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Title:

### **Liposome-mediated Targeting of Finasteride to the Hair Follicles**

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## **Liposome-mediated targeting of finasteride to the hair follicles**

### **Abstract**

**Introduction:** Targeted delivery of finasteride, indicated orally in the treatment of alopecia and some other pilosebaceous disorders, to the hair follicles can increase the concentration of the drug at pilosebaceous units (PU) while reducing its systemic side effects.

**Methods:** Finasteride-entrapped multilamellar liposomes/niosomes (MLVs) were prepared by the film formation and reverse evaporation methods. The MLVs were characterized with regard to the size, drug entrapment efficiency, and release behavior in various media and drugs permeability through different membranes. *In vivo* deposition of <sup>3</sup>H-finasteride into PU and other compartments of the hamster ear was determined following topical application of finasteride-MLVs and a hydroalcoholic finasteride solution (HA) by scraping method. The decrease in the area of the ear and flank sebaceous glands after twice a day/4 weeks topical application was quantitatively determined by planimetry of histological sections of skin.

**Results:** Finasteride permeation through hamster flank skin was faster from finasteride-HA than from finasteride-vesicles (0.13 vs. 0.025-0.058  $\mu\text{g}/\text{cm}^2\cdot\text{h}$ , respectively), ( $p < 0.05$ ). The  $T_{50\%}$  release from liquid-state liposomes/niosomes into artificial sebum at 37 °C ranged from 2.5 to 7.5 h. Un-extruded charged MLVs comprising liquid-state amphiphiles of Brij97, dimyristoyl phosphatidylcholine (DMPC), Brij97:Brij76 (1:1 mole ratio) deposited 2.05-2.5% of the applied dose to the

PU. This was significantly higher than 0.35-0.92%, which was deposited by extruded vesicles (100, 400 nm) of the same liquid-state amphiphiles, gel-state vesicles, or finasteride-HA. Selected finasteride-MLVs, HA of finasteride and a hydroalcoholic progesterone solution (positive control) significantly decreased the size of PU of ear and flank organ located under application site (36-48%,  $p < 0.01$ ), while HA of finasteride exhibited a systemic effect as determined by measuring the PU size in untreated side of the flank ( $p < 0.05$ ).

**Conclusion:** Both *in vivo* deposition and *in vivo* bioassay studies demonstrated the potential of charged liquid-state MLVs for effective delivery of finasteride to the PU.

**Key words:** Finasteride, niosome, liposome, deposition and targeting, pilosebaceous unit, hamster ear and flank

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## List of abbreviations

<b>Chol</b>	<b>Cholesterol</b>
<b>CPM</b>	<b>Count Per Minute</b>
<b>CPP</b>	<b>Critical Packing Parameter</b>
<b>DCP</b>	<b>Dicethyl phosphate</b>
<b>DHT</b>	<b>Dihydro testosterone</b>
<b>DMPC</b>	<b>Dimyristoyl phosphatidylcholine</b>
<b>DPM</b>	<b>Disintegration Per Minute</b>
<b>DPPC</b>	<b>DIPALMITOYLPHOSPHATIDYLECHOLINE</b>
<b>DSC</b>	<b>Differential Scanning Calorimetry</b>
<b>EE</b>	<b>Entrapment Efficiency</b>
<b>EO</b>	<b>Ethylene Oxide</b>
<b>Fin.</b>	<b>Finasteride</b>
<b>GDL</b>	<b>Glyceryl di-laurate</b>
<b>GDS</b>	<b>Glyceryl di-stearate</b>
<b>HA</b>	<b>Hydroalcoholic solution</b>
<b>HSDSC</b>	<b>High sensitivity differential scanning calorimetry</b>
<b>LOD</b>	<b>Limit Of Detection</b>
<b>LOQ</b>	<b>Limit Of Quantitation</b>
<b>LUV</b>	<b>Large Unilamellar Vesicles</b>
<b>m. r.</b>	<b>Molar ratio</b>
<b>MLV</b>	<b>Multi Lamellar Vesicles</b>
<b>Mol%</b>	<b>Mole percent</b>
<b>NSVs</b>	<b>Non-ionic surfactant vesicles</b>
<b>PBS</b>	<b>Phosphate Buffered Saline</b>
<b>PC</b>	<b>Egg Phosphatidylcholine</b>
<b>PG</b>	<b>Propylene Glycol</b>
<b>POE</b>	<b>Polyoxyethylene</b>
<b>Prog.</b>	<b>Progesterone</b>
<b>PU</b>	<b>Pilosebaceous unit</b>
<b>RT</b>	<b>Room Temperature</b>
<b>SC</b>	<b>Stratum Corneum</b>
<b>SA</b>	<b>stearylamine</b>
<b>T</b>	<b>Testosterone</b>
<b>T<sub>c</sub></b>	<b>Transition Temperature</b>
<b>TEM</b>	<b>Transmission Electron Microscopy</b>

**References**

1. Lauer AC, Lieb LM, Ramachandran C, Flynn GL, Weiner N. Transfollicular drug delivery. *Pharm Res* 1995;12(2):179-86.
2. Gupchup GV, Zata J. Targeted delivery to pilosebaceous structures. *Cosmetics and Toiletries* 1997;112:79-88.
3. Osborne DW, Hatzenbuehler DA. The Influence of skin surface lipid on topical formulations. In: Osborne DW, Amann AH, editors. *Topical Drug Delivery Formulations*. New York: Marcel Dekker Inc., 1990.
4. Grams YY, Bouwstra JA. Penetration and distribution of three lipophilic probes in vitro in human skin focusing on the hair follicle. *J Controlled Release* 2002;83:253-62.
5. Lauer AC, Ramachandran C, Lieb LM, Niemiec SM, Weiner N. Targeted delivery to the pilosebaceous unit via liposomes. *Adv Drug Deliv Rev* 1996;18:311-24.
6. Niemiec SM, Ramachandran C, Weiner N. Influence of nonionic liposomal composition on topical delivery of peptide drug into pilosebaceous units: an in vivo study using the hamster ear model. *Pharm Res* 1995;12(8):1184-8.
7. Weiner N. Targeted follicular delivery of macromolecular via liposomes. *Int J Pharm* 1998;162:29-38.

8. Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. *Pharm Res* 1993;10(12):1738-44.
9. Sumian CC, Pitre FB, Gauthier BE, Bouclier M, Mordon SR. A new method to improve penetration depth of dyes into the follicular duct: potential application for laser hair removal. *J Am Acad Dermatol* 1999;41:172-5.
10. Tata S, Weiner N, Flynn G. Relative influence of ethanol and propylene glycol cosolvents on deposition of minoxidil into the skin. *J Pharm Sci* 1994;83:1508-10.
11. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev Res* 2000;50:406-15.
12. Wu HL, Ramachandran C, Weiner N, Roessler BJ. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. *Int J Pharm* 2001;220:63-75.
13. Lieb LM, Limmatta AP, Bryan RN, Krueger GG. Description and definition of a transfollicular route of permeant for topically applied agents to human scalp skin. *J Invest Dermatol* 1995;104:655.



14. Hueber F, Wepierre J, Schaefer H. Role of transepidermal and transfollicular routes in percutaneous absorption of hydrocortisone and testosterone: in vivo study in the hairless rat. *Skin Pharmacol* 1992;5:99-107.
15. Hueber F, Besnard M, Schaefer H, Wepierre J. Percutaneous absorption of estradiol and progesterone in normal and appendage-free skin of the hairless rat: lack of importance of nutritional blood flow. *Skin Pharmacol* 1994;7:245-56.
16. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: I. In vitro evaluation using fluorescent techniques with the hamster ear model. *J Invest Dermatol* 1992;99:108-13.
17. Lieb LM, Liimatta AP, Bryan RN, Brown BD, Krueger GG. Description of the intrafollicular delivery of large molecular weight molecules to follicles of human scalp *Skin In Vitro*. *J Pharm Sci* 1997;86(9):1022-9.
18. Lieb LM, Flynn G, Weiner N. Follicular (pilosebaceous unit) deposition and pharmacological behavior of cimetidine as a function of formulation. *Pharm Res* 1994;11(10):1419-23.
19. Li L, Lishko V, Hoffman RM. Liposomes can specifically target entrapped melanin to hair follicles in histocultured skin. *In Vitro Cell Dev Biol* 1993;29A:192-4.

20. Vaughan CD. Using solubility parameters in cosmetics formulations. *J Soc Cosmet Chem* 1985;36:319-33.
21. Nicolau G, Baughman RA, Tonelli A, McWilliams W, Schiltz J, Yacobi A. Deposition of viprostol (a synthetic PGE2 vasodilator) in the skin following topical administration to laboratory animals. *Xenobiotica* 1987;17:1113-20.
22. Lauer AC, Elder JT, Weiner ND. Evaluation of the hairless rat as a model for in vivo percutaneous absorption. *J Pharm Sci* 1997 Jan;86(1):13-8.
23. Hueber F, Schaefer H, Wepierre J. Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin. *Skin Pharmacol* 1994;7:237-44.
24. Fabin B, Touitou E. Localization of lipophilic molecules penetration rat skin in vivo by quantitative autoradiography. *Int J Pharm* 1991;74:59-65.
25. Mills OH, Kligman AM. A human model for assaying comedolytic substances. *Br J Dermatol* 1982;107:543-8.
26. Bojar RA, Cutcliffe AG, Graupe K, Cunliffe WJ, Holland KT. Follicular concentration of azelaic acid after a single topical application. *Br J Dermatol* 1993;129(4):399-402.
27. Kao J, Helman J. In vitro percutaneous absorption in mouse skin: influence of skin appendages. *Tox Appl Pharmacol* 1988;94:93-103.

28. Green PG, Hinz RS, Kim A, Szoka FC, Guy RH. Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm Res* 1991;8:1121-7.
29. Cullander C, Guy RH. Sites of iontophoretic current flow into the skin: identification and characterization with the vibrating probe electrode. *J Invest Dermatol* 1991;97:55-64.
30. Burnette RR, Ongpipattanakul B. Characterization of the pore transport properties and tissue alteration of excised human skin during iontophoresis. *J Pharm Sci* 1988;77:132-7.
31. Lauer AC. Percutaneous drug delivery to the hair follicle. In: Bronaugh RL, Maibach HI, editors. *Percutaneous Absorption Drug-Cosmetic-Mechanisms-Methodology*. 3rd ed. New York: Marcel Dekker, Inc.; 1999. p. 427-49.
32. Barry BW. Drug delivery routes in skin: a novel approach. *Adv Drug Deliv Rev* 2002;54(Suppl. 1):S31-S40.
33. Grams YY, Alaruikka S, Lashley L, Caussin J, Lynne W, Bouwstra JA. Permeant lipophilicity and vehicle composition influence accumulation of dyes in hair follicles of human skin. *Eur J Pharm Sci* 2003;18:329-36.
34. Plewig G., Luderschmidt C. Hamster ear model for sebaceous glands. *J Invest Dermatol* 1977;68:171-6.

35. Illel H, Schaefer H. Transfollicular percutaneous absorption: skin model for quantitative studies. *Acta Derm Venerol (Stockh)* 1988;68:427-30.
36. Illel H, Schaefer H, Wepierre J, Doucet O. Follicles play an important role in percutaneous absorption. *J Pharm Sci* 1991;80:424-7.
37. Touitou E, Meidan VM, Horwitz E. Methods for quantitative determination of drug localized in the skin. *J Controlled Release* 1998;56:7-21.
38. Liao S, Lin J, Dang MT, Zhang H, Kao YH, Fukuchi J, et al. Growth suppression of hamster flank organs by topical application of catechins, alizarin, curcumin, and myristoleic acid. *Arch Dermatol Res* 2001;293(4):200-5.
39. Weissmann A, Bowden J, Frank B, Horwitz SN, Frost P. Morphometric studies of the hamster flank organ: an improved model to evaluate pharmacologic effects on sebaceous glands. *J Invest Dermatol* 1984;82(5):522-5.
40. Chen C, Puy LA, Simard J, Li X, Singh SM, Labrie F. Local and systemic reduction by topical finasteride or flutamide of hamster flank organ size and enzyme activity. *J Invest Dermatol* 1995;105(5):678-82.
41. Matias JR, Orentreich N. The hamster ear sebaceous glands. I. Examination of the regional variation by stripped skin planimetry. *J Invest Dermatol* 1983;81(1):43-6.

42. Bernard E, Dubios J, Wepierre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. *J Pharm Sci* 1997;86(5):573-8.
43. Touitou E, Levi-Schaffer F, Dayan N, Alhaique F, Riccierr F. Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers. *Int J Pharm* 1994;103:131-6.
44. Li L, Lishko VK, Margolis LB, Hoffman RM. Product-delivering liposomes specifically target hair follicles in histocultured intact skin. *In Vitro Cell Dev Biol* 1992;28A:679-81.
45. Rougier A, Lotte C. The stripping technique. In: Shaw VP, Maibach HI, editors. *Topical drug bioavailability, bioequivalence and penetration*. New York: Plenum Press; 1993. p. 163-82.
46. Balsari AL, Morelli D, Menard S, Veronesi U, Colnaghi MI. Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. *FASEB J* 1994;8:226-30.
47. Chen C, Li X, Singh SM, Labrie F. Activity of 17beta-(N-alkyl/arylformamido) and 17beta-[(N-alkyl/aryl) alkyl/arylamido]-4-methyl-4-aza-5alpha-androstan-3-ones as 5alpha-reductase inhibitors in the hamster flank organ and ear. *J Invest Dermatol* 1998;111(2):273-8.

48. Matias JR, Malloy VL, Orentreich N. Synergistic antiandrogenic effects of topical combinations of 5 alpha-reductase and androgen receptor inhibitors in the hamster sebaceous glands. *J Invest Dermatol* 1988;91(5):429-33.
49. Lu B, Federoff HJ, Wang Y, Goldsmith LA, Scott G. Topical application of viral vectors for epidermal gene transfer. *J Invest Dermatol* 1997;108:803-8.
50. Niemiec SM, Ramachandran C, Weiner N. Perifollicular transgenic expression of human interleukin-1 receptor antagonist protein following topical application. *J Pharm Sci* 1997;86:701-8.
51. Li L, Lishko V, Hoffman RM. Liposome targeting of high molecular weight DNA to the hair follicles of histocultured skin: a model for gene therapy of the hair growth processes. *In Vitro Cell Dev Biol* 1993;29A:258-60.
52. Schreier H, Bouwstra JA. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *J Controlled Release* 1994;30:1-15.
53. Plessis JD, Ramachandran C, Weiner N, Muller DG. The Influence of lipid composition and lamellarity of liposomes on the physical stability of liposomes upon storage. *Int J Pharm* 1996;127:273-8.
54. Musial W, Kubis A. Preliminary assessment of alginic acid as a factor buffering triethanolamine interacting with artificial sebum. *Eur J Pharm Biopharm* 2003;55:237-40.

55. Bandak S, Ramu A, Barenholz Y, Gabuzon A. Reduced UV-induced degradation of doxorubicin encapsulated in polyethyleneglycol-coated liposomes. *Pharm Res* 1999;16(6):841-6.
56. Boswell GW, Buell D, Bekersky I. AmBisome (liposomal amphotericin B): a comparative review. *J Clin Pharmacol* 1998;38(7):583-92.
57. Gabizon A, Goren D, Cohen R, Barenholz Y. Development of liposomal anthracyclines: from basics to clinical applications. *J Controlled Release* 1998;53:275-9.
58. Manosroi A, Manosroi J. Microencapsulation of human insulin DEAE-dextran complex and the complex in liposomes by the emulsion non-solvent addition method. *J Microencapsul* 1997;14:761-8.
59. Manosroi A, Wongtrakul P, Manosroi J, Sakai H, Sugawara F, Yuasa M, et al. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. *Colloids Surf B Biointerfaces* 2003;30:129-38.
60. Tsuchihashi M, Harashima H, Kiwada H. Development of a pharmacokinetic/pharmacodynamic (PK/PD)-simulation system for doxorubicin in long circulating liposomes in mice using peritoneal P388. *J Controlled Release* 1999;61(1-2):9-19.

61. Agarwal R, Katare OP, Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *Int J Pharm* 2001;228:43-52.
62. Touitou E, Junginger HE, Weiner N, Nagai T, Mezei M. Liposomes as carriers for topical and transdermal delivery. *J Pharm Sci* 1994;83(9):1189-203.
63. Varshosaz J, Pardakhty A, Hajhashemi V, Najafabadi AR. Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. *Drug Deliv* 2003;10:251-62.
64. Vanlerberghe G, Morancais JL. Niosomes in perspective. *S T P Pharma Sciences* 1996;6(1):5-11.
65. van Hal DA. Nonionic surfactant vesicles for dermal and transdermal drug delivery. Ph.D. Thesis. Leiden University, The Netherlands; 1994.
66. Lawrence MJ, Chauhan S, Lawrence SM, Barlow DJ. The formation, characterization and stability of non-ionic surfactant vesicles. *S T P Pharma Sciences* 1996;6(1):49-60.
67. Hofland HEJ. Vesicles as transdermal drug delivery system Ph. D. Thesis. Leiden University, The Netherlands; 1992.



68. Bouwstra JA, van Hal DA, Hofland HEJ, Junginger HE. Preparation and characterization of nonionic surfactant vesicles. *Colloids Surf A: Physicochem Eng Aspects* 1997;123-124:71-80.
69. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int J Pharm* 1998;172(33):70.
70. Uchegbu IF, Double JA, Turton JA, Florence AT. Distribution, metabolism and tumoricidal activity of doxorubicin in sorbitan monostearate (Span 60) niosomes in the mouse. *Pharm Res* 1995;12:1019-24.
71. Bouwstra JA, Hofland HEJ. Niosomes. In: Kreuter J, editor. *Colloidal drug delivery systems*. New York: Marcel Dekker; 1994. p. 191-217.
72. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). *Int J Pharm* 1994;105:1-6.
73. Azmin MN, Florence AT, Handjani-vila RM, Stuart JFB, Vanlerberghe G, Whittaker JS. The effect of non-ionic surfactant vesicles (niosome) entrapment on the absorption and distribution of methotrexate in mice. *J Pharm Pharmacol* 1985;37:237-42.

74. Kiwada H, Niimura H, Kato Y. Tissue distribution and pharmacokinetic evaluation of the targeting efficiency of synthetic alkyl glycoside vesicles. *Chem Pharm Bull (Tokyo)* 1985;33:2475-82.
75. Assadullahi TP, Hider RCMAJ. Liposome formation from synthetic polyhydroxy lipids. *Biochem Biophys Acta* 1991;1083:271-6.
76. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes-nonionic surfactant vesicles. *J Pharm Pharmacol* 1985;37:863-8.
77. Chauhan S, Lawrence MJ. The preparation of polyoxyethylene containing non-ionic surfactant vesicles. *J Pharm Pharmacol* 1989;41:6P.
78. Montserrat K, Gratzel M, Tundo P. Light-induced charge injection in functional crown ether vesicles. *J Am Chem Soc* 1980;102:5527-9.
79. Darwish IA, Uchegbu IF. The evaluation of crown ether based niosomes as cation containing and cation sensitive drug delivery systems. *Int J Pharm* 1997;159:207-13.
80. Uchegbu IF, McCarthy D, Schatzlein A, Florence AT. Phase transitions in aqueous dispersions of the hexadecyl diglycerol ether (C16G2) non-ionic surfactants, cholesterol and cholesteryl poly-24-oxyethylene ether: vesicles, tubules, discosomes and micelles. *STP Pharm Sci* 1996;6(1):33-43.

81. Arunothayanun P, Uchegbu IF, Craig DQM, Turton JA, Florence AT. In vitro/in vivo characterisation of polyhedral niosomes. *Int J Pharm* 1999;183:57-61.
82. Uchegbu IF, Duncan R. Niosomes containing N-(2-hydroxypropyl) methacrylamide copolymer-doxorubicin (PK1): effect of preparation and choice of surfactant on niosome characteristics and a preliminary study of body distribution. *Int J Pharm* 1997;155:7-17.
83. Baillie AJ, Coombs GH, Dolan TF, Laurie J. Non-ionic surfactant vesicles, niosomes, as a delivery system for anti-leishmanial drug, sodium stibogluconate. *J Pharm Pharmacol* 1986;38:502-5.
84. Dimitrijevic D, Lamandin C, Uchegbu IF, Shaw AJ, Florence AT. The effect of monomers and of micellar and vesicular forms of non-ionic surfactants (Solulan C24 and Solulan 16) on Caco-2 cell monolayers. *J Pharm Pharmacol* 1997;49:611-6.
85. Hofland HEJ, Geest R, Bodde HE, Junginger HE, Bouwstra JA. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. *Pharm Res* 1994;11(5):659-64.
86. Hofland HEJ, Bouwstra JA, Verhoef JC, Buckton G, Chowdry BZ, Ponec M, et al. Safety aspects of non-ionic surfactant vesicles-a toxicity study related to

- physicochemical characteristics of non-ionic surfactants. *J Pharm Pharmacol* 1992;44:287-94.
87. Kiwada H, Niimura H, Fujisaki Y, Yamada S, Kato Y. Application of synthetic alkyl glycoside vesicles as drug carriers. I: preparation and physical properties. *Chem Pharm Bull (Tokyo)* 1985;33:753-9.
88. Gianasi E, Cociancich F, Uchegbu IF, Florence AT, Duncan R. Pharmaceutical and biological characterisation of a doxorubicin-polymer conjugate (PK1) entrapped in sorbitan monostearate Span 60 niosomes. *Int J Pharm* 1997;148:139-48.
89. Rowe RC, Sheskey PJ, Weller PJ. Handbook of pharmaceutical excipients. 4th ed. London, UK: The Pharmaceutical Press and American Pharmaceutical Association; 2003.
90. Carafa M, Santucci E, Alhaique F, Coviello T, Murtas E, Riecceiri FM, et al. Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. *Int J Pharm* 1998;160:51-9.
91. Santucci E, Carafa M, Coviello T, Murtas E, Riecceiri FM, Alhaique F, et al. Vesicles from polysorbate 20 and cholesterol- A simple preparation and a characterisation. *STP Pharm Sci* 1996;6:29-32.

92. Uchegbu IF, Schatzlein A, Vanlerberghe G, Morgatini N, Florence AT.  
Polyhedral non-ionic surfactant vesicles. *J Pharm Pharmacol* 1997;49:606-10.
93. New RRC. *Liposomes a practical approach*. New York: Oxford University Press; 1990.
94. Moser P, Marchandavier M, Labrude P, Handjani-vila RM, Vigneron C.  
Haemoglobin niosomes.1. Preparation, physicochemical and oxygen-carrying properties and stability. *Pharm Acta Helv* 1989;64:192-202.
95. Chopineau J, Lesier S, Ollivon M. Vesicle formation by enzymatic processes. *J Am Chem Soc* 1994;116:11582-3.
96. Talsma H, Steenbergen MJv, Borchert jCH, Cromelin DJA. A novel technique for the one-step preparation of liposomes and non-ionic surfactant vesicles without the use of organic solvents. Liposome formation in a continuous gas stream: The bubble method. *J Pharm Sci* 1994;83:276-80.
97. Stafford S, Baillie AJ, Florence AT. Drug effects on the size of chemically defined non-ionic surfactant vesicles. *J Pharm Pharmacol* 1988;40:26P.
98. Ganesan MG, Weiner ND, Flynn GL, Ho N.F.H. Influence of liposomal drug entrapment on percutaneous absorption. *Int J Pharm* 1984;20:139-54.

99. Ford JL, Timmins P. The use of thermal analysis in the study of liposomes. In: Ford JL, Timmins P, editors. Pharmaceutical thermal analysis. Chichester: Elis Horwood Limited; 1989. p. 259-78.
100. Grabiél-Madelmont C, Lesieur S, Ollivon M. Characterization of loaded liposomes by size exclusion chromatography. *J Biochem Biophys Methods* 2003;56:189-217.
101. Rogerson A, Cummings J, Willmott N, Florence AT. The distribution of doxorubicin in mice following administration in niosomes. *J Pharm Pharmacol* 1988;40:337-42.
102. Archer JS, Chang RJ. Hirsutism and acne in polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynecol* 2004;18(5):737-54.
103. Beigi A, Sobhi A, Zarrinkoub F. Finasteride versus cyproterone acetate-estrogen regimens in the treatment of hirsutism. *Int J Gynaecol Obstet* 2004;87:29-33.
104. Ranjan M, Diffley P, Stephen G, Price D, Walton TJ, Newton RP. Comparative study of human steroid 5 $\alpha$ -reductase isoforms in prostate and female breast skin tissues: sensitivity to inhibition by finasteride and episteride. *Life Sci* 2002;71:115-26.

105. Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S. Activity of the type 1  $5\alpha$ -reductase exhibits regional differences in isolated sebaceous glands and whole skin. *J Invest Dermatol* 1995;105:209-14.
106. Chen W, Zouboulis CC, Orfanos CE. The  $5\alpha$ -reductase system and its inhibitors: recent development and its perspective in treating androgen-dependent skin disorders. *Dermatology* 1996;193:177-84.
107. Luu-The V. Characterization, expression and immunohistochemical localization of  $5\alpha$ -reductase in human skin. *J Invest Dermatol* 1994;102:221-6.
108. Harris GS, Kozarich JW. Steroid  $5\alpha$ -reductase inhibitors in androgen-dependent disorders. *Curr Opin Chem Biol* 1997;1:254-9.
109. Itami S, Kurata, Sonada T, Takayasu S. Characterization of  $5\alpha$ -reductase in cultured human dermal papilla cells from beard and occipital scalp hair. *J Invest Dermatol* 1991;96:57-60.
110. Dallob AL, Sadick NS, Unger W, Lipert S, Geissler LA, Gregoire SL, et al. The effect of finasteride, a  $5\alpha$ -reductase inhibitor, on scalp skin testosterone and dihydrotestosterone concentrations in patients with male pattern baldness. *J Clin Endocrinol Metab* 1994;79:703-6.
111. Rittmaster RS, Uno H, Povar ML, Mellin TN, Loriaux DL. The effect of *N,N*-diethyl-4-methyl-3-oxo-4-aza- $5\alpha$ -androstane- $17\beta$  carboxamide, a  $5\alpha$ -reductase

- inhibitor and antiandrogen, on the development of baldness in the stumptail macaque. *J Clin Endocrinol Metab* 1987;65:188-93.
112. Bakshi RK, Oatel GF, Rasmusson GH. 7 $\beta$ -Methyl-4-aza-cholestan-3-one (MK386) and related 4-azasteroids as selective inhibitors of human type 1 5 $\alpha$ -reductase. *J Med Chem* 1994;37:3871-4.
113. Martindale: The complete drug reference. 34th ed. London, UK: Pharmaceutical Press; 2005.
114. The Merck index. 13th ed. Whitehouse Station, NJ, USA: Merck and Co., INC.; 2001.
115. Clark's analysis of drugs and poisons. 3rd ed. London: Pharmaceutical press; 2004.
116. Ptacek P, Macek J, Klima J. Determination of finasteride in human plasma by liquid-liquid extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 2000;738:305-10.
117. Constanzer ML, Matuszewski BK, Bayne WF. High-performance liquid chromatographic method for the determination of finasteride in human plasma at therapeutic doses. *J Chromatogr B Biomed Appl* 1991;566:127-34.
118. Carlin JR, Christofalo P, Vandenheuvcl WJA. High-performance liquid chromatographic determination of N-(2-methyl-2-propyl)-3-oxo-4-aza-5 $\alpha$ -



- androst-1-ene-17 $\beta$ -carboxamide, a 4-azastroid, in human plasma from a phase I study. *J Chromatogr B Biomed Appl* 1988;427:79-91.
119. Syed AA, Amshumali MK. LC determination of finasteride and its application to storage stability studies. *J Pharm Biomed Anal* 2001;25:1015-9.
120. Takano T, Hata S. High-performance liquid chromatographic determination of finasteride in human plasma using direct injection with column switching. *J Chromatogr B Biomed Appl* 1996;676:141-6.
121. Carlucci G, Mazzeo P. Finasteride in biological fluids: extraction and separation by a graphitized carbon cartridge and quantification by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 1997;693:245-8.
122. Lunn G, Schmuff NR. HPLC methods for pharmaceutical analysis. New York, NY, USA: John Wiley and sons, Inc.; 1997.
123. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. *Anal Chem* 1998;70:882-9.
124. Guarna A, Danza G, Bartolucci G, Marrucci A, Dini S, Serio M. Synthesis of 5,6,6- [2H3] finasteride and quantitative determination of finasteride in human

137. Radiation safety handbook office of research safety northwest university.  
2002. Report No.: HSE 901-14.
138. Radiation safety manual prepared for office of health and safety centers for  
disease control (CDC). 1999.
139. Technical information. [T-1652-NUC-88-7]. 1988. California, USA, Beckman  
Instruments, Inc.
140. Scintillation system catalog: LS 6000 series supplies and accessories. [BR-  
8127A]. 1996. California, USA, Beckman Instruments, Inc.
141. Papahadjopoulos D, Szoka F, inventors; Method of encapsulating biologically  
active materials in lipid vesicles. United States patent 4 235 871. 1980.
142. MacDonald RC, MacDonald RI, Taleshita K, Subbarao NK, Hu L. Small-  
volume extrusion apparatus for preparation of large, unilamellar vesicles.  
Biochem Biophys Acta 1991;1061:297-303.
143. Data presentation and interpretation. In: Allen T, editor. Particle size  
measurement, powder sampling and particle size measurement. 5th ed. London:  
Chapman & Hall; 1997. p. 44-111.
144. Particle characterisation. In: Seville JPK, Tuzan U, Clift R, editors. Processing  
of particulate solids. 1st ed. London: Chapman & Hall; 1997. p. 1-52.

145. Bonina F, Montenegro L. Comparison of different separative techniques in the quantitative determination of active compound enclosed in liposomal systems. *Int J Cosmet Sci* 1994;16:183-97.
146. Fildes F.J.T., Oliver JE. Interaction of cortisol-21-palmitate with liposomes examined by differential scanning calorimetry. *J Pharm Pharmacol* 1978;30:337-42.
147. Montenegro L, Panico AM, Bonina F. Quantitative determination of hydrophobic compound entrapped in dipalmitoylphosphatidylcholine liposomes by differential scanning calorimetry. *Int J Pharm* 1996;138:191-7.
148. Costa P, Lobo JMS. Influence of dissolution medium agitation on release profile of sustained-release tablets. *Drug Dev Ind Pharm* 2001;27(8):811-7.
149. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci* 2001;13:123-33.
150. Lu DR, Abu-Izza K, Mao F. Nonlinear data fitting for controlled release devices: an integrated computer program. *Int J Pharm* 1996;129:243-51.
151. Washington C. Drug release from microdisperse systems: a critical review. *Int J Pharm* 1990;58:1-12.

152. Plessis JD, Ramachandran C, Weiner N, Muller DG. The influence of particle size of liposomes on the deposition of drug into skin. *Int J Pharm* 1994;103:177-82.
153. Nasser B, Florence AT. Some properties of extruded non-ionic surfactant micro-tubes. *Int J Pharm* 2003;254:11-6.
154. Hao Y, Zhao F, Li N, Yang Y, Li K. Studies on a high encapsulation of colchicine by a niosome system. *Int J Pharm* 2002;244:73-80.
155. Szoka F, Olson F, Heath TVW, Mayhew E, Papahadjopoulos D. Preparation of unilamellar liposomes of intermediate size (0.1-0.2  $\mu\text{m}$ ) by a combined of reverse phase evaporation and extrusion through polycarbonate membranes. *Biochim Biophys Acta* 1980;601:559-71.
156. Jin AJ, Huster D, Gawrisch K, Nossal R. Light scattering characterization of extruded lipid vesicles. *Eur Biophys J* 1999;28:187-99.
157. Berger N, Sachse A, Bender J, Schubert R, Brandl M. Filter extrusion of liposomes using different devices: comparison of liposome size, encapsulation efficiency, and process characteristics. *Int J Pharm* 2001;223:55-68.
158. Benatti CR, Tiera MJ, Feitosa E, Olofsson G. Phase behaviour of synthetic amphiphile vesicles investigated by calorimetry and fluorescence methods. *Thermochim Acta* 1999;328:137-42.

159. Engbert JBFN, Hoekstra D. Vesicle-forming synthetic amphiphiles. *Biochim Biophys Acta* 1995;1241:323-40.
160. Taylor KMG, Morris RM. Thermal analysis of phase transition behaviour in liposomes. *Thermochim Acta* 1995;248:289-301.
161. McCauley, Brittain HG. Thermal methods of analysis. In: Brittain HG, editor. *Physical characterization of pharmaceutical solids*. New York: Marcel Dekker, Inc.; 1995. p. 241.
162. Montenegro L, Panico AM, Ventmiglia A, Bonina VP. In vitro retinoic acid release and skin permeation from different liposome formulations. *Int J Pharm* 1996;133:89-96.
163. Shah VP, Skelly JP. Practical consideration in developing a quality control (in vitro release) procedure for topical drug products. In: Shah VP, Maibach HI, editors. *Topical drug bioavailability, bioequivalence, and penetration*. New York: Plenum Press; 1993. p. 107-16.
164. Saarinen-Savolainen P, Jarvinen T, Taipale H, Urtti A. Methods for evaluating drug release from liposomes in sink conditions. *Int J Pharm* 1997;159:27-33.
165. Manconi M, Sinico C, Valenti D, Loy G, Fadda AM. Niosomes as carriers for tretinoin. I. Preparation and properties. *Int J Pharm* 2002;234:237.

166. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. *J Pharm Pharmacol* 1999;51:1123-34.
167. Roberts MS, Cross SE. Skin transport. In: Walters KA, editor. *Dermatological and transdermal formulations*. New York: Marcel Dekker, Inc.; 2002.
168. Fleisher D, Niemiec SM, Oh CK, Ramachandran C, Weiner N. Topical delivery of growth hormone releasing peptide using liposomal systems: an in vitro study using hairless mouse skin. *Life Sci* 1995;57(13):1293-7.
169. Egbaria K, Weiner N. Liposomes as a topical drug delivery system. *Adv Drug Deliv Rev* 1990;5:287-300.
170. Tschan T, Steffen H, Supersaxo A. Sebaceous-gland deposition of isotretinoin after topical application: an in vitro study using human facial skin. *Skin Pharmacol* 1997;10(126):134.
171. Walters KA, Walker M, Olejnik O. Non-ionic surfactants. Effects on hairless mouse skin permeability characteristics. *J Pharm Pharmacol* 1987;40:525-9.
172. Han I, Kim M, Kim J. Enhanced transfollicular delivery of adriamycin with a liposome and iontophoresis. *Exp Dermatol* 2004;13(2):86-92.

173. Balsari AL, Morelli D, Menard S, Veronesi U. Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. *Res Commun* 1994;8:226-31.
174. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. *J Invest Dermatol* 2004;123:168-76.
175. Weiner N, Martin F, Riaz M. Liposomes as a drug delivery system. *Drug Dev Ind Pharm* 1989;15:1523-54.
176. Bernard E, Dubios JL, Wepierre J. Percutaneous absorption of a new antiandrogen included in liposomes or in solution. *Int J Pharm* 1995;126:235-43.
177. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. In vitro skin permeation of estradiol from various proniosome formulations. *Int J Pharm* 2001;215:91-9.
178. Fang JY, Hong CT, Chiu WT, Wang YY. Effect of liposomes and niosomes on skin permeation of enoxacin. *Int J Pharm* 2001;219:61-72.
179. Valjakka-Koskela R, Kirjavainen M, Monkkonen J. Enhancement of percutaneous absorption of naproxen by phospholipids. *Int J Pharm* 1998;175:225-30.
180. Sarpotdar PP, Zatz JL. Percutaneous absorption enhancement by nonionic surfactants. *Drug Dev Ind Pharm* 1986;12:1625-47.

## دارورسانی هدفمند فیناستراید به فولیکول مو توسط سیستم های لیپوزومی

### خلاصه پایان نامه

**مقدمه:** دارورسانی هدفمند فیناستراید به فولیکول مو می تواند غلظت دارو را در پیلوسباسه افزایش دهد و اثرات جانبی سیستمیک آنرا کم نماید.

**روشها:** نیوزوم و لیپوزوم حاوی دارو به دو روش تشکیل فیلم و تبخیر فاز معکوس تهیه شد و ویژگی آنها از نظر اندازه ذره ای، کفایت احتباس، آزادسازی در محیط های فسفات بافرسالین و سبوم مصنوعی، همچنین نفوذ دارو از غشای پوستی در برون تن بررسی گردید. مطالعه درون تنی استقرار فیناستراید نشاندار ( $^3\text{H}$ -finasteride) در پیلوسباسه و دیگر بخشهای گوش هامسترپس از استعمال موضعی وزیکول های حاوی فیناستراید و محلول هیدروالکلی آن به طریقه جدا کردن فولیکولی (scraping) انجام شد. همچنین تاثیر استعمال فرآورده به میزان دو بار در روز به مدت ۴ هفته بر مساحت غدد سباسه گوش و فلانک هامستر به روش تعیین سطح مقطع بافتی پوست مطالعه گردید.

**نتایج:** سرعت نفوذ فیناستراید از پوست ناحیه فلانک از محلول هیدروالکلی سریعتر از وزیکولهای حاوی دارو بود (  $0.16 \mu\text{g}/\text{cm}^2.\text{h}$  در برابر  $0.025-0.058 \mu\text{g}/\text{cm}^2.\text{h}$ ). وزیکولهای حالت مایع بارداری  $2/5-7/5$  ساعت  $50\%$  از دارو را به درون سبوم مصنوعی آزاد کردند.

استعمال وزیکولهای اکستروند نشده متشکل از آمفی فیل های حالت مایع بریج ۹۷، DMPC و مخلوط ۱:۱ بریج ۷۶: بریج ۹۷، منجر به استقرار  $2/5-2/05\%$  ازدز استعمال شده در واحد



پیلوسباسبه گردید، که بطور بارزی نسبت به وزیکولهای مشابه اکستروُدشده (۴۰۰ و ۱۰۰ nm)، وزیکولهای حالت ژل و نیز محلول هیدروالکلی، استقرار بالاتری را موجب گردید (۰/۹۲-۰/۳۵٪). وزیکولهای دارای بیشترین استقرار و محلولهای هیدروالکلی فیناستراید و پروژسترون (کنترل مثبت) بطور مشهودی اندازه غدد سباسبه گوش و ناحیه فلانک را در سمت استعمال شده کاهش دادند (۳۶-۴۸٪،  $p < 0/05$ )، در حالیکه محلول هیدروالکلی باعث کاهش اندازه این غدد در سمت استعمال شده و سمت استعمال نشده فلانک گردید، که می تواند نشان دهنده اثر سیستمیک آن باشد.

**بحث و نتیجه گیری:** مطالعات درون تنی استقرار فولیکولی دارو و کاهش اندازه غدد سباسبه هامسترپس از تجویز موضعی، می تواند نشان دهنده قابلیت وزیکولهای حالت مایع بارداربرای دارورسانی موثر فیناستراید به واحد پیلوسباسبه باشد.

#### **لغات کلیدی:**

فیناستراید، نیوزوم، لیپوزوم، دارورسانی هدفمند، واحد پیلوسباسبه، گوش و فلانک هامستر