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Design and Evaluation of Controlled Transdermal Drug Delivery of Testosterone

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Background

TDD is a painless method of delivering drugs systemically by applying a drug formulation onto intact and healthy skin (Han T et al. 2015).TDD has many advantages over other conventional routes of drug delivery (Donenelly et al.2012).It can improve patient compliance due to the reduction of dosing frequencies and is also suitable for patients who are unconscious or vomiting, or those who rely on self-administration (Prausnitz M et al.2008).Transdermal delivery provides a leading edge over injectables and oral routes by increasing patient compliance and avoiding first pass metabolism, respectively (Selvam et al. 2010).The success of transdermal delivery system in pharmaceutical market is evident from the fact that currently, more than 35 transdermal drug delivery products are approved in the USA for wide variety of pathophysiological conditions including hypertension, angina pectoris, motion sickness, female menopause, and male hypogonadism,(Barry 2001).The formulation of drugs into a transdermal drug delivery system requires a selection of physicochemical and biological properties (Rani et al. 2011).Testosterone is considered to be a suitable candidate for transdermal delivery due to the hydrophobic nature (log P o/w=3.3) and low molecular weight (M.W.=288 g/mol) of testosterone which are favorable factors for transdermal delivery (Sitruk-ware R et al.1989).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 (Mazur A et al.1998)..Male hypogonadism is defined as low testosterone levels with serum total testosterone <10–12 nmolL-1 (~2.88–3.46 ngml-1) (Nieschlag E et al.2004).

Normal aging
Older men generally have lower testosterone levels than younger men do. As men age, there's a slow and continuous decrease in testosterone production. The rate that testosterone declines varies greatly among men. As many as 30% of men older than 75 have a testosterone level that is below normal, according to the American Association of Clinical Endocrinologists. Whether or not treatment is necessary remains a matter of debate.[Harman S.M et al.2001]

Pathophysiology of Testosterone and Hypogonadism

Regardless of the age or comorbid conditions, obesity is associated with hypogonadism. The Baltimore Longitudinal Study of Aging found that testosterone decreased by 10 ng/dL per 1-kg/m2 increase in body mass

index.(Harman S.M et al.2001).Testosterone is available in several preparations which include subcutaneous implants (Handelsman DJ.et al.1990), oral, sublingual preparations and scrotal transdermal patches (Korenman SG.et al.1987), non-scrotal transdermal patches, and testosterone gel (Nieschlag E.2006).Testosterone replacement therapy is the primary treatment option for hypogonadism. Ideally, the therapy should provide physiological testosterone levels, typically in the range of 300 to 800 ng/dL. According to the guidelines from the American Association of Clinical Endocrinologists,[AACE Diabetes 2007] updated in 2002, the goals of therapy are to:

1.Restore sexual function, libido, well-being, and behavior

2.Produce and maintain virilization

3.Optimize bone density and prevent osteoporosis

4.In elderly men, possibly normalize growth hormone levels

5.Potentially affect the risk of cardiovascular disease

6. In cases of hypogonadotropic hypogonadism, restore fertility
[Bhasin S.et al.2006]

Hypogonadism is a medical term for decreased functional activity of the gonads. The gonads (ovaries or testes) produce hormones (testosterone, estradiol, antimullerian hormone, progesterone, inhibin B, activin) and gametes (eggs or sperm).(Yialamas MA et al.2003).Howell et al. reported that hypogonadism was seen in 30% of the men with cancer and 90% of these gentlemen had germinal epithelial failure.(Howell SJ et al.1999)The interest in developing alternative formulations for the transdermal delivery of testosterone remains a desirable objective.(Thomas BJ et al.2004).The goal of our study was to formulate and characterize the transdermal patches of testosterone in order to explore the feasibility of this route of administration to provide a once daily formulation for the drug. This would not only reduce its frequency of administration but would also contribute toward increasing its bioavailability and patient compliance.

Methods

Materials

Testosterone (purity assay 99.8%) was obtained as gift sample from Wyeth labs Ltd. The pressure sensitive adhesive (Cotran no. 9871),polyethylene heat sealable backing membrane (sctotchpack no. 1022) were received as gift sample from 3M Co. U.S.A. . The release liner was obtained as a gift sample from Johnson and Johnson

Ltd. Both the backing membrane and release liner were die-cuttable, printable occlusive and low strength films.ethylcellulose was procured as gift sample from lyka labs ltd .PVP K25 was obtained from SD fine chemicals .PEG 6000 was purchased from SD fine chemicals .Eudragit RL100 and Eudragit rs100 was obtained from zaveri & co limited . dibutyl phthalate was obtained from S.D fine chemicals.

Equipments

UV spectra were recorded on Perkin Elmer lambda 15 UV/visible spectrophotometer in the UV/visible range of 190 to 600 nm. Franz diffusion cell assembly was procured from Permegear, Inc., USA. Infrared spectrophotometer FT-IR-8300 was obtained from Perkin Elmer PE RX 1 FTIR spectrophotometer.

A DSC-41, Differential scanning calorimeter, Shimadzu corporation, Kyoto, Japan. equipped with a floppy disk drive FDD 1A and a recorded C-126A chromatopak was used during the studies.15 mg of sample (adhesive matrix patch) was weighed on a sico popular balance and placed in a aluminum cell. Reference cell as well as sample coll were crimpod and placed in the oven and heated from 20tc to 220C at a rato of 10 CImin. A DSC thermogram of 15 mg of Testosterone was obtained in a similar way.

Preformulation studies

Physicochemical characterization of testosterone

1. Determination of melting point

Small quantity of testosterone was taken in capillary tube (fused at one end) and placed in melting point apparatus, and the melting temperature was recorded. Three separate measurements were taken for the purpose, and their average value was obtained.

2.Partition coefficient determination

Partition coefficient of testosterone was determined in octanol-water system (Wells 2002). The two phases were taken in a 1:1 v/v ratio and mutually saturated in a water bath shaker at 37 °C, and the two phases were then separated. Ten milligrams of the drug was added to a mixture of 20 ml of pre-saturated organic phase and 20 ml of pre-saturated water and shaken for 10 min. The flasks were then kept at 37 °C for 24 h with intermittent shaking. The mixture was subsequently centrifuged to separate the aqueous and non-aqueous phases. The two phases were separately analyzed for duloxetine spectrophotometrically. The partition coefficient of the drug "Ko/w" was then calculated from the ratio of drug concentration in octanol and aqueous phase.

Drug–polymer interaction studies

1. **Fourier-transform-infrared spectroscopy**

Fourier−transform−infrared spectroscopy (FTIR) was employed to analyze the pure drug , physical mixture of testosterone and polymer as well as the drug loaded transdermal patches employing KBr pellets method. All samples were scanned from 100 to 4000 cm−1 .

2.Differential scanning calorimetry

Possible interactions between the drug and the utilized polymer were analyzed from DSC thermograms of the pure drug testosterone and the formulation (polymer + drug) obtained using the A DSC-41, Differential scanning calorimeter, Shimadzu corporation,Kyoto, Japan. equipped with a floppy disk drive FDD 1A and a recorded C-126A chromatopak was used during the studies.15 mg of sample (adhesive matrix patch) was weighed on a sico popular
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Fabrication of transdermal patches

Transdermal films containing testosterone were casted on glass slide using brass ring by solvent evaporation method using ethylcellulose , PVP k25 ,Eudragit RL 100 and Eudragit RS100 , PEG 6000 in presence and absence of a plasticizer. DBT was used as a plasticizer in all cases. Table 1 shows the formulae and composition for the different types of formulated patches. Testosterone (60 mg) was dissolved in dichloromethane for EC:PVP and EC:PEG600 as solvent and for EURL100: EU RS100 acetone was usee as the solvent . Accurately weighed quantity of the dibutyl phthalate was added to the solution of polymer and stirred well .Then, with the help of syringe and needle, the solution was poured into a brass ring of 5.0-cm diameter placed on the glass surface and delivered some drug adhesive and polymer solution over the backing membrane inside the brass ring . add the remaining at the center allow to semi dry and remove the brass ring .The solvent was allowed to evaporate for 6 h in a thermostatically controlled oven at 60 °C. Place release liner and pack in self sealable plastic bag.

Table 1 Formulae and composition of formulated transdermal systems of testosterone patches

PATCH CODE	ADHESIVE POLYMER CONCENTRATION	DRUG LOADING	ENHANCER	ENHANCER %
A3T2E0	30	2	-	-
A5T4PE2	50	4	POLYETHYLENE GLYCOL 400	20
A5T8PE2	50	8	POLYETHYLENE GLYCOL 400	20
A5T10PE2	50	10	POLYETHYLENE GLYCOL 400	20
P5T4CD3	50	4	CARDOMOM OIL	20
P7T4CD3	50	4	CARDOMOM OIL	30
P7T4LM2	50	4	D-LIMONINE	20
P5T4LM3	70	4	D-LIMONINE	30

EU10T4CD3	50	4	CARDOMOM OIL	30
EU10T6CD3	100	6	CARDOMOM OIL	30
EU10T4IM	100	4	ISOPROPYL MYRISTATE	30
EU10T6IM3	100	6	ISOPROPYL MYRISTATE	30
F35T1PE3	100	1	PEG-400	30
F35T4PE3	100	4	PEG-400	30
F35T4CD3	100	4	CARDOMOM OIL	30
F35T4LM3	100	4	D-LIMONINE	30

EVALUATION:

Evaluation of adhesive matrix transdermal patches

1. Peel adhesion is the force required to remove and adhesive coating from a test substrate .a single coat was applied to stainless steel plate . the patch was then pulled at 180 angle typically to measure the force . the force was expressed in grams /inch width of the patch

2. Rolling ball tack test

Tack is the ability of a polymer to adhere to a substrate with
itle contact pressure .Tack is affected by the molecular weight and the composition of polymer. Here the distance of ball is measured with
The patches . The less tacky the adhesive, further the ball will travel.

3. Shear strength test

Shear strength is also the measurement of cohesive strength of Adhesive polymer This can be influenced by molecular weight, the cross- linking the composition of polymer, as well as the amount of tackifier added. Here, the maximum load required To pull the patch off the plate is recorded. Greater the weight, more is strength of adhesive,

4. Hold bar test

This test primarily measures the cohesive strength of the adhesive
polymer. A highly cohesive pressure sensitive adhesive is desirable for
best performance adhesiveness to prevent splitting of the adhesive on removing the patch from another object. In this test, time for failure was measured. The
longer the time, the greater the cohesive strength.

Evaluation of polymer matrix transdermal patches

1. Physical appearance

All the prepared patches were visually inspected for color, clarity, flexibility, and smoothness.

2. Thickness of the patch

The thickness of the drug loaded patches was measured by using a screw gage micrometer at three different points on the patches. Average values and standard deviation values of the three readings were calculated for each drug loaded patch.

3. Uniformity of weight

The patches were subjected to weight variation test by weighing all the patches on a digital weighing machine. The determinations were carried out in triplicate for each formulation. Average weight and standard deviation values were then calculated.

4. Flatness study

Flatness study was conducted to appraise that the prepared transdermal patches possess a smooth surface and shall not constrict with time. Three longitudinal strips were cut from the film at three different portions. The length of each strip was measured and the variation in length because of non−uniformity in flatness by determining percent constriction, with 0% constriction equivalent to 100% flatness. Percent constriction was obtained as (l1 − l2)/l1 × 100. Here, l1 is the initial length of each strip, and l2 is the final length of each strip.

5. Folding endurance

This test was carried out to check the efficiency of the plasticizer and the strength of the patch prepared using different polymers. The folding endurance is defined as the number of folds required to break any polymeric patch. The folding endurance was measured manually by repeatedly folding a small strip of the film (2 × 2 cm) at the same place until it broke. The number of times the patch could be folded at the same place without breaking/cracking gave the value of folding endurance. Three patches of each type were taken for the test.

6. Tensile strength:

The definition of the tensile strength as per the ASTM testing machine was used for the measurement of Tensile strength at break is the maximum tensile stress ntained by the specimen during a tension test:
given by:

$$\text{Tensile strength} : \frac{W}{tXb} \times (LXAL)$$

Where
W=Break force in Kg.
T=Thickness of the film
B=Width of the film
L=Original length of film

$$AL=\frac{Lf-L}{L}$$

Lf=Length of film at break

The tensile strength was expressed in Kg Cm (Table 6.13). The instrument consists of a fixed jaw and a movable jaw which slides along with guide bars of the frame. The frame with guide bar is clamped in aerical position. The instrument provides for recording and printing of the
stress-strain values.The full scale load was kept at 10 Kg.
Guage length=10 mm
Cross head spoed =20-200 mm. Per minute
Chart speed=20 mm per minute

A load range of of the 1, cm. 0-1000 gm. was deployed on the y-axis. The movement of the gm. Hence the on the chart by the pointer would be equal to 100 breaking load is given by the (value x 1000) gms. strips of 10 cm length were cuts using a guide (ruler) and specimen razor blade. The thickne Procedure The samples thickness of the film sample were measured The samples were mounted between a jaw of instron initial distances between the jaw was fixed. The mounted tester. The specimen imens were then strained at the constant rate of the jaw separation and the resultant breaking load was recorded automatically. (Table 6.13)

7. Percent elongation at break:

The percent elongation at break may be defined as the % change in the length when the specimen breaks gniion

$$\text{Percent elongation at break} = \frac{La - Lb}{Lb} \times 100$$

In the instron tensile strength tester as explained above on the chart, the Y-axis gives the breaking load and X-axis give the percent elongation at break. The arrangements for the load displayed are such that for every 2 minute movement on the x-axis, the elongation of the film is by 1 mm

8. Modulus of elasticity:

It is an important characteristic which can be obtained as the ratio of the tensile strength to stress (elongation at break).

9. Percentage moisture absorption/water vapor absorption

The percent moisture absorption test was carried out to check the physical stability and integrity of the films in high humid conditions. The prepared films (3.14 cm2) were individually weighed accurately and exposed to 85 ± 5% relative humidity in a desiccator containing 100 ml of saturated solution of potassium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72 h. The percent moisture absorption was determined from the following formula:

% moisture uptake =(Final weight – Initial weight) /Initial weight X 100

10. Percentage moisture content

This test was also carried to check the integrity of films under dry conditions. The individual transdermal films (of specified area) were kept in a desiccator containing fused anhydrous calcium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72 h. The percentage moisture content was determined by using the following formula:

% Moisture content= (Initial weight – Final weight) / Initial weight X 100

11. Water vapor transmission

Water vapor transmission rate (WVTR) is defined as the quantity of moisture transmitted through unit area of film in unit time. Glass vials of equal volume and diameter were used as transmission cells. The cells were washed properly and dried in oven. Then, about 1 g of anhydrous fused calcium chloride was placed in each vial, and the patch was fixed over the brim of the vial with the help of an adhesive tape. These vials were then weighed and placed in desiccators containing saturated solution of potassium chloride to maintain 84% relative humidity. These cells were removed from the desiccators and weighed after 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th day. The water vapor transmission rate was determined as follows:

W: V: T: W L/S Where W is the weight of water vapors transmitted, L is the thickness of patch and S is the surface area exposed in square centimeter.

12. Surface pH

Patches were kept in contact with 0.5 ml of double distilled water for 1 h in glass tubes and were allowed to swell. A combined glass electrode was brought near the surface of patch and pH readings were taken after allowing an equilibration period of 1 min.

13. Drug content determination

Three to six patches were transferred to a 100 ml volumetric flask 20 ml methanol added to it . the flask kept on mechanical shaker for 4 hours . after 4 hours the methanol was replaced with by another 20 ml and extraction was continued for 4 hours . the same procedure was repeated for 8 hours and extraction was continued for 24 hours . The combined extract was collected and volume made upto 100 ml . From this solution 1 ml was taken and made up to 10 ml . the solution was injected into the column and drug content was determined by HPLC

14. In vitro drug release

Diffusion cell was employed for the in vitro characterization of transdermal formulations. This is a reliable method for the prediction of drug transport across the skin from topical formulations. The receptor compartment of the diffusion cell was filled with 40% PEG 400 in distilled water and in vitro drug release studies were carried out using synthetic cellophane membrane. The prepared formulations were applied on to the membrane in the donor compartment and were uniformly spread onto the cellophane membrane. The assembly was

constantly maintained at 37.0 ± 2.0 °C at 50 rpm. Samples (1.0 ml aliquots) were then withdrawn at suitable time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 12, and 24 h) and replenished with an amount of medium.
The samples were diluted with acetonitrile and injected into HPLC system

15. Drug permeation/ex vivo studies

A .Male wistar rats

Drug permeation studies were carried out using the skin of male Wistar rats(hindustan lever research divisom , bombay). The rats were sacrificed by spinal dislocation. The skin samples were cut, removed, and washed with normal saline. Adhering fat and connective tissue were removed using blunt-ended forceps. The skin was kept in normal saline solution for 6 h. The hairs from the skin of the rat were shaved carefully to avoid peripheral damage.the thickness was measured using screwgauge and was found to be between 260-350 micrometer .For all the studies the skin was frozen at 15 0c and thawed in water half and hour before mounting on the diffusion cell .

The receptor compartment of the Franz diffusion cell was filled with PEG 400 (40%w/w) The prepared formulations were applied over the skin of the rat in the donor compartment. The temperature of the assembly was constantly maintained at 37 ± 2 °C and the stirring rate controlled at 50 rpm. Samples (1 ml aliquots) were withdrawn at suitable time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 24 h) and replaced with the same amount of fresh medium to maintain the receptor phase. The samples were analyzed spectrophotometrically at λmax of 225 nm.

B.Human cadaver skin

Excised human cadaver skin (plastic surgery dept ,B Y L nair hospital , mumbai) was obtained from the abdominalm site of the body . the skin sample was stored at 4 0C in a sealed bag and washed thoroughly with the distilled water to remove any subcutaneous fatty tissue . it was then immersed in peg 400 SOLUTION (40%) AT 370oC for equilibrium before using for permeation studies .

16.Stability studies

The patches stored at refrigeration , room temperature 25/60% RH ,40 0C /75% RH ,room temperature /56% RH and room temperature /75% RH for 1 week upto 48 week did not show any change in the appearance and drug content

17.In-vivo studies

Invivo studies was conducted on 55 day old male wistar rats were housed .they were given access to food and water .weights were recorded periodically . the serum testosterone levels were assayed by raioimmunoassay before and 20 days after castration by radioimmunoassay to ascertainn the fall in hormone levels range due to removal of testis . the animals divided into two groups 10 of each one receiving the adhesive matrix patch and

the other receiving the polymer matrix patch . control and testosterone patches were applied on abdominal site of rat for 24 hours and replaced with the new patch . the blood sample collected from orbital sinus method .

18.Skin irritation studies

Ethical clearance for the handling of experimental animals was obtained from the Institutional Animal Ethical Committee (IAEC), Panjab University, Chandigarh, and the studies were conducted as per approved protocol. The albino Wistar rats were housed in cages, with free excess to standard laboratory diet and water. The dorsal abdominal skin of the rats was shaved carefully avoiding peripheral damage, before 24 h of conducting the study. Transdermal patch was applied onto the nude skin and covered with a non-sensitizing microporous tape. A aqueous solution of formalin was applied as standard skin irritant. The animals were applied with new patch each day up to 7 days. The formulation was removed after 7 days; score of erythema was recorded and was compared with standard. The score of erythema was read and recorded by the Draize scoring method (Draize et al. 1944) as score 0 for no erythema, score 1 for very slight erythema (light pink), score 2 for welldefined erythema (dark pink), score 3 for moderate to severe erythema (light red), and score 4 for severe erythema (dark red).

Results and discussion

1.Physicochemical characterization of testosterone

The melting point of testosterone was found to be in the range of 154°C (melting point literature 155 °C)and the octanol/water partition coefficient of duloxetine was found to be 3.28 (partition coefficient literature 3.3) (http://www.drugbank.ca/drugs/DB00476).
Melting point : 155 °C (https://www.drugbank.ca/drugs/DB00624)
Log P: 3.3 (https://pubchem.ncbi.nlm.nih.gov)

2.Drug–polymer interaction studies

The stability of a formulation amongst other factors depends on the compatibility of the drug with the excipients. Hence, any possible physical or chemical drug– excipient interactions were assessed by FTIR and DSC

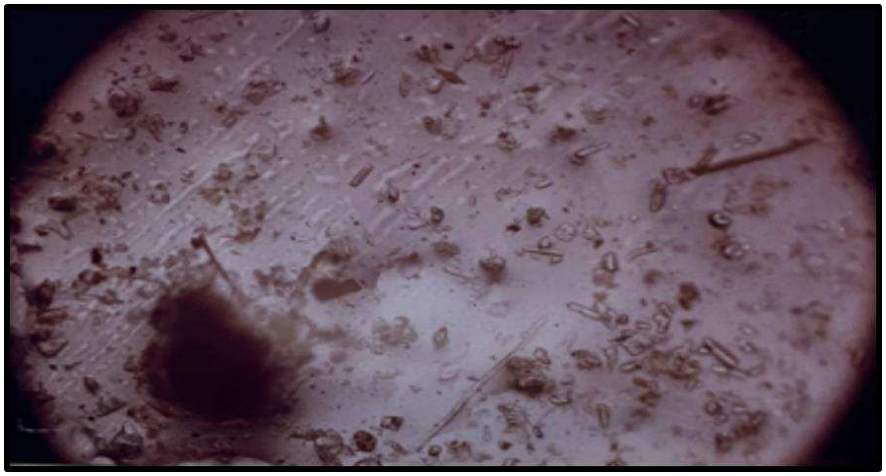
3.Fourier-transform-infrared spectroscopy

The FTIR spectra for the drug, polymers, physical mixtures of drug and polymer, and the transdermal formulations are displayed

In . Salient spectral data for the drug and the polymers is as follows: duloxetine HCl: IR (KBr: v, cm−1): 1579.05 (aromatic alkene), 1463.34 (thiophene ring), 1233.53 (C–O bond stretching). Hydroxypropyl methylcellulose (HPMC): IR (KBr: v, cm−1): 3450.48 (O–H stretching), 2934.77 (C–H stretching, aliphatic), 1393.58 (C–O–C, stretching, dialkyl)) The characteristic absorption bands of the drug as well as polymers were found to be present at their usual positions in the formulations. This indicated the absence of any drug–polymer interaction in the formulation signifying their mutual compatibility.

4.Differential scanning calorimetry

The DSC thermograms for the pure drug and the formulation (drug + polymer) are shown in Fig. 3. The DSC thermogram of testosterone exhibited an endothermic peak In the DSC thermogram of the formulation, there was no appearance of any new peaks. Moreover, there was no change in peak shape and its onset. These results indicated that the chemical integrity of the drug was preserved and that there was no interaction between the drug and the polymer.. Thus, testosterone was found to be compatible with the polymer, suggesting that such combination polymer could be used for the preparation of the various transdermal patches.below picture is the crystallization observed in the polymer matrix patches when high drug loading was done so low concentrtaion of drug was found to be suitable .



Evaluation of adhesive properties of the patch

1.Peel Adhesion test

50 mg of adhesive
encentration with and without drug in patch did not leave any residue on the
pate indicated the desirable peel adhesion for TDDS.

2. Rolling ball tack test

Patch 50 mg of adhesive concentration without drug showed 10.8 mms of
mg of adhesive concentration was found to be very much tacky as the
avelled lowest distance compared to other adhesive concentration.
ut in presence of drug and enhancer it showed tackiness as that
mg and 40 mg of adhesive concentration. Thus the proper balance of cohesiveness and wettability was obtained with 50 mg patch

3. Shear strength test

These test indicated that patch without drug with 50
mgof adhesive concentration possessed higher strength, but inclusion of
enhancer did not reduce it to a very low level

4. Hold bar test

Patches containing drug and 30 mg & 40 mg of adhesive showed maximum cohesive
sirength, and the patch containing drug and 50 mg of adhesive showed
desired cohesive strength which was found to be similar to that of
patch without drug.

Evaluation of transdermal patches
EC:PEG600 COMPOSITION

Sr. No	Patch Code	Thickness variation (mm) Mean ± SD	Weight variation (mg) Mean ± SD	Drug content mg/patch Mean ± SD	Content uniformity
1	F35 T1 E0	170.3 ± 2.382	47.06 ± 2.056	0.966 ± 0.193	0.992 ± 0.121
2	F40 T1 E0	172.3 ± 2.061	53.56 ± 2.129	0.993 ± 1.156	0.994 ± 0.074

3	F45 T1 E0	172.7 ± 2.935	56.55 ± 1.304	0.973 ± 0.298	0.967 ± 0.073
4	F35 T2 E0	175 ± 2.647	48.16 ± 1.968	1.984 ± 0,014	1.957 ± 0.04
5	F35 T4 E0	177.3 ± 1.067	50.33 ± 2.067	3.978 ± 0.058	3.984 ± 0.083
6	F35 T1 LM3	177.48 ± 1.469	57.31 ± 2.267	0.951 ± 0.108	0.972 ± 0.231
7	F35 T1 CD3	178.09 ± 2.556	57.77 ± 2.058	0.992 ± 0.007	0.997 ± 0.056
8	F35 T1 PE2	181.3 ± 2.194	59.31 ± 1.977	0.971 ± 0.192	0.984 ± 0.196
9	F35 T4 LM3	181.4 ± 2.746	59.39 ± 2.139	3.966 ± 0.096	3.958 ± 0.061
10	F35 T4 CD3	181.9 ± 1.866	59.39 ± 2.139	3.971 ± 0.171	3.993 ± 0.118
11	F35 T4 PE3	180.6 ± 2.195	59.46 ± 2.150	3.968 ± 0.063	3.951 ± 0.068
12	F35 T4 PE1	179.6 ± 2.061	49.6 ± 1.118	3.972 ± 0.028	3.972 ± 0.42
13	F35 T4 PE2	180.4 ± 1.830	51.28 ± 1.830	3.993 ± 0.36	3.939 ± 0.139
14	F35 T4 CD2	188.3 ± 1.977	51.79 ± 2.702	3.951 ± 0.007	3.898 ± 0.058
15	F35 T4 CD2	188.3 ± 1.977	51.79 ± 2.702	3.951 ± 0.007	3.898 ± 0.058

EC:PVP COMPOSITION

Sr.No	**Patch Code**	**Thickness variation (mm) Mean ± SD**	**Weight variation (mg) Mean ± SD**	**Drug content mg/patch Mean ± SD**	**Content uniformity**
1	P5 T1 E0	183.8±1.939	66.30 ± 1.011	0.993± 0.115	0.951± 0.163
2	P7 T1 E0	226.6±1.959	92.18±1.145	0.986±0.206	0.964±0.215
3	P9 T1 E0	235.4±1.9586	101.36±0.965	0.951±0.45	0.989±0.115
4	P7 T2 E0	212.0±2.097	93.21±1.246	1.947±0.110	1.943±0.116
5	P7 T4 E0	212.6±2.129	95.43±1.054	3.836±0.163	3.942±0.215

6	P7 T4 PG2	237.6±2.727	115.32±1.336	3.943±0.128	3.988±0.212
7	P7 T4 PG3	236.8±2.531	125.29±1.501	3.991±0.110	3.997±0.110
8	P7 T4 PG4	238±2	135.26±1.246	3.964±0.215	3.968±0.128
9	P7 T4 LM2	233.6±1.2	115.29±1.369	3.988±0.116	3.965±0.108
10	P7 T4 LM3	233.7±1.738	125.38±1.08	3.936±0.231	3.989±0.188
11	P7 T4 LM4	235.4±1.958	135.20±1.261	3.989±0.121	0.996±0.188
12	P7 T1 LM3	231±1.859	113.15±1.065	0.996±0.310	1.964±0.208
13	P7 T42 LM3	232.58±1.762	114.16±1.834	1.945±0.188	3.961±0.161
14	P7 T4 CD2	221.72±.086	109.31±0.637	3.977±0.116	3.974±0.121
15	P7 T4 CD1	223.4±2.438	99.40±0.834	3.994±0.215	3.972±0.182
16	P7 T4 CD3	224±2.804	119.28±0.541	3.983±0.15	1.969±0.212
17	P7 T2 CD3	226.1±1.839	114.63±0.438	1.983±0.236	0.960±0.217
18	P7 T1 CD3	228.4±1.796	113.39±0.938	0.927±0.312	2±0.032
19	P5 T2 E0	232.9±2.935	67.34±1.192	2.001±0.008	3.972±0.146
20	P5 T4 E0	233.06±2.067	69.46±1.631	3.911±0.936	6.006±0.018
21	P5 T6 E0	234.16±1.817	71.12±1.328	5.998±0.948	3.993±0.050
22	P5 T4 PG1	189.12±2.118	94.28±2.009	3.961±0.002	3.948±0.132
23	P5 T4 PG2	189.46±1.656	79.32±1.192	3.9721±0.107	3.894±0.072
24	P5 T4 PG4	189.93±2.432	84.46±1.820	3.964±0.177	3.948±0.132
25	P5 T1 PG3	139.38±2.118	89.29±1.838	0.986±0.321	0.991±0.05
26	P5 T2 PG3	187.38±2.118	81.12±1.683	1.991±0.161	1.989±0.09
27	P5 T2 IM1	187.69±1.190	82.19±1.682	1.948±0.163	1.999±0.082
28	P5 T2 IM2	188.59±2.746	72.29±1.782	1.966±0.009	1.957±0.102
29	P5 T2 IM3	190.06±1.169	77.21±1.693	1.939±0.039	1.976±0.177

30	P5 T2 IM4	189.03±1.196	82.31±2.071	1.971±0.093	1.972±0.139
31	P5 T1IM3	190.13±1.961	87.03±2.002	0.975±0.312	0.969±0.127
32	P5 T2 IM4	191.53±1.150	81.38±1.071	3.9480±0.198	3.982±0.138
33	P5 T2 LM1	191.78±2.38	84.28±1.381	1.999±0.361	1.971±0.071
34	P5 T2 LM1	191.86±1.384	72.71±1.721	1.957±0.028	1.993±0.061
35	P5 T2 LM3	191.99±2.324	77.79±2.107	3.981±0.162	3.953±0.18
36	P5 T2 LM4	191.08±1.428	82.91±1.184	1.9760±.169	1.978±0.132
37	P5 T1 LM3	191.93±2.061	87.44±1.977	0.963±0.073	0.974±0.183
38	P5 T4 LM3	191.16±2.641	81.32±1.071	3.976±0.192	3.973±0.094
39	P5 T2 CD1	191.16±2.061	86.36±2.224	1.094±0.067	1.084±0.098
40	P5 T2 CD2	191.04±2.118	72.42±2.047	1.972±0.028	1.911±0.093
41	P5 T2 CD3	191.48±2.513	77.32±1.361	1.984±0.029	1.911±0.138
42	P5 T2 CD4	191.81±1.566	82.58±1.194	0.984±0.021	0.961±0.058
43	P5 T4 CD3	192.48±2.077	87.46±1.931	3.961±0.030	3.951±0.024
44	P5 T1 CD3	191.38±1.830	81.96±2.138	0.987±0.048	0.9790±0.038

ADHESIVE PATCH COMPOSITION

Sr.No	Patch Code	Thickness variation (mm) Mean ± SD	Weight variation (mg) Mean ± SD	Drug content mg/patch Mean ± SD	Content uniformity
1	A3T2E0	138.33±1.67	32.96±0.66	1.968±0.005	1.938±0.009
2	A4T2E0	141.32±2.66	42.33±0.17	1.987±0.037	1.929±0.014
3	A5T2E0	145.33±2.66	52.18±0.15	1.921±0.011	1.999±0.062
4	A7T2E0	147.99±1.63	76.30±0.65	1.919±0.046	1.961±0.037
5	A5T1E0	139.33±1.33	51.38±0.72	0.969±0.003	0.916±0.037
6	A5T2E0	145.33±2.66	53.08±0.15	1.969±0.048	1.897±0.006
7	A5T4E0	147.99±1.63	54.31±0.52	3.961±0.003	3.697±0.05
8	A5T8E0	151.33±1.33	58.31±0.17	7.900±0.093	7.923±0.08
9	A5T10E0	153.66±1.33	60.60±0.65	9.997±0.006	9.921±0.011
10	A5T4CD3	145.33±2.66	70.92±0.65	3.979±0.013	4.103±0.004
11	A5T8CD3	147.99±1.63	73.96±0.66	7.919±0.046	7.961±0.018
12	A5T10CD3	151.33±1.33	75.49±0.15	9.972±0.048	9.897±0.006
13	A5T4PG2	145.33±2.66	64.41±0.72	4.005±0.005	4.976±0.008
14	A5T8PG2	147.99±1.66	68.09±0.17	7.928±0.004	7.697±0.051
15	A5T10PG2	151.33±1.33	69.36±0.72	9.919±0.046	9.980±0.003
16	A5T4PE2	147.33±1.32	64.16±0.65	9.997±0.006	9.921±0.011
17	A5T8PE2	149.99±1.63	60.64±0.17	7.914±0.036	7.938±0.037
18	A5T10PE2	151.66±1.33	69.81±0.52	9.932±0.06	9.983±0.007

1.Physical parameters

All the patches were evaluated for their physical parameters (weight, thickness, folding endurance, diameter, area, flatness, and surface pH), and they were found to be flexible, uniform, smooth, and transparent (Table 2). All the formulations were uniform in their weight, thickness, folding endurance, and diameter, with low SD values. The weight of the prepared transdermal patches for different type of formulations ranged between 183.7 ± 1.939 mg and 226.9 ± 1.959 mg, but within a formulation, all the patches showed low standard deviation values. The thickness of the patches varied from 1.00 ± 0.0019 mm to 2.513 ± 0.0015 mm. Low standard deviation values in the film thickness measurements ensured uniformity of the patches which further indicated the reproducibility of the procedure followed for the preparation of the patches. Folding endurance values varied between 300.0 ± 1.96 and 306.6 ± 1.68. The flatness study showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness. Thus, no amount of constriction was observed which indicated that all patches had smooth flat surface which would be maintained when the patches are applied to the skin.

2.Percent moisture absorption/water vapor absorption studies

The prepared patches showed minimal moisture absorption rates ranging from 0.0045 to 0.0075 % thus ensuring general stability and protection from microbial contamination. There was a statistically significant difference ($P < 0.05$) as assessed by paired t test, and increase in the polymer concentration increased the moisture absorption capacity. The variation in polymer type or inclusion of the plasticizer dibutyl phthalate did not significantly alter the moisture absorption rates.

3.Moisture loss studies

Moisture loss studies were carried out in order to determine the stability of the prepared patches under dry ambient conditions. The results obtained are given in Table 4. The percent moisture loss for the prepared transdermal patches was found to range from 1.84 to 7.34, reflecting a low moisture loss in all the prepared transdermal films with formulation EC:PVP showing minimum loss and formulation EURL100:EURS100 showing maximum moisture loss. The results for all the prepared transdermal patches reflected a low moisture loss for all the prepared transdermal films. Moisture loss was found to be significantly higher ($P < 0.05$) in patches formulated with EURL100;EURS100 compared with that in EC:PVP however, the values for patches formulated with EC:PVP,EC:PEG6000 were found to be similar (assessed by paired t test). Further, the patches formulated with PEG 400 had significantly lower moisture loss indicating that the plasticizer will help the formulation remain stable and will also make it less brittle during long-term storage particularly under dry conditions.

4.Water vapor transmission studies

Water vapor transmission studies were carried out to determine the permeability characteristics of the transdermal patches. The water vapor transmission rate for the prepared patches ranged from 0.0011 to 0.0015 mg cm/ cm2 24 h (Table 5) indicating that all the formulations were permeable to water vapor. The low water vapor transmission (WVT) rates again emphasize the stability aspects on long-term storage. No statistically significant difference was seen with the change in type and concentration of the polymer.

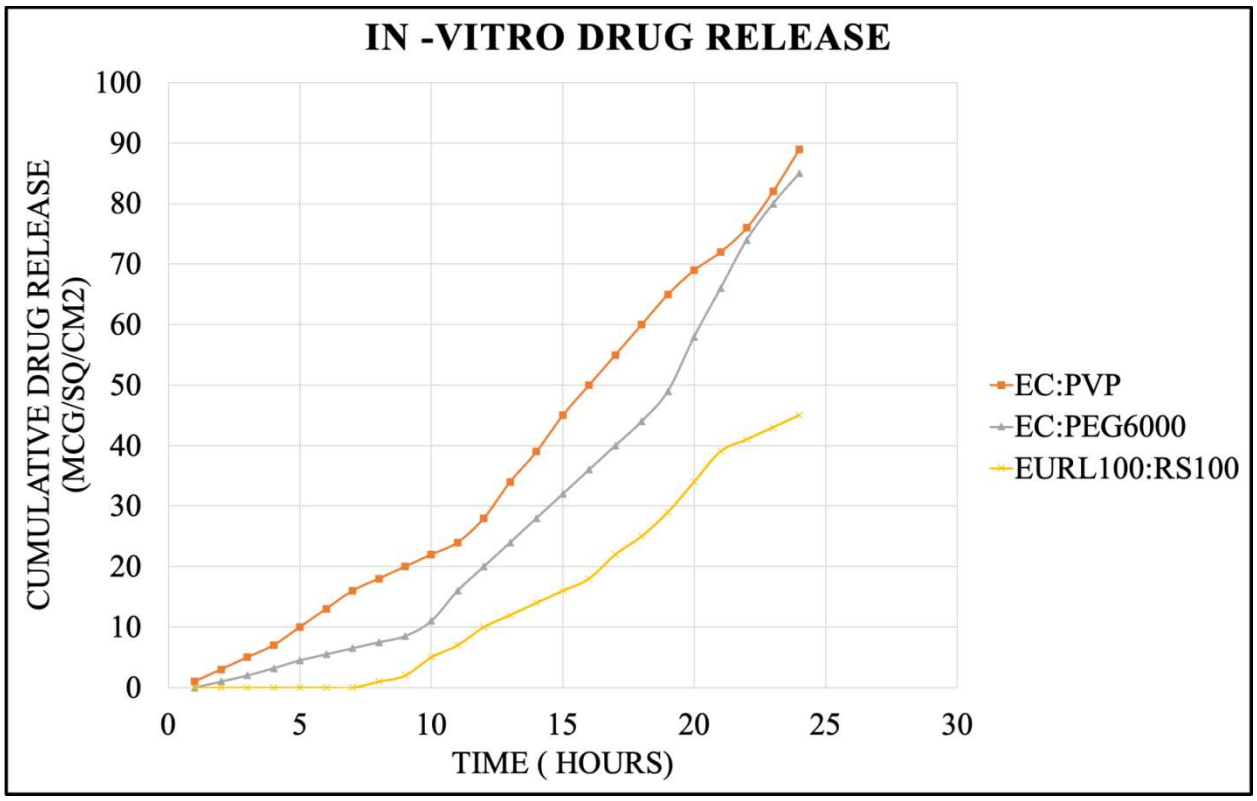
5.Drug content

Drug content analysis was performed for all the prepared transdermal systems by following standard method, and the results are displayed figuratively in Fig. 4. A near uniform drug content was noted for the prepared transdermal films ranging from 97 to 99.73%. This suggests that the process employed to prepare the films was capable of affording uniform drug content and minimum variability.

6.In vitro release studies

The in vitro release profile is an important tool that predicts in advance how a drug will behave in vivo. Release studies are required for predicting the reproducibility of rate and duration of drug action. The in vitro drug release profiles of the formulations prepared from the EC:PVP and EC:PEG600 , EURL100: EU RS100 .

The cumulative percentage of the drug released in 24 h was found to be satisfactory for all types of transdermal films and drug release ranged from 80.77 to 95.59%.whereas eudragit showed poor drug release . The films returned very good permeability coefficient and flux values . foprmulatyed EC:PVP patches for in vitro kinetic release was found to decrease with increase in the polymer concentration because testosterone solubility in matrix increase and unable to diffuse with the increase in drug loading there was decrease in drug release further studied with DSC studies and it was found with high drug loading there was crystallization and hence low 4 mg/patch drug loading was used and penetration enhancer had no effect on the drug release .The formulation patches with EURL100:EURS100 displayed an overall lower drug release in 24 h compared to other patches, and this was statistically assessed by paired t test. However the release of testosterone was found to be less and it showed lag period this could be due to high solubility of testosterone in eudragit matrix . there was plateau level reach in drug release with increase in the loading dose of testosterone from 1mg/patch to 6 mg/patch and hence lower concentration 4mg/patch leading to a little increase drug release was used . also there was not significant increase in the drug release with the addition of the penetration enhancer cardomom oil, D-limonine Formulated patches EC:PEG6000 it was found there was decrease in drug release with 45 mg/patch and hence 40mg/patch gave desired drug release and also it showed increase in drug release with increase in testosterone from 1mg/patch to 4mg/patch and hence no increase with the addition of the penetration enhancer .
Hence EC:PVP and EC:PEG6000 used good drug release upto 24 hours without any lag .

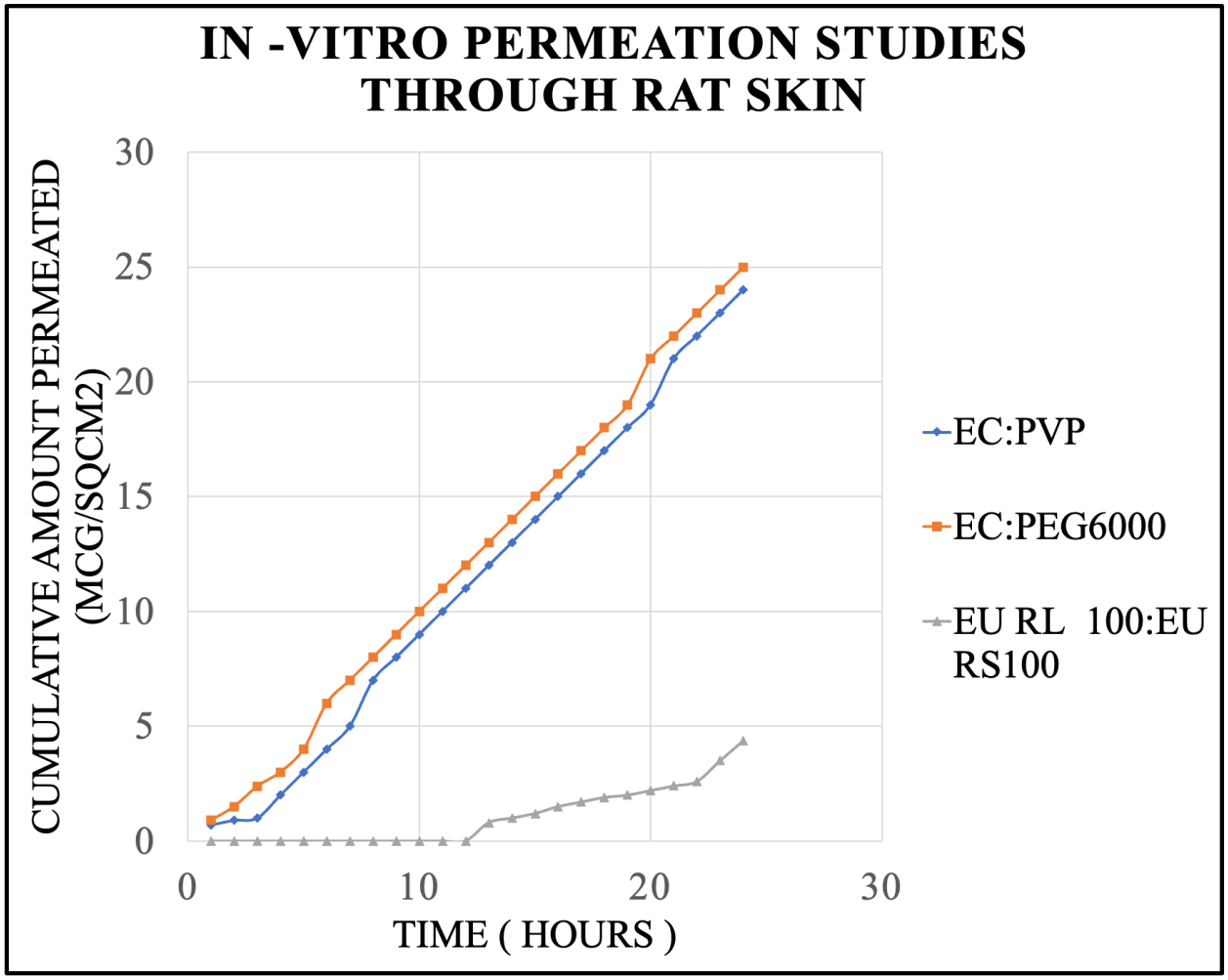


7.Ex vivo permeation

Formulated EC:PVP Patches containing 50 mg/patch polymer concentration was selected for further human skin .patches were found to follow zero order kinetics through human skin .permation rate was found to increase in the order PG>IPM>D-limonine>cd . cardamom oil was found to be the best enhancer for transdermal drug delivery through ethyl cellulose polymer matrix .

Formulated EURL100:EURS100 it is concluded that the permeation rate through human skin was found to be significantly low which is not sufficient to deliver the testosterone through the system to achieve desired plasma level . even the addition of enhancer could not increase the permeation rate of testosterone .

Formulated EC:PEG6000 HENCE IT CAN BE CONCLUDED THAT CARDOMOM OIL , d-limonine and PEG400 help in enhancing the flux of testosterone through skin and PEG 400 showed best enhancement factor



8.Stability studies

Two formulation such as ethyl cellulose polymer matrix containing 4mg/patch of testosterone and 30% d-limonine and Cardomom oil , second EC : PEG 6000 with PEG patchs containing 4 mg/patch of testosterone showed excellent physicochemical properties and high in-vitro permeation of testosterone through rat and human skin however eudrgait patches showed poor physical and mechanical properties and significantly low in vitro permeation of testosterone patches .

The parameters used for the evaluation was appearance , weight variation and drug content and in-vitro permeation rate and these studies were done in triplicate .shelf life was calculated with the Arrhenius plot .results given in additional file .

.none of the patch showed incraesse in the weight at these humidty condidtion thus it can be concluded and said that there was negligible moisture uptake by the patches .polyethylene laminated aluminium foil can act as good moisture vapour transmission barrier and can be used as packaging these patches

the shelf life of the formulated transdermal patch was found be to be around 5.22 years

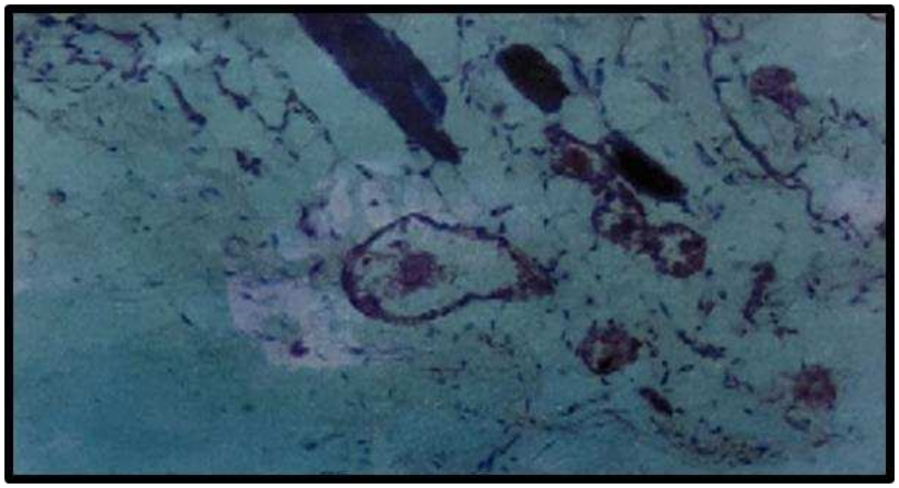
9.In vivo studies

The results indicated that average 1.805 mg of adhesive matrix patch and 0.708 mg of testosterone patch from polymer matrix patch was retained. In other words 89.08% of in vivo testosterone in comparison to that of in vitro permeation data was obtained with adhesive matrix patch . 94.84% of in vitro permeation of testosterone was obtained in comparison to that of in vitro permeationn data obtained from polymer matrix patch . thus good invitro and invivo correlation was obtained .

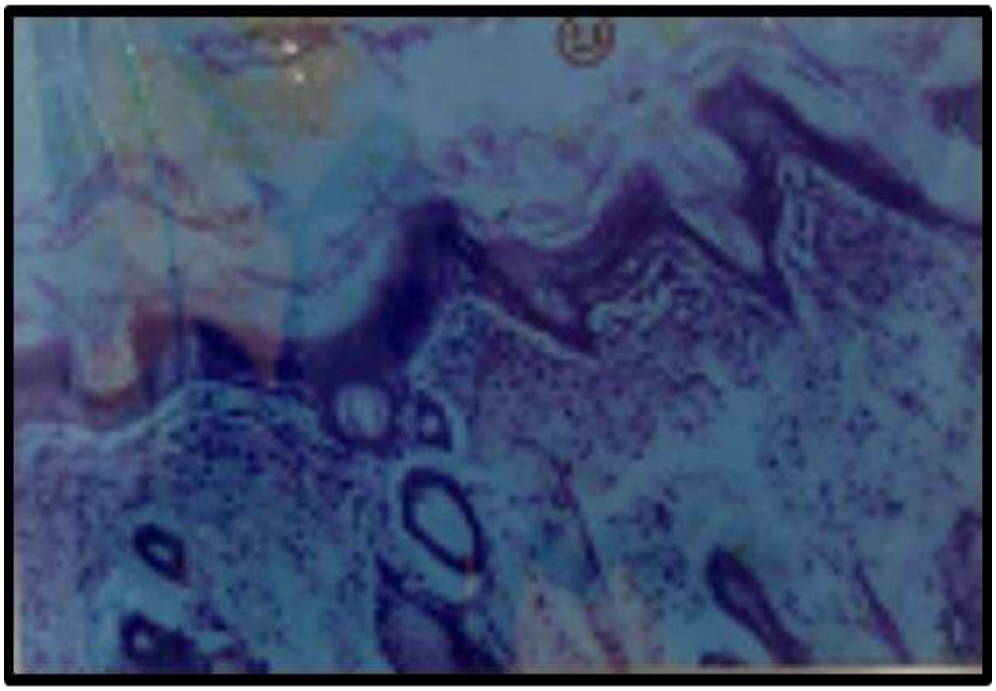
10.Hypersensitivity reactions

TDDSs are intended for topical application, hence, the prime assurance needed for such formulations is their biocompatibility with the skin (site of action), i.e., their use must not lead to any kind of inflammatory reactions. To assess the potential of patches to cause skin irritation or sensitization, hypersensitivity tests were carried out on the rat's skin for the selected formulation. The animals were observed for 7 days for the development of signs of erythema (redness and flushing of skin) and edema (papules and wheals). The score of erythema was read and recorded by the Draize scoring method as score 0 for no erythema, score 1 for mild erythema (barely perceptible- light pink), score 2 for moderate erythema (dark pink) and edema, score 3 for moderate to severe erythema and moderate edema, and score 4 for severe erythema (extreme redness) and edema. None of the formulated transdermal patches were shown to demonstrate edema formation in comparison with the standard formalin group for 7 days. The formulated Adhesive nad polymer matrix patches showed very slight erythema reaction as compared to the control patch This suggested the non-allergenic and non-irritant profile for the developed transdermal films

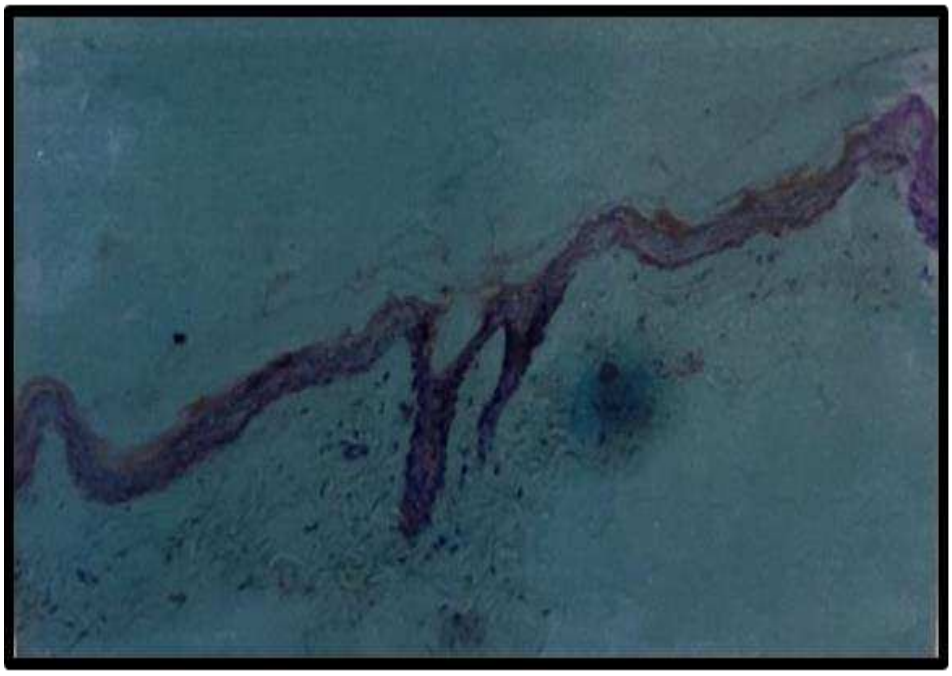
Control patch



Adhesive matrix patch



Polymer matrix patch



Conclusions

Transdermal films of testosterone have been successfully formulated as once daily formulation by solvent evaporation technique. Evaluation of the prepared films in terms of physical appearance, weight uniformity, thickness uniformity, surface pH, flatness test, water vapor absorption, water vapor transmission, and drug content uniformity suggest that the method employed for formulation of the transdermal patches was reproducible and ensured excellent quality and uniformity in patch characteristics with minimum variability. Further, in vitro and ex vivo drug release studies for all the formulations showed that drug release equivalent to first dose of the drug was obtained in 2.0–3.0 h and nearly complete release (94%) was achieved in 24 h. These results show that transdermal delivery of duloxetine hydrochloride can have good potential applications in therapeutic arena offering advantages in terms of reduced dosing frequency, improved patient compliance, non-invasive characteristics, improved bioavailability, and easy termination of therapy. The required chronic administration of testosterone should further accentuate the aforesaid advantages

REFERENCES:

AACE diabetes mellitus clinical practice guidelines task force. (2007). Endocrine Practice, 13, 3. https://doi.org/10.1016/s1530-891x(20)46419-5

Bhasin, S., Cunningham, G. R., Hayes, F. J., Matsumoto, A. M., Snyder, P. J., Swerdloff, R. S., & Montori, V. M. (2006). Testosterone therapy in adult men with androgen deficiency syndromes: An Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism, 91(6), 1995-2010. https://doi.org/10.1210/jc.2005-2847

Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci. 2001;14:101–14.

Donnelly, R. F., Singh, T. R., Garland, M. J., Migalska, K., Majithiya, R., McCrudden, C. M., Kole, P. L., Mahmood, T. M., McCarthy, H. O., & Woolfson, A. D. (2012). Hydrogel-forming microneedle arrays for enhanced Transdermal drug delivery. Advanced Functional Materials, 22(23), 4879-4890. https://doi.org/10.1002/adfm.201200864

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. The Journal of Clinical Endocrinology & Metabolism, 86(2), 724-731. https://doi.org/10.1210/jcem.86.2.7219

HANDELSMAN, D. J., CONWAY, A. J., & BOYLAN, L. M. (1990). Pharmacokinetics and pharmacodynamics of testosterone pellets in man. The Journal of Clinical Endocrinology & Metabolism, 71(1), 216-222. https://doi.org/10.1210/jcem-71-1-216

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. The Journal of Clinical Endocrinology & Metabolism, 86(2), 724-731. https://doi.org/10.1210/jcem.86.2.7219

Han, T., & Das, D. B. (2015). Potential of combined ultrasound and microneedles for enhanced transdermal drug permeation: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, *89*, 312–328. https://doi.org/10.1016/j.ejpb.2014.12.020

Howell, S. J., Radford, J. A., Ryder, W., & Shalet, S. M. (1999). Testicular function after cytotoxic chemotherapy: Evidence of Leydig cell insufficiency. Journal of Clinical Oncology, 17(5), 1493-1493. https://doi.org/10.1200/jco.1999.17.5.1493

Korenman, S. G., Viosca, S., Garza, D., Guralnik, M., Place, V., Campbell, P., & Davis, S. S. (1987). Androgen therapy of hypogonadal men with transscrotal testosterone systems. The American Journal of Medicine, 83(3), 471-478. https://doi.org/10.1016/0002-9343(87)90757-1

Mazur, A., & Booth, A. (1998). Testosterone and dominance in men. Behavioral and Brain Sciences, 21(3), 353-363. https://doi.org/10.1017/s0140525x98001228

Nieschlag, E., Behre, H., Bouchard, P., Corrales, J., Jones, T., Stalla, G., Webb, S., & Wu, F. (2004). Testosterone replacement therapy: Current trends and future directions. Human Reproduction Update, 10(5), 409-419. https://doi.org/10.1093/humupd/dmh035

Nieschlag, E. (2006). Testosterone treatment comes of age: New options for hypogonadal men. Clinical Endocrinology, 65(3), 275-281. https://doi.org/10.1111/j.1365-2265.2006.02618.x

Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. *Nature Biotechnology*, *26*(11), 1261-1268. https://doi.org/10.1038/nbt.1504

Rani S, Saroha K, Syan N. Transdermal patches a successful tool in transdermal drug delivery system: an overview. Der Pharmacia Sin. 2011;2(5):17–29.

Selvam RP, Singh AK, Sivakumar T. Transdermal drug delivery systems for antihypertensive drugs - A review. Int J Pharm Biomed Res. 2010;1(1):1–8.

Sitruk-Ware, R. (1989). Transdermal delivery of steroids. Contraception, 39(1), 1-20. https://doi.org/10.1016/0010-7824(89)90012-7

Thomas, B. J., & Finnin, B. C. (2004). The transdermal revolution. Drug Discovery Today, 9(16), 697-703. https://doi.org/10.1016/s1359-6446(04)03180-0

Yialamas, M. A., & Hayes, F. J. (2003). Androgens and the ageing male and female. Best Practice & Research Clinical Endocrinology & Metabolism, 17(2), 223-236. https://doi.org/10.1016/s1521-690x(03)00018-6