

REFERENCES

- Aboul-Enein, H. Y. 1977. Analytical Profile of Drug Substances and Excipients, vol 12. New Jersey: Academic Press.
- Agarwal, R., Katare, O. P., and Vyas, S. P. 2001. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. International Journal of Pharmaceutics 228: 43-52.
- Arunothayanun, P., Bernard, M. S., Craig, D. Q. M., Uchegbu, I. F., and Florence, A. T. 2000. The effect of processing variables on the physical characteristic of non-ionic surfactant vesicles (niosomes) formed from hexadecyl diglycerol ether. International Journal of Pharmaceutics 201: 7-14.
- Arunothayanun, P., Turton, J. A., Uchegbu, I. F., and Florence, A. T. 1998. Preparation and in vitro/in vivo evaluation of leuteinizing hormone releasing hormone (LHRH)-loaded polyhedral and spherical/tubular niosomes. Journal of Pharmaceutical Sciences 88: 34-38.
- Arunothayanun, P., Uchegu, I. F., Craig, D. Q. M., Turton, J. A., and Florence, A. T. 1999. In vitro/in vivo characterization of polyhedral niosomes. International Journal of Pharmaceutics 183: 57-61.
- Asavisanu, L. M. 1997. A double-blinded control trial of effectiveness of 10% propylthiouracil lotion compared with lotion base in patients with psoriasis vulgaris at university hospital. Master Thesis, Faculty of Medicine, Chulalongkorn University.
- Ayala-Bravo, H. A., Q., et al. 2003. Effects of sucrose oleate and sucrose laurate on *in vivo* human stratum corneum permeability. Pharmaceutical Research 20: 1267-1273.
- 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. Journal of Pharmacy and Pharmacology 37: 863-868.
- Bajue, W. T. 2003. Psoriasis: diagnosis and management. Dermatology 9: 54-59.
- Baker, J. N. W. 1991. The pathophysiology of psoriasis. Lancet 338: 227-230.

- Barlow, D. J., Lawrence, M. J., and Timmins, P. A. 2000. Molecular modeling of surfactant vesicles. In Uchegbu, I. F. (ed.), Synthetic surfactant vesicles: Niosomes and other non-phospholipid vesicular systems, pp.12. Amsterdam: Harwood academic publishers.
- Barry, B. W. 2001. Is transdermal drug delivery research still important today? Drug Delivery Today 6: 967-971.
- Betageri, G. V., and Parson, D. L. 1992. Drug encapsulation and release from multilamellar and unilamellar liposomes. . International Journal of Pharmaceutics 81: 235-241.
- Betz, G., Imboden, R., and Imanidis, G. 2001. Interaction of liposome formulations with human skin in vitro. International Journal of Pharmaceutics 229: 117-129.
- Betz, G., Nowbakht, P., Imboden, R., and Imanidis, G. 2001. Heparin penetration into and permeation through human skin from aqueous and liposomal formulations in vitro. International Journal of Pharmaceutics 228: 147-159.
- Bouwstra, J. A., and Hofland, H. E. J. 1994. In Kreuter J. (ed.), Colloidal drug delivery systems, pp. 191-217. New York: Marcel Dekker.
- Bouwstra, J. A., and Honeywell-Nguyen, P. L. 2002. Skin structure and mode of action of vesicles. Advanced Drug Delivery Reviews 54 (suppl. 1): S41-S55.
- Bouwstra, J. A., Honeywell-Nguyen, P. L., Gooris, G. S., and Ponec, M. 2003. Structure of the skin barrier and its modulation by vesicular formulations. Progress in Lipid Research 42: 1-36.
- Bouwstra, J. A., van Hal, D. A., Hofland, H. E. J., and Junginger, H. E. 1997. Preparation and characterization of nonionic surfactant vesicles. Colloids and Surfaces A: Physicochemical and Engineering Aspects 123: 71-80.
- Bowen, J. L., and Heard, C. M. 2006. Film drying and complexation effects in the simultaneous skin permeation of ketoprofen and propylene glycol from simple gel formulations. International Journal of Pharmaceutics 307: 251-257.
- Brain, K. R., Walters, K. A., and Watkinson, A. C. 2002. Methods for studying percutaneous absorption. In Walters, K. A., (ed.), Dermatological and transdermal formulations, New York: Maecel Dekker.

- Carafa, M., et al. 1998. Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. International Journal of Pharmaceutics 160: 51-59.
- Carafa, M., Marianecchi, C., Lucania, G., Marchei, E., and Santucci, E. 2004. New vesicular ampicillin-loaded delivery systems for topical application: characterization, in vitro permeation experiments and antimicrobial activity. Journal of Controlled Release 95: 67-74.
- Carafa, M., Santucci, E., and Lucania, G. 2002. Lidocaine-loaded non-ionic surfactant vesicles: characterization and in vitro permeation studies. International Journal of Pharmaceutics 231: 21-32.
- Cevc, G. 2004. Lipid vesicles and other colloids as drug carriers on the skin. Advanced Drug Delivery Reviews 56: 675-711.
- Cevc, G., and Blume, G. 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochimica et Biophysica Acta 1104: 226-232.
- Cevc, G., Blume, G., and Schatzlein, A. 1997. Transfersomes-mediated transepidermal delivery improves the region-specificity and biological activity of corticosteroids in vivo. Journal of Controlled Release 45: 211-226.
- Cevc, G., Gebauer, D., Stieber, J., Schatzlein, A., and Blume, G. 1998. Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochimica et Biophysica Acta 1368: 201-215.
- Cevc, G., Schatzlein, A., and Blume, G. 1995. Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. Journal of Controlled Release 36: 3-16.
- Cevc, R., Schatzlein, A. and Richardsen, H. 2002. Ultradefordable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. Biochimica et Biophysica Acta 1564: 21-30.
- Chopineau, J., Lesieur, S., and Ollivon, M. 1994. Vesicle formation by enzymatic processes. Journal of American Chemistry and Society 116: 11582-11583.

- Chowdhury, M. M. U., and Marks, R. 2001. Oral propylthiouracil for the treatment of resistant plaque psoriasis. Journal of Dermatological Treatment 12: 81-85.
- Dalvi, U. G., and Zatz, J. L. 1981. Effect of nonionic surfactants on penetration of dissolved benzocaine through hairless mouse skin. Journal of The Society of Cosmetic Chemists 32: 87-94.
- De Jong, E. M. G. J. 1997. The course of psoriasis. Clinics in Dermatology 15: 687-692.
- Desai, T. R., and Finlay, W. H. 2002. Nebulization of niosomal all-trans-retinoic acid: An inexpensive alternative to conventional liposomes. International Journal of Pharmaceutics 241: 311-317.
- Devaraj, et al. 2002. Release studies on niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol. Journal of Colloid and Interface Science 251: 360-365.
- Dimitrijevic, D., Lamandin, C., Uchegbu, I. F., Shaw, A. J., and Florence, A. T. 1997. The effect of monomers and of micellar and vesicular forms of nonionic surfactants (SolulanC24 and Solulan16) on Caco-2 cell monolayers. Journal of Pharmacy and Pharmacology 49: 611-616.
- Dipiro, J. T., et al. (eds), 2002. Pharmacotherapy: A pathophysiologic approach, 5th ed. New York: Medical Publish Division.
- Downton, S. M., Hu, Z., Ramachandran, C., Wallach, D. F. H., and Weiner, N. 1993. Influence of liposomal composition on topical delivery of encapsulated cyclosporine A I. An *in vitro* study using hairless mouse skin. S. T. P. Pharma Sciences 3: 404-407.
- du Plessis, J., Ramachandraj, C., Weiner, N. D., and Muller, D. G. 1994. The influence of particle size of liposomes on the disposition of drug into the skin. International Journal of Pharmaceutics 103: 277-282.
- El Maghraby, G. M. M., Williams, A. C., and Barry, B. W. 1999. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. Journal of Pharmacy and Pharmacology 51: 1123-1134.

- El Maghraby, G. M. M., Williams, A. C., and Barry, B. W. 2000a. Skin delivery of oestradiol from lipid vesicles: important of liposome structure. International Journal of Pharmaceutics 204: 159-169.
- El Maghraby, G. M. M., Williams, A. C., and Barry, B. W. 2000b. Oestradiol skin delivery of from ultradeformable liposomes: refinement of surfactant concentration. International Journal of Pharmaceutics 196: 63-74.
- El Maghraby, G. M. M., Williams, A. C., and Barry, B. W. 2001. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in vitro. Journal of Pharmacy and Pharmacology 53: 1069-1077.
- El Maghraby, G. M. M., Williams, A. C., and Barry, B. W. 2004. Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes. International Journal of Pharmaceutics 276: 143-161.
- Elias, A. N., and Barr, R. J. 1995. Low-dose oral propylthiouracil in the treatment of plaque psoriasis. International Journal of Dermatology 34: 519-520.
- Elias, A. N., Barr, R. J., Rohan, M. K., and Danganan, K. 1995. Effect of orally administered antithyroid thioureylenes on PCNA and P53 expression in psoriatic lesion. International Journal of Dermatology 34: 280-283.
- Elias, A. N., et al. 1994. A controlled trial of topical propylthiouracil in the treatment of patients with psoriasis. Journal of the American Academy of Dermatology 29: 78-81.
- Elias, A. N., Goodman, M. M., and Rohan, M. K. 1993b. Serum ICAM-1 concentrations in patients with psoriasis treated with antithyroid thioureylenes. Clinical and Experimental Dermatology 18: 526-529.
- Elias, A. N., Goodman, M. M., and Rohan, M. K. 1993c. Effect of propylthiouracil and methimazole on serum levels of interleukin-2 receptors in patients with psoriasis. International Journal of Dermatology 32: 537-540.
- Elias, A. N., Goodman, M. M., Liem, W. H., and Barr, R. J. 1993a. Propylthiouracil in psoriasis: Results of open trial. Journal of the American Academy of Dermatology 29: 78-81.

- Endo, M., Yamamoto, T., and Ijuin, T. 1996. Effect of nonionic surfactant on the percutaneous absorption of tenoxicam. Chemical and Pharmaceutical Bulletin 44: 865-867.
- Fang, J. Y., Hong, C. T., Chiu, W. T., and Wang, Y. Y. 2001. Effect of liposomes and niosomes on skin permeation of enoxacin. International Journal of Pharmaceutics 219: 61-72.
- Fang, J. Y., Hwang T. L., and Leu, Y. L. 2003. Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. International Journal of Pharmaceutics 250: 313-325.
- Fang, J. Y., Sung, K. C., Lin, H. H., and Fang C. L. 1999. Transdermal iontophoretic delivery of enoxacin from various liposome-encapsulated formulations. Journal of Controlled Release 60: 1-10.
- Fang, J. Y., Wu, P. C., Huang, Y. B., and Tsai, Y. H. 1995. In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. International Journal of Pharmaceutics 126: 119-128.
- Femenia-Font, A., Balaguer-Fernandez, C., Merino, V., Rodilla, V., and Lopez-Castellano, A. 2005. Effects of chemical enhancers on the in vitro percutaneous absorption of sumatripan succinate. European Journal of Pharmaceutics and Biopharmaceutics 61: 50-55.
- Fleisher, D., et al. 1995. Topical delivery of growth hormone releasing peptide using liposomal systems: An *in vitro* study using hairless mouse skin. Life Sciences 57: 1293-1297.
- Florence, A. T. 1993. Nonionic surfactant vesicles: Preparation and characterization. Liposome Technology, vol 1, London: CRC Press.
- Flynn, G. L. and Smith, R. W. 1972. Membrane diffusion III: Influence of solvent composition and permeant solubility on membrane transport. Journal of Pharmaceutical Sciences 61: 61-66.
- Foco, A., Gasperlin, M., and Kristl, J. 2005. Investigaion of liposomes as carriers of sodium ascorbyl phosphate for cutaneous photoprotection. International Journal of Pharmaceutics 291: 21-29.

- Fresta, , M., and Puglisi, G. 1997. Corticosteroid dermal delivery with skin-lipid liposomes. Journal of Controlled Release 44: 141-151.
- Friedman, E. S., Friedman, P. M., Cohen, D. E., and Washenik, K. 2002. Allergic contact dermatitis to topical minoxidil solution: Etiology and treatment. Journal of the American Academy of Dermatology 46: 309-312.
- Gaikwad, S. Y., Jagtao, A. G., Ingle, A. D., Ra, S. G., and Gude, R. P. 2000. Antimetastatic efficacy of niosomal pentoxifylline and its combination with activated macrophages immune B16F10 melanoma model. Cancer Biotherapy and Radiopharmaceuticals 15:605-615.
- Ganesan, M. G., Weiner, N. D., Flynn, G. L., and Ho, N. F. H. 1984. Influence of liposomal drug entrapment on percutaneous absorption. International Journal of Pharmaceutics 20: 139-154.
- Gianasi, D., Cociancich, F., Uchegbu, I. F., Florence, A. T., and Duncan, R. 1997. Pharmaceutical and biological characterization of a doxorubicin-polymer conjugate (PK1) entrapped in sorbitan monostearate Span 60 niosomes. International Journal of Pharmaceutics 148: 139-148.
- Guenin, E. P., and Zatz, J. L. 1995. Interaction of skin surfactant vesicle components. Journal of the Society and Cosmetic Chemistry 46: 77-84.
- Guinedi, A. S., Mortada, N. D., Mansour, S., and Hathout, R. M. 2005. Preparation and evaluation of reverse-phase evaluation and multilamellar niosomes as ophthalmic carriers of acetazolamide. International Journal of Pharmaceutics 306: 71-82.
- Handjani-vila, R. M., Ribier, A., Rondot, B., and Vanlerberghe, G. 1979. Dispersion of lamellar phase of non-ionic lipids in cosmetic products. International Journal of Cosmetic Science 1: 303-314.
- Hao, Y., Zhao, F., Li, N. A., Yang, Y., and Li, K. 2002. Studies on a high encapsulation of colchicine by a niosome system. International Journal of Pharmaceutics 244: 73-80.
- Haran, G., Cohen, R., bar, L. K., and Barenholz, Y. 1993. Transmembrane ammonium sulphate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochimica et Biophysica Acta 1151:201-215.

- Harvey, R. D., Heenan, R. K., Barlow, D. J., and Lawrence, M. J. 2005. The effect of electrolyte on the morphology of vesicles composed of the dialkyl polyoxyethylene ether surfactant 2C₁₈E₁₂. Chemistry and Physics of Lipids 133: 27-36.
- Ho, N. F. H., Ganesen, M. G., Weiner, N. D., and Flynn, G. L. 1985. Mechanisms of topical delivery of liposomally entrapped drugs. Journal of Controlled Release 2: 61-65.
- Hofland, H. E. J., Bouwstra, J. A., Ponec, M., Spies, F., Verhoef, J. C., and Junginger, H. E. 1991. Interactions of non-ionic surfactant vesicles with cultured keratinocytes and human skin in vitro: a survey of toxicological aspects and ultrastructural changes in stratum corneum. Journal of Controlled Release 16: 155-168
- Hofland, H. E. J., Bouwstra, J. A., Verhoef, J. C., Buckton, G., Chowdry, B. Z., Ponec, M., and Junginger, H. E. 1992. Safety aspects of non-ionic surfactant vesicles: A toxicity study related to the physicochemical characteristics of non-ionic surfactant. Journal Pharmacy Pharmacology 44: 287-294.
- Hofland, H. E. J., et al. 1993. Nonionic surfactant vesicles: a study of vesicle formation, characterization, and stability. Journal of Colloids and Interface Sciences 161: 366-376.
- Hofland, H. E. J., van den Geest, R., Bodde, H. E., Junginjer, H. F., and Bouwstra, J. A. 1994. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharmaceutical Research 11: 659-664.
- Honeywell-Nguyen, P. L., and Bouwstra, J. A. 2003. The in vitro transport of pergolide from surfactant-based elastic vesicles through human skin: a suggested mechanism of action. Journal of Controlled Release 86: 145-156.
- Honeywell-Nguyen, P. L., Arenja, S., and Bouwstra, J. A. 2003. Skin penetration and mechanisms of action in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulations. Pharmaceutical Research 20: 1619-1625.
- Honeywell-Nguyen, P. L., de Graaff, A. M., Groenink, H. W. W., and Bouwstra, J. A. 2002. The in vivo and in vitro interactions of elastic and rigid vesicles with human skin. Biochimica et Biophysica Acta 1573: 130-140.
- Honeywell-Nguyen, P. L., Frederik, P. M., Bomans, P. H. H., Junginger, H. E., Bouwstra, J. A. 2002. Transdermal delivery of pergolide from surfactant-based

- elastic and rigid vesicles: characteristic and in vitro transport studies. Pharmaceutical Research 19: 991-997.
- Honeywell-Nguyen, P. L., Groenink, H. W. W., de Graaff, A. M., and Bouwstra, J. A. 2003. The in vivo transport of elastic vesicle into human skin: effects of occlusion, volume and duration of application. Journal of Controlled Release 90: 243-255.
- Jacobi, U., Taube, H., Schafer, U. F., Sterry, W., and Lademann, J. 2005. Comparison of four different in vitro systems to study the reservoir capacity of the stratum corneum. Journal of Controlled Release 103: 61-71.
- Jayaraman, S. C., Ramachandran, C., and Weiner, N. 1996. Topical delivery of erythromycin from various formulations: An in vivo hairless mouse study. Journal of Pharmaceutical Sciences 85: 1082-1084.
- Junginger, H. E., Hofland, H. E. J., and Bouwstra, J. A. 1991. Liposomes and niosomes: Interactions with human skin. Cosmetics and Toiletries 106: 45-50.
- Kamath, M. P., Shenoy, B. D., Tiwari, S. B., Karki, R., Udupa, N., and Korian, M. 2000. Prolong release biodegradable vesicular carrier for rifampicin-formulation kinetics of release. Indian Journal of Experimental Biology 38: 113-118.
- Kibbe, A. H., (ed.), 2000. Handbook of Pharmaceutical Excipients, Washington: American Pharmaceutical Association.
- Kirjavanen, M. et al. 1996. Interaction of liposomes with human skin in vitro-the influence of lipid composition and structure. Biochimica et Biophysica Acta 1304: 179-180.
- Kiwada, H., Niimura, H., Fujisaki, Y., Yamada, S., and Kato, Y. 1985. Application of synthetic alkyl glycoside vesicles as drug carriers. I. Preparation and physical properties. Chemical and Pharmaceutical Bulletin 33: 753-759.
- Knepp, V. M., Szoka, F. C. Jr., and Guy, R. H. 1990. Controlled drug release from a novel liposomal delivery system. II. Transdermal delivery characteristics. Journal of Controlled Release 12: 25-50.
- Kose, K., Utas, S., Yazici, C., Akdas, A., and Kelestimur, F. 2001. Effect of propylthiouracil on adenosine deaminase activity and thyroid function in patients with psoriasis. British Journal of Dermatology 144: 1121-1126.

- Kunida, h., Ushio, N., Nakano, A., and Miura, M. 1993. Three-phase behavior in a mixed sucrose alkanoate and polyethyleneglycol alkyl ether system. Journal of Colloid and Interface Science 159: 37-44.
- Ladbrooke, B. D., and Chapman, D. 1969. Thermal analysis of lipids, proteins and biological membranes a review and summary of some recent studies. Chemistry of Physical Lipids 3: 304-367.
- Law, S. L., Jang, T. F., Chang, P., and Lin, C. H. 1994. Release characteristics of mitoxantrone-containing liposomes. International Journal of Pharmaceutics 103: 81-85.
- Li G. L., Danhof, M., Bouwstra, J. A. 2001. Effect of elastic liquid-state vesicle on apomorphine iontophoresis transport through human skin in vitro. Pharmaceutical Research 18: 1627-1630.
- Lichtenstein, A., and Margalit, R. 1995. Liposome-encapsulated silver sulfadiazine (SSD) for the topical treatment of infected burns: thermodynamics of drug encapsulation and kinetics of drug release. Journal of Inorganic Biochemistry 60: 187-198.
- Liden, K. G., and Weinstein, G. D. 1999. Psoriasis: current perspectives with an emphasis on treatment. The American Journal of Medicine 107:595-605.
- Lieb, L. M., Flynn, G., and Weiner, N. 1994. Follicular (pilosebaceous unit) deposition and pharmacological behavior of cimetidine as a function of formulation. Pharmaceutical Research 11: 1419-1423.
- Lopez, A., Llinares, F., Cortell, C., Herraiz, M. 2000. Comparative enhancer effects of Span[®] 20 with Tween[®] 20 and Azone[®] on the in vitro percutaneous penetration of compounds with different lipophilicities. International Journal of Pharmaceutics 202: 133-140.
- Manconi, M., et al. 2003. Niosomes as carriers for tretinoin II. Influence of vesicular incorporation on tretinoin photostability. International Journal of Pharmaceutics 260: 261-272.
- Manconi, M., Sinico, C., Valenti, D., Lai, F., and Fadda, A. M. 2006. Niosomes as carriers for tretinoin III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. International Journal of Pharmaceutics 311:11-19.

- Manconi, M., Sinico, C., Valenti, D., Loy, G., and Fadda, A. M. 2002. Niosomes as carriers for tretinoin. I. Preparation and properties. International Journal of Pharmaceutics 234: 237-248.
- Manosroi, A. et al. 2005. The entrapment of kojic oleate in bilayer vesicles. International Journal of Pharmaceutics 198: 13-25.
- Manosroi, A., et al. 2003. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloids and Surfaces B: Biointerfaces 30: 129-138.
- Margalit, R., Okon M., Yerushalmi, N., and Avidor, E. 1992. Bioadhesive liposomes as topical drug delivery systems: molecular and cellular studies. Journal of Controlled Release 19: 275-288.
- Marro, D., Guy, R. H., and Delgado-Charro, M. B. 2001. Characterization of the iontophoretic permselectivity properties of human and pig skin. Journal of Controlled Release 70: 213-217.
- Martin, A., (ed), 1993. Physical Pharmacy. Philadelphia: Lea & Febiger.
- Megrab, N. A., Williams, A. C., and Barry, B. W. 1995. Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water cosolvent systems. International Journal of Pharmaceutics 116: 101-112.
- Mezei, M., Sager, R. W., Stewart, W. D., and Deruyter, A. L. 1966. Dermatitic effect of nonionic surfactants I. Journal of Pharmaceutical Sciences 55: 584-590.
- Moffat, A., Osselton, D. M., and Widdop, B. 2004. Clarke's Analysis of Drugs and Poisons. London: Pharmaceutical Press.
- Montenegro, L., Panico, A. M., Ventimiglia, A., and Bonina, F. P. 1996. In vitro retinoic acid release and skin permeation from different liposome formulations. International Journal of Pharmaceutics 133: 89-96.
- Nacht, S. 1995. Encapsulation and other topical delivery systems. Cosmetics and Toiletries 110: 25-45.
- Namdeo, A., and Jain, N. K. 1999. Niosomal delivery of 5-fluorouracil. Journal of Microencapsulation 16: 731-740.

- Naresh, R. A. R., Pillai, G. K., Udupa, N., and Chandrashekar, G. 1994. Anti-inflammatory activity of noisome encapsulated diclofenac sodium in arthritic rats. Indian Journal of Pharmacology 26: 46-48.
- New, R. R. C. 1997. Liposomes: A practical approach, New York: Oxford University Press.
- Niemiec, S. M., Ramachandran, C., and Weiner, N. 1995. Influence of nonionic liposomal composition on topical delivery of peptide drugs into pilosebaceous units: An in vivo study using the hamster ear model. Pharmaceutical Research 12: 1184-1188.
- Ogiso, T., Niinaka, N., and Iwaki, M. 1996. Mechanism for enhancement effect of lipid disperse system on percutaneous absorption. Journal of Pharmaceutical Sciences 85: 57-64.
- Ohta, M., Ramachandran, C., and Weiner, N. D. 1996. Influence of formulation type on the deposition of glycolic acid and glycerol in hairless mouse skin following topical *in vivo* application. Journal of the Society of Cosmetic Chemists 47:97-107.
- Okuyama, et al. 1999. Influence of non-ionic surfactants, pH and propylene glycol on percutaneous absorption of piroxicam from cataplasm. International Journal of Pharmaceutics 186: 141-148.
- Park, E. S., Chang, S. Y., Hahn, M., and Chi, S. C. 2000. Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. International Journal of Pharmaceutics 209: 109-119.
- Parthasarathi, G., Udupa, N., and Pillai, G. K. 1993. Formulation and in vitro evaluation of vincristine encapsulation niosomes. Indian Journal of Pharmaceutical Sciences 56: 90-94.
- Perez-Cullell, N., et al. 2000. Influence of the fluidity of liposome compositions on percutaneous absorption. Drug Delivery 7: 7-13.
- Philippot, J. R., and Schuber, F. 1995. Liposomes as tools in basic research and industry, USA: CRC Press.
- Pillai, G. K., and Salim, M. L. D. 1999. Enhanced inhibition of platelet aggregation in-vitro by noisome-encapsulated indomethacin. International Journal of Pharmaceutics 193: 123-127.

- Plookchit Chetratanont. 2002. Effects of formulation and preparation method on drug entrapment of minoxidil niosomes. Master's Thesis, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.
- Ratana Rattanatraiphop. 2000. Effects of formulation factors on physical properties and in vitro biological activity of propylthiouracil liposomes. Master Thesis, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.
- Reddy, D. N., and Udupa, N. 1993. Formulation and evaluation of oral and transdermal preparations of flurbiprofen and piroxicam incorporated with different carriers. Drug Development and Industrial Pharmacy 19: 843-852.
- Roson, M. J., (ed.), 1989. Surfactants and Interfacial Phenomena, New York: John Wiley and Son.
- Ross, J. S., and Shah, J. C. 2000. Reduction in skin permeation of *N, N*-diethyl-*m*-tolbuamide (DEET) by altering the skin/vehicle partition coefficient. Journal of Controlled Release 67: 211-221.
- Ruckmani, K., Jayakar, B., and Ghosal, S. K. 2000. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemias: encapsulation, storage, and in vitro release. Drug Development and Industrial Pharmacy 26: 217-222.
- Saarinen-Savolainen, P., Jarvinen, T., Taipale, H., and Urtti, A. 1997. Methods for evaluating drug release from liposomes in sink conditions. International Journal of Pharmaceutics 159:27-33.
- Saras, M., Gally, J., Vincent, M., Ollivaon, M., and Lesieur, S. 1994. Micelle vesicle transition of nonionic surfactant cholesterol assemblies induced by octyl glucoside-A time resolved fluorescence study of dehydroergosterol. Journal of Colloid and Interface Science 167: 159-171.
- Sarpotdar, P. P., and Zatz, J. L. 1986. Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin in vitro. Journal of Pharmaceutical Sciences 75: 176-181.

- Scalf, L. A., and Fowler, J. F. 2000. Peristomal allergic contact dermatitis due to Gantrez in Stomahesive paste. Journal of the American Academy of Dermatology 42: 355-356.
- Schmook, F. P., Meingassner, J. G., and Billich, A. 2001. Comparison of human or epidermis model with human and animal skin in in-vitro percutaneous absorption. International Journal of Pharmaceutics 215: 51-56.
- Schreier, H., and Bouwstra, J. A. 1994. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. Journal of Controlled Release 30: 1-15.
- Sentjurc, M., Vrhovnik, K., and Kristl, J. 1999. Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. Journal of Controlled Release 59: 87-97.
- Shahiwala, A., and Misra, A. 2002. Studies in topical application of niosomally entrapped nimesulide. Journal of Pharmacy and Pharmaceutical Sciences 5: 220-225.
- Shin, S. C., Cho, C. W., and Oh, I. J. 2001. Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins. International Journal of Pharmaceutics 222: 199-203.
- Sinico, C., Manconi, M., Peppi, M., Lai, F., Valenti, D., and Fadda, A. M. 2005. Liposomes as carriers for dermal delivery of tritinin: in vitro evaluation of drug permeation and vesicle-skin interaction. Journal of Controlled Release 103: 123-136.
- Skalko, N., Cajkovic, M., and Jalsenjak, I. 1992. Liposomes with clindamycin hydrochloride in the therapy of Acne vulgaris. International Journal of Pharmaceutics 85: 97-101.
- Talsma, H., Steenberg, M. J. V., Borchert, J. C. H., and Crommelin, D. J. A. 1994. A novel technique for the one-step preparation of liposomes and nonionic surfactant vesicles without the use of organic solvents. Journal of Pharmaceutical Science 83: 276-280.

- Toutou, E., Dayan, N., Bergelson, L., Godin, B., and Eliaz, M. 2000. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release 65: 403-418.
- Toutou, E., Junginger, H. E., Weiner, N. D., Nagai, T., Mezei, M. 1994. Liposomes as carriers for topical and transdermal delivery. Journal of Pharmaceutical Sciences 83: 1189-1203.
- Trotta, M., Peira, E., Carlotti, M. E., and Gallarate, M. 2004. Deformable liposomes for dermal administration of methotrexate. International Journal of Pharmaceutics 270: 119-125.
- Uchegbu, I. F., and Duncan, R. 1997. Niosomes containing N-(2-hydroxypropyl) methacrylamide copolymer-doxorubicin (PK1): Effect of method of preparation and choice of surfactant on niosome characteristics and a preliminary study of body distribution. International Journal of Pharmaceutics 155:7-17.
- Uchegbu, I. F., and Florence, A. T. 1995. Non-ionic surfactant vesicles (Niosomes): physical and pharmaceutical chemistry. Advances in Colloid and Interface Sciences. 58: 1-55.
- Uchegbu, I. F., and Vyas, S. P. 1998. Non-ionic surfactant based vesicles (niosomes) in drug delivery. International Journal of Pharmaceutics 172: 33-70.
- Uchegbu, I. F., Bouwstra, J. A., and Florence, A. T. 1992. Large disk-shaped structures (Discomes) in nonionic surfactant vesicle to micelle transitions. Journal of Physical Chemistry 96: 19-27.
- Uchegbu, I. F., Double, 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. Pharmaceutical Research 12: 1019-1024.
- Uchegbu, I. F., Schatzlein, A., Vanlerberghe, G., Morgatini, N., and Florence, A. T. 1997. Polyhedral non-ionic surfactant vesicles. Journal of Pharmacy and Pharmacology 49: 606-610.
- Udupa, N., Chandraprakash, K. S., Umadevi, P., and Pillai, G. K. 1993. Formulation and evaluation of methotrexate niosomes. Drug Development and Industrial Pharmacy 19: 1331-1342.



- United States Pharmacopeial Convention: The United States Pharmacopeia 25–The National Formulary 20. Mack Publishing: Pennsylvania. 2002.
- van den Bergh, B. A. I., Bouwstra, J. A., Junginger, H. E., and Wertz, P. W. 1999. Elasticity of vesicles affects hairless mouse skin structure and permeability. Journal of Controlled Release 62: 367-379.
- van den Bergh, B. A. I., de Vries, S., and Bouwstra, J. A. 1998. Interactions between liposomes and human stratum corneum studied by freeze-substitution electron microscopy. International Journal of Pharmaceutics 167: 57-67.
- van den Bergh, B. A. I., Vroom, J., Gerritsen, H., Junginger, H. E., and Bouwstra, J. A. 1999. Interaction of elastic and rigid vesicles with human skin in vitro: electron microscopy and two-photon excitation microscopy. Biochimica et Biophysica Acta 1461: 155-173.
- van den Bergh, B. A. I., Wertz, P. W., Junginger, H. E., and Bouwstra, J. A. 2001. Elasticity of vesicles assessed by electron spin resonance, electron microscopy and extrusion measurements. International Journal of Pharmaceutics 217: 13-24.
- van Kuijk, M. E. M. J., Janssen, J., Cullander, C., Junginger, H. E., and Bouwstra, J. A. 1998. A cross-section device to improve visualization of fluorescent probe penetration into the skin by confocal laser scanning microscopy. Pharmaceutical Research 15: 352-356.
- van Kuijk, M. E. M. J., Junginger, H. E., and Bouwstra, J. A. 1998. Interactions between liposomes and human skin in vitro, a confocal laser scanning microscopy study. Biochimica et Biophysica Acta 1371: 31-39.
- van Kuijk, M. E. M. J., Mougín, L., Junginger, H. E., and Bouwstra, J. A. 1998. Application of vesicles to rat skin in vivo: a confocal laser scanning microscopy study. Journal of Controlled Release 56: 189-190.
- Verma, D. D., Verma, S., Blume, G., and Fahr, A. 2003. Particle size of liposomes influences dermal delivery of substance into skin. International Journal of Pharmaceutics 258: 141-151.
- Wallach, D. F. H., and Philippot, J. R. 1993. New type of lipid vesicle: Novasome. In: Gregoriadis, G. (ed.), Liposome Technology, vol 12, pp. 141-156. Boca Raton, FL: CRC Press.

- Waranuch, N., Ramachandran, C., and Weiner, N. D. 1997. Effect of lipid composition on topical delivery of cyclosporine-A from nonionic liposomal formulations: An in vitro study with hairless mouse skin. Journal of Liposome Research 7: 261-271.
- Waranuch, N., Ramachandran, C., and Weiner, N. D. 1998. Controlled topical delivery of cyclosporine-A from nonionic liposomal formulations: mechanistic aspect. Journal of Liposome Research 8: 225-238.
- Weiner, N., Martin, F., and Riaz, M. 1989. Liposomes as a drug delivery system. Drug Development and Industrial Pharmacy 15: 1523-1554.
- Wester, R. C., and Maibach, H. I. 1990. In vitro testing of topical pharmaceutical formulations. In Osborne, E. W., and Amann, A. H., (eds.), Topical drug delivery formulations, New York: Marcel Dekker.
- Wester, R. C., Melendres, J., Sedik, L., Maibach, H., and Riviere, J. E. 1998. Percutaneous absorption of salicylic acid, theophylline, 2, 4-dimethylamine, diethyl hexyl phthalic acid, and p-aminobenzoic acid in the isolated perfused porcine skin flap compared to man in vivo. Toxicology and Applied Pharmacology 151: 159-165.
- Williams, A. C., and Barry, B. W. 2004. Penetration enhancers. Advanced Drug Delivery Reviews 56: 603-618.
- Wu, P. C., Huang, Y. B., and Tsai, Y. H. 1997. In vitro percutaneous absorption of captopril. International Journal of Pharmaceutics 148: 41-46.
- Yamane, M.A., Williams, A. C., and Barry, B. W. 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. Journal of Pharmacy and Pharmacology 47: 978-989.
- Yokomiza, Y., and Sagitani, H. 1996. The effect of phosphates on the percutaneous penetration of indomethacin through the dorsal skin of guinea pig in vitro. 2. The effects of the hydrophobic group in phospholipids and a comparison with general enhancers. Journal of Controlled Release 42: 37-46.
- Yoshioka, T., and Florence, A. 1994. Vesicle (noisome)-in-water-in-oil (v/w/o) emulsions: an in vitro study. International Journal of Pharmaceutics 108: 117-123.

- Yoshioka, T., Sternberg, B., and Florence, A. T. 1994. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60, and 80) and a sorbitan triester (Span 85). International Journal of Pharmaceutics 105: 1-6.
- Yu, H. Y., and Liao, H. M. 1996. Triamcinolone permeation from different liposome formulations through rat skin in vitro. International Journal of Pharmaceutics 127: 1-7.
- Zellmer, S., Pfeil, W., and Lasch, J. 1995. Interaction of phosphatidylcholine liposomes with human stratum corneum. Biochimica et Biophysica Acta 1237: 176-182.

APPENDICES

APPENDIX A

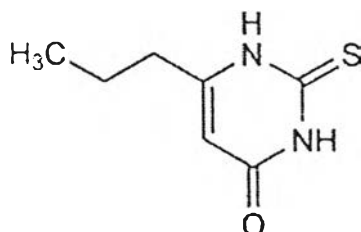
Molecular Structure and Physical Properties of Propylthiouracil (PTU)

(Aboul-Enein, 1977; Moffat, 2004)

1. Molecular structure

1.1 Empirical: C₇H₁₀N₂OS

1.2 Structural:



1.3 Molecular weight: 170.23

2. Physical properties

2.1 Melting range: 219-221 °C

2.2 Log P: 1.0

2.3 Solubility:

PTU is sparingly soluble in water (1:900 at 20 °C), soluble in 100 parts boiling water, in 60 parts of ethanol, in 60 parts of acetone, practically insoluble in ether, chloroform, benzene, freely soluble in aqueous solutions of ammonia and alkali hydroxide. A saturated aqueous solution is neutral or slightly acidic to litmus.

2.4 Ultraviolet spectrum:

PTU in neutral methanol absorbs ultraviolet radiation at 275 nm (a_m 15800) and at 214 nm (a_m 15600). In alkaline medium, it shows maxima at 315.5 nm (a_m 10900), 260 nm (a_m 10700) and at 207.5 nm (a_m 15400).

2.5 Stability:

PTU is a relatively stable compound at room temperature. It is recommended that it should be kept in a well-closed containers protected from light.

APPENDIX B

Molecular Structure and Physical Properties of Some Selected Materials

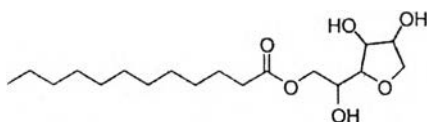
(Kibbe, 2000)

Properties of some selected materials

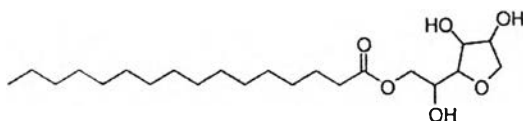
Material	Formula	Property
Cholesterol	$C_{27}H_{46}O$	MW: 386.67 MP: 147-150 °C BP: 360 °C
Span [®] 20	$C_{18}H_{34}O_6$	MW: 346 HLB: 8.6
Span [®] 40	$C_{22}H_{42}O_6$	MW: 403 MP: 44-48 °C HLB: 6.7
Span [®] 60	$C_{24}H_{46}O_6$	MW: 431 MP: 53-57 °C HLB: 4.7
Brij [®] 52	$C_{22}H_{45}O_3$	MW: 357 MP: 33 °C HLB: 5.3
Brij [®] 76	$C_{38}H_{78}O_{11}$	MW: 710 MP: 38 °C HLB: 12.4
Solulan [®] C24	-	MW: 1,443 HLB: 8-9 Cloud point: 88-95 °C
Glyceryl disterate	$C_{39}H_{76}O_5$	MW: 636 HLB: 2.4 MP: 55-60 °C
Sucrose laurate ester (L-595)	-	HLB: 5.0
PEG-8-L	$C_{29}H_{58}O_{10}$	MW: 552 HLB: 13.0 MP: 12 °C

The structure of Span[®] 20, Span[®] 40, Span[®] 60, Brij[®] 52, Brij[®] 76, Solulan[®] C24, GDS, sucrose laurate ester, and PEG-8-L

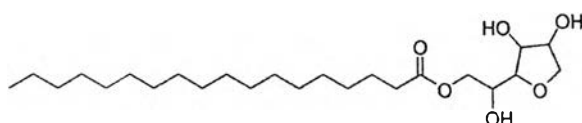
1. Span[®] 20



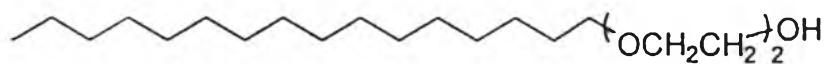
2. Span[®] 40



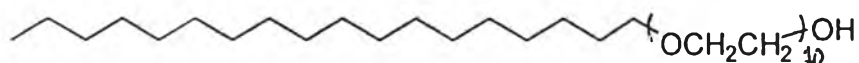
3. Span[®] 60

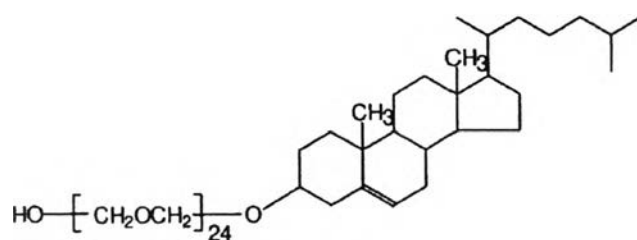


4. Brij[®] 52

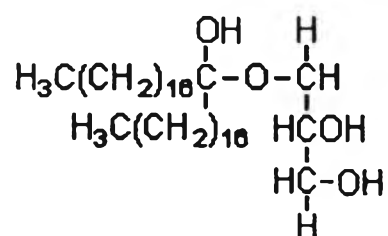


5. Brij[®] 76

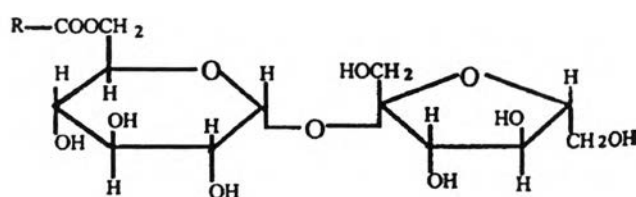


6. Solulan[®] C24

7. Glyceryl distearate (GDS)

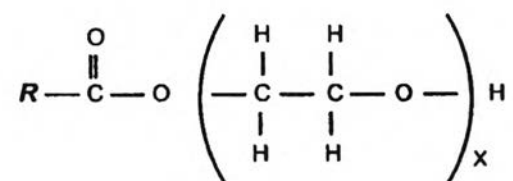


8. sucrose laurate ester



$$R = \text{C}_{12}$$

9. PEG-8-L



$$R = \text{C}_{12} \text{ and } X = 8$$

APPENDIX C

Validation of UV Spectroscopic Method
(The United States Pharmacopieal Convention, 2002)

Validation for the Quantitative Determination of PTU in Isopropanol by UV Spectroscopy

1. Specificity

Under the UV absorption spectrophotometric method used, the absorbance of PTU must not be interfered by the absorbance of other components in the sample. The blank vesicular suspension (without PTU) and PTU vesicular suspension were prepared. The UV spectrum from UV spectrophotometer of the blank vesicular suspension was compared with the spectra of the PTU vesicular suspension.

2. Linearity

Eight standard solutions of PTU ranging 1.0 to 10.0 $\mu\text{g/mL}$ were prepared and analyzed. Linear regression analysis of the absorbance versus their concentrations was performed. The linearity was determined from the coefficient of determination.

3. Accuracy

PTU at 2.5, 5.5 and 8.5 $\mu\text{g/mL}$ and surfactant/cholesterol mixtures 100 mg/mL were prepared. Three sets of each concentration were prepared. Each individual sample was analyzed by UV spectrophotometry at 275 nm, and percent analytical recovery of each sample was calculated.

4. Precision

4.1 Within run precision

The within run precision was evaluated by analyzing six sets of the three standard solutions of PTU in six intervals of time in the same day. The mean, standard deviation (SD) and the coefficient of variation (%CV) of each standard solution were determined.

4.2 Between run precision

The between run precision was evaluated by comparing each concentration of five sets of standard solutions were prepared and analyzed in different days. The mean, standard deviation (SD) and the coefficient of variation (%CV) of each standard solution were determined.

Validation for the Quantitative Determination of PTU Solution in Isopropanol by UV Spectrophotometry

The validation of the analytical method is the process by which performance characteristics of the method are established to meet the requirements for the intended analytical parameters. The analytical parameters used for the assay validation were specificity, linearity, accuracy and precision.

1. Specificity

The specificity of an analytical method is its ability to measure the given analyte accurately and specificity in the presence of other components in the sample. The UV absorption spectra (Figure C1-C16) indicated that the wavelength 275 nm was the optimal wavelength giving the highest sensitivity without interference of surfactants, cholesterol and Solulan[®] C24 which showed no absorbance at the wavelength 200-400 nm.

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. The linearity is usually expressed in term of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The standard curves of PTU in water and phosphate buffer pH 7.4 diluted with isopropanol were shown in Figures C17 and C18, respectively. The standard curves were found to be linear with coefficient of determination 0.9999 and 0.9999, respectively. These results indicated that UV spectrophotometric method was acceptable for quantitative analysis of PTU in the range studied. The equations of standard curves according to Beer's Law were used for calculating the concentration of PTU.

3. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. The percentages of analytical recovery of

each PTU concentration in water and phosphate buffer pH 7.4 are shown in Table C1 and C2. All the percentage analytical recovery of all drug concentrations in water with a mean of and a %CV of, and in phosphate buffer pH 7.4 with a mean of and a %CV of, indicated the high accuracy of this method. Thus, it could be used for analysis of PTU in all concentrations used.

4. Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation).

The precision of the analysis of PTU in water and phosphate buffer pH 7.4 by UV spectrophotometric method was determined both within run precision and between run precision as illustrated in Tables C3-C6. All percentage coefficient of variation values were lower than 2.00%, indicating that of the UV spectrophotometric method used were precise for quantitative analysis of PTU in the range studied.

In conclusion, the analysis of PTU in water and phosphate buffer pH 7.4 by UV spectrophotometric method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for determination of the content of PTU.

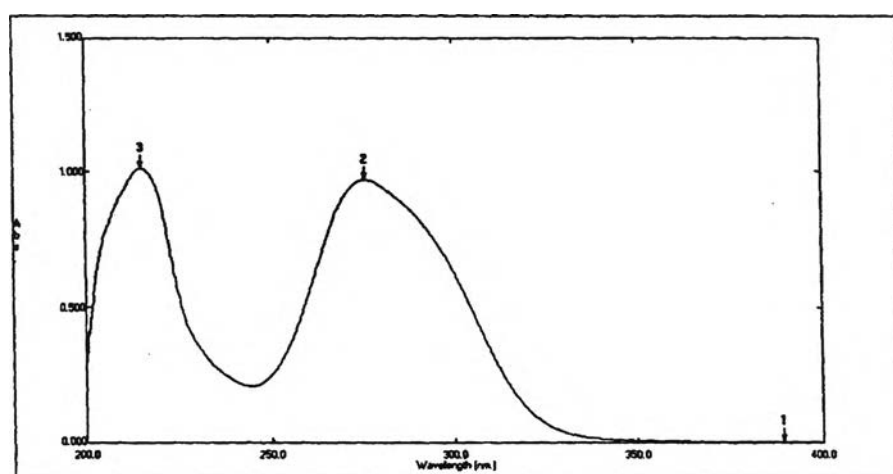


Figure C1 Absorption spectrum of PTU in water diluted with isopropanol

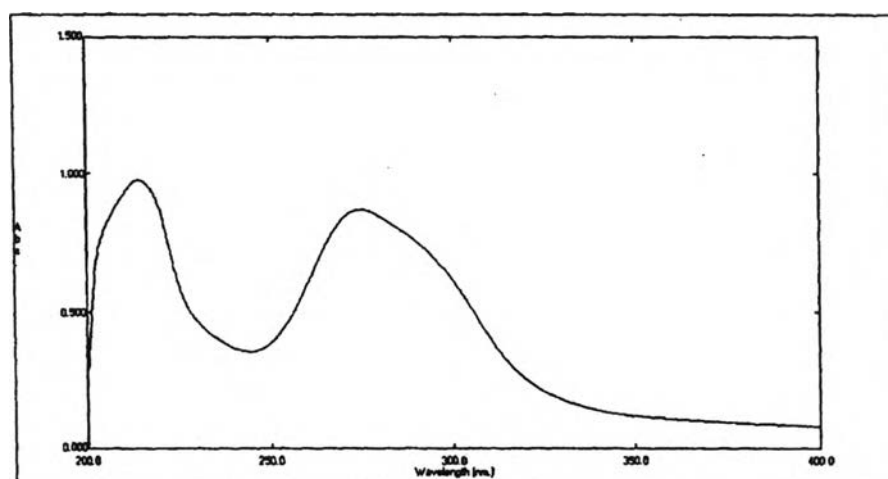


Figure C2 Absorption spectrum of PTU in phosphate buffer pH 7.4 diluted with isopropanol

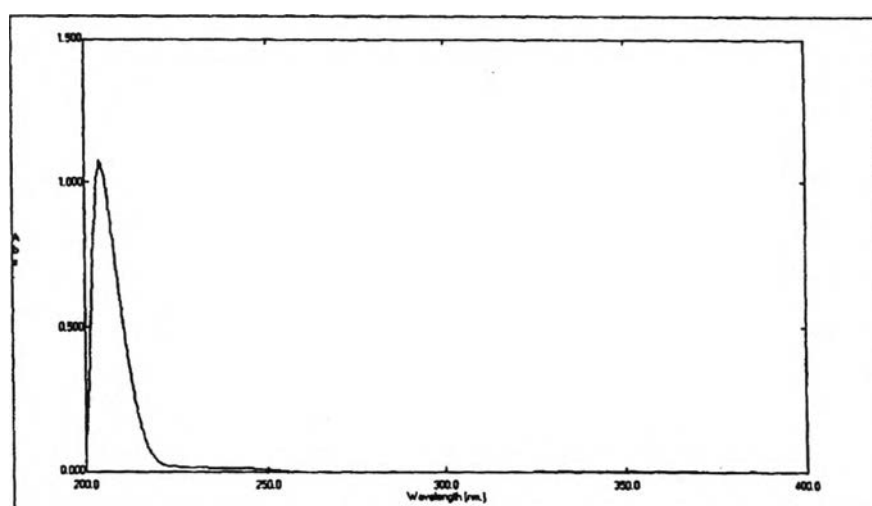


Figure C3 Absorption spectrum of Brij® 52:CHO:Solulan® C24 without PTU in water diluted with isopropanol

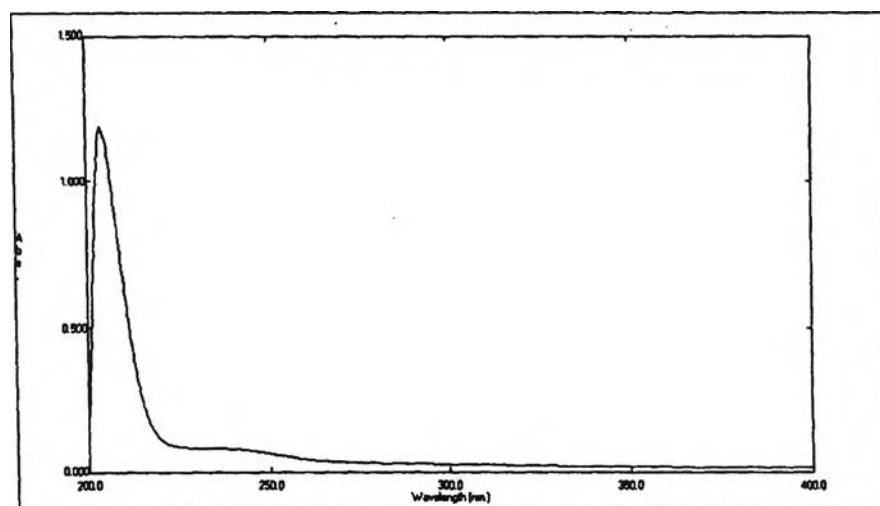


Figure C4 Absorption spectrum of Brij[®] 52:CHO:Solulan[®] C24 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

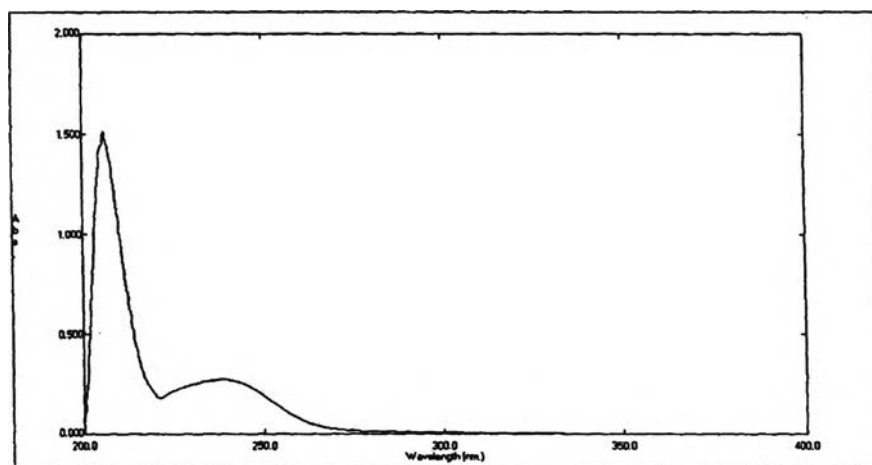


Figure C5 Absorption spectrum of Brij[®] 76:CHO:Solulan[®] C24 without PTU in water diluted with isopropanol

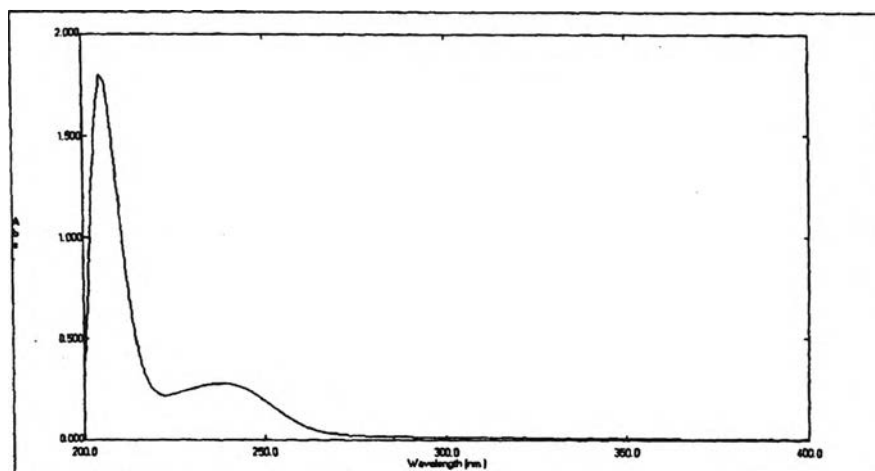


Figure C6 Absorption spectrum of Brij[®] 76:CHO:Solulan[®] C24 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

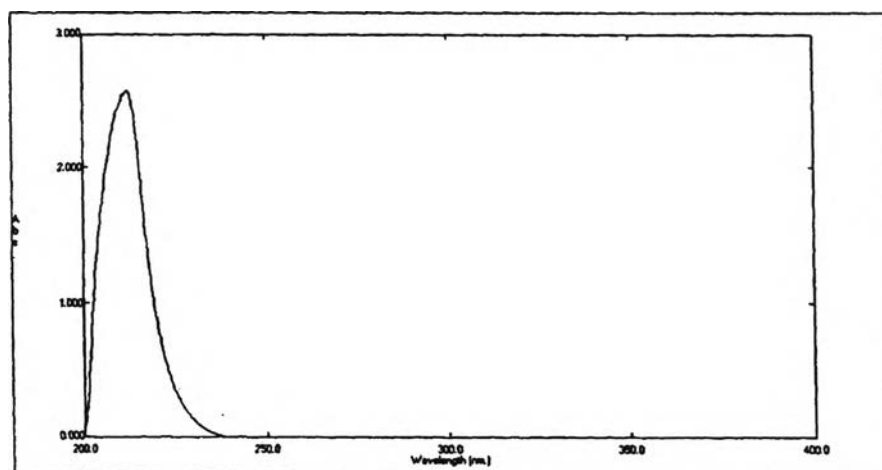


Figure C7 Absorption spectrum of GDS:CHO:Brij[®] 76 without PTU in water diluted with isopropanol

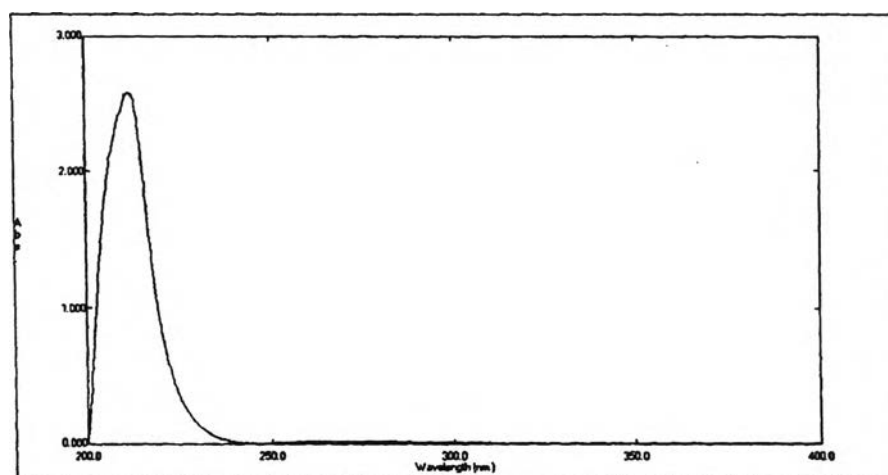


Figure C8 Absorption spectrum of GDS:CHO:Brij® 76 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

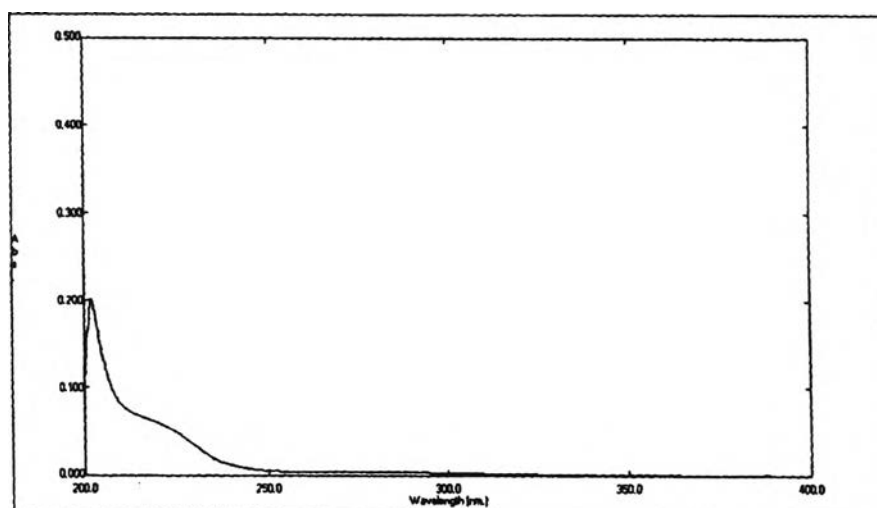


Figure C9 Absorption spectrum of L-595:PEG-8-L without PTU in water diluted with isopropanol

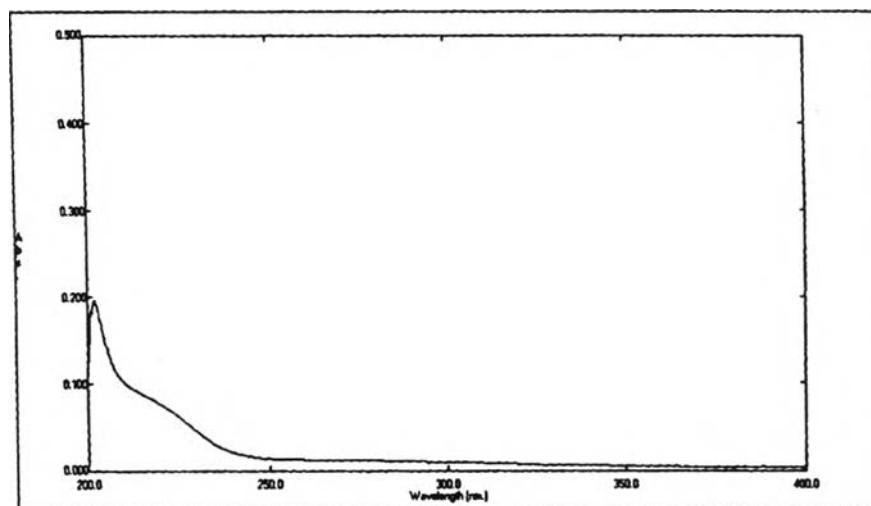


Figure C10 Absorption spectrum of L-595:PEG-8-L without PTU in phosphate buffer pH 7.4 diluted with isopropanol

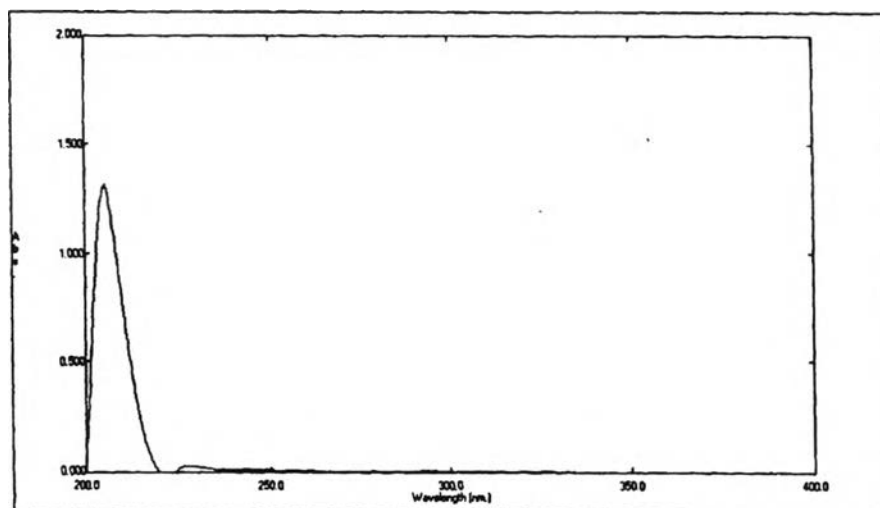


Figure C11 Absorption spectrum of Span® 20:CHO:Solulan® C24 without PTU in water diluted with isopropanol

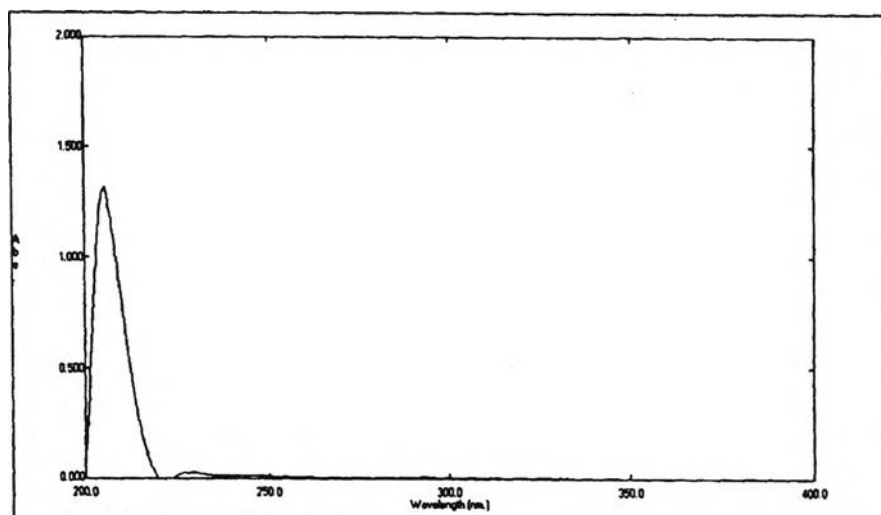


Figure C12 Absorption spectrum of Span[®] 20:CHO:Solulan[®] C24 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

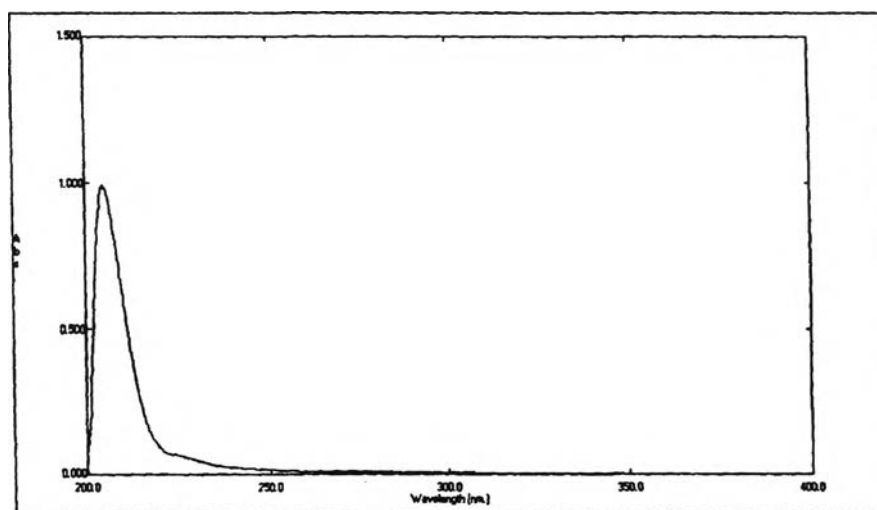


Figure C13 Absorption spectrum of Span[®] 40:CHO:Solulan[®] C24 without PTU in water diluted with isopropanol

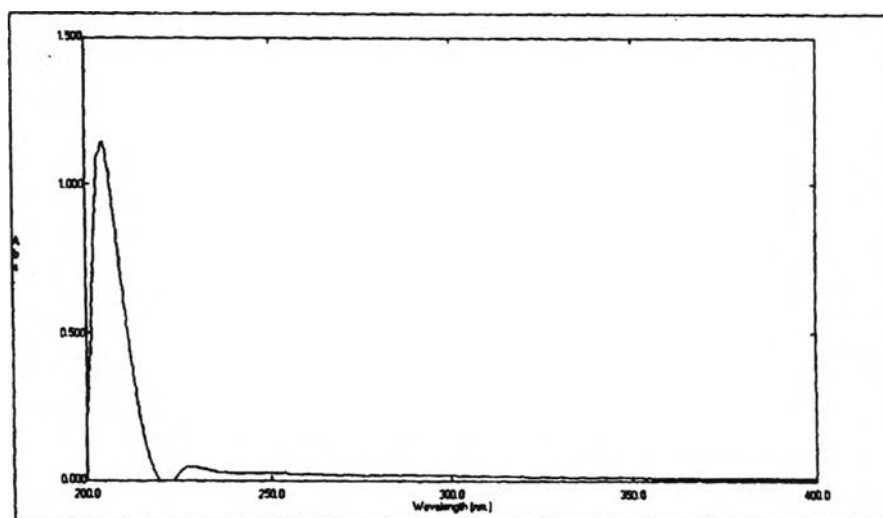


Figure C14 Absorption spectrum of Span[®] 40:CHO:Solulan[®] C24 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

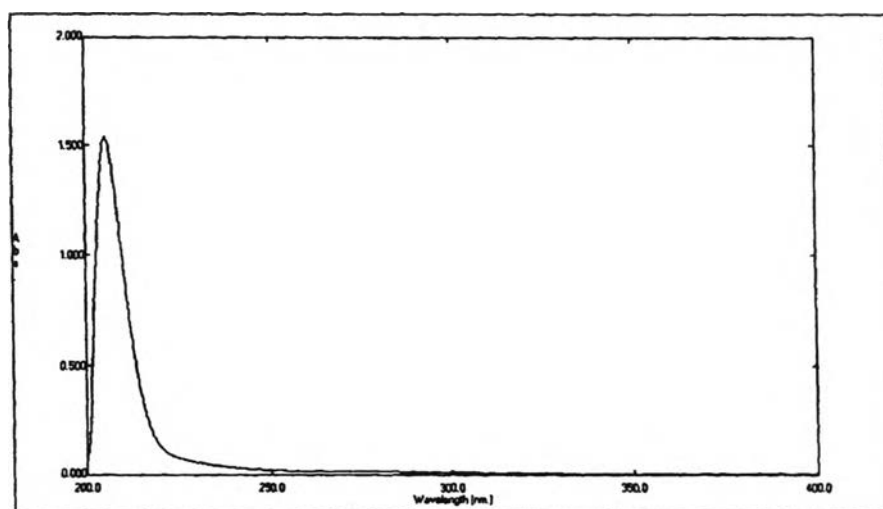


Figure C15 Absorption spectrum of Span[®] 60:CHO:Solulan[®] C24 without PTU in water diluted with isopropanol

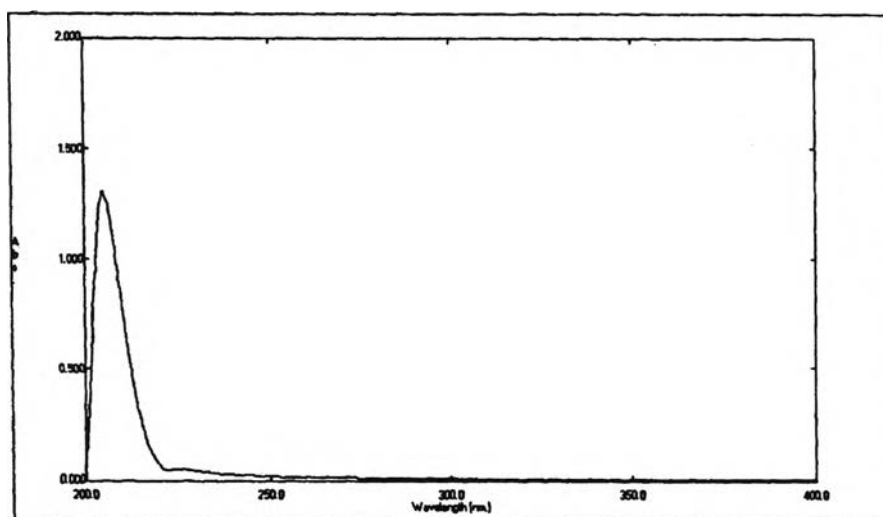


Figure C16 Absorption spectrum of Span[®] 60:CHO:Solulan[®] C24 without PTU in phosphate buffer pH 7.4 diluted with isopropanol

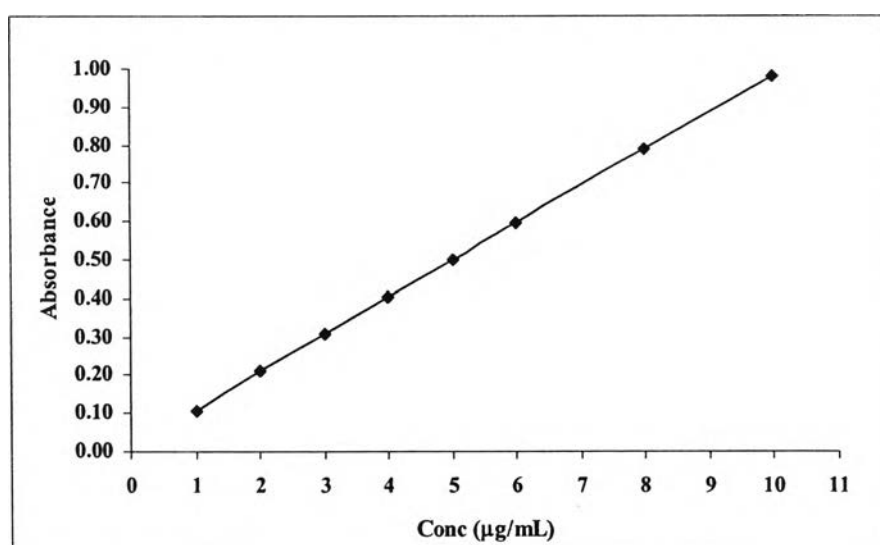
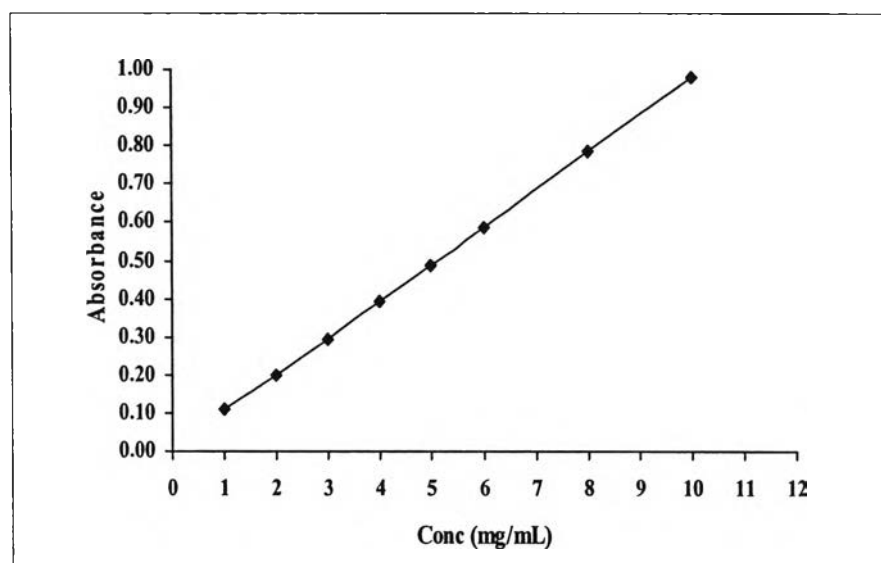


Figure C17 A representative of standard curve of standard solution of PTU in water diluted with isopropanol

Where $y = 0.0157 + 0.0967x$; $r^2 = 0.9999$

$y = \text{absorbance}$, $x = \text{PTU concentration } (\mu\text{g/mL})$



FigureC 18 A representative of standard curve of standard solution of PTU in phosphate buffer pH 7.4 diluted with isopropanol

Where $y = 0.0046 + 0.0972x$; $r^2 = 0.9999$

y = absorbance, x = PTU concentration ($\mu\text{g/mL}$)

Table C1 The percentages of analytical recovery of PTU in water diluted with isopropanol by UV spectrophotometric method

Actual concentration of PTU ($\mu\text{g/mL}$)	Calculated concentration of PTU ($\mu\text{g/mL}$)	% Analytical recovery
2.50	2.48	99.20
	2.48	99.20
	2.48	99.20
	2.57	102.80
	2.47	98.80
	2.47	98.80
5.50	5.48	99.64
	5.48	99.64
	5.36	97.45
	5.59	101.64
	5.38	97.82
	5.38	97.82
8.50	8.56	100.71
	8.51	100.12
	8.36	98.35
	8.76	103.06
	8.38	98.56
	8.43	99.18

Mean % Recovery = 99.56

SD = 1.60

%CV = 1.60

Table C2 The percentages of analytical recovery of PTU in phosphate buffer pH 7.4 diluted with isopropanol by UV spectrophotometric method

Actual concentration of PTU ($\mu\text{g/mL}$)	Calculated concentration of PTU ($\mu\text{g/mL}$)	% Analytical recovery
2.50	2.50	100.00
	2.52	100.80
	2.44	97.60
	2.41	97.40
	2.46	98.40
	2.44	97.60
5.50	5.72	104.00
	5.62	102.18
	5.55	100.91
	5.40	98.18
	5.66	102.91
	5.55	100.91
8.50	8.55	100.59
	8.49	99.88
	8.33	98.00
	8.53	100.35
	8.49	99.88
	8.46	99.53

Mean % Recovery = 99.95

SD = 1.88

%CV = 1.89

Table C3 The within run precision of PTU in water diluted with isopropanol by UV spectrophotometric method

Conc.($\mu\text{g/mL}$)	Calculated Conc.($\mu\text{g/mL}$)						Mean	SD	%CV
	1	2	3	4	5	6			
2.50	2.4536	2.4089	2.4313	2.4031	2.3978	2.4219	2.4644	0.0182	0.74
5.50	5.7397	5.6592	5.5821	5.4369	5.6939	5.5821	5.6157	0.1073	1.91
8.50	8.5705	8.5388	8.3777	8.3909	8.5488	8.3560	8.4638	0.0986	1.16

Table C4 The within run precision of PTU in phosphate buffer pH 7.4 diluted with isopropanol by UV spectrophotometric method

Conc.($\mu\text{g/mL}$)	Calculated Conc.($\mu\text{g/mL}$)						Mean	SD	%CV
	1	2	3	4	5	6			
2.50	2.5295	2.4934	2.4728	2.5429	2.4388	2.5408	2.5030	0.0421	1.68
5.50	5.4902	5.4794	5.3959	5.4866	5.4794	5.4143	5.4576	0.413	0.76
8.50	8.6406	8.6290	8.6060	8.4908	8.3954	8.3834	8.5242	0.1173	1.38

Table C5 The between run precision of PTU in water diluted with isopropanol by UV spectrophotometric method

Conc.($\mu\text{g/mL}$)	Calculated Conc.($\mu\text{g/mL}$)					Mean	SD	%CV
	Day1	Day2	Day3	Day4	Day5			
2.50	2.5009	2.4644	2.5115	2.4557	2.4867	2.4838	0.0236	0.95
5.50	5.4466	5.6157	5.4608	5.5377	5.5158	5.5153	0.0676	1.23
8.50	8.4663	8.4638	8.4908	8.5322	8.5341	8.4974	0.0343	0.40

Table C6 The between run precision of PTU in phosphate buffer pH 7.4 diluted with isopropanol by UV spectrophotometric method

Conc($\mu\text{g/mL}$)	Calculated Conc.($\mu\text{g/mL}$)					Mean	SD	%CV
	Day1	Day2	Day3	Day4	Day5			
2.50	2.4617	2.5030	2.4885	2.4829	2.5104	2.4893	0.0189	0.76
5.50	5.5818	5.4576	5.4693	5.5093	5.5680	5.5172	0.0563	1.02
8.50	8.4721	8.5242	8.5095	8.6293	8.6633	8.5597	0.0822	0.96

APPENDIX D

Validation of HPLC Method
(The United States Pharmacopieal Convention, 2002)

Validation for the Quantitative Determination of PTU in PBS pH 7.4 Solution by HPLC Method

1. Specificity

Under the HPLC method used, the peak chromatogram of PTU must not be interfered by the peak chromatogram of other components in the sample. The blank vesicular suspension (without PTU) and PTU vesicular suspension were prepared. The chromatogram of the blank vesicular suspension was compared with chromatogram of the PTU vesicular suspension.

2. Linearity

Eight standard solutions of PTU ranging of 0.05 to 10.0 $\mu\text{g/mL}$ for PBS system and 0.10 to 10.0 $\mu\text{g/mL}$ for methanol system, were prepared and analyzed. Linear regression analysis of the absorbance versus their concentrations was performed. The linearity was determined from the coefficient of determination.

3. Accuracy

PTU at 0.15, 5.0 and 8.5 $\mu\text{g/mL}$ for PBS system and 0.30, 5.0 and 8.5 $\mu\text{g/mL}$ and surfactant/cholesterol mixtures 100 mg/mL were prepared. Three sets of each concentration were prepared. Each individual sample was analyzed by HPLC method, and percent analytical recovery of each sample was calculated.

4. Precision

4.1 Within run precision

The within run precision was evaluated by analyzing six sets of the three standard solutions of PTU in six intervals of time in the same day. The mean, standard deviation (SD) and the coefficient of variation (%CV) of each standard solution were determined.

4.2 Between run precision

The between run precision was evaluated by comparing each concentration of five sets of standard solutions were prepared and analyzed in different days. The mean, standard deviation (SD) and the coefficient of variation (%CV) of each standard solution were determined.



Validation for the Quantitative Determination of PTU in PBS pH 7.4 Solution by HPLC Method

The validation of the analytical method is the process by which performance characteristics of the method are established to meet the requirements for the intended analytical parameters. The analytical parameters used for the assay validation were specificity, linearity, accuracy and precision.

1. Specificity

The specificity of an analytical method is its ability to measure the given analyte accurately and specificity in the presence of other components in the sample. The chromatograms (Figure D1-D8) indicated that the conditions used was the optimal condition giving the highest sensitivity without interference of surfactants, cholesterol and Solulan[®] C24 which showed no peak chromatograms at the peak of internal standard and PTU. The retention time of PTU and theophylline were about 5.1 and 8.9, respectively. Thus these two peaks were completely separated from each other.

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. The linearity is usually expressed in term of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The standard curves of PTU solution diluted with PBS and methanol were shown in Figures D9-D10, respectively. The standard curves were found to be linear with coefficient of determination 0.9999 and 0.9999, respectively. These results indicated that HPLC method was acceptable for quantitative analysis of PTU in the range studied. The equations of standard curves according to Beer's Law were used for calculating the concentration of PTU.

3. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy may often be expressed as percent recovery by

the assay of known, added amount of analyte. The percentages of analytical recovery of each PTU concentration in PBS and methanol are shown in Table D1 and D2. All the percentage analytical recovery of all drug concentrations in water with a mean of and a %CV of, and in phosphate buffer pH 7.4 with a mean of and a %CV of, indicated the high accuracy of this method. Thus, it could be used for analysis of PTU in all concentrations used.

4. Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation).

The precision of the analysis of PTU in PBS and methanol by HPLC method was determined both within run precision and between run precision as illustrated in Tables D3-D6. All percentage coefficient of variation values were lower than 2.00%, indicating that of the UV spectrophotometric method used were precise for quantitative analysis of PTU in the range studied.

In conclusion, the analysis of PTU in water PBS and methanol by HPLC method method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for determination of the content of PTU.

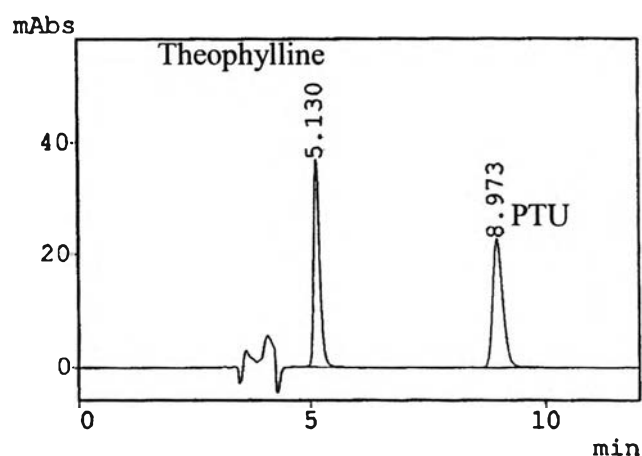


Figure D1 HPLC chromatogram of PTU (3.0 $\mu\text{g}/\text{mL}$) and theophylline (5.0 $\mu\text{g}/\text{mL}$) in methanol

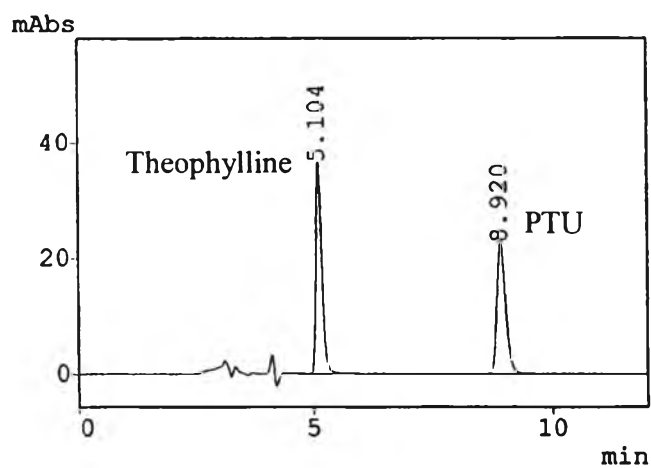


Figure D2 HPLC chromatogram of PTU (3.0 $\mu\text{g/mL}$) and theophylline (5.0 $\mu\text{g/mL}$) in PBS

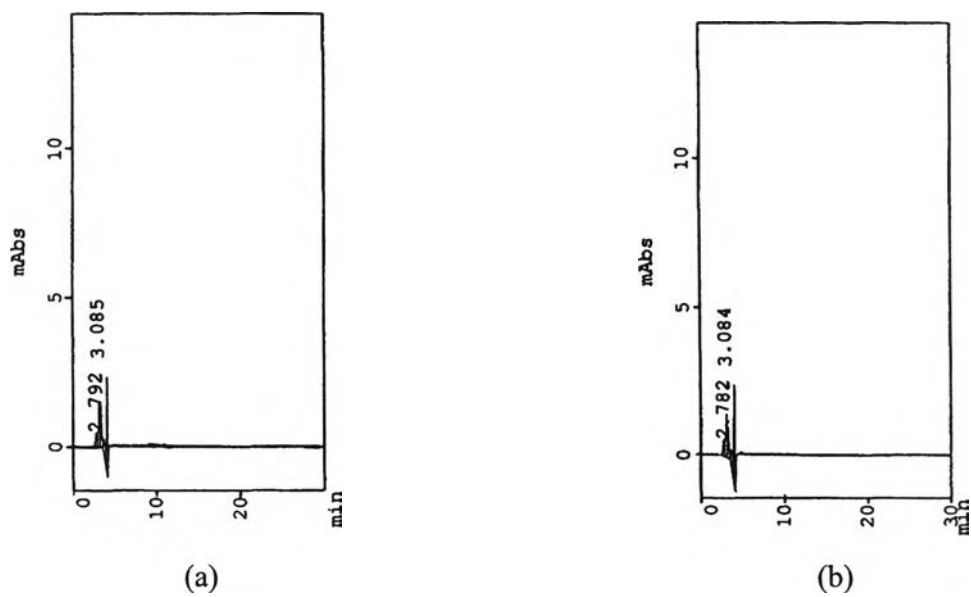


Figure D3 HPLC chromatogram of (a) = GDS:CHO:Brij[®] 76 in PBS
(b) = L-595:PEG-8-L in PBS

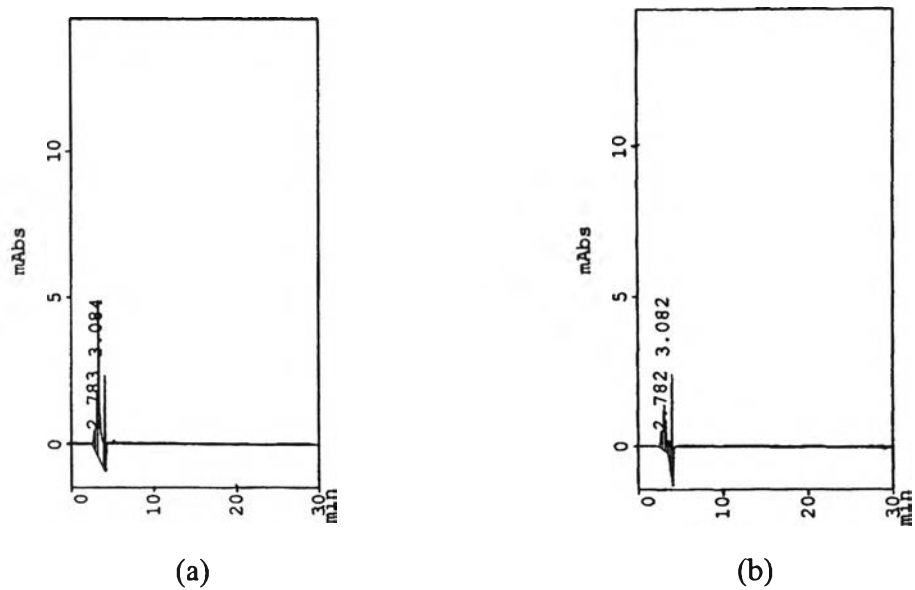


Figure D4 HPLC chromatogram of (a) = Span[®] 40:CHO:Solulan[®] C24 in PBS
(b) = Span[®] 20:CHO:Solulan[®] C24 in PBS

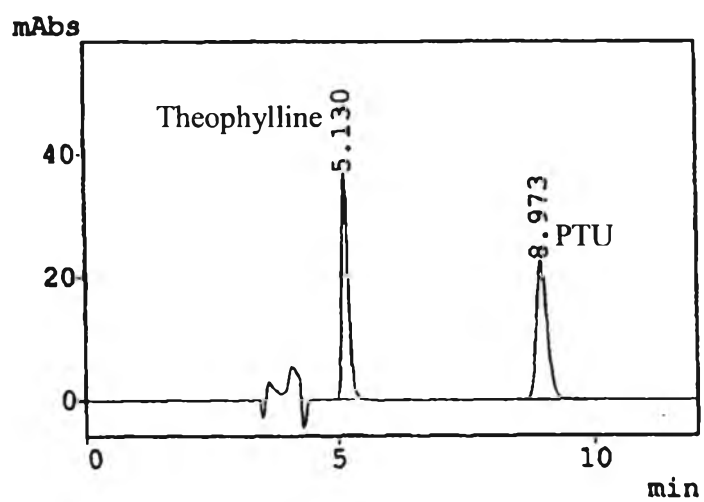


Figure D5 HPLC chromatogram of PTU (3.0 $\mu\text{g}/\text{mL}$) and theophylline (5.0 $\mu\text{g}/\text{mL}$)
in GDS:CHO:Brij[®] 76 in methanol

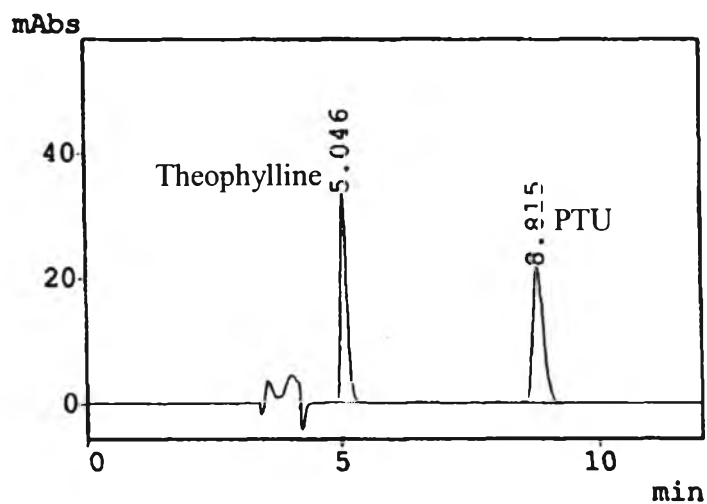


Figure D6 HPLC chromatogram of PTU (3.0 $\mu\text{g}/\text{mL}$) and theophylline (5.0 $\mu\text{g}/\text{mL}$) in L-595:PEG-8-L in methanol

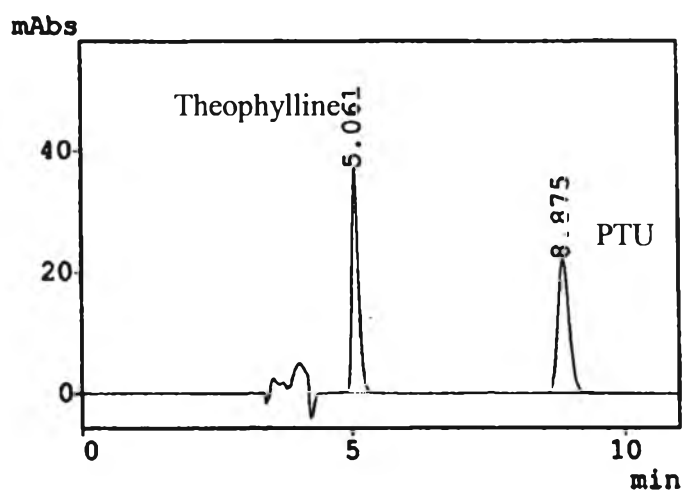


Figure D7 HPLC chromatogram of PTU (3.0 $\mu\text{g}/\text{mL}$) and theophylline (5.0 $\mu\text{g}/\text{mL}$) in Span[®] 40:CHO:Solulan[®] C24 in methanol

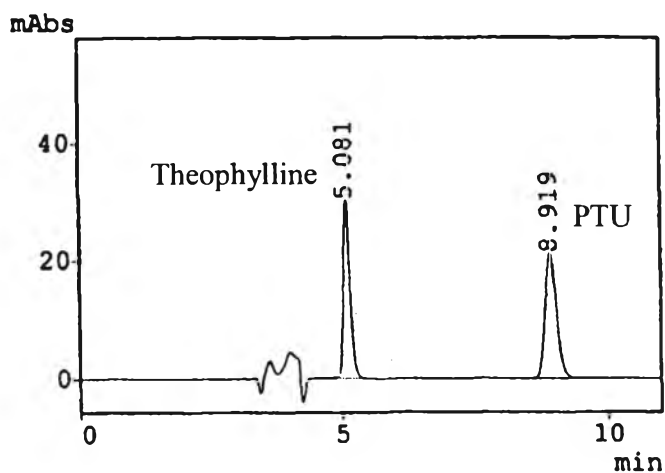


Figure D8 HPLC chromatogram of PTU (3.0 $\mu\text{g/mL}$) and theophylline (5.0 $\mu\text{g/mL}$) in Span[®] 20:CHO:Solulan[®] C24 in methanol

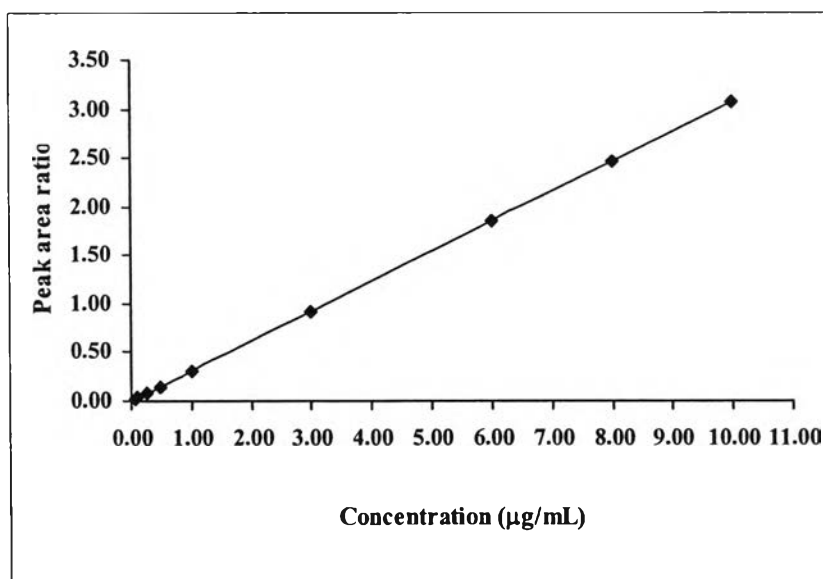


Figure D9 A representative of standard curve of standard solution of PTU diluted with PBS

Where $y = 0.3083x - 0.0024$; $r^2 = 0.9999$
 $y = \text{absorbance}$, $x = \text{PTU concentration } (\mu\text{g/mL})$

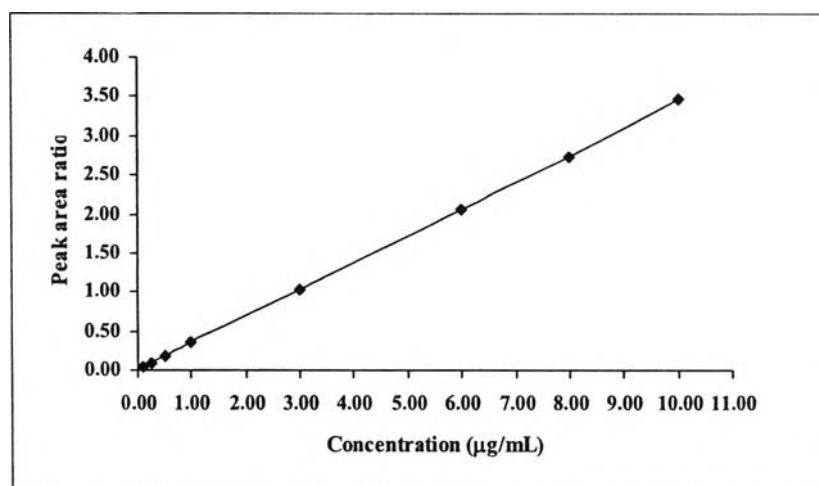


Figure D10 A representative of standard curve of standard solution of PTU diluted with methanol

Where $y = 0.3432x + 0.0062$; $r^2 = 0.9999$

$y =$ absorbance, $x =$ PTU concentration ($\mu\text{g/mL}$)

Table D1 The percentages of analytical recovery of PTU in water diluted with PBS pH 7.4

Actual concentration of PTU ($\mu\text{g/mL}$)	Calculated concentration of PTU ($\mu\text{g/mL}$)	% Analytical recovery
0.15	0.153	101.96
	0.152	101.17
	0.153	101.20
	0.149	99.03
	0.154	102.37
	0.147	97.82
5.00	4.921	98.41
	4.949	98.98
	5.015	100.29
	4.994	99.88
	4.970	99.40
	4.991	99.82
8.50	8.399	98.81
	8.542	100.49
	8.564	100.75
	8.417	99.03
	8.454	99.46
	8.485	99.82

Mean % Recovery = 99.92

SD = 1.22

%CV = 1.22

Table D2 The percentages of analytical recovery of PTU diluted with methanol

Actual concentration of PTU ($\mu\text{g/mL}$)	Calculated concentration of PTU ($\mu\text{g/mL}$)	% Analytical recovery
0.30	0.306	101.83
	0.294	98.05
	0.298	99.34
	0.297	99.07
	0.296	98.55
	0.305	101.66
5.00	5.028	100.55
	4.945	98.90
	4.935	98.70
	4.998	99.96
	5.033	100.6
	5.012	100.25
8.50	8.551	100.60
	8.469	99.64
	8.641	101.66
	8.349	98.22
	8.670	102.01
	8.380	98.59

Mean % Recovery = 99.90

SD = 1.31

%CV = 1.31

Table D3 The within run precision of PTU diluted with PBS pH 7.4 by HPLC method

Conc.($\mu\text{g}/\text{mL}$)	Calculated Conc.($\mu\text{g}/\text{mL}$)						Mean	SD	%CV
	1	2	3	4	5	6			
0.15	0.1499	0.1514	0.1501	0.1467	0.1498	0.1479	0.1493	0.0017	1.12
5.00	5.1065	5.2126	5.0555	5.0502	5.0583	5.0877	5.0952	0.0615	1.21
8.50	8.6389	8.6476	8.3157	8.6187	8.6151	8.5864	8.5704	0.1266	1.48

Table D4 The within run precision of PTU diluted with methanol by HPLC method

Conc.($\mu\text{g}/\text{mL}$)	Calculated Conc.($\mu\text{g}/\text{mL}$)						Mean	SD	%CV
	1	2	3	4	5	6			
0.30	0.3055	0.3051	0.3000	0.3037	0.3055	0.2992	0.3028	0.0032	1.06
5.00	5.0276	4.9913	4.9756	4.9955	5.0753	5.0920	5.0543	0.0516	1.02
8.50	8.5509	8.4721	8.5228	8.4638	8.4663	8.5720	8.5007	0.0618	0.73

Table D5 The between run precision of PTU diluted with PBS pH 7.4 by HPLC method

Conc.($\mu\text{g}/\text{mL}$)	Calculated Conc.($\mu\text{g}/\text{mL}$)					Mean	SD	%CV
	Day1	Day2	Day3	Day4	Day5			
0.15	0.1491	0.1510	0.1521	0.1499	0.1485	0.1501	0.0014	0.96
5.00	5.0100	4.9769	4.9290	5.0570	4.9938	4.99933	0.0468	0.94
8.50	8.3529	8.3667	8.5486	8.3358	8.3297	8.3867	0.916	1.09

Table D6 The between run precision of PTU diluted with methanol by HPLC method

Conc($\mu\text{g/mL}$)	Calculated Conc.($\mu\text{g/mL}$)					Mean	SD	%CV
	Day1	Day2	Day3	Day4	Day5			
0.30	0.2980	0.2972	0.3028	0.2949	0.3038	0.2993	0.0038	1.26
5.50	4.9348	4.9981	4.9830	4.9349	4.9567	4.9615	0.0285	0.57
8.50	8.6413	8.3486	8.5227	8.6478	8.6582	8.5637	0.1323	1.54

Appendix E

Photographs of PTU Niosomes

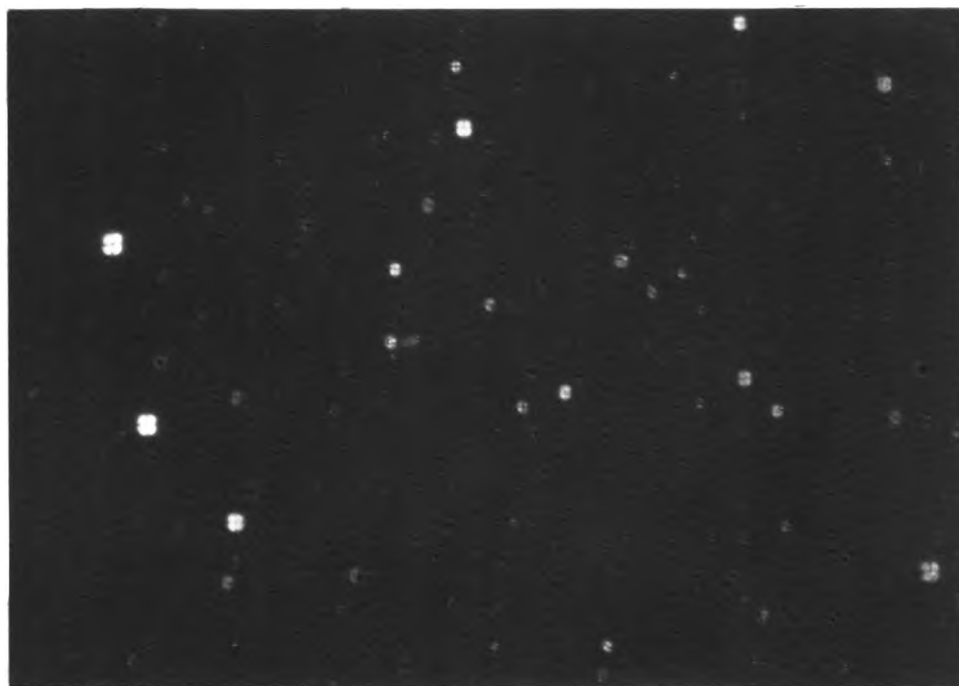


Figure E1 Polarized-light microscopic image of the Span[®] 20:CHO:Solulan[®] C-24 vesicles (x 400)

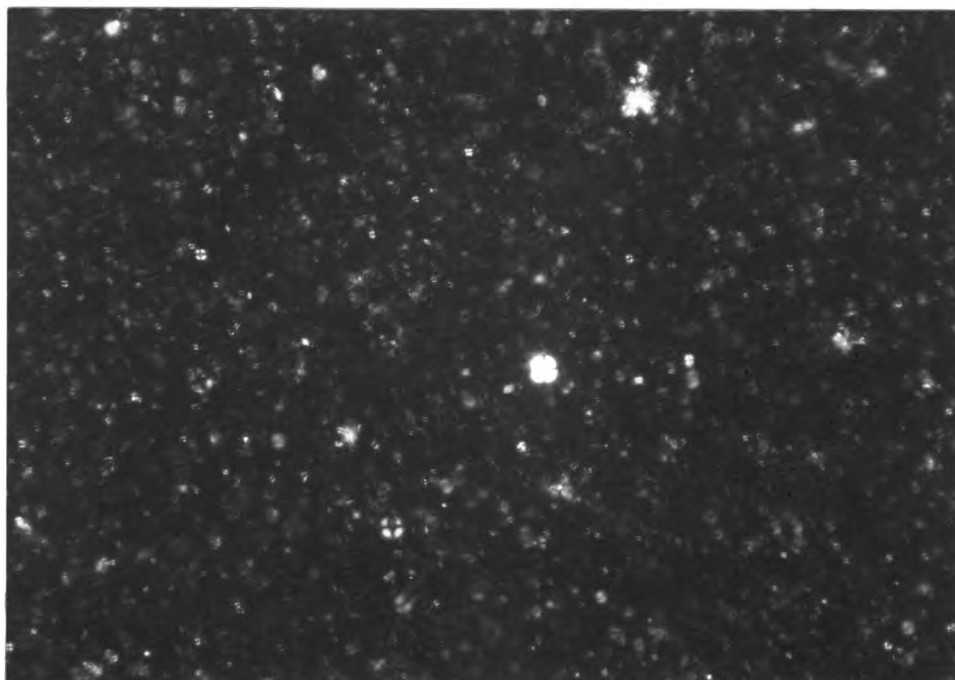


Figure E2 Polarized-light microscopic image of the Span[®] 40:CHO:Solulan[®] C-24 vesicles (x 400)





Figure E3 Polarized-light microscopic image of the GDS:CHO:Brij[®] 76 vesicles (x 400)

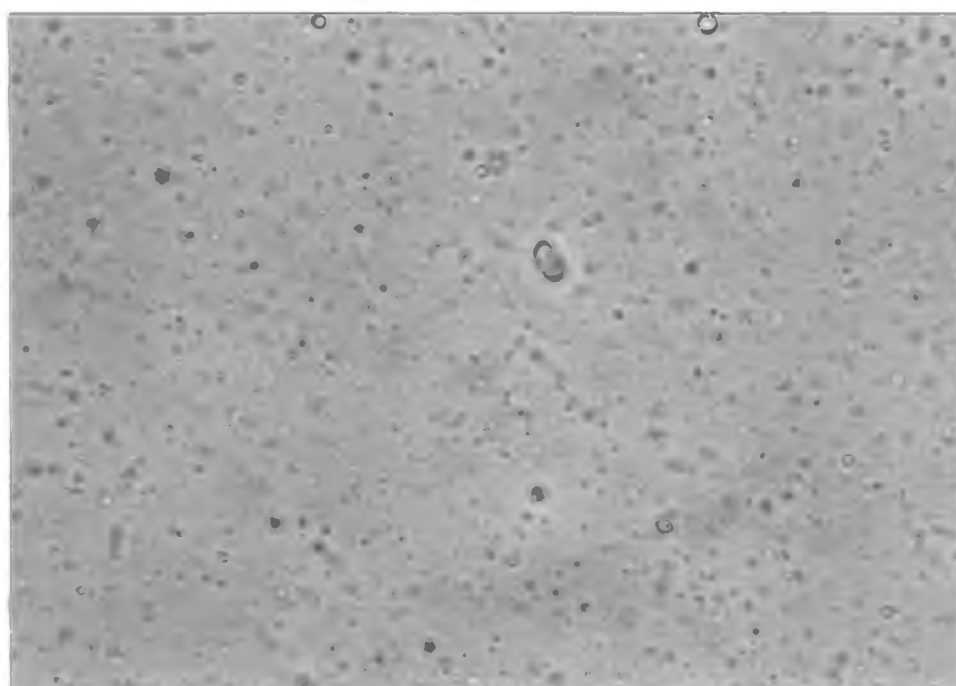


Figure E4 Photograph of niosomes prepared by Span[®] 20:CHO:Solulan[®] C-24 vesicles (x 1000)

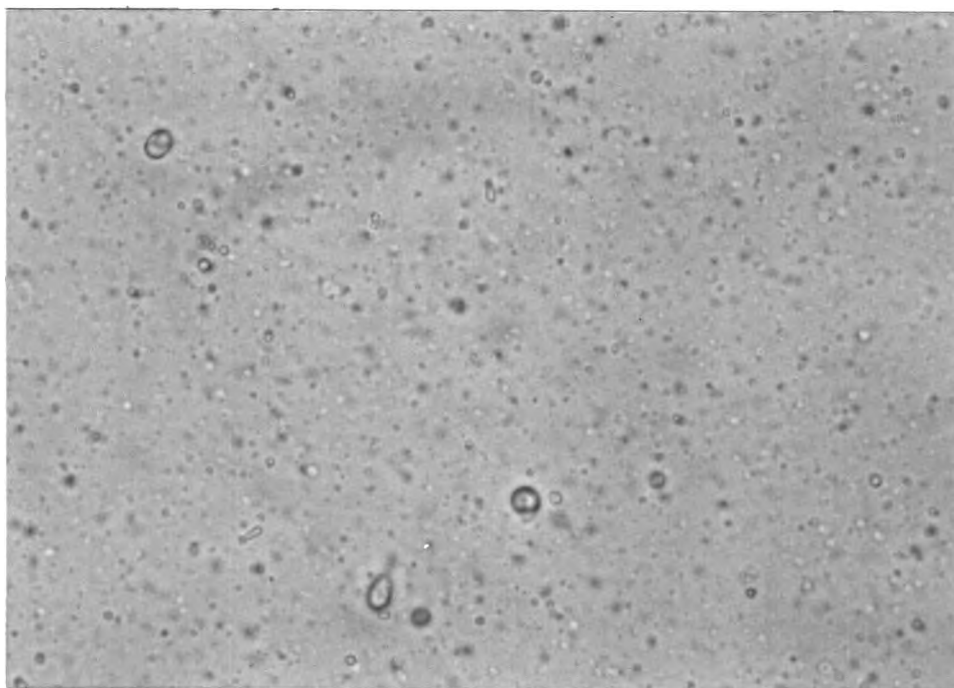


Figure E5 Photograph of niosomes prepared by Span[®] 40:CHO:Solulan[®] C-24 vesicles (x 1000)

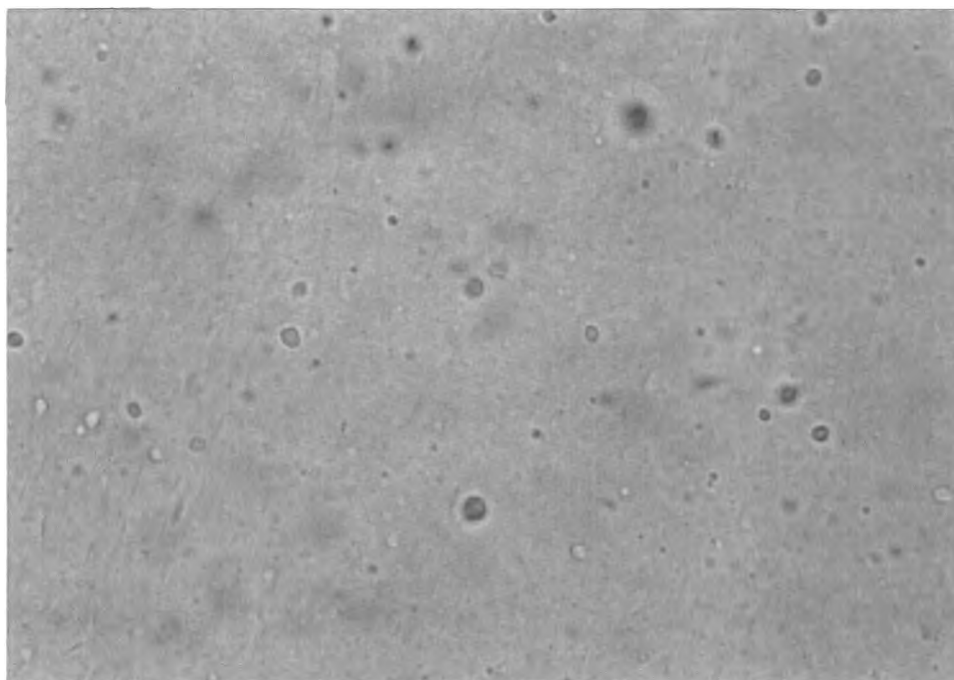
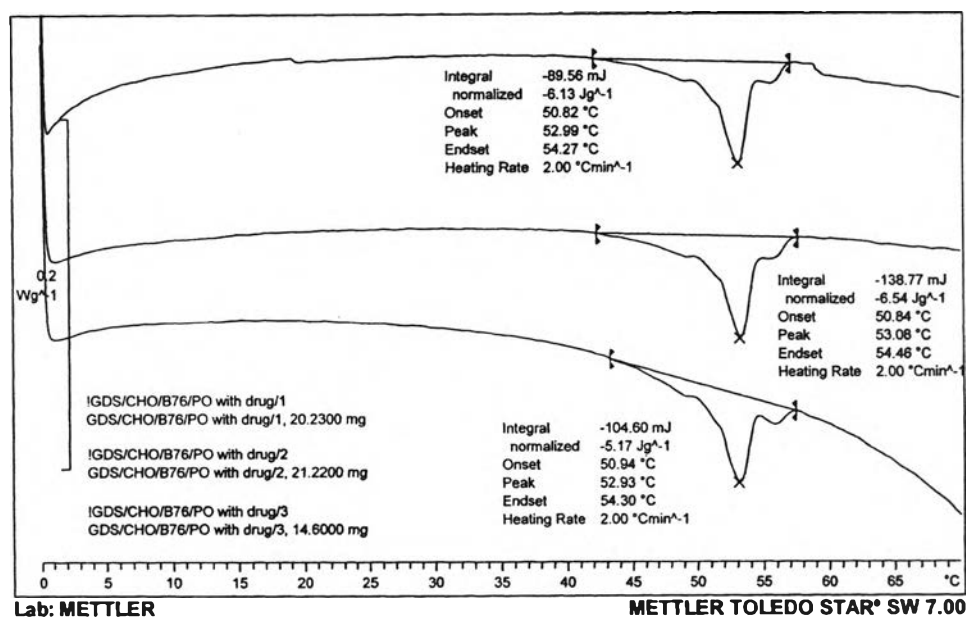
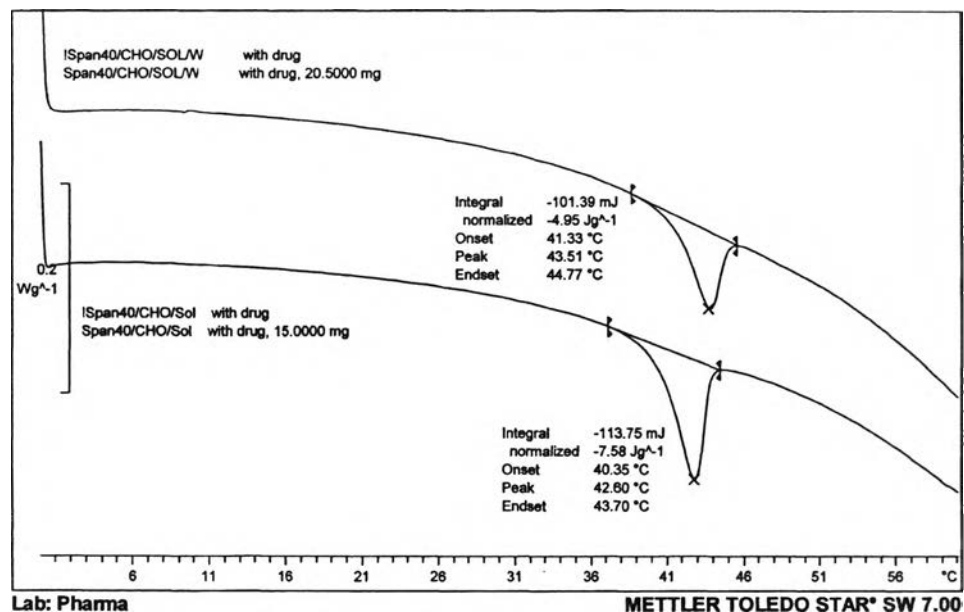


Figure E6 Photograph of niosomes prepared by GDS:CHO:Brij[®] 76 vesicles (x 1000)

APPENDIX F

DSC Thermogram of PTU Niosomes

Figure F1 DSC thermogram of GDS:CHO:Brij[®] 76 niosomesFigure F2 DSC thermogram of Span[®] 40:CHO:Solulan[®] C24 niosomes

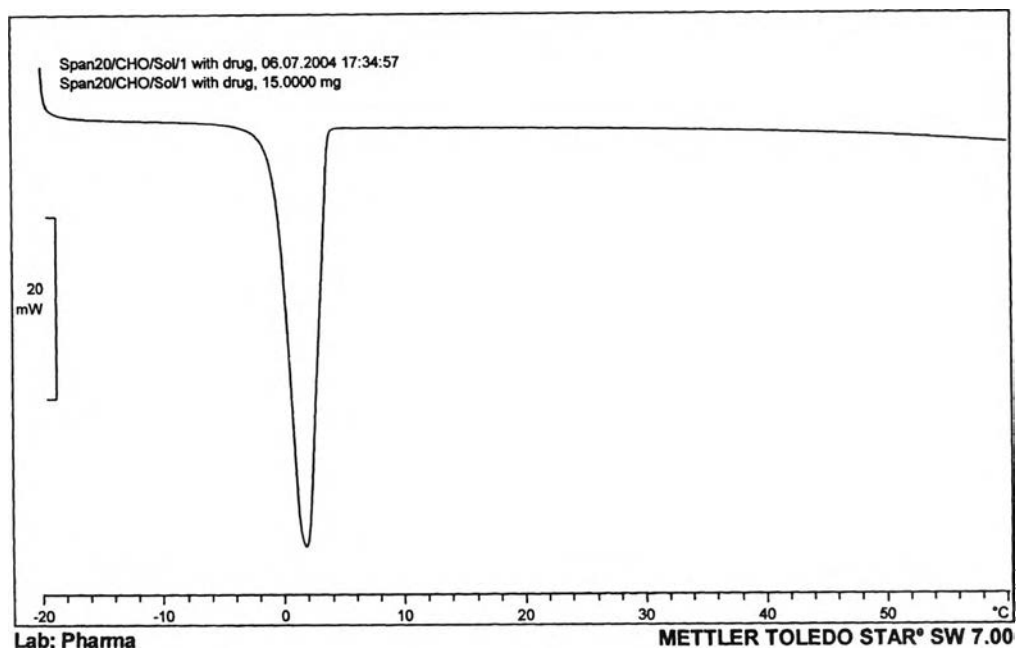


Figure F3 DSC thermogram of Span[®] 20:CHO:Solulan[®] C24 niosomes

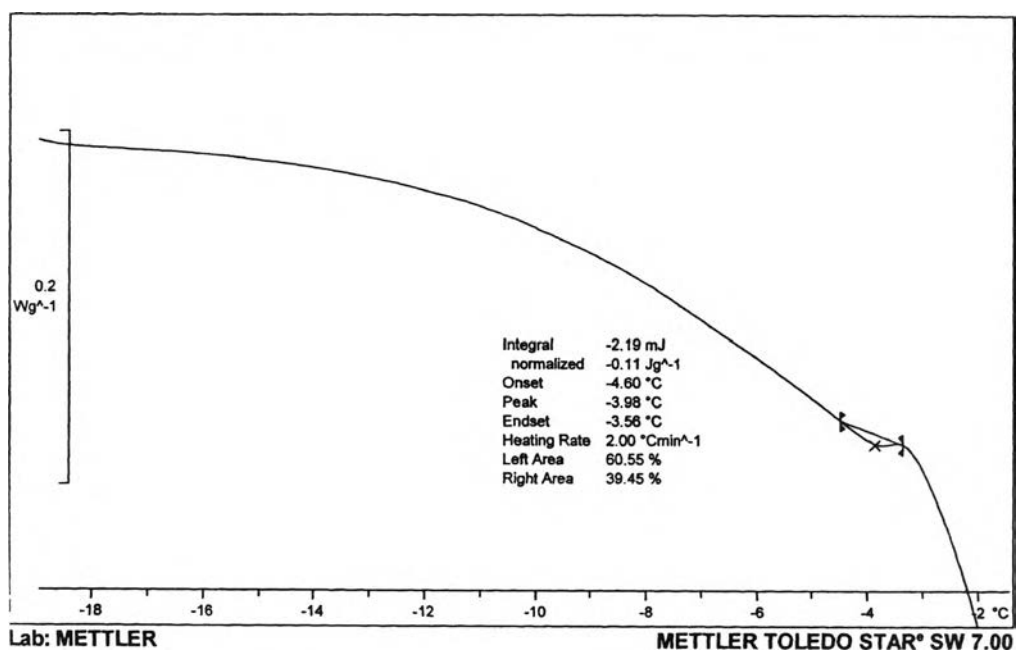


Figure F4 DSC thermogram of L-595:PEG-8-L niosomes

VITA

Ms Waraporn Suwakul was born on January 5, 1955 in Nakornpathom, Thailand. She received the Master of Sciences in Pharmacy from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok in 1983. Since graduation, she has worked as a faculty member at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok. She entered the doctorate program in Pharmaceutics at Chulalongkorn University in 2000.

