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Research Article

PREPARATION AND CHARACTERIZATION OF MINOXIDIL LOADED NIOSOME CARRIER FOR EFFECTIVE FOLLICULAR DELIVERY

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Abstract

Minoxidil is a drug known to stimulate hair growth, the treatment of androgenic alopecia could be improved by drug accumulation into the hair follicles. This work investigated *in vitro* the potential of niosomal formulations to achieve perfect follicular delivery. Sixteen niosomal formulations were prepared by thin film hydration technique based on full-factorial design and their physicochemical properties and follicular permeation were evaluated. Minoxidil niosomal formulations (2%) with Low particle size and suitable drug loading and slow release pattern were applied on human hair follicule. Percentage of drug permeated through human follicules after 52 hrs ($\%P_{52}$) was estimated. Most of niosomal formulations increased %P52 in comparison with control that highest enhancement ration was 3 folds and provided by formulation 1. Our finding suggested 4 parameters that controlled %P52: higher drug release, using sodium lauryl sulfate as surfactant, higher amount of surfactant and solid lipid increased %P52.

Keywords: Minoxidil, Niosome, Follicular delivery, Alopecia

Introduction

Penetration of topically applied compounds may occur via the stratum corneum as well as via skin appendages, i.e., sweat glands and hair follicles (1.2). Initially, skin appendages were not considered to be significant transdermal penetration routes, as evidence suggested that they accounted for only approximately 0.1% of the skin surface area (3). These calculations, however, did not take into the account that the hair follicles represent invaginations, which extend deep into the dermis with a significant increase in the actual surface area available for penetration. With a rich perifollicular vascularisation and changes in the differentiation pattern along the follicular duct, the follicle possesses distinct characteristics which favor penetration, and multiple studies suggest that the follicular penetration route may be especially relevant for hydrophilic and high molecular weight molecules, as well as by particle-based drug delivery systems (4.5.6.7).

Direct delivery of some active drugs through the cell membrane into cells was generally inefficient and often faced with many problems, such as enzymolysis, hydrolysis, severely toxic-side effects and so on. Niosome, one kind of colloidal particles, can encapsulate these active drugs, and brings a very promising way to increase drug bioavailability, prevent drug degradation, reduce drug toxic effects and transport drugs to the target sites (8). Moreover, niosome attracts much attention because of its advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost and convenient storage of non-ionic surfactants, and large numbers of surfactants available for the design of niosomes (9).

Minoxidil, , is the only topical medical treatment with proven efficacy for the treatment of Androgenetic alopecia (AGA). AGA is hereditary and is the progressive, androgendependent thinning of scalp hair, which follows a definite pattern. The US Food and Drug Administration approved treatments for AGA are oral finasteride at a dose of 1mg per day and topical solutions of 2 and 5% minoxidil (10). Little is known of

the effect of minoxidil on normal human hair growth and studies have been limited mainly to the response of androgenetic alopecia to topical minoxidil. Recently reported that minoxidil stimulates hair growth in human by prolonging anagen through proliferative and antiapoptotic effects on dermal papilla cells of human hair follicles (11). Minoxidil have been reported for its poor skin penetration ability, which limits minoxidil usefulness as a potent drug in the use of hair growth treatment. Minoxidil is poorly soluble in water and most of the water immiscible organic solvents such as chloroform .Therefore, it has been formulated for topical use in an ethanol-based solution containing ethanol, propylene glycol and water (12).

Whether due to the tendency of the drug to crystallize in ethanol-based formulations or other factors, the minoxidil formulation shows relatively inefficient uptake by the skin. Further, evaporation of ethanol, when the formulation is applied to the skin, leaves a viscous propylene glycol/water residue which may be objectionable to many users. Moreover, typical side effects of the topical treatment with ethanol-based minoxidil formulations include irritative dermatitis going along with pruritus, erythema, scaling and dryness occur at the onset of therapy (13). In some cases, allergic contact dermatitis or exacerbation of seborrhoic dermatitis has been reported. While most of the patients with allergic contact dermatitis described in the literature showed a positive sensitization to the vehicle substance propylene glycol evaluated by patch testing, reactions to the active ingredient minoxidil are rare (14). Since most of the conventional topical minoxidil formulations consist of propylene glycol–water–ethanol solution, to minimize the side effects and to improve the therapeutic efficiency, new dermatological preparations with free of organic solvents and propylene glycol is required.

Vesicular system, both liposomes and niosomes are uni- or multilamellar spheroidal structures composed of amphiphilic molecules assembled into bilayers. They are considered primitive cell models, cell-like bioreactors and matrices for bioencapsulation. In the recent years, nonionic surfactant vesicles known as niosomes received great attention as an alternative potential drug delivery system to conventional liposomes. Moreover, compared to phospholipid vesicles, niosomes offer higher chemical and physical stability (15) with lower cost and greater availability of surfactant classes (16). Niosomes have been reported to enhance the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug and improve penetration of the trapped substances across the skin. In addition, these systems have been reported to decrease side effects and to give a considerable drug release (17). They are thought to improve the horny layer properties both by reducing transepidermalwater loss and by increasing smoothness via replenishing lost skin lipids(18). Moreover, it has been reported in several studies that compared to conventional dosage forms, vesicular formulations exhibited an enhanced cutaneous drug bioavailability (19). In this work, the effects of minoxidil entrapped niosomes on the drug penetration in Follicular membrane and no follicular membrane were investigated by in vitro permeation experiments, and compared with those of control minoxidil solution 2mg/mL (propylene glycol–water–ethanol at 20:30:50, v/v/v) . Niosomal formulations were prepared by thin film-hydration (TFH) method using cholesterol , lecithin , oleic acid and ionic surfactants sodium lauryl sulfate (SLS) and Oleoyl macrogol-6 glycerides EP (Labrafil), and hydroxypropyl methylcellulose (HPMC). This paper focuses on the properties of thin film-hydrated niosomes as potential new minoxidil carriers for effective skin delivery.

Materials and Methods

Chemicals

Cholesterol, oleic acid, lecithin, sodium lauryl sulfate (SLS) and Oleoyl macrogol-6 glycerides EP (Labrafil)*, and hydroxypropyl methylcellulose (HPMC) were purchased from Merck (Germany). Minoxidil (MW 209.25, 99% purity) was provided by Merc (Germany) . Chloroform were supplied by D. C. chemical Co. Ltd. (Seoul, South Korea). All other materials and solvents used in this study were of analytical grade.Freshly double distilled water was use in the formulation .

Minoxidil assay

The amount determination of Minoxidil was carried out by UV spectrophotometry (BioWaveII, WPA) at λmax= 288 nm.

Vesicle preparation

Thin film-hydration method. Accurately weighed quantities of the surfactant (SLS or Labrafil) and cholesterol, lecithin, oleic acid in different molar ratios,were dissolved in 25mL of chloroform in a round-bottom flask (Agarwal et al., 2001) The organic solvents were removed under vacuum in a rotary evaporator at 40 ◦C for 30 min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the surfactant film was carried out using 10mL of distilled water which is containing 0.1mg powder Minoxidil and 2 mg HPMC.

Then, the vesicle suspension was sonicated in 3 cycles of 3min “on”1min “off” or 3 cycles of 3min “on”1min “off” leading to the formation of multilamellar niosomes. The

niosomal suspensionwas left to mature overnight at 4 ◦C and stored at refrigerator temperature for further studies.

Content of drug in vesicles

In order to increase the stability of the prepared vesicles placed at 4 ° C , then 10 ml of vesicles formulations at 20,000 rpm and a temperature of -10 ° C for half an hour, centrifuged and the surface layer was separated , Again precipitated by adding 5 ml of distilled water and centrifuged again, and the above procedure, the supernatant again collected and the supernatant phase and distilled water to a certain volume level has increased and the amount of drug by machine downloaded specified, the value is determined. Due to the amount of drug used in the formula using the following formula to calculate the percentage of the drug is trapped. And the following formula is used to calculate the amount of load.

$$EE\% = \frac{total\ \ drug\ - diffused\ \ drug}{total\ \ drug} \times 100$$

Characterization of minoxidil niosomes

Particle size

Particle size of vesicles after dilution with distilled water at 25 °C, is done by machine particle size analyzer . The mean droplet size of samples was determined at 25 ºC by SCATTER SCOPE 1 QUIDIX (South Korea) . Each sample was measured three times.

Physical stability of minoxidil niosomes

To investigate the stability, vesicles at 30 º C and 65% relative humidity for 3 months and the change in particle size and loading amount of drug will be reviewed.

Investigation of drug release from vesicles

Franz diffusion cells (area 3.16 cm2) with a cellulose membrane were used to determine the release rate of minoxidil from different niosome formulations. The cellulose(molecular weight G12 000) membrane was first hydrated in distilled water at 25 ºC for 24 hours. The membrane was then clamped between the donor and receptor chambers of the cell diffusion. Diffusion cell was filled with 25 ml of water . The receptor medium was constantly stirred by externally driven magnetic beads at 300 rpm throughout the experiment. 5 ml of solution containing minoxidil loaded niosome was placed in donor compartment. At predetermined time intervals(0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24h),a 2ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor medium. Samples were analyzed by UV visible spectrophotometer (BioWaveII, WPA) at 288 nm. The results were plotted as cumulative released drug percentage versus time.

Permeation of hair follicle drug formulations due to vesicles

Diffusion cells fabricated in-house were utilized for the permeation studies.

follicular hair membrane (area=0.00275005125 cm2)and no-follicular hair membrane (3.14cm^2) were placed between donor and receptor chambers of the cells .The donor phase was filled with a 2ml Minoxidil niosom sample while the receptor compartment was filled with 25 ml water . The diffusion cell was placed and clamped in a water bath 37 ± 0.5 oC placed on a magnetic stirrer with a heater. The receptor chambers were stirred continuously with the help of magnetic bead at 300rpm. At predetermined time intervals (0.5, 1, 2, 3,…….,56 h), 2 ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor solution. Samples were analyzed by UV visible spectrophotometer (BioWaveII, WPA) at 288 nm. The results were plotted as cumulative permeated drug percent versus time. The solution of minoxidil was used as positive control in this work.

Experimental design for preparation of liposomes and permeation

Several parameters influence on final properties of niosome and permeation through hair follicle. Full-factorial design was used concerning with 4 variables at 2 levels. solid/liquid lipid ratio (S/L), lipid/ surfactant ratio (L/S), type of surfactant (S) and sonication time (S.T) were chosen as independent variables (Table 1). Dependent variables were include drug loading, particle size, drug release and permeation parameters through human follicular skin. The effects of independent variables were evaluated. The intensity of variables interaction on each response were estimated through simultaneous multiple regression.

Results

Loading capacity of niosomes

Determination of loading capacity is inevitable for evaluating therapeutic efficiency. Table 2 illustrates the loading capacity.

Table 1. Independent variables and levels

Variable	Low level	High level
solid/ liquid lipid	9	19
Lipid/ surfactant	10	20
Type of surfactant	Sodium lauryl sulfate	Labrafil
Sonication time (min)	3	5

Table 2.Loading capacity of niosomes, mean±S.D. (n=3).

Formulation No.	% drug loading
1	64 ± 3.4
2	54 ± 3.5
3	60 ± 2.5
4	71.8 ± 3.3
5	67.5 ± 1.9
6	58.9 ± 2.1
7	81.8 ± 3.9
8	48.4 ± 2.8
9	66.7 ± 3
10	61.8 ± 4.4
11	64.5 ± 3.7
12	72.4 ±3
13	63.1 ± 2.9
14	41.4 ± 3.7
15	62.3 ± 2.8
16	68.7 ± 1.9

Factorial and variance analysis were performed in order to evaluate the impact of independent variables on loading efficiency (LE).

Results illustrate that solid-liquid lipid ratio and lipid-surfactant ratio have a significant impact on LE, however, an increase in liquid lipid and surfactant leads to increase of drug loading. In addition, minoxidil is naturally hydrophobic compound while the niosomes have lipophilic and hydrophilic nature. Maximum loading capacity of 82% belonging to formulation one indicates that the main location for drug loading is bilayers membrane.

Liposomes particle size distribution

Particle size would critically influence on liposome formulations, apart from permeation and cumulative properties. Table 3 presents the results of mean particle size and polydispersity index. Through this experiment, the influence of independent variables was also studied on niosomes mean particle size. The following equation demonstrates the regression between independent variables and particle size of niosomes *(Equation-1)*

Particle size= 180 – 3.66 (S/L) + 2.15 (L/S) – 3.3 (S)- 4.8 (S.T)

The above equation indicates that all variables have a significant impact on mean particle size. Practically an increase in solid lipid, sonication time and using sodium lauryl sulfate as surfactant leads to lower growth in particle size. As liposome particle size ranges from 100 to 190 nm, it seems that method preparation was appreciated for niosome preparation.

Table 3. Liposomes mean particle size and polydispersity index prepared by both procedures (mean ± SD) n=3

Formulation No.	Mean particle size (nm)	Poly dispersity index
1	110 ± 15	0.4
2	123 ± 13	0.6
3	128 ± 15	0.52
4	120.8±17	0.38
5	135 ± 9	0.29
6	134 ± 9	0.51
7	149 ± 15	0.47
8	130 ± 10	0.52
9	152 ± 13	0.43
10	185 ± 14	0.33
11	179 ±13	0.42
12	156± 15	0.38
13	178 ± 14	0.27
14	136 ± 12	0.55
15	162 ± 14	0.41
16	122 ± 15	0.44

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Drug release from niosomes

The recent experiment was carried out in phosphate buffer pH=7 and drug release was followed during 52 hours. In order to determine the effect of independent variables on drug release, percentage of drug released after 8 hours (R8) and 48 hours (R48) were measured. Negligible drug released observed after 2-4 hrs and so R8 concerns with rapid release of the component whereas R48 indicates slow release rate (table 4).

Table 4. Different parameters regarding drug release from niosomes (mean±SD, n=3)

Formulation No.	%R8	%R48
1	30.5 ± 2.15	78.2 ± 2.3
2	19.8 ± 3.2	68.2 ± 1.5
3	15 ± 0.4	52.8 ± 0.8
4	13.1 ± 2.11	54.3 ±2.25
5	16.1 ± 2.33	57.9 ±4.2
6	8.1 ± 1.65	48.1 ± 0.75
7	12.1 ± 0.33	49.3 ± 1.27
8	14.47 ± 3.5	51.5 ± 3.6
9	10.36 ± 0.2	39.7 ± 0.5
10	7.43 ± 1.3	33.6 ± 0.3
11	9.5 ± 0.17	48.8 ± 1.2
12	15.2 ± 2.5	48.1 ± 2.77
13	5.8 ± 1.35	34.7 ± 1.1
14	3.9 ± 0.12	40.27 ± 2.4
15	6 ± 0.3	39.2 ± 1.2
16	9.2 ± 2.1	31.7 ± 1.8

As it can be observed, maximum amount of %R8 is 30.5% that indicated slow release pattern. L/S ratio had a significant and inverse impact on drug release after 8 hours. The results illustrate that an increase in mentioned variable leads to a decrease in drug release rate. This variable was the reasons for decreasing particle size. A maximum amount of R48 is 78.22% that belongs to formulation 1 same as %R8. S/L ratio and S.T had direct and L/S indirect and significant impact on %r48. It seems that by increase in surfactant content, the partitioning of drug toward outer lipid matrix promotes and S.T with decreasing particle size and increasing surface area improves %R48.

Release profile from niosomes follows a one-steps process that illustrates a slow release profile. In order to evaluate drug release mechanism from niosomes, release profile in three kinetic models including: zero, first-order and Higuchi models were studied. Correlation coefficients and velocity constants in three situations for all formulations indicate that Higuchi model had more consistency concerning with release profile. Accordingly, the main mechanism for control release of drug is diffusion which is strongly depends on concentration gradient between inside and outside environment of nanoparticles.

Minoxidil niosomal permeation studies from human hair follicule

In order to evaluate the effect of different formulations on minoxidil permeation, static diffusion cell and isolated human hair follicule were used. The amount and percentage of permeated drug was measured during 52 hours. These parameters were measured on the basis of infinite dose considering sink condition. The results demonstrate that less than 10% of drug in donor phase, permeate through human follicule while the maximum concentration in receiver phase was not more than 10% of drug saturation concentration in receiver phase thus sink condition and steady state was maintained. Percentage of drug permeated 52 hours (%P52) and follicular permeation profile t for all formulation in comparison with 2% aqueous solution of minoxidil as control shown in table 5 and figure 1.

Table 5. Presentation of %P52 parameters of minoxidil through human follicule hair comparing transfersomes with control groups (Mean±S.D, n=3)

Formulation No.	%P52
1	79.7 ± 6.65
2	74.1 ± 3.87
3	66.8 ± 3.79
4	55.5 ± 4.18
5	56.3 ± 5.11
6	67.6 ±6.25
7	26.9 ±2.88
8	49.8 ± 2.91
9	54.2 ± 1.51
10	26.9 ±2.23
11	30.5 ± 2.42
12	29.9 ± 1.95
13	35 ± 4.2
14	50.8 ± 4.19
15	36.2 ± 3.18
16	56.65 ± 4.45
Control	25.1 ± 2.37

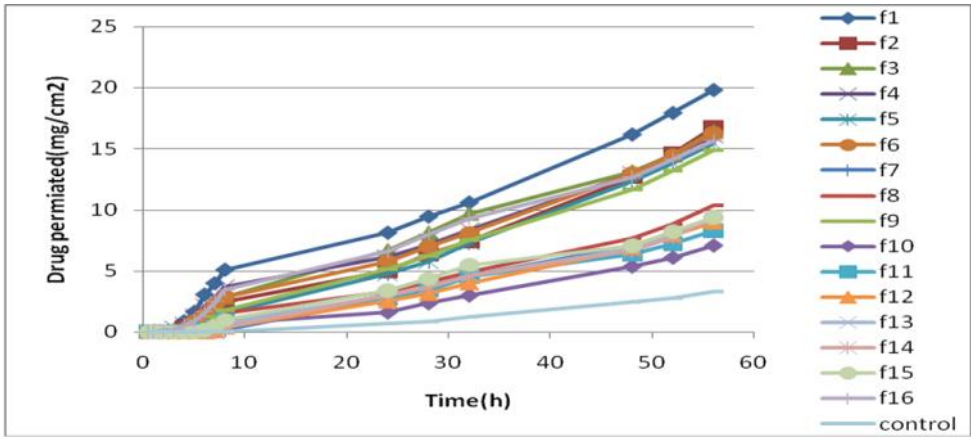

Fig 1. Minoxidil niosomal formulations permeated through human follicule hair comparison with control

The enhancement ratio for %P52 (ER_P) that obtained by different niosomes in comparison with control are sown in table 6.

Table 6: The calculated Effects of niosomes on %P52 in comparison with 2% aqueous solution as control (Mean ± Standard deviation, N=3)

Formulation No.	ER_P
1	3.2±0.22
2	2.96±0.21
3	2.67 ±0.3
4	2.22±0.23
5	2.25±0.17
6	2.7±0.20
7	1.07±0.11
8	1.99±0.14
9	2.17±0.15
10	1.07±0.09
11	1.22±0.11
12	1.2±0.13
13	1.4±0.09
14	2.03±0.14
15	1.45±0.12
16	2.27±0.19

The results suggest that the %P52 of minoxidil through human follicule hair was significantly increased ($p < 0.05$) relative to all niosomes excepting formulations 7, 10, 11 and 12. Maximum ER_P (3.2) obtained by formulation 1.. Another aspect of permeation studies is concerned with independent variables influences. Regression analysis between independent variables and %P52 indicates significant and indirect relation is confirmed with L/S ratio and direct with S/L and S. the similar patter observed for %R48. Therefore it seems that drug permeation through human follicules is controlled by drug release pattern. High amount of surfactant and low particle size of niosomes increased minoxidil permeation through follicules.

Discussion and Conclusion

In the present work attempts have been made to prepare the niosome by using minoxidil, which is used for Alopecia. Sixteen niosomal formulations were prepared by thin film hydration technique based on full-factorial design. Properties of niosomes such as the particle size and its distribution, the drug entrapment efficiency, the drug release behavior and drug permeation through human follicule hair were investigated. Niosomes demonstrated particle size range of 110-190 nm and maximum 82% drug loading. Low particle size and suitable drug loading help to localized minoxidil into the hair follicules. It seems that the main barrier for minoxidil penetration through hair follicule was the partitioning into the bulge region in follicule. This finding was according to physicochemical properties of minoxidil as hydrophobic compound. Significant and direct impact of surfactant amount on particle size and loading is similar but no any significant correlation was observed between particle size and loading. This finding suggested that the effect of surfactant on drug loading is not related to particle size. It seems that surfactant improves drug solubility on niosome. Main location for minoxidil loading in niosome is bilayers membrane. But with additional amount of surfactant higher amount of drug dissolved in membrane and aqueous core. Drug loading is only controlled by drug solubility. Drug release pattern demonstrates slow release profile. High drug solubility in niosome is the reason for this behavior. Slow release pattern is a good property for minoxidil because it helps to drug localization in the follicule. Highest drug release belongs to formulation 1 with lowest particle size. Therefore surface area and particle size are parameters that affect on drug release. In the other hand, follicular drug delivery by niosomes evaluated with percentage of drug that permeated through follicules after 52 hrs (%P52). Highest %P52 was 80% that provided by formulation 1. Our finding suggested 4 parameters that controlled %P52: higher drug release, using sodium lauryl sulfate as surfactant, higher amount of surfactant and solid lipid increased %P52. Most of niosomal formulations increased %P52 in comparison with control that

highest enhancement ration was 3 folds and provided by formulation 1. In conclusion, formulation 1 with perfect physicochemical properties and good stability is the best formulation that increased minoxidil follicular delivery by improves drug partitioning into the aqueous canal in human hair.

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References

1. J. Bouwstra, G. Pilgram, G. Gooris, H. Koerten, M. Ponec, New aspects of the skin barrier organization, Skin Pharmacol. Appl. Skin Physiol. 14 (Suppl. 1)(2001) 52–62.
2. E.H. Choi, S.H. Lee, S.K. Ahn, S.M. Hwang, The pretreatment effect of chemical skin penetration enhancers in transdermal drug delivery using iontophoresis, Skin Pharmacol. Appl. Skin Physiol. 12 (1999) 326–335.
3. H. Schaefer, T. Redelmeier, Skin Barrier: Principles of Percutaneous Absorption, Karger, Basel, 1996, p. 56.
4. E.A. Essa, M.C. Bonner, B.W. Barry, Human skin sandwich for assessing shunt route penetration during passive and iontophoretic drug and liposome delivery, J. Pharm. Pharmacol. 54 (2002) 1481–1490.
5. S. Dokka, S.R. Cooper, S. Kelly, G.E. Hardee, J.G. Karras, Dermal delivery of topically applied oligonucleotides via follicular transport in mouse skin, J. Invest. Dermatol. 124 (2005) 971–975.
6. R. Toll, U. Jacobi, H. Richter, J. Lademann, H. Schaefer, U. Blume-Peytavi, Penetration profile of microspheres in follicular targeting of terminal hair follicles, J. Invest. Dermatol. 123 (2004) 168–176.
7. T. Ogiso, T. Shiraki, K. Okajima, T. Tanino, M. Iwaki, T. Wada, Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of penetration between scalp and abdominal skins in vitro, J. Drug Target. 10 (2002) 369–378.
8. Azmin, M.N., Florence, A.T., Handjani-Vila, R.M., Stuart, J.F.B., Vanlerberghe, G., Whittaker, J.S., 1985. The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J. Pharm. Pharmacol. 37, 237–242.
9. Vora, B., Khopade, A.J., Jain, N.K., 1998. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Control. Release 54, 149–165.
10. Price, V.H., 1999. Treatment of hair loss. N. Engl. J. Med. 341, 964–973.
11. Han, J.H.,Kwon, O.S., Chung, J.H., Cho, K.H., Eun, H.C., Kim, K.H., 2004. Effect of minoxidil on proliferation and apoptosis in dermal papilla cells of human hair follicle. J. Dermatol. Sci. 34, 91–98.
12. Tata, S., Flynn, G.L., Weiner, N.D., 1995. Penetration of minoxidil from ethanol/ propylene glycol solutions: effect of application volume and occlusion. J. Pharm. Sci. 84, 688–691.
13. Williams, A.C., Barry, B.W., 2004. Penetration enhancer. Adv. Drug Deliv. Rev. 56,603–618.
14. Hagemann, T., Schlutter-Bohmer, B., Allam, J.P., Bieber, T., Novak, N., 2005. Contact Dermat. 53, 53–55.
15. Vora, B., Khopade, A.J., Jain, N.K., 1998. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Control. Release 54, 149–165.
16. Manconi, M., Sinico, C., Valenti, D., Lai, F., Fadda, A.M., 2006. Niosomes as carriers for tretinoin III A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. Int. J. Pharm. 311, 11–19.
17. Shahiwala, A.,Misra, A., 2002. Studies in topical application of niosomally entrapped nimuslide. J. Pharm. Sci. 5, 220–225.
18. Junginger, H.E., Hofland, H.E.J., Bouwstra, J.A., 1991. Liposomes and niosomes interactions with human skin. Cosmet. Toil. 106, 45–50.
19. Manconi, M., Sinico, C., Valenti, D., Lai, F., Fadda, A.M., 2006. Niosomes as carriers for tretinoin III A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. Int. J. Pharm. 311, 11–19.

