Follicular (Pilosebaceous Unit) Deposition and Pharmacological Behavior of Cimetidine as a Function of Formulation

Linda Margaret Lieb,[1] Gordon Flynn,[1] and Norman Weiner[1,2]

Received March 22, 1994; accepted May 26, 1994

The effect of formulation on cimetidine delivery to the pilosebaceous unit and other skin phases was studied. *In vitro* and *in vivo* deposition determinations as well as a pharmacodynamic antiandrogenic sebaceous gland bioassays were made. A complex variety of factors influence how the formulation affects both the degree of drug deposition and its pharmacological activity in the pilosebaceous unit. When cimetidine was applied in formulations at pH values where it was predominately unionized, the thermodynamic driving force proved the dominant factor in influencing the extent of drug deposition into the pilosebaceous unit. Although more cimetidine was deposited into the pilosebaceous unit *in vivo* from the phospholipid-based liposomal formulation when cimetidine was ionized, this formulation was also the only one devoid of significant antiandrogenic action. Of great importance, it is clear from the studies that deposition from complex formulations, such as liposomes, where bilayer/drug interactions can persist in the skin, may give a false impression of the activity of a drug within a tissue. Moreover, data for cimetidine in 50% alcohol solution show that one can maintain local effects while reducing systemic activity by simply manipulating drug concentration in the application.

KEY WORDS: follicular deposition; pilosebaceous unit; cimetidine; liposomes; topical application; formulation effects; antiandrogen.

INTRODUCTION

It would seem plausible that the treatment of follicular disease states can be greatly enhanced by specific drug targeting. Examples of disease states involving the pilosebaceous units are acne, alopecia androgenetica (male pattern hair loss) and alopecia areata. Previously we described an *in vitro* methodology for determining the deposition of a polar marker, carboxyfluorescein, into hamster ear pilosebaceous units (1) and showed the degree of carboxyfluorescein deposition into pilosebaceous units is greatly influenced by formulation factors. Liposomes provided greater deposition than the other formulations tested.

In this study, we further explore effects of formulation on pilosebaceous unit deposition by: i) determining whether the hamster ear model can be used to differentiate cimetidine deposition into pilosebaceous unit from various formulations after a single *in vivo* application; and ii) exploring whether or not pharmacokinetic-pharmacodynamic behaviors in the hamster ear model are consistent with the deposition of this drug. Cimetidine was chosen for a number of reasons: i) it is available as a radiolabeled compound, thus providing requisite analytical sensitivity; ii) cimetidine is a known antiandrogen (2-12) and is expected to pharmacologically stimulate the pilosebaceous unit, resulting in an antiandrogenic effect which can be quantified in the hamster ear sebaceous gland (13); and iii) as a weak base (pKa ~ 7.0), cimetidine can be formulated to yield various states of ionization, affording study of the effects of drug charge on deposition and activity.

MATERIALS AND METHODS

Materials

Phosphatidylcholine and phosphatidylserine were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). d-α-Tocopherol was purchased from Kodak (Rochester, NY). Glycerol dilaurate, POE-10 stearyl ether, cholesterol, (N,N-bis[2-hydoxyethyl])-glycine (BICINE, pKa = 8.3), (2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES, pKa = 7.5) and potassium pthalate (pKa = 4.0) were all purchased from Sigma Chemical Co. (St. Louis, MO). ^{3}H-Cimetidine, specific activity 250μCi/125 μl, was obtained from Amersham. Cold cimetidine was obtained from Sigma. Ecolite scintillation cocktail (ICN Biomedical, Inc., Irvine, CA) was used to mix the aqueous skin compartments for assay. All other chemicals were obtained from Fischer Scientific (Springfield, NJ).

Male Syrian golden hamsters (10–14 weeks, Charles River Laboratories) were used. The hamsters were maintained at a photoperiod of 14 hr of light and 10 hr of darkness (14,15). Food and water were provided *ad libitum*.

Preparation of Cimetidine-Containing Formulations

Phospholipid based liposomal formulations were prepared by standard methods (16–17) and contained phosphatidylcholine:cholesterol:phosphatidylserine at a mole ratio of 1.0:0.5:0.1. The latter lipid was included to impart sufficient charge to the liposome to prevent flocculation (17).

Nonionic (Novasome™) liposomal formulations were prepared by separately heating the lipid and aqueous phases (containing cimetidine) to 60°C. The aqueous phase was then added to the lipid phase and the mixture was allowed to cool with mixing. The Novasome contained glyceryl dilaurate: cholesterol:POE-10 stearyl ether, at a weight ratio of 57:15: 28 and the total lipid concentration of both liposomal formulations was 15 mg/ml.

In Vitro Deposition Studies

Franz diffusion cells having a diameter of 5 mm, an application area of about 0.20 cm^2, and a 4 ml receiver compartment (Crown Glass, Somerville, NJ) were used. Hamsters were sacrificed and their ears excised at the base. The whole ear was mounted, ventral side up with the medial section exposed to treatment, on the cell and the receiver compartment was filled with buffer in such manner that the liquid surface was near but not actually in contact with the ear's facing surface (1).

The cells were attached to a heating block and held at

[1] College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065.

[2] To whom correspondence should be addressed.

0724-8741/94/1000-1419$07.00/0

38°C to provide a membrane temperature of 32°C, as verified with a temperature probe. Thirty μl of formulation were applied. At the end of 24 hours, the cells were dismantled, the ears were processed into their respective compartments (see below), and the samples analyzed. Four runs were performed on each formula.

At appropriate time points, the cell was dismantled and the surface of each whole ear membrane was washed thoroughly with 10 ml of buffer. The surface was then gently padded with a Kimwipe® to remove the formulation residue. Each ear membrane was separated into the following compartments via either peeling or gentle scraping: (1): the epidermis, the ventral dermis, and the cartilage/dorsal (nontreated) side. The sebaceous glands in the ventral dermis were removed by scraping. In this procedure, a dull scalpel is dragged across the underside of the ventral dermis. Care is taken to scrape with the minimum force necessary to dislodge the pilosebaceous material as evidenced by the appearance of its milky contents. The scraping procedure is considered complete when the areas of the ear previously occupied by the sebaceous glands appear void under light microscopy. Aliquots of each of the compartments including skin rinses and the donor phase of the diffusion cell were individually collected and mixed with 15 ml of Ecolite scintillation cocktail and assayed.

In Vivo Deposition Studies

Based on the results of the *in vitro* deposition studies, three formulations, each containing 3% cimetidine, were tested. Syrian male hamsters, at least 12 weeks in age, were anesthetized with 1 ml of a 10 mg/ml pentobarbital solution in normal saline. The animals were then given booster shots of 0.5 ml as needed throughout the experimental period. The hamsters were placed on their backs (Flat Back Method) inside a plastic cage to allow the ears to lie flat and facilitate formula application. Thirty μl of formulation were applied very slowly on the ventral mid section of the ear. Care was exercised here to avoid any formulation reaching the edges of the ear since this might spill over and contaminate the dorsal side. The approximate area covered was 0.5–0.6 cm^2. After 12 hours, the hamsters were sacrificed by i.p. injection of 5 ml saturated Urethane and their ears were excised. Each ear membrane was mounted on a board, and pieces of adhesive tape, 2 cm wide and 4 cm long, were used to strip the ventral side of the ear (15–20 strips were sufficient to fully remove the stratum corneum). Each ear remnant was further separated into ventral dermis, and cartilage/dorsal compartments. The sebaceous glands in the ventral dermis were removed using the scraping technique. All the samples were assayed via liquid scintillation counting.

Bioassay for Sebaceous Gland Activity

This procedure is based on a method reported by Matias et al (13). The same formulations as used for the *in vivo* deposition study were tested in the bioassay study. In addition, a 5% hydroalcoholic solution of cimetidine was prepared and compared directly against the 3% solution. A 5% solution of spironolactone (Sigma, St. Louis, MO) in acetone was used as a positive control.

Twenty-five μl of a given formulation were applied twice daily to the right ventral ear of a hamster for a period of four weeks. The left ear served as the control for systemic activity. A separate group of animals received placebo formulations. Each formulation was tested on 3–4 animals. At the treatment period's end, the hamsters were terminated and their ears were excised and stripped (separated from cartilage). To image the sebaceous gland, the ventral ear skin was stained for 3 hours with 0.1% (w/v) Sudan Black B in propylene glycol, rinsed and left standing overnight in a solution of propylene glycol; pH = 7.4 buffer (85:15; v/v) and then fixed by soaking over a second night in 10% buffered formalin. A 3-mm punch biopsy of the medial zone was then taken and fixed, dermis side up, on a microslide. The slide containing the hamster ear skin section was examined in phase contrast bright light using a Leitz Fluovert FS microscope with a 32X lens. Each sebaceous gland image was stored digitally in an IBM computer and the image processed for quantitative planimetry (surface area) using a Quantex QX-7 image processor. Nine sebaceous gland measurements were taken for each ear. Each measurement was compared statistically with the placebo-treated ear.

Data Analysis

Results were based on the comparison of the percentage applied dose found in the various strata. Mean values and standard errors were determined for deposition in each tissue stratum analyzed for each formulation tested. Statistical comparisons for significance were performed by the independent t-test method (one-paired).

RESULTS

Mean percentages of the dose of cimetidine applied to the surface of the ear and found in its isolated compartments at 24 hr in the *in vitro* experiments are presented in Table 1. Data for seven different formulations are tabulated. Standard errors of the mean (n = 4) are bracketed next to their respective percents recovered. Compartmental recoveries

Table I. Percentage of Applied Dose of 0.5% Cimetidine from Various Formulations Deposited into Pilosebaceous Unit, Dermis and Dorsal Strata of Syrian Male Hamster Ear 24 Hours after *in vitro* Topical Application. Values in Parentheses Denote Standard Errors (n = 4)

Formulation	Pilosebaceous unit	Dermis	Dorsal region
Aqueous solution (pH = 8.3)	2.4 (0.5)	2.1 (0.4)	0.5 (0.1)
Phospholipid liposome (pH = 8.3)	1.1 (0.2)	2.5 (0.5)	0.31 (0.2)
Nonionic liposome (pH = 8.3)	1.5 (0.2)	1.3 (0.2)	1.1 (0.1)
50% alcohol solution (pH = 7.4)	1.1 (0.1)	1.5 (0.2)	0.5 (0.2)
Aqueous solution (pH = 5.5)	1.3 (0.3)	1.2 (0.2)	0.3 (0.1)
Phospholipid liposome (pH = 5.5)	1.2 (0.1)	0.9 (0.1)	0.3 (0.1)
Nonionic liposome (pH = 5.5)	1.7 (0.3)	1.2 (0.3)	1.3 (0.6)

ranged from a fraction of a percent to 2.4%. The variability in results in terms of standard errors of the mean ranges from a little under 10% to something over 50%. It will be noticed that no data are presented for the epidermal compartment. The reason for this is that the epidermis is scraped off as a single layer, stratum corneum intact, and it is next to impossible to fairly apportion the total material associated with the epidermis between that which is adsorbed to the surface and that which is actually in the tissue. The dorsal region includes cimetidine in the ear's cartilage since this too cannot be quantitatively isolated.

Results of the *in vivo* experiments are tabulated in Table 2. Values are provided in terms of the milligrams of cimetidine found in each of six identified compartments, cage wash through pilosebaceous units, for each of three vehicles tested. Standard errors of the mean (n = 3) associated with these values are recorded in brackets. Mass balances are organized in the last row of the table.

Bioassay data are shown in Tables 3 and 4. Table 3 presents the decreases observed in the size of the sebaceous gland relative to placebo treatment in the Syrian hamster ear following the application of either cimetidine or spironolactone. Results are presented for both the treated ear and the untreated ear. The results of Matias et al. (13) are also shown for comparison. In our studies, the cimetidine was administered in 50% ethanol, 25 μL of a 5% solution twice daily for a total of 20 days. The spironolactone and progesterone were administered in neat acetone. No statistical differences were noted between the reductions of sebaceous gland area at the treated and contralateral ear sites with either cimetidine or spironolactone, but in all cases, the reductions were highly significant relative to the placebo treatment.

In Table 4, the relative anti-androgenicities of three formulations containing 3% cimetidine in 50% ethanol, nonionic liposomes and phospholipid liposomes are given. As in the previous instance, 25 μL of these three formulations were applied twice a day for a period of 20 days. The 3% cimetidine formulations in the ethanolic and nonionic liposomal vehicles were active, but now only at the local level. Cimetidine formulated at 3% in the phospholipid liposomal preparation proved to be inactive.

Table II. Micrograms of Applied Dose of 3% Cimetidine (Total = 900 mcg) from Various Formulations Deposited into Various Compartments of Syrian Male Hamster Ear 12 Hours after *in vivo* Topical Application. Values in Parentheses Denote Standard Errors (n = 3)

Compartment	50% alcohol solution (pH = 7.4)	Nonionic liposome (pH = 5.5)	Phospholipid liposome (pH = 5.5)
Cage wash	0.5 (0.5)	0.4 (0.1)	0.0 (0.0)
Surface stratum corneum	908 (44)	778 (27)	669 (38)
Strips 3 to end	26.1 (2.2)	94.7 (3.2)	161.8 (7.3)
Dorsal region	0.0 (0.0)	0.1 (0.0)	0.3 (0.3)
Dermis	0.0 (0.0)	0.4 (0.2)	0.2 (0.1)
Pilosebaceous unit	0.1 (0.0)	0.3 (0.1)	0.6 (0.3)
Mass balance (%)	106.8 (1.5)	99.2 (3.8)	98.5 (4.6)

DISCUSSION

The literature contains very little information regarding the mechanism and the kinetics of follicular permeation. In the present investigations we have evaluated depositions of cimetidine within and around this gland using as our model the large and purportedly representative sebaceous apparatus of the Syrian hamster ear. The nature of the vehicle and vehicle pH are numbered among the principal variables we dealt with. Several previous investigators have examined the effect vehicle pH exerts on drug deposition into skin structures. Wallace and Barnett (18), for example, concerned themselves with the effect of pH on methotrexate's penetration into hairless mouse skin. They developed compartmental models for the process and concluded that transport into and through the tissue involves parallel diffusional pathways. It is their claim that a shunt pathway predominated at high pH where the drug was virtually totally ionized. In their view, the shunt was a combined interappendageal and intercellular route. Similarly, an analysis of early transient (nonstationary state) diffusion of ibuprofen into human skin was said to be substantially (≈25%) dependent on shunts or an actual appendageal path (19), with the ionized form of ibuprofen presumed to be the species diffusing by way of the appendages. In each of these investigations the shunt mechanism was inferred through data patterns only; the tissues being examined did not lend themselves to isolation and independent assay of the putative phases.

Differences in concentrations in the skin's isolatible compartments following topical application have been measured directly on occasion. Correoller et al. (20) developed two mathematical models to quantitatively estimate linoleic acid deposition from acetone solution into rat sebaceous structures. They found the sebaceous concentration of linoleic acid in the upper epidermis to be four to six times higher than in the remaining tissue and twice higher in the follicular duct than in the deeper gland. These, of course, are to some degree expected trends resulting from diffusion concentration gradients being set up across the barrier phases.

The pilosebaceous unit itself is a highly heterogeneous structure and, when considering targeted delivery into hair follicles, one first must consider what precise area within the follicle one wishes the drug to reach. Formulation strategies for follicular delivery have included: i) the use of volatile organic solvents, such as ethanol, to either partially dissolve and draw a fraction of sebum out of the follicular duct or to soften that material which is in the duct (13) and ii) the inclusion of wetting agents to assure the vehicle makes good contact with the sebaceous medium across the vent of the duct (20,21). More recently, liposomes have been proposed for follicular targeting. On drying, liposomes develop into a structured film which seemingly fills the follicular openings, intimately mixes with the follicular contents, and fosters drug diffusion to the depths of the gland. The present studies are among the first to specifically delve into such factors.

The combined results of the *in vitro* and *in vivo* initiatives here teach us that a complex of formulation attributes determine deposition and the actual pharmacological effectiveness of drugs acting on the pilosebaceous unit. For the relatively simple case of the aqueous systems, the solution at pH 8.3, where the drug is predominately unionized, induced

Table III. Percent Decrease from Placebo Treated Ears of Male Syrian Hamster Ear Sebaceous Gland Mean Surface Area after Twice Daily Application of 25 μl of Various 5% Formulations of Antiandrogen Drugs for a Total of 20 Days. Cimetidine is Formulated in 50% Ethanol in Water While Spironolactone and Progesterone are Formulated in Neat Acetone

Drug	Treated or contralateral ear	Percent decrease from placebo treated ear	p value from placebo treatment (t-test)	Source
Cimetidine	Treated	23	$p < 0.025$	This study
Cimetidine	Contralateral	37	$p < 0.01$	This study
Spironolactone	Treated	33	$p < 0.005$	This study
Spironolactone	Contralateral	40	$p < 0.01$	This study
Spironolactone	Treated	31	$p < 0.01$	Reference 13
Spironolactone	Contralateral	27		Reference 13
Progesterone	Treated	58	$p < 0.001$	Reference 13
Progesterone	Contralateral	0		Reference 13

more cimetidine into all the hamster ear compartments tested than did the aqueous formulation at a pH where the drug was predominately ionized. This result comes as no surprise given the lipoidal nature of the sebaceous substance. The thermodynamic activity of cimetidine's free base is in fact far greater at pH 8.3, where the aqueous medium is near the saturation point, than at lower pH. What is not so obvious is that the simple, alkaline solution of cimetidine should be the most efficient formulation for depositing cimetidine into the follicular substances of all those we tested. Apparently the thermodynamic driving force was high enough to generally set it apart in this regard. The bioassays add several important new dimensions to the work. The test is one which quantitatively establishes the antiandrogenic activity of a drug in terms of decreased sebaceous gland size. Data in Table 3 show that cimetidine applied as a 5% hydroalcoholic solution (50:50) twice a day to the hamster ear in 25 μl dosages suppressed androgenic function both locally and systemically. To be more specific, the sebaceous glands in both the ear receiving the applications and the control ear were reduced in size relative to ears on animals treated identically with vehicle only.

This appears to be the first demonstration of cimetidine's antiandrogenicity in a topical assay. The data for spironolactone, a known, potent antiandrogen, serve as the positive control in these studies. Using neat acetone as its solvent and the identical treatment regimen, 5% spironolactone also proves to be both locally and systemically active. These results are in excellent agreement with an earlier study of Matias, et al. (13), who developed the procedure. Matias, et al. also showed the assay is capable of distinguishing between local and systemic antiandrogenicity by demonstrating progesterone's activity was strictly at the application site (Table 3).

Based on the results of the *in vivo* deposition studies, one might confidently expect the phospholipid liposome to be at least as pharmacologically active as the hydroalcoholic solvent and the nonionic liposomal formulation tested against it. However, this expectation is not met (Table 4). Rather, when cimetidine is formulated in these vehicles at 3% strength, and the formulations are applied according to the standard regimen, only the hydroalcoholic solution and the nonionic liposomes suppressed the growth of the sebaceous gland. For the present study, the effects were strictly local. One interesting aspect of this result is that it establishes a split in local and systemic activity in the hydroalcoholic solution strictly based on formulation concentration, apparently the first such demonstration of its kind. The nonionic liposomes appeared equipotent with the hydroalcoholic solution.

The inactivity of the phospholipid liposomal formulation in the bioassay is at first puzzling given the substantial deposition of the cimetidine into deeper tissues with this formulation *in vivo*. However, the phospholipid liposomes contain an appreciable fraction of phosphatidylserine, a negatively charged molecule, capable of forming an ion pair with

Table IV. Percent Decrease from Placebo Treated Ear of Male Syrian Hamster Ear Sebaceous Gland Mean Surface Area after Twice Daily Application of 25 μl of 3% Cimetidine Formulation for a Total of 20 Days

Formulation	Treated or contralateral ear	Percent decrease from placebo treated ear	p value from placebo treatment (t-test)
50% ethanol soln.	Treated	29	$p < 0.01$
50% ethanol soln.	Contralateral	8	NS
Nonionic liposomes	Treated	31	$p < 0.001$
Nonionic liposomes	Contralateral	6	NS
Phospholipid liposomes	Treated	3	NS
Phospholipid liposomes	Contralateral	0	NS

cimetidine at the pH of the formulation. We suspect it may be this ion pair which is deposited and not free cimetidine, in which case the drug would be present but not in an active form. Irrespective of the actual mechanism, this is of great consequence since these results demonstrate that attempts to optimize formulations based on in vitro or in vivo drug deposition analyses of skin tissues can yield false impressions of the activity of the drug within that tissue.

ACKNOWLEDGMENTS

This work was supported by grants from The National Institutes of Health; Al 22303 and Alopex Pharmaceuticals, Inc.

REFERENCES

1. L. M. Lieb, C. Chandrasekharan, K. Egbaria, and N. Weiner. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: I. In vitro evaluations using fluorescent techniques with the hamster ear model. *J. Invest. Dermatol.* **99**:108–113 (1992).
2. G. F. Delle Fave. Gynaecomastia with cimetidine. *Lancet* **2**:174–176 (1976).
3. M. M. Wolfe. Impotence on cimetidine treatment. *N. Engl. J. Med.* **300**:94–96 (1979).
4. D. H. Van Thiel. Hypothalmic-pituitary-gonadal dysfunction in men. *N. Engl. J. Med.* 300:1012–1015 (1979).
5. S. J. Winters, J. L. Banks, and D. L. Loriaux. Cimetidine is an antiandrogen in the rat. *Gastroenterology* 76:504–508 (1979).
6. J. W. Funder and J. E. Mercer. Cimetidine, a histamine H_2 receptor antagonist, occupies androgen receptors. *J. Clin. Endocrinol. Metab.* **48**:189–191 (1980).
7. S. Baba. In vivo studies on the antiandrogenic effects of cimetidine versus cyproterone acetate in rats. *Prostate* 2:163–174 (1981).
8. F. Lyons, J. Cook, and S. Shuster. Inhibition of sebum excretion by an H_2 blocker. *Lancet* 2:1376 (1979).
9. R. A. Vigersky. Treatment of hirsute women with cimetidine. *N. Engl. J. Med.* 303:1042 (1980).
10. H. Aram. Treatment of female androgenetic alopecia with cimetidine. *Int. J. Dermatol.* **26**:128–130 (1987).
11. J. V. Hennessy. Comparative antiandrogenic potency of spironolactone and cimetidine: Assessment by the chicken cockscomb topical bioassay. *Proc. Soc. Exp. Biol. Med.* **182**:443–447 (1986).
12. M. Gloor, H. Wirth, and U. Swoboda. Is sebosupression by cimetidine an antiandrogenic effect? *Acta. Derm. Venerol. (Stockh.)* **61**:262–264 1981.
13. J. R. Matias, V. Malloy, and N. Orentreich. A comparative study of the effects of percutaneously administered 5-α-reductase and androgen receptor inhibitors on hamster sebaceous glands. *Ann. N.Y. Acad. Sci.* 425:454–456 (1985).
14. G. Plewig, and C. Luderschmidt. Hamster ear model for sebaceous glands. *J. Invest. Dermatol.* **68**:171–176 (1977).
15. J. Hoffman. Photoperiod, pineal, melatonin and reproduction in hamsters. *Prog. Brain. Res.* 52:397–415 (1979).
16. A. D. Bangham, M. M. Standish, and J. C. Watkins. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* **13**:238–252 (1965).
17. F. Szoka and D. Papahadjopoulos. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Biochemistry* 75:4194–4198 (1978).
18. S. M. Wallace and G. J. Barnett. Pharmacokinetic analysis of percutaneous absorption: Evidence of parallel penetration pathways for methotrexate. *J. Pharmacokinet. Biopharm.* 6:315–325 (1978).
19. J. C. Keister and G. B. Kasting. The use of transient diffusion to investigate transport pathways through skin. *J. Controlled Release* 4:111–117 (1986).
20. M. Corroller. Sebaceous accumulation of linoleic acid following topical application in the hairless rat and its mathematical treatment. *Pharmacol. Skin* **1**:111–120 (1987).
21. S. Rothman. Percutaneous absorption. In *Physiology and Biochemistry of the Skin.* The University of Chicago Press, Chicago, 1954, pp. 26–59.
22. S. Rothman. The principles of percutaneous absorption. *J. Lab. Clin. Med.* **28**:1305–1321 (1943).
23. L. M. Lieb. Formulation factors affecting follicular (pilosebaceous route) drug delivery as evaluated with the hamster model. Dissertation, University of Michigan (1994).
24. C. Artmann. Skin penetration of liposomes from soy bean phospholipids. *Communication I.F.S.C.C.*, New York (1990).
25. K. Walters, M. Walker, and J. Olejnik. Non-ionic surfactant effects on hairless mouse skin permeability characteristics. *J. Pharm. Pharmacol.* 40:525–529 (1987).
26. J.-C. Tsai, G. L. Flynn, N. Weiner, and J. J. Ferry. Effect of minoxidil concentration on the deposition of drug and vehicle into the skin. *Int. J. Pharm.* **96**:111–117 (1993).