

## *In vitro* and *in vivo* evaluation of a novel testosterone transdermal delivery system (TTDS) using palm oil base

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### ARTICLE INFO

#### Article type:

Original article

#### Article history:

Received: Mar 31, 2015

Accepted: Aug 6, 2015

#### Keywords:

HAMIN™

Skin permeation

Testosterone

Transdermal delivery-system

### ABSTRACT

**Objective (s):** Transdermal preparations for testosterone are becoming popular because of their unique advantages such as avoidance of first-pass effect, convenience, improved bioavailability, and reduction of systemic side effects. A novel testosterone transdermal delivery system (TTDS) was developed using a palm oil base called HAMIN™ (a commercial product) and tested using *in vitro* and *in vivo* skin permeability test methods.

**Materials and Methods:** The physical characteristics of the formulation such as particle size and viscosity were determined by using Franz diffusion cell and Brookfield viscometer, respectively. *In vivo* skin permeability test was performed on healthy rabbits through the skin. Testosterone in serum was analyzed using the validated Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique.

**Results:** *In vitro* study showed that the cumulative amount of testosterone diffused was between 40 to 1400 ngcm<sup>-2</sup> over a period of five hr after application of TTDS through the artificial Strat-M™ membrane. In the *in vivo* rabbit skin permeability test, the results indicated that testosterone was well absorbed with a mean C<sub>max</sub> and T<sub>max</sub> of 60.94 ngml<sup>-1</sup> and 2.29 hr after application of TTDS while no increase was observed in placebo treatment. Particle size analysis ranged from 79.4 nm to 630.0 nm for placebo and 97 to 774.0 nm for TTDS.

**Conclusion:** The formulation was successfully prepared using HAMIN™, which has demonstrated great potential for topical delivery of testosterone.

#### ► Please cite this article as:

Didi EMH, Chik Z, Noordin MI, Mohamed Z. *In vitro* and *in vivo* evaluation of a novel testosterone transdermal delivery system (TTDS) using palm oil base. Iran J Basic Med Sci 2015; 18:1167-1175.

## Introduction

Testosterone is one of the primary naturally occurring androgens in man. The androgens have a key role in the production of secondary sexual characteristics in men (1). Androgen treatment has been shown to relieve symptoms of androgen deficiency (2). Male hypogonadism is defined as low testosterone levels with serum total testosterone <10–12 nmolL<sup>-1</sup> (~2.88–3.46 ngml<sup>-1</sup>) (3). Testosterone deficiency or hypogonadism demonstrates symptoms such as impotence, exhaustion, upset mood, and weakening of secondary sexual characteristics (4-6). Testosterone replacement therapy may be indicated by improving serum testosterone levels (4, 7).

Testosterone is available in several preparations which include subcutaneous implants (8), oral, sublingual preparations and scrotal transdermal patches (9), non-scrotal transdermal patches, and testosterone gel (2). Among numerous administration means for replacing testosterone, transdermal delivery

system (TDS) provides more advantages compared to oral and intramuscular application. Occurrence of high hepatic first-pass metabolism in the liver following oral administration requires that many testosterone doses be administered, hence the need for potentially painful injections that may lead to supra-physiological testosterone serum concentrations (10). However, a negative aspect of TDS is that only small amounts of drug manage to be delivered through the stratum corneum. Thus, drugs that are given transdermally must be effective even at small doses (11). The interest in developing alternative formulations for the transdermal delivery of testosterone remains a desirable objective (12). Testosterone is considered to be a suitable candidate for transdermal delivery due to the hydrophobic nature (log P<sub>o/w</sub>=3.3) and low molecular weight (M.W.=288 g/mol) of testosterone which are favorable factors for transdermal delivery (13).

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In the current study, the suitability of a paration from domestic palm oil called HAMIN™ to perform as a base for topical delivery of a testosterone cream formulation was investigated. Palm oil derives from the fruit of the *Elaeis guineensis* tree (14). Lately, the use of palm oil has been explored extensively in the pharmaceutical industry for a variety of applications due to its good characteristics such as incorporation of long-chain triglyceride, being non-toxic, relatively low cost, and high thermal, high productivity and oxidative stability (15). However, only a few publications mention palm oil as an alternative in cream formulation for drug delivery (16-18). To date, there have been no reports on the application of palm oil in transdermal delivery of testosterone, especially in Malaysia. General product quality tests such as classification, substance uniformity, particle size, and viscosity need to be conducted for topical drug products. In this work, we assessed the *in vitro* transfer of testosterone cream through an artificial membrane, of the prepared testosterone transdermal delivery system (TDDS) using HAMIN™ as a base, following which, *in vivo* skin permeability test using rabbits was conducted to initially assess the TDDS to deliver the therapeutic amount of testosterone through the skin. *In vivo* skin permeability test on animals is a good indicator for the skin absorption properties prior to its assessment in humans.

## Materials and Methods

### Chemicals

All chemicals were HPLC grade. Methanol was obtained from JT Baker, USA while diethyl ether was obtained from VWR International Ltd (England). Activated charcoal was provided by R&M Chemicals (Essex UK) while ethyl acetate and formic acid were obtained from Fisher Scientific. Testosterone standard and prednisone as internal standard were procured from Sigma-Aldrich, USA. Xylazine and ketamine were purchased from Troy Laboratories PTY Limited, Australia. Strat-M™ membranes were purchased from Phenomenex, USA. The palm oil base which contains a mixture of hydrogenated palm kernel stearin, hydrogenated palm oil stearic acid, and glyceryl monostearate was obtained from the Department of Pharmacy, University of Malaya, Kuala Lumpur, Malaysia. Span 20 and tween 80 were obtained from the R&M Chemical, while deionized water was used for cream preparation.

### Animals

New Zealand white strain rabbits weighing 1.2-1.5 kg were obtained from the Animal House, University of Malay, Kuala Lumpur, Malaysia. The animals were sheltered in a controlled environment (23 °C ± 2 °C), hygienic room with a 12 hr light and dark cycle, and also were allowed to get unlimited

water and food. The experimental procedures and animal welfare followed the care and use of animals and related internal ethical guidelines. Efforts were made to avoid animal suffering and to cut down on the number of rabbits used. The animals were maintained in individual wire cages, given solid pellet diet including food and water. They were housed in an animal holding room during the period of the study.

### Instruments

The LC-MS/MS instrument consisted of an LC-10A UFLC pump with an SIL-HT automatic auto-injector (Shimadzu, Kyoto Japan) and an API 3200 Mass Spectrometer system (Applied Biosystems, USA). The LC-MS/MS system was operated using Analyst software (version 1.4.2). Nitrogen was used as the nebulizer, auxiliary, collision, and curtain gas. Particle size was measure using Dynamic Light Scattering model ELSZ-0 (Otsuka Electronics, Japan), Viscometer used to measure viscosity was RVDV-II Model (Brookfield, USA) and permeation study was analyzed by Franz diffusion cell, (Permegear, USA) consisting of a 5 ml calibrated receptor compartment with a sampling port.

### LC-MS/MS parameter

For this study, the analytical column used was a Phenomenex, Gemini-NX C18 (150 mm length x 2.1 mm internal diameter, particle size 5 µm) and Phenomenex, Gemini-NX C18 guard column (4 mm internal diameter x 2.0 mm length). The mobile phase used was 50 mM ammonium acetate (pH 5.8) in pump A, while mobile phase B was 100% acetonitrile. The flow rate was set at 0.35 mlmin<sup>-1</sup> and a gradient elution at room temperature was used. The gradient program started with 45% B, then ramped to 95% B at 4.00 min and held until 6.00 min. The mobile phase was then brought back to 45% B at 6.10 min and this state was held until 9.00 min. Sample injection volume was 15 µl. An API 3200 tandem mass spectrometer fitted with an electrospray ionization (ESI) probe and run in the positive ionization mode, was used to perform mass spectral analysis. Analytes were then quantified by multiple reactions monitoring (MRM). The measurement of parent and daughter ions simultaneously were enhanced by MRM for each selected compound. The protonated ion [M+H]<sup>+</sup> for the selected analyte was picked up by the first quadrupole (Q1) and targeted into the collision cell (Q2) where it would be fragmented to smaller product ions during mass spectral analysis. For each [M+H]<sup>+</sup> precursor ion, a distinct product ion was elected by the third quadrupole (Q3). The precursor ion intensity was constantly monitored and the data was kept in the computer system of the mass spectrometer. For quantitative analysis, the

**Table 1.** Dosage amount of ingredients in placebo and testosterone transdermal delivery system (TDDS) cream

Composition	Placebo (g)	TDDS (g)
Palm oil (HAMIN) -mixture of hydrogenated palm kernel stearin, hydrogenated palm oil stearic acid, and glyceryl monostearate	7.00	7.00
Span 20	2.25	2.25
Tween 80	2.66	2.66
Testosterone	-	1.00
Water	8.09	7.09
Total	20.00	20.00

precursor ion was  $m/z$  289.27 while fragment ions monitored for testosterone were  $m/z$  97.10 and  $m/z$  109.10. Precursor ion  $m/z$  369.64 and fragment ions  $m/z$  147.00 and  $m/z$  171.00 were monitored for the internal standard (IS), prednisone. The spectrum and molecular structure of testosterone are shown in Figure 1. This method was validated based on the bioanalytical method validation procedures as outlined by the United States Food and Drug Administration, USFDA (19).

#### TDDS preparation

To formulate TDDS, palm oil was used as the oil phase while the mixture of Tween 80 and span 20 were used as surfactants. Homogenization technique was used in this preparation. The solid palm oil base was first heated at 60 °C in a water bath to form a liquid, and surfactant combinations were later added into the mixture. The mixture was homogenized using IKA T-18 Ultra Turrax Digital Homogenizer at 4000 rpm. Testosterone (1 g) was then added to the mixture to obtain 5%. Deionized water which was heated separately to 60°C and added into the mixture until the total weight was 20 g and the homogenization process was continued until a semi-solid form was reached. The formulation was allowed to cool unassisted at ambient temperature. The above procedures were used to prepare placebo with no testosterone added, which act as a control. The chemical compositions of the TDDS and placebo are listed in Table 1.

#### Particle size measurements

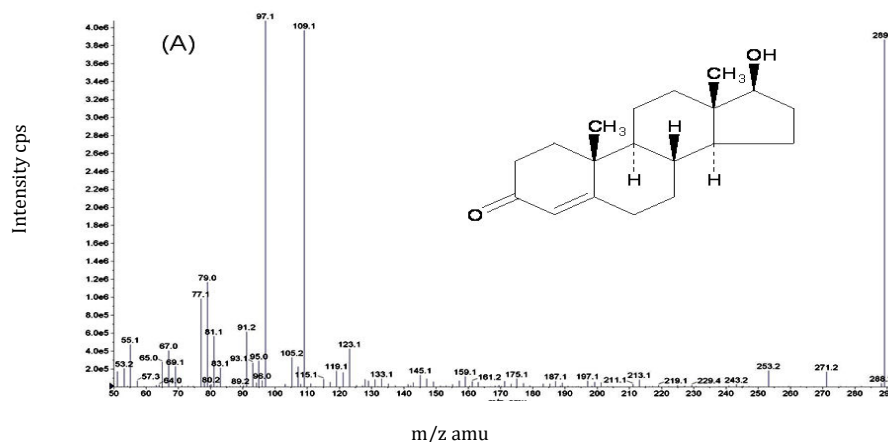
The particle size of the emulsion was analyzed using Dynamic Light Scattering (DLS) particle size analyzer (ELSZ-0, Otsuka Electronics, Japan). The measurement was carried out at a scattering angle of 165 ° at 25 °C. The emulsions were dissolved with deionized water to a measured concentration at which the DLS analyzer can analyze the scattering light intensity accurately.

#### Rheological test

The viscosity of the prepared testosterone formulations were calculated using a Brookfield Viscometer (Brookfield Model RVDV-II Middleboro, USA) by use of spindle no 52. The spindle speed rate was amplified in ascending (0.01–0.1 rpm) to descending speed order (0.1–0.01 rpm). The viscosity was measured from the viscometer display.

#### In vitro permeability test

The permeation of TDDS was done through the artificial Strat-M™ membrane and determined by Franz diffusion cell. The receptor compartment was filled up with receptor medium 50 mM ammonium acetate and making sure that all the bubbles under the membrane were removed. The whole apparatus was fixed on a magnetic stirrer and the solution was continuously stirred using magnetic stirring bar (11xg). A quantity of 1 g of TDDS formulation was applied topically to the surface of the membrane and

**Figure 1.** Chemical structure and mass spectrum for testosterone obtained from LC-MS/MS

spread evenly to achieve complete coverage. The donor compartment was enclosed with parafilm and the temperature was kept at  $35\pm 1$  °C by a water jacket. The diffusion area was  $0.81\text{ cm}^2$ . Samples (500  $\mu\text{l}$ ) were taken out from the sampling port at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 hr time intervals, and 500  $\mu\text{l}$  of fresh solution was immediately injected into the sampling port to replace samples which were drawn earlier. The concentration of drug in each sample was measured by LC-MS/MS. The cumulative amount of testosterone from receiver solutions across the synthetic membranes after application of TDDS over 5 hr was plotted.

#### **In vivo skin permeability test**

This is a pilot study to investigate the ability of a TDDS to deliver the drug effectively through rabbit skin using HAMIN™ for topical delivery of testosterone and placebo as a control. New Zealand white rabbits were used because these animals are very docile and non-aggressive and hence they were easy to handle thereby allowing us to make observations. Furthermore, it is widely bred and very economical compared with larger animals such as pigs or monkeys (20). In addition, the use of rabbits for experimentation is in the small animal category hence it comes under the purview of the institutional animal ethics committee which we have in place. The study was approved by the Animal Care and Use Committee (ACUC), Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, (ethics reference no. FAR/17/02/2012/DEMH(R)).

#### **Animal model**

Seven adult male rabbits received two treatments each. They received placebo in the first treatment and TDDS in the second. The rabbits were anesthetized with a mixture of ketamine and xylazine, which was administered intramuscularly. The fur of the rabbits over the dorsum was removed using electric hair clippers. One gram of either placebo or TDDS preparation were applied to the shaved area of the rabbit abdomen ( $16\text{ cm}^2$ ) and gently massaged. Each of the rabbits was left to recover for one week after each treatment. One milliliter of blood samples were taken from the marginal vein of the ear into plain tubes periodically at times of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 4 hr after administration. Blood was left for 10 to 15 min before centrifugation at 7378xg for 5 min and the serum was transferred into cryovial tubes and stored at  $-20^\circ\text{C}$  prior to analysis. The cannula was flushed with approximately 200  $\mu\text{l}$  of heparinized 0.9% NaCl injectable solution (10 units  $\text{ml}^{-1}$ ) immediately after each blood sampling to prevent occlusion. Specimens were thawed in room temperature before analysis (21).

#### **Sample preparation**

Sample preparation was carried out using liquid-liquid extraction. The frozen serum was thawed at room temperature ( $25\pm 1$  °C). The thawed serum was vortexed to ensure complete mixing of content. To each 100  $\mu\text{l}$  serum sample, 50  $\mu\text{l}$  of IS (containing 500  $\text{ngml}^{-1}$  of prednisone) was added followed by 800  $\mu\text{l}$  mixture of diethyl ether: ethyl acetate (50:50 v/v). The mixture was vortexed for 20 sec and centrifuged for 5 min at 16162xg. The aqueous layer was discarded and the solvent layer was transferred to a fresh microcentrifuge tube and evaporated to dryness under a nitrogen stream. The extract was dissolved in 100  $\mu\text{l}$  of 70% methanol-water mixture, vortexed then centrifuged for 2 min at 7378xg. The supernatant was then transferred to 2 ml vials with inserts. A volume of 15  $\mu\text{l}$  was then injected into the LC-MS/MS instrument.

#### **Pharmacokinetic analysis**

Pharmacokinetic parameters were measured by a non-compartmental analysis using WinNonlin Professional, ver. 5.3 (Pharsight Corporation, Mountain View, California). The total area under the serum concentration-time curve from time zero to the last measured time, in serum ( $\text{AUC}_{0-t}$ ), was determined using the trapezoidal rule method. The peak serum concentration ( $C_{max}$ ) and time to reach the maximum concentration ( $T_{max}$ ) were determined from the experimental data directly. The statistical significance of the differences for  $\text{AUC}_{0-t}$  between placebo and TDDS of pharmacokinetic parameters were compared using analysis of paired t-test at the probability level of  $P < 0.05$ . All statistical analyses of data were performed using SPSS ver. 22.

## **Result**

#### **Particle size measurements using DLS**

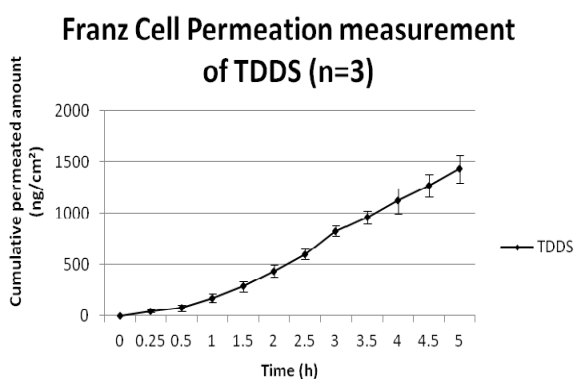
Both TDDS and placebo particle size were measured using DLS in order to study the influence of particle size on viscoelastic properties of a semi-solid testosterone structure, and the particle sizes were calculated using ELS-Z software version 3.01. Both TDDS and placebo were prepared using a homogenization technique that revealed particle sizes, which ranged from 79.4 nm to 630.0 nm for placebo and 97 to 774.0 nm for TDDS. The results show that the placebo contains smaller particle sizes compared to TDDS.

#### **Rheological test**

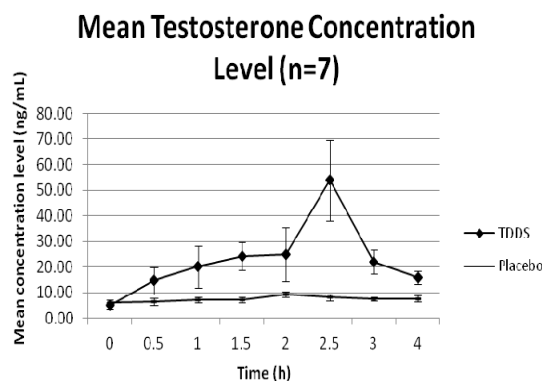
In the viscosity test, shear stress was first increased from 0.01 to 0.1 rpm and then decreased from 0.1 to 0.01 rpm to check possible effects of viscosity. Placebo and TDDS had similar flow curves, but the TDDS had greater pseudoplastic loops than placebo, which means that the formulated TDDS is highly viscous compared to placebo. Shear stress can

**Table 2.** Viscosity of placebo and rheological behavior

Viscosity (Pa s)	Speed (RPM)	Torque (%)	Shear Stress (D/cm2)	Shear Rate (1/Sec)
367114.00	0.01	3.68	73.42	0.02
347270.00	0.03	10.46	208.36	0.06
208362.00	0.06	12.60	250.03	0.12
177355.75	0.08	14.35	283.77	0.16
161728.60	0.10	16.26	323.46	0.20
173635.00	0.08	14.03	277.82	0.16
195132.67	0.06	11.83	234.16	0.12
277816.00	0.03	8.38	166.69	0.06
575476.00	0.01	5.83	115.10	0.02



**Figure 2.** Cumulative amounts of testosterone transdermal delivery system (TDDS) permeated after 5 hr in Strat M™ membrane



**Figure 3.** Mean serum concentration levels of testosterone after application of testosterone transdermal delivery system (TDDS) and placebo in healthy rabbits (n=7)

only be increased up to 0.05 rpm due to high viscosity in the TDDS. From the rheological test, viscosities of both TDDS and placebo are shown in Tables 2 and 3.

**In vitro permeability test**

The aim of the *in vitro* experiments in transdermal delivery is to evaluate the penetration of testosterone through the skin. The cumulative amount of testosterone diffused was between 40 to 1400 ngcm<sup>-2</sup> and increased linearly as time progressed. The cumulative amounts of testosterone diffusion per unit area across the membrane were shown in Figure 2.

**LC-MS/MS method validation for serum testosterone analysis**

Based on the mean percentage of coefficient of variation (%CV) for three quality control samples, the intra-day precision and inter-day precision of serum samples ranged from 2.19% to 9.03% and from 3.34% to 7.16%, respectively. When assessed by means of the three quality control samples, the accuracy for intra-day and inter-day for serum samples ranged from 95.15% to 100.86% and

from 96.5% to 100.29%, respectively. The results from this data clearly showed that the method that was developed for the measurement of testosterone in serum has good precision, accuracy and reproducibility for the quantification of testosterone.

**In vivo skin permeability test**

The validated LC-MS/MS method was used to examine the testosterone levels in the rabbit serum. The pharmacokinetic parameter was calculated by using the non-compartmental method. The mean serum concentrations versus time profiles of testosterone after application of TDDS and placebo to rabbits are shown in Figure 3. The pharmacokinetic parameters are shown in Table 5. The mean *C<sub>max</sub>* and *AUC<sub>0-t</sub>* were 60.94 ngml<sup>-1</sup> and 5670.02 ngml<sup>-1</sup>h, respectively for the rabbit that received TDDS. Meanwhile, the mean *C<sub>max</sub>* and *AUC<sub>0-t</sub>* were 9.75 ngml<sup>-1</sup> and 30.10 ngml<sup>-1</sup>h, respectively for the rabbit that received placebo. The application of TDDS showed an increase in serum testosterone in all rabbits, and the differences in *AUC<sub>0-t</sub>* values between TDDS and placebo are statistically significant (*P*<0.05).



**Table 3.** Viscosity of testosterone transdermal delivery system (TDDS) and rheological behavior

Viscosity (Pa s)	Speed (RPM)	Torque (%)	Shear Stress (D/cm <sup>2</sup> )	Shear Rate (1/Sec)
1468456.00	0.01	14.85	293.69	0.02
997161.00	0.02	20.13	398.86	0.04
820218.67	0.03	24.82	492.13	0.06
734228.00	0.04	29.55	587.38	0.08
670727.20	0.05	33.84	670.73	0.10
821045.50	0.04	33.06	656.84	0.08
1021966.00	0.03	30.89	613.18	0.06
1403963.00	0.02	28.29	561.59	0.04
2460656.00	0.01	24.80	492.13	0.02

## Discussion

*In vitro* and *in vivo* bioavailability studies have been done to study transdermal permeation characteristics and the percutaneous absorption of TDDS in animal models. Rabbits have been chosen as animal models in this study as they are easily available large animals and have been reported to be the most suitable animal model candidates due to the closest permeability characteristics, after pigs and monkeys, to the human skin (22, 23). Skin from pigs and rhesus monkeys were reported to have permeability properties closest to human skin but because of the restricted use of these creatures and their availability these are rarely used (24). Many TDDSs have been developed and marketed globally under the brand name of Testoderm®, Androgel®, Testim® and Androlone® (25) and have undergone extensive research to indicate that physiological androgen replacement pattern is safe and can provide many clinical benefits. Palm oil has been widely used as a base in cosmetic and pharmaceutical industries in Malaysia (26-28). In this study, HAMIN™, which is a palm oil base that has been formulated by the University of Malaya researchers was chosen as the base of the TDDS formulation, following reports of it being a good vehicle for pharmaceutical use (29). The semi-solid dosage and finished base are offered at a competitive price instead of the normal expensive price charged for pharmaceutical and cosmetic products that use organic base ingredients. HAMIN™ is providing not only an esthetically-acceptable value product but is also easy to process during manufacturing (29).

In order to support clinical studies to determine the pharmacokinetics of testosterone in serum, a liquid chromatography-tandem mass spectrometry was used because of the known sensitivity of the instrument to detect low concentrations of testosterone. The specificity was obtained by using multiple reaction monitoring (MRM) in the API3200 LC-MS/MS system, which is able to select specific m/z and ignores all other fragments. In this mode, selected transitions between the precursor ion and a single fragment are monitored (Figure 1). Both sensitivity and signal to noise ratio were increased whereas the spectra specific for selected precursor showed less chemical noise or interference. This is important for the analysis of testosterone to avoid wrong interpretation due to the contamination and matrix effect if the system was to be less specific.

The physical characteristics such as particle size and viscosity of testosterone semi-solid phase have a major impact on the formation of a semi-solid testosterone structure. Rolland *et al* reported that particle sizes greater than 10 μm remain on the skin surface, whereas particles between 3–10 μm are concentrated in the hair follicles, and particles smaller than 3 μm penetrate both the follicles and stratum corneum (30). Homogenization can help to disperse materials in solutions better in order to obtain a more homogeneous mixture with smaller particle sizes due to rupture of the larger particles into smaller ones. In addition rheological properties and the type of vehicle such as ointment, water-in-oil cream, oil-in-water cream and gel, water and surfactant content, concentration and particle size of

**Table 4.** Pharmacokinetic profiles of testosterone obtained from *in vivo* skin permeability test after application of testosterone transdermal delivery system (TDDS) to rabbits (n=7)

Animal	Product	Mean $AUC_{0-t}$ (ngml <sup>-1</sup> *h) ±SD	Mean $C_{max}$ (ngml <sup>-1</sup> ) ±SD
Rabbit	Placebo (control)	23.1025±7.50	9.28±3.00
(n=7)	TDDS	5670.02±841.58	60.94±38.70

active ingredient, and its solubility will influence drug release from the semi-solid formulation. Results of viscosity measurement showed that the formulations can be characterized by pseudoplastic flow, in which viscosity decreases with increased shear rate. Pseudoplasticity can be displayed by the manner which is similar to squeezing a ketchup bottle in which the energy makes it go from thick like nectar to streaming like water. Kamel *et al* reported the same plastic characteristic of the prepared formulation for testosterone solid lipid microparticle TDS (31). A rheological property of viscosity is important as the semi-solid dosage form can affect drug delivery. Changes in the viscoelastic behavior can be attributed to the presence of the interaction of different particles as these have different particle sizes and particle size distributions.

The application of *in vitro* techniques was first carried out to develop the right formulations for TDDS before undergoing *in vivo* skin permeability test (32). The utilization of Franz diffusion cell to evaluate skin permeability has advanced into an essential research procedure, giving a clearer picture on the relationships between drug, formulation and skin (33). Franz diffusion cells are usually used with excised animal or human skin. In any case, when biological skin is not readily available, synthetic membranes are used. In this study, the selection of synthetic Strat-M™ membrane was made since it was reported that Strat-M™ membrane correlates more closely with human skin even though there are many types of commercial synthetic membranes existing in the market (34, 35). *In vitro* studies were performed to determine whether the formulated TDDS was able to deliver the drug through the membrane before being tested using the *in vivo* method. The results from *in vitro* permeability study showed that the formulated TDDS was able to deliver testosterone through the membrane and its absorption increases over a period of 5 hr. Based on this observation, it was then applied to *in vivo* study to assess the performance of the formulated TDDS in the animal models.

*In vivo* experimentations are easy to carry out as the procedure offers flexibility in adapting the model to many factors related to preliminary or feasibility studies during the development of TDS (23). An animal skin membrane that is very comparable in characteristics to the human skin is required to replace human skin for *in vivo* permeation and for topical bioavailability studies. Animals and humans have huge differences in terms of appendage openings per unit area, thickness of skin, porosity of skin, and structure, and these aspects influence the percutaneous absorption of drugs (22). Placebos, which act as a control, were used in this experiment, as testosterone is an endogenous compound. Figure

2 shows the level of testosterone in the serum of rabbits after the application of TDDS and placebo. The results showed a steady increase in testosterone levels in the serum after the application of TDDS and reaching maximum concentration mean  $C_{max}$  of 60.94 ng/ml at 2.5 hr post-application. The  $T_{max}$  was achieved at 2.5 hr probably due to the delay in drug absorption through the skin after the application of TDDS. This above result shows that our TDDS testosterone was able to deliver a significant amount of testosterone through rabbit skin as compared to the placebo. This study has demonstrated positive *in vitro* and *in vivo* results for the transdermal delivery of testosterone using HAMIN™. Another study by Kim *et al* using a different animal model (Sprague-Dawley rat) to compare reservoir-type TDS of testosterone (experimental patch 5 mg) and Androderm (commercial product 5 mg) showed comparable testosterone concentration profiles in which concentration of testosterone significantly increased after applying the testosterone patch and reached the maximum concentration (~300 ng/ml) within 3 hr after application of the patch (36). These *in vivo* studies allow the determination of the rate of permeation across the skin and drug penetration in the skin as well as determining important characteristics involved in preliminary studies in the development of transdermal drug delivery systems. The result from this study showed that the TDDS formulation has successfully delivered testosterone systemically in the animal model.

## Conclusion

The results obtained in both *in vitro* and *in vivo* studies demonstrated that HAMIN™ base has the potential to be used in future development for transdermal drug delivery systems. Successful transdermal drug application using HAMIN™ will be more efficient with the improvement of the formulation. Consequently, this formulation may be applicable to other new drug products to be developed for transdermal delivery using HAMIN™ in future studies.

## Acknowledgment

The results reported in this paper are based on a student thesis. The authors gratefully acknowledge the financial support by the University of Malaya (Kuala Lumpur, Malaysia) PJP grant, FS152/2007C and UM/MOHE High Impact Grant (H-20001-E0005) to carry out this work. We would like to thank Ms Hairin Taha for her editorial assistance.

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