.5263	8.5177	0.0149	59.2680	58.6200	58.8387	58.9089	0.3297	67.4266	0.0123	94.61

Chol = cholesterol, SM52 = simulsol® M52

Table 10(IV). The amount of saquinavir mesylate in 15 ml of niosomes prepared from Brij®30: Cholesterol: Simulsol® M52 (mole ratio 70:0:30) in water at 37°C

Concentration of lipid/ surfactants (mM)	Amount of entrapped drug (mg)					Amount of free drug (mg)					Total solubilized drug (mg)	Entrapment efficiency (EE)	%recovery
	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD			
60	8.5006	8.5263	8.5263	8.5177	0.0149	59.2680	58.6200	58.8387	58.9089	0.3297	67.4266	0.0093	96.96
120	6.2253	6.2185	6.1946	6.2128	0.0161	101.6810	102.7065	100.1759	101.5211	1.2729	107.7339	0.0034	88.67
180	6.2019	6.2138	6.2303	6.2153	0.0143	120.1006	122.2370	120.3990	120.9122	1.1570	127.1275	0.0023	84.42
300	6.1880	6.0916	6.2244	6.1680	0.0686	158.2615	159.2430	156.7133	158.0726	1.2754	164.2406	0.0013	84.03

Table 11(IV). Percentage of saquinavir mesylate released from niosomal pellets dispersed in water

Time (h)	Amount of drug release (%)				
	n1	n2	n3	Mean	SD
0.5	68.09	65.13	40.46	57.89	15.17
1	65.13	67.10	60.20	64.14	3.56
2	83.88	73.02	84.87	80.59	6.57
4	95.72	69.08	71.05	78.62	14.85
6	88.81	79.93	75.98	81.58	6.57
8	93.75	48.35	87.83	76.64	24.68
12	95.72	61.18	94.73	83.88	19.66

Table 12(IV). Percentage of saquinavir mesylate released from niosomal pellets dispersed in 0.1N hydrochloric acid

Time (h)	Amount of drug release (%)				
	n1	n2	n3	Mean	SD
1	-23.27	14.76	-69.26	-4.26	26.89
2	-66.42	-39.74	-106.15	-53.08	18.87
4	-74.36	-45.98	-147.60	-60.17	20.07
6	-48.82	-24.41	-143.62	-36.61	17.26
8	-22.71	-28.95	-147.59	-25.83	4.42
12	-37.47	-36.33	-156.11	-36.90	0.80

Table 13(IV). Percentage of saquinavir mesylate released from niosomal pellets dispersed in 0.1N phosphate buffer pH 6.8

Time (h)	Amount of drug release (%)				
	n1	**n2**	**n3**	**Mean**	**SD**
1	2.29	10.15	6.33	6.26	3.93
2	15.41	17.26	14.75	15.81	1.30
4	61.03	61.23	66.38	62.88	3.03
6	51.47	45.23	58.34	51.68	6.56
8	32.28	48.35	40.93	40.52	8.05
12	30.35	27.88	26.96	28.39	1.75

APPENDIX V

Percentage amount of drug released from proniosomes

Table 1(V). Percentage of saquinavir mesylate (SQV) released from capsule A (capsule filled with a mixture of SQV and lactose) in water at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	18.79	16.93	19.69	18.47	1.41
0.33	30.77	36.47	34.60	33.95	2.90
0.50	44.94	53.07	49.88	49.30	4.10
1	72.83	71.35	67.77	70.65	2.60
2	85.85	85.22	82.96	84.68	1.52
4	90.18	90.95	90.88	90.67	0.43
6	95.92	94.17	95.32	95.14	0.89
8	97.07	95.40	96.97	96.48	0.94
12	97.98	96.72	98.14	97.62	0.78
24	93.67	97.54	96.81	96.01	2.05

Table 2(V). Percentage of saquinavir mesylate (SQV) released from capsule A (capsule filled with a mixture of SQV and lactose) in 0.1N hydrochloric acid at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	0	0	0	0	0
0.33	0	0	0	0	0
0.50	25.11	13.79	17.76	18.89	5.74
1	30.67	16.03	22.47	23.06	7.34
2	38.23	26.23	33.24	32.56	6.03
4	49.15	34.51	38.91	40.86	7.51

Table 3(V). Percentage of saquinavir mesylate (SQV) released from capsule A (capsule filled with a mixture of SQV and lactose) in phosphate buffer pH 6.8 at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	3.36	2.14	2.78	2.69	0.59
0.33	5.84	6.17	4.87	5.88	0.66
0.50	8.91	10.10	7.72	9.30	1.16
1	17.42	18.33	16.92	17.49	0.70
2	31.34	29.32	31.37	29.68	1.15
4	51.47	42.33	47.58	45.90	4.49
6	59.65	49.26	49.29	53.29	5.86
8	71.06	57.82	56.35	63.06	7.93
12	92.64	81.90	75.70	85.40	8.39
24	93.85	86.46	77.66	88.23	7.93

Table 4(V). Percentage of saquinavir mesylate (SQV) released from capsule B (capsule filled with a mixture of recrystallized SQV and lactose) in water at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	13.11	11.81	12.79	12.57	0.68
0.33	24.73	20.26	21.52	22.17	2.31
0.50	30.05	28.13	28.00	28.73	1.15
1	37.38	38.10	35.09	36.86	1.57
2	41.55	39.35	39.14	40.02	1.34
4	46.90	44.90	45.49	45.76	1.03
6	48.17	46.20	46.98	47.12	0.99
8	52.37	47.42	44.93	48.24	3.79
12	54.92	45.55	49.96	50.14	4.69
24	55.86	57.22	49.21	54.10	4.29

Table 5(V). Percentage of saquinavir mesylate (SQV) released from capsule B (capsule filled with a mixture of recrystallized SQV and lactose) in 0.1N hydrochloric acid at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	0	0	0	0	0
0.33	0	0	0	0	0
0.50	14.19	11.89	8.64	11.57	2.79
1	17.87	13.77	11.37	14.34	3.29
2	30.74	25.48	23.17	26.46	3.88
4	40.44	34.33	32.34	35.71	4.22

Table 6(V). Percentage of saquinavir mesylate (SQV) released from capsule B (capsule filled with a mixture of recrystallized SQV and lactose) in phosphate buffer pH 6.8 at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	0.57	0.84	1.21	0.83	0.32
0.33	2.04	0.81	3.89	3.01	0.92
0.50	3.02	1.20	6.11	4.57	1.52
1	7.20	7.69	11.66	9.32	2.19
2	16.56	21.38	19.69	17.93	1.57
4	35.05	39.95	28.03	29.66	4.02
6	39.59	42.55	32.44	35.01	3.52
8	41.38	46.42	39.51	40.13	1.32
12	55.81	50.27	52.92	53.19	1.41
24	60.45	58.21	55.86	57.51	2.47

Table 7(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-C (capsule filled with granules of SQV niosomes prepared from Brij®30: cholesterol: Simulsol®M52 (mole ratio 70:0:30) and lactose in water at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.08	31.93	31.17	32.50	31.55	0.54
0.17	48.34	42.84	44.37	45.59	3.89
0.33	65.68	57.66	59.58	61.67	5.67
0.50	74.09	70.16	72.39	72.13	2.78
1	80.88	77.29	79.63	79.08	2.54
2	84.88	83.53	83.62	84.21	0.95
4	87.79	84.72	86.99	86.25	2.17
6	90.61	91.12	89.52	90.87	0.36
12	91.47	84.96	89.23	88.22	4.61

Table 8(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-C (capsule filled with granules of SQV niosomes prepared from Brij®30: cholesterol: Simulsol®M52 (mole ratio 70:0:30) and lactose in 0.1N hydrochloric acid at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	58.32	75.91	70.95	68.40	9.07
0.33	77.40	84.57	72.04	78.00	6.29
0.50	82.23	90.59	84.10	85.64	4.39
1	88.22	94.41	91.24	91.29	3.10
2	91.34	100.52	96.64	96.17	4.61
4	95.15	102.84	100.29	99.43	3.92

Table 9(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-C (capsule filled with granules of SQV niosomes prepared from Brij®30: cholesterol: Simulsol®M52 (mole ratio 70:0:30) and lactose in phosphate buffer pH 6.8 at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.08	38.62	20.86	29.57	29.68	8.88
0.17	68.10	53.15	61.31	60.85	7.49
0.33	79.64	71.99	78.13	76.59	4.05
0.50	87.83	87.39	88.30	87.84	0.46
1	90.57	91.29	91.29	91.05	0.42
2	95.75	94.65	93.92	94.77	0.92
4	97.22	97.16	97.55	97.31	0.21
6	98.81	98.03	98.43	98.42	0.39
12	98.94	95.53	101.45	98.64	2.97

Table 10(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-D (capsule filled with granules of a mixture of SQV and lipid/ surfactants (mole ratio 70:0:30) and lactose in water at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.08	26.06	18.29	20.00	21.45	4.08
0.17	47.15	34.76	37.62	39.84	6.49
0.33	73.37	72.22	66.02	70.54	3.95
0.50	85.84	81.18	77.94	81.65	3.97
1	94.07	81.69	83.54	86.43	6.68
2	97.26	99.15	88.33	94.91	5.78
4	98.39	102.30	91.93	97.54	5.23
6	101.54	105.03	98.14	101.57	3.45
12	94.34	103.94	95.47	97.92	5.25

Table 11(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-D (capsule filled with granules of a mixture of SQV and lipid/ surfactants (mole ratio 70:0:30) and lactose in 0.1N hydrochloric acid at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.17	55.62	43.89	47.95	49.15	5.96
0.33	80.30	69.59	73.01	74.30	5.47
0.50	88.54	87.42	88.17	88.04	0.57
1	99.79	96.17	96.48	97.48	2.00
2	100.76	100.27	101.48	100.84	0.61
4	100.16	101.02	100.89	100.69	0.46

Table 12(V). Percentage of saquinavir mesylate (SQV) released from capsule SQV-D (capsule filled with granules of a mixture of SQV and lipid/ surfactants (mole ratio 70:0:30) and lactose in phosphate buffer pH 6.8 at 37°C

Time (h)	Percentage drug release				
	n1	n2	n3	Mean	SD
0.08	39.80	54.42	40.18	44.80	8.33
0.17	55.96	63.90	53.23	57.70	5.54
0.33	69.86	79.38	68.61	72.62	5.89
0.50	84.85	88.14	79.05	84.01	4.60
1	90.01	93.76	86.31	90.03	3.72
2	94.07	97.34	90.59	94.00	3.37
4	100.11	99.53	99.61	99.75	0.32
6	102.32	102.70	100.99	102.01	0.90
12	103.78	103.50	99.89	102.39	2.17

Table 13V. Area under the curves of dissolution profiles of saquinavir mesylate released from capsule A, B, SQV-C and SQV-D in water, 0.1N hydrochloric acid and phosphate buffer pH 6.8 up to 4 h (n=3), Mean (SD)

AUC (%h)	Medium	Formulation			
		SQV-C	SQV-D	A	B
n1	Water	320.11	363.73	296.82	153.59
n2		308.93	357.26	298.89	147.22
n3		314.46	330.43	291.83	145.58
Mean (SD)		314.50 (5.59)	350.48 (17.66)	295.84 (3.63)	148.80 (4.23)
n1	0.1N HCl	348.35	378.32	137.87	104.68
n2		381.37	371.61	90.47	86.84
n3		365.55	375.03	111.54	78.50
Mean (SD)		365.09 (16.51)	374.99 (3.35)	113.29 (23.75)	90.01 (13.38)
n1	PBS pH6.8	341.80	331.98	116.05	66.73
n2		333.46	350.26	104.81	78.46
n3		338.28	317.78	111.17	69.19
Mean (SD)		337.85 (4.19)	333.34 (16.28)	110.68 (5.63)	71.46 (6.18)

AUC = Area under the curve, A= capsule filled with a physical mixture of SQV and lactose, B = capsule filled with a physical mixture of R-SQV and lactose, SQV-C = capsule filled with SQV-NL and SQV-D = capsule filled with SQV-AL

Table 14(V). Area under the curves of dissolution profiles of saquinavir mesylate released from capsule A, B, SQV-C and SQV-D in water, 0.1N hydrochloric acid and phosphate buffer pH 6.8 up to 12 h (n=3), Mean (SD)

AUC 24h	Medium	Formulation			
		SQV-C	SQV-D	A	B
n1	Water	1044.78	1151.30	1066.01	563.77
n2		1013.01	1191.52	1057.82	517.86
n3		1027.25	1101.34	1060.55	519.74
Mean (SD)		1028.34 (15.91)	1148.05 (45.18)	1061.46 (4.17)	533.79 (25.98)
n1	0.1N HCl	NA	NA	NA	NA
n2		NA	NA	NA	NA
n3		NA	NA	NA	NA
Mean (SD)		NA	NA	NA	NA
n1	PBS pH6.8	1126.28	1140.82	685.28	416.73
n2		1107.56	1161.76	582.93	443.32
n3		1125.48	1110.35	577.79	386.49
Mean (SD)		1119.77 (10.58)	1137.64 (25.85)	615.33 (60.63)	415.51 (28.44)

AUC = Area under the curve, NA = not applicable, A= capsule filled with a physical mixture of SQV and lactose, B = capsule filled with a physical mixture of R-SQV and lactose, SQV-C = capsule filled with SQV-NL and SQV-D = capsule filled with SQV-AL

Table 15(V). Area under the curves of dissolution profiles of saquinavir mesylate released from capsule A, B, SQV-C and SQV-D in water, 0.1N hydrochloric acid and phosphate buffer pH 6.8 up to 24 h (n=3), Mean (SD)

AUC 24h	Medium	Formulation			
		SQV-C	SQV-D	A	B
n1	Water	NA	NA	2215.95	1228.46
n2		NA	NA	2223.39	1134.48
n3		NA	NA	2230.23	1114.74
Mean (SD)		NA	NA	2223.19 (7.14)	1159.23 (60.77)
n1	0.1N HCl	NA	NA	NA	NA
n2		NA	NA	NA	NA
n3		NA	NA	NA	NA
Mean (SD)		NA	NA	NA	NA
n1	PBS pH6.8	NA	NA	1804.23	1114.29
n2		NA	NA	1593.09	1094.21
n3		NA	NA	1497.95	1039.20
Mean (SD)		NA	NA	1631.76(156.76)	1082.57 (38.87)

AUC = Area under the curve, NA = not applicable, A= capsule filled with a physical mixture of SQV and lactose, B = capsule filled with a physical mixture of R-SQV and lactose, SQV-C = capsule filled with SQV-NL and SQV-D = capsule filled with SQV-AL

Table 16(V). Percentage of saquinavir mesylate content in proniosome capsule SQV-C (capsule filled with SQV-NL) after stored at 25°C for 4 months

Time (month)	mg per capsule				
	n1	n2	n3	Mean	SD
0	8.70	8.76	8.73	8.73	0.03
1	9.01	8.74	8.71	8.82	0.17
2	8.33	8.65	8.41	8.46	0.17
3	7.65	7.98	7.98	7.87	0.19
4	5.78	5.64	5.90	5.77	0.13

Table 16(V). Percentage of saquinavir mesylate content in proniosome capsule SQV-C (capsule filled with SQV-NL) stored at 45°C for 4 months

Time (month)	mg per capsule				
	n1	n2	n3	Mean	SD
0	8.70	8.76	8.73	8.73	0.03
1	8.66	8.66	8.48	8.60	0.10
2	7.39	7.52	7.55	7.49	0.08
3	7.33	7.65	7.64	7.54	0.18
4	5.91	5.74	5.64	5.76	0.14

VITA

Miss Rattana Srichisak was born on May 9, 1978 in Trat province, Thailand. She received the Bachelor of Science in Pharmacy from Faculty of Pharmaceutical Sciences, Silpakorn University, Nakhon Pathom, Thailand. After graduation, she continued her studying in the Master's Degree in Industrial Pharmacy Program in the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Her research "Preparation and characterization of niosomes encapsulating saquinavir mesylate" was accepted for the poster session in the 2005 AAPS annual meeting and exposition, Tennessee, USA.