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RESEARCH PAPER

Development of a Novel Soft Hydrogel for the Transdermal Delivery of Testosterone

Na-Mi An,[1] Dae-Duk Kim,[1] Young-Hee Shin,[2] and Chi-Ho Lee[1,*]

[1]College of Pharmacy, Pusan National University, Pusan, Korea
[2]College of Pharmacy, Kyungsung University, Pusan, Korea

ABSTRACT

A soft hydrogel formulation for the transdermal delivery of testosterone (TS) was developed, and the effect of various skin-permeation enhancers was studied in vitro and in vivo. Testosterone was incorporated into a polyvinyl alcohol (PVA)-based soft hydrogel with polyisobutylene (PIB) and various skin-permeation enhancers (dodecylamine, HPE101, oleic acid, or lauric acid). In vitro rat-skin permeation of TS from the soft hydrogel was investigated using Keshary-Chien diffusion cells for 24 hr at 37°C. In vivo plasma-concentration profiles of TS after applying the soft hydrogel on the dorsal skin of rat were determined using a commercial radioimmunoassay kit. The formulated soft hydrogel formed a thin film on the skin within 2 to 3 min after application and remained in a dried-film state for at least 24 hr. Addition of PIB into the hydrogel to increase the adhesion resulted in a negligible reduction in the skin-permeation rate of TS. However, rat-skin permeation of TS increased with the addition of permeation enhancers both in vitro and in vivo. Dodecylamine at the concentration of 3% was the most effective among tested. Plasma concentration of TS significantly increased for at least 24 hr with the addition of dodecylamine. These results suggest the feasibility of the development of a soft hydrogel formulation for the transdermal delivery of TS.

Key Words: Testosterone; Permeation enhancer; Transdermal delivery; Soft hydrogel.

INTRODUCTION

Testosterone (TS) is the primary androgenic hormone secreted by the testis in men, while only small amounts of TS are synthesized in the ovary and adrenal in women.[1] Prior to puberty, the concentration of testosterone in plasma is low (less than 20 ng/dl), but in the adult male a 15- to 50-times

*Correspondence: Chi-Ho Lee, College of Pharmacy, Pusan National University, Pusan 609-735, Korea; Fax: 82-51-513-6754; E-mail: chiho@hyowon.pusan.ac.kr.

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increase is observed with the rate of production ranging from 2.5 to 11 mg per day.[2] As a therapeutic agent for men, TS is used for substitutional therapy for climacteric symptoms or for hypogonadism. It has also been employed to facilitate development of adult masculine characteristics when the adolescent process has been delayed. For women, TS therapy may be efficacious when administered with estrogens in the treatment of menopause. Testosterone also finds widespread application in the palliative treatment of breast cancer in women.[3]

The most common treatment for TS deficiency has been to replace the missing TS through injections or oral administrations. In recent years, researchers have studied a variety of different methods for TS administration, including transdermal-delivery system,[4] topical spray and topical aerosol,[5,6] sublingual tablets,[7] and subcutaneous implants.[8] Among these, the transdermal route is considered to be safer and more effective than injection methods. Avoidance of hepatic first-pass elimination, decrease in side effects, and the relative ease of drug-input termination in problematic cases, as well as maintaining suitable plasma concentration for longer duration through a noninvasive zero-order delivery, are the well-documented advantages of this route of administration.[9] Nevertheless, transdermal drug delivery has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum. Penetration enhancers, prodrugs, iontophoresis, and phonophoresis have been used as methods to increase the skin permeation rate of various drugs.[10] Among them, penetration enhancers are one of the most convenient methods and have shown relatively high effects.

Polyvinyl alcohol (PVA)-based soft hydrogel is a novel transdermal drug-delivery system developed in this laboratory. It is in a semisolid phase in a sealed tube but forms a thin film within 2 to 3 min when applied to the skin (Fig. 1). It can, therefore, prevent drugs from being washed out and release drugs from the dried film for a long period of time. In our previous study, an optimum formulation of PVA hydrogel was studied using the computer-optimization technique with indomethacin as a model drug.[11] In this study, a soft hydrogel of TS was formulated and the effect of polyisobutylene (PIB) as an adhesive agent and various skin-permeation enhancers on the permeation of TS across the excised rat skin were examined in vitro and in vivo.

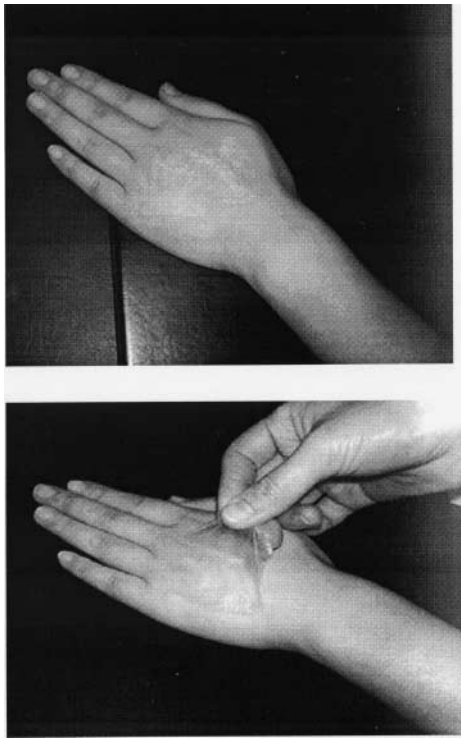

Figure 1. Morphology of soft hydrogel after 2 to 3 min of application on the skin.

MATERIALS AND METHODS

Materials

Testosterone, oleic acid, lauric acid, dodecylamine, and PEG400 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1-(2-(Decylthio)ethyl)azacyclopentan-2-one (HPE-101) was obtained from Hisamitsu Pharmaceutical Co. (Tokyo, Japan). PIB and PVA were gifts from Amarans Cosmetic Co. (Seoul, South Korea). Tween 60, span 60, and propylene glycol (PG) were purchased from Junsei Chemical Co. (Tokyo, Japan), and poly(1-vinylpyrrolidone-co-acrylic acid) from Aldrich Chemical Co. (Milwaukee, WI). All the reagents were of analytical grade or higher.

Fabrication of the Soft Hydrogel

Various amounts of PIB and a permeation enhancer were added into 100 g of 3% TS soft hydrogel formulation and fabricated as follows: PVA (9.0 g), H_2O (35.0 mL), and ethanol (4.0 g) were

mixed uniformly in beaker A and heated in a 50°C water bath for 3 hr. In beaker B, PEG400 (5.0 g), PG (4.0 g), glycerin (2.5 g), tween 60 (2.0 g), H_2O (10.0 mL), and ethanol (4.0 g) were mixed uniformly. In beaker C, PVAA (0.5 g) was mixed uniformly with 14.7 mL of H_2O. Beakers A, B, and C were placed in a 85°C oven for 1 hr, and then contents of beaker B and C were poured into beaker A. Various amounts of PIB (1.0–6.0 g) dissolved in liquid paraffin (1:1 ratio) was added in beaker A and mixed using a homogenizer at 800 rpm for 30 min in a 60°C water bath. Testosterone (3.0 g), a skin-permeation enhancer (0–5.0 g), span 60 (0.3 g), and ethanol (6.0 g) were mixed in beaker D at 60°C and then poured into beaker A. The mixture of A, B, C, and D in beaker A was thoroughly mixed with a homogenizer in a 60°C water bath for an additional hour. After sonicating for about 2 min, the soft hydrogel was finally stored in a sealed tube and kept in the refrigerator until used for further experiments.

In Vitro Study

In vitro skin permeation of TS across the rat skin was conducted using Keshary-Chien permeation cells (surface area of 2.14 cm^2) at 37°C. Male Sprague Dawley (250–300 g) rats obtained from Dae-Han Laboratory Animal Research Center Co. (Taejeon, Korea) were humanely sacrificed in a CO_2 chamber. The dorsal hair was removed with a clipper and full-thickness skin (about 16 cm^2) was surgically removed from the dorsal site of each rat. After carefully removing the subcutaneous fat and washing with normal saline, the skin specimen was cut into appropriate sizes. Soft hydrogel of various compositions (0.2 g) was applied to the stratum corneum side of the skin and then mounted between the donor and receptor cells (stratum corneum side facing the donor cells). The receptor half-cells were filled with isotonic phosphate buffer (IPB) solution containing 40% (v/v) PEG400 to maintain sink condition (12.0 mL). At predetermined time intervals, 0.5 mL of receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. Samples were kept in a freezer (−20°C) until analyzed by HPLC.

HPLC Analysis of Testosterone

The TS concentrations were determined using a HPLC system (Waters 746) with a UV detector (Waters M510). A Waters ODS column (5 μm, 125 × 4 mm) was used as the analytical column at ambient temperature. An acetonitrile–acetate buffer (pH 4.0, 0.1 M) combination (60:40) was used as the mobile phase at a flow rate of 1.0 mL/min. The wavelength of the UV detector was set at 242 nm. Injections of 20 μL were made for all solutions to be analyzed. Retention time of TS was about 6 min.

In Vivo Study

Rats weighing about 250 g were lightly ether anesthetized and dorsal hair was removed with a hair clipper. After one day, each rat was kept in a metabolic holder and under light ether anesthesia, soft hydrogel (1.5 g) was applied on the dorsal skin of rat (surface area = 4 × 5 cm^2). At predetermined time intervals, blood samples (about 150 μL) were obtained for 24 hr from the tail vein without anesthesia using a heparinized 1-mL syringe. The blood was immediately centrifuged for 5 min at 3000 rpm and the plasma was kept at −20°C until analyzed.

Plasma concentration of TS was measured by radioimmunoassay (RIA) using reagents and protocol supplied by the Diagnostic Products Corporation (DPC, Los Angeles, California). Fifty microliters of plasma was used for assay. The lower limit of detection was 0.2 ng/mL.

RESULTS AND DISCUSSION

Effect of Polyisobutylene (PIB) as an Adhesive Agent

In our preliminary studies, the PVA-based soft hydrogel showed a poor bioadhesion force once a thin film was formed after drying. Thus, various concentrations of PIB was added in the formulation to increase the adhesiveness of the soft hydrogel. PIB is known to increase the bioadhesive potential by reducing the surface energy and/or water-wetting angle when added in the adhesive polymer.[12] Moreover, it is also known that the release rate of various drugs with different functional groups is not changed since PIB has no reactive functional groups among its polymer components.[13]

As shown in Fig. 2, the rat skin-permeation rate of TS showed a slightly decreasing tendency as the concentration of PIB in the soft hydrogel increased up to 6%. However, it was not statistically significant.

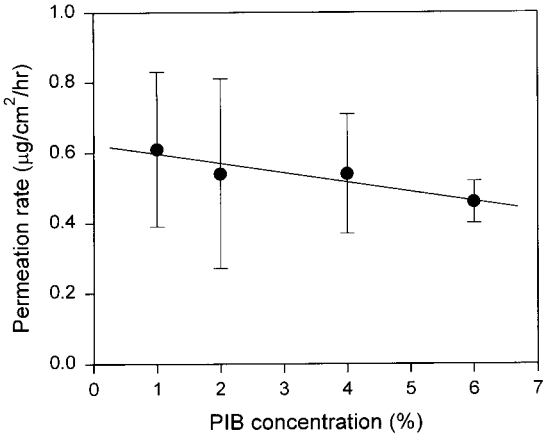

Figure 2. Effect of PIB concentration on the skin-permeation rate of TS delivered from soft hydrogel containing 3% TS without skin-permeation enhancer. Each value is the mean ± SD of three determinations.

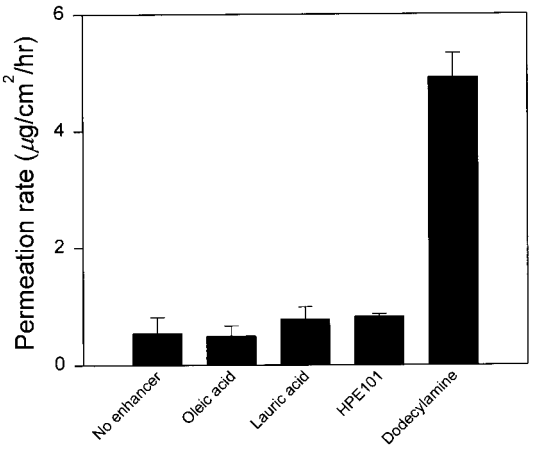

Figure 3. Effect of various skin-permeation enhancers (3%) on the skin-permeation rate of TS from soft hydrogel containing 2% PIB. Each value is the mean ± SD of three determinations.

The addition of 2% PIB already resulted in the desirable adhesive properties with the insignificant reduction in TS permeation rate. However, it was not possible to measure the peeling strength of the soft hydrogel since it was too fragile after forming a film.

Effect of Skin-Permeation Enhancers

The effect of various skin permeation enhancers on the skin permeation rate of TS was investigated using the soft hydrogel containing 2% PIB and 3% enhancer (i.e., dodecylamine, HPE-101, lauric acid, or oleic acid). The permeation rates of TS were calculated from the slope of the linear portion of the cumulative amount permeated vs. time in hour. As shown in Fig. 3, dodecylamine was the most effective enhancer among those tested. The permeation rate of TS from the soft hydrogel without an enhancer was 0.54 (±0.27) μg/cm²/hr, and that containing 3% dodecylamine was about 10 times greater, being 4.92 (±0.41) μg/cm²/hr. On the other hand, not much enhancing effect was observed with oleic acid, lauric acid, and HPE-101.

To estimate the effect of the dodecylamine concentration on the skin-permeation rate of TS, various concentrations (1%, 3%, and 5%) of dodecylamine were added into the soft hydrogel containing 2% PIB. Figure. 4 shows the effect of dodecylamine concentration on the skin-permeation profiles of TS. Skin-permeation rate of TS increased proportionally

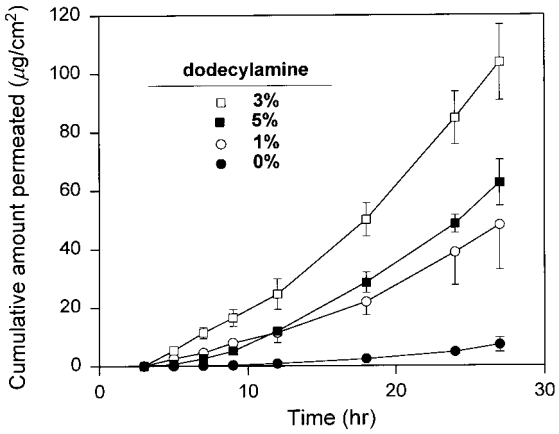

Figure 4. Skin-permeation profiles of TS delivered from soft hydrogel containing 2% PIB and various concentrations of dodecylamine. Each value is the mean ± SD of three determinations.

as the concentration of dodecylamine increased up to 3% in the soft hydrogel (Table 1). However, TS permeation rate decreased when 5% dodecylamine was added in the soft hydrogel. Similar parabolic dose-dependent enhancement effects of skin-permeation enhancers were reported in previous studies.[14–16] Addition of more than 3% dodecylamine dramatically increased the viscosity of soft hydrogel, changing it to a gummy state, which probably resulted in the reduction of the thermodynamic activity of TS, thereby decreasing the skin-permeation rate.

Table 1. Rat skin-permeation rate of TS delivered from soft hydrogel containing 2% polyisobutylene and various concentrations of dodecylamine.

Dodecylamine concentration (%)	Permeation rate $\mu g/cm^2/hr$ (±SD)	Lag time hour (±SD)
0	0.54 (0.27)	7.67 (0.59)
1	2.28 (0.75)	5.92 (1.54)
3	4.92 (0.41)	6.09 (0.57)
5	3.37 (0.26)	8.82 (0.57)

Each value is the mean (±SD) of three determinations.

Fatty acids and amines are known to have a potent skin permeation-enhancing effect for various drugs.[9,17] These effects appear to involve the disruption of lipid bilayers that are filling the extracellular spaces of the stratum corneum. Unsaturated fatty acids particularly affect the fluidity of lipids in the intercellular layers of the stratum corneum because of their resemblance in structure to the lipids.[18] Dodecylamine is a typical unsaturated fatty amine, which is known to increase the skin-permeation rate of various drugs.[19] Addition of dodecylamine in propylene glycol significantly increased the skin-permeation rate of TS by increasing the partitioning of TS on the skin, thereby increasing the permeability coefficient.[19] A recent study from this laboratory also demonstrated that dodecylamine synergistically increased the skin-permeation rate of TS when added in an ethanol/water cosolvent system.[20]

In Vivo Effect of Dodecylamine

Figure 5 shows the plasma TS concentration vs. time profiles following the application of the soft hydrogel containing 2% TS and 2% PIB. Basal plasma TS-concentration profile of rats without the soft hydrogel application was also observed as a control, which was within the normal level of 3–10 ng/mL as reported in the literature.[21] Plasma concentration of TS significantly increased after applying the TS soft hydrogel, and reached maximum concentration within 1 hr after the application. High plasma concentration of TS was maintained for at least 24 hr, probably due to the sustained release of TS from the soft hydrogel. Dodecylamine significantly increased the plasma concentration of TS, which is consistent with the in vitro skin-permeation study. Figure 6 shows the area under the curve

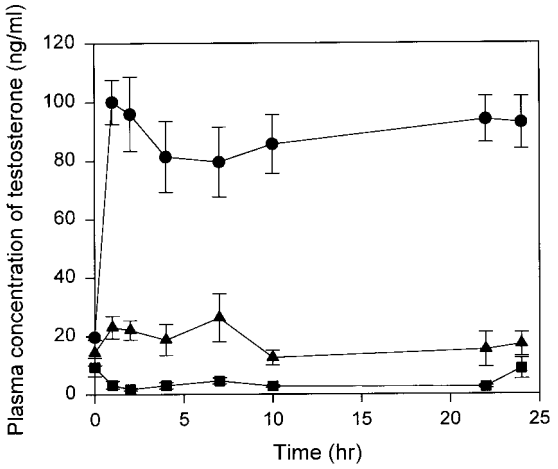

Figure 5. Plasma-concentration profiles of TS after application of soft hydrogel on the abdominal skin of rat. Each hydrogel contained 3% TS and 2% PIB. Each value is the mean ± SD of 3 to 6 determinations. ■, basal plasma concentration of TS; ▲, without dodecylamine; ●, with 3% dodecylamine.

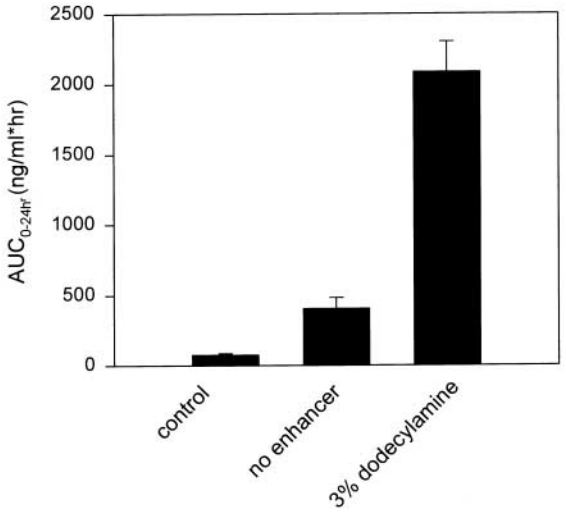

Figure 6. Area under the curve (AUC) values calculated from the plasma-concentration profiles of TS for 24 hr by the trapezoidal rule. Each hydrogel contained 3% TS and 2% PIB. Each value is the mean ± SD of 3 to 6 determinations.

(AUC) values for 24 hr ($AUC_{0\sim24h}$) calculated from the plasma concentration profiles of TS by the trapezoidal rule. The AUC value significantly increased from 77.73 (±8.67) ng·hr/mL to 407.29 (±76.71) ng·hr/mL when 3% dodecylamine was added in the soft hydrogel.

CONCLUSIONS

Polyvinyl alcohol-based novel soft hydrogel formulation for TS transdermal delivery was successfully prepared. The soft hydrogel formed a thin film on the skin within 2 to 3 min after application and remained in a dried-film state on the skin for at least 24 hr. While PIB did not significantly affect the skin-permeation rate of TS, dodecylamine was the most effective skin-permeation enhancer for TS in both the in vitro and in vivo studies. These results suggest that the development of a soft hydrogel formulation for the controlled transdermal delivery of TS is feasible, and further clinical study is under way in this laboratory.

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REFERENCES

1. Hardman, J.G.; Limbird, L.E., Eds. In *A Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 9th Ed.; Macmillan: London, 1996; 1459–1485.
2. Rosenfield, R.L. Role of androgens in growth and development of the fetus, child, and adolescent. Adv. Pediatr. **1972**, *19*, 172–213.
3. Gennaro, A.R., Ed. *Remington: The Science and Practice of Pharmacy*, 20th Ed.; Lippincott Williams & Wilkins: Philadelphia, 2000; 1391.
4. Satyannrana, S.; Ganaga, S.; Singh, J. Transport through rat skin from transdermal patch formulations. Pharmazie **1993**, *48*, 467–468.
5. Morgan, T.M.; Reed, B.L.; Finnin, B.C. Enhanced skin permeation of sex hormones with novel topical spray vehicles. J. Pharm. Sci. **1998**, *87* (1), 1213–1218.
6. Morgan, T.M.; Parr, R.A.; Reed, B.L.; Finnin, B.C. Enhanced transdermal delivery of sex hormones in swine with a novel topical aerosol. J. Pharm. Sci. **1998**, *87* (1), 1219–1225.
7. Salehian, B.; Wand, C.; Alexander, G.; Davidson, T.; McDonald, V.; Berman, N.; Dudley, R.E.; Zikl, F.; Verdloff, R.S. Pharmacokinetics, bioefficacy and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate—a clinical research center study. J. Clin. Endocr. Metab. **1995**, *80* (12), 3567–3575.
8. Kesler, D.J.; Christenson, D.C.; Wallace, M.E. The effect of esterification on their release of testosterone and estradiol from silicone implants. Drug Dev. Ind. Pharm. **1996**, *22* (3), 275–279.
9. William, A.C.; Barry, B.W. Skin permeation enhancers. Crit. Rev. Ther. Drug **1992**, *9*, 305–353.
10. Sugibayashi, K.; Nakyama, S.; Seki, S.; Hosoya, K.; Morimoto, Y. Mechanism of skin penetration-enhancing effect by laurocapram. J. Pharm. Sci. **1992**, *81*, 58–64.
11. Lee, C.H.; Lee, K.J.; Park, A.J.; Shin, Y.H. Studies on controlled release of indomethacin from PVA hydrogel. Arch. Pharm. Res. **1993**, *16*, 43–49.
12. Guo, J. Investigation of the surface properties and bioadhesion of buccal patches. J. Pharm. Pharmacol. **1994**, *46*, 647–650.
13. Kokubo, T.; Sugibayashi, K.; Morimoto, Y. Interaction between drugs and pressure-sensitive adhesives in transdermal therapeutic systems. Pharm. Res. **1994**, *11*, 104–107.
14. Xiong, G.L.; Quan, D.; Maibach, H.I. Effects of penetration of penetration enhancers on in vitro percutaneous absorption of low molecular weight heparin through human skin. J. Cont. Rel. **1996**, *42*, 289–296.
15. Hou, S.Y.E.; Flynn, G.L. Enhancement of hydrocortisone permeation of human and hairless mouse skin by 1-dodecylazacycloheptane-2-one. J. Invest. Dermatol. **1989**, *93*, 774–779.
16. Lambert, W.J.; Higuchi, W.I.; Knutson, K.; Krill, S.L. Dose-dependent enhancement effects of azone on skin permeability. Pharm. Res. **1989**, *6*, 798–803.
17. Cooper, E.R. Increased skin permeability for lipophilic molecules. J. Pharm. Sci. **1984**, *73*, 1153–1156.
18. Kim, D.D.; Kim, J.L.; Chien, Y.W. Mutual hairless rat skin permeation enhancing effect of ethanol/water system and oleic acid. J. Pharm. Sci. **1996**, *85*, 1191–1195.
19. Anungst, B.J.; Blake, J.A.; Hussain, M.A. Contributions of drug solubilization, partitioning, barrier disruption, and solvent permeation

to the enhancement of skin permeation of various compounds with fatty acids and amines. Pharm. Res. **1990**, *7*, 712–718.

20. Kim, M.K.; Zhao, H.; Lee, C.H.; Kim, D.D. Formulation of resevoir-type testosterone transdermal delivery system. Int. J. Pharm. **2001**, *219*, 51–59.
21. Royland, J.E.; Weber, L.J.; Fitzpatrick, M. Testes size and testosterone levels in a model for weightlessness. Life Sci. **1994**, *54*, 545–554.