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Original Article

FORMULATION AND DEVELOPMENT OF TRANSDERMAL DRUG DELIVERY SYSTEM OF ETHINYLESTRADIOL AND TESTOSTERONE: *IN VITRO* EVALUATION

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ABSTRACT

Objective: The combination therapy of ethinylestradiol and testosterone in post-menopausal females has shown improved sexual response and libido. The present studies were designed to develop a suitable matrix-type transdermal drug delivery system (TDDS) of ethinylestradiol and testosterone using the polymer chitosan.

Methods: Five formulations (ET1 to ET5) were developed by varying the concentration of polymer and keeping the drug load constant. Physical parameters and drug excipient interaction studies were evaluated in all the formulations. *In vitro* skin permeation profiles of ethinylestradiol and testosterone from various formulations were simultaneously characterized in a thermostatically controlled modified Franz Diffusion cell using HPLC. Based on the physical parameters and *in vitro* skin permeation profile formulation ET3 containing 30 mg/ml of chitosan was found to be the best and chosen for further studies. Optimized formulation was subjected to *in vivo* pharmacokinetic analysis in rats using ELISA.

Results: Stability profile of patch formulation ET3 depicted stability up to 3 mo. One week skin irritation evaluation in rats indicated that formulation ET3 was nonirritating. Combination transdermal patch across rat skin showed a maximum release of 92.936 and 95.03 % in 60 h with a flux of 2.088 and 21.398 µg/cm^{2}h for ethinylestradiol and testosterone respectively.

Conclusion: The net result of this study is the formulation of a stable, non-irritating transdermal patch of ethinylestradiol and testosterone, with good bioavailability and can be used as Estrogen Replacement Therapy (ERT) in postmenopausal women.

Keywords: Estradiol, Testosterone, Transdermal matrix patches, Chitosan, Formulation development

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INTRODUCTION

The concept of delivering drugs through the skin for systemic treatment of diseased states is gaining increasingly great importance due to its advantages [1]. The advantages of TDDS are bypassing of hepatic first-pass metabolism, enhancement of therapeutic efficiency, prolonged duration of action of potent drugs with short plasma half-life and maintenance of steady plasma or serum level of the drug. However, transdermal delivery is limited to drugs having low doses, low melting points, and molecular weights and solubility of greater than 1 m g/ml in both water and mineral oil [2].

Ethinylestradiol and testosterone both play a significant role in regulating female sexual function. The most common complaints associated with decreased estrogen and/or testosterone levels are decreased desire and libido, vaginal dryness and lack of sexual arousal in postmenopausal females [3-13]. The estimated proportion of women who can be classified as having low sexual desire ranges from 7 to 33%, depending on the population studied and the definition being used [8, 14]. Due to a hormonal deficiency in postmenopausal women, Hypoactive Sexual Desire Disorder (HSDD) is commonly reported [3, 15-17]. Estrogen replacement therapy improves sexual function in females in conjunction with testosterone. In plasma, testosterone is largely bound to Serum Hormone Binding Globulin (SHBG). Estrogen Replacement Therapy (ERT) increases SHBG production: therefore, it is plausible that postmenopausal women treated with exogenous estrogen have less available free testosterone than untreated women [18]. This may subsequently lead to a decrease in sexual function in women who are treated with ERT [18-21]. Postmenopausal women who have failed to correct their sexual function with ERT alone can improve their sexual response treated additionally with testosterone [3, 10, 19-22]. Moreover, ethinylestradiol and testosterone possess most of the ideal physicochemical and biological properties to be formulated into a transdermal patch type delivery system like small t½, (in minutes), small daily dose in (in µg/day) [10, 23] and hepatic first-pass effect upon oral administration. But so far, no work related to the development of transdermal patches of ethinylestradiol and testosterone in combination has been reported. The present investigation was designed to develop a suitable matrix patch [24] type TDDS for ethinylestradiol and testosterone employing varied amounts of chitosan. Chitosan is widely acknowledged to be non-toxic even at relatively high concentrations *in vivo* and *in vitro*.

Chitosan is a cationic polymer that exhibits several unique properties, which are desirable features of TDDS. Chitosan may act as penetration enhancer to improve the permeability of drugs across the underlying tissue by its effects on tight junctions [25-28]. The aim of the present study was to optimize the concentration of polymer for a suitable delivery system in terms of *in vitro* and *in vivo* skin permeation of drugs and to find out the best possible concentration of polymer, which may be chosen for further studies.

MATERIALS AND METHODS

Materials

Chitosan and ethinylestradiol were received as a gift sample from Central Cochin Fisheries Ltds., Cochin and Ontop Pharmaceuticals ltd., Bangalore. Testosterone was purchased from Sigma Aldrich, India. methanol (HPLC grade) (SD Fine Chemical Ltd, Mumbai, disodium hydrogen phosphate A. R grade, potassium dihydrogen orthophosphate (CDH (P) Ltd New Delhi), gum acacia (Pioneer Chemical Co., Delhi) were also procured. All chemicals were used as received without any further purification.

Preparations of films

Transdermal films of ethinylestradiol (2.5 mg) and testosterone (25 mg) were prepared by varying the concentration of chitosan (20, 25, 30, 35 and 40 mg/ml) and keeping the drug load constant. Bioadhesive film was prepared by a solvent casting method. The

chitosan was accurately weighed and transferred to a l 0 ml beaker; to this 5 ml of 4%, (v/v) lactic acid (as a solvent and permeation enhancer) was added. The beaker was kept on magnetic stirrer to dissolve the polymer. The mixture was stirred continuously stirred to prevent the formation of lumps of polymer and stirring was continued till a clear solution was obtained. To this 25 mg of testosterone was added and the stirring was continued. After 24h of stirring. 2.5 mg of ethinylestradiol was added, and the stirring was continued for another 24 h. The resulting solution was then transferred onto the Teflon coated mould (15 cm^2). The mould was covered with an inverted funnel to control the rate of evaporation of the solvent system and kept undisturbed overnight for drying. The dried patches were kept in desiccators until use.

Drug-polymer interaction study

The physicochemical compatibility between ethinylestradiol and testosterone and polymers used in the films was studied by using Fourier transform-infrared (FT-IR-8400, Shimadzu Co., Japan) spectroscopy. The pellatization was done by the KBr pellet method. The FT-IR spectra were recorded in the wavelength region between 4000 and 400 cm^{-1}. The spectra obtained for ethinylestradiol and testosterone and physical mixtures of ethinylestradiol and testosterone with polymers were compared.

Differential scanning calorimetry

About 5 mg of sample was weighed and crimped into an aluminum pan and analyzed at scan range from 0 °C–300 °C at the heating rate of 5 °C/min under a nitrogen flow of 25 ml/min

Physical characteristics of the prepared films

The thickness of each patch was measured at different sites using Membrane Thickness Gauge, and the average thickness was calculated. Patches were evaluated for their physical appearance and graded as opaque/transparent/smooth/wrinkled/moist/dry/flexible/tough/sticky/non-sticky.

Each patch was cut into six patches of 1 cm^2 area and weighed. The average weight was calculated, and percentage deviation of each 1 cm^2 patch from average weight was determined. Folding endurance was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value. Flatness was determined as thee longitudinal strips were cut out from each film. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction with 0% constriction equivalent to 100% flatness [29].

Percentage of constriction = $(I_1-I_2)/I_2\times100$

Where, l_1= initial length of each strip and

I_2= final length of each strip

Percentage moisture absorption

The films were weighed accurately and placed in the desiccator containing 100 ml of saturated solution of potassium chloride, which maintains 84.34% RH at 25 °C. After 3 d the films were taken out and weighed. The percentage moisture absorption was calculated using the following formula [2]-

Percentage of moisture content = X-Y/Y × 100

Where, X = initial weight, Y = final weight.

Drug content uniformity

An accurately cut patch of 1 cm^2area was taken and added to the beaker containing 10 ml saline solution. The beaker was kept for 24 h with occasional shaking. The samples were analyzed for drug content using HPLC at 244 and at 280 nm for testosterone and ethinylestradiol respectively [39].

***In vitro* skin permeation studies**

The *in vitro* skin permeation studies of ethinylestradiol and testosterone from TDDS though rat skin was conducted using a modified Franz Diffusion cell. Protocols for all animal experiments were approved by the Institutional Animal Ethics Committee. Methanol: phosphate Buffer saline, pH 7.4 in the ratio of 7.3 was used as a receptor fluid in the receptor compartment of the cell. Rat's abdominal skin was excised, hair was removed using scissors, and fatty tissues attached to dermis were removed carefully [40, 41]. The skin was mounted between donor and receiver compartment of the diffusion cell having capacity 20 ml, with the epidermis facing upward into the donor compartment. The test film of 1.5 cm^2area was placed on the acclimatized skin. The bathing solution in receiver compartment was agitated with a magnetic stirrer at a temperature of 37±1 °C maintained thermostatically. Samples (l ml in each case) were withdrawn at regular intervals, and fresh receptor fluid was added to maintain a constant volume of receptor fluid. The Samples were analyzed at 280 and 244 nm, for ethinylestradiol and testosterone respectively using HPLC (LC Solutions Shimadzu, Japan) and drug contents were determined from the calibration curve. Calibration curves for both the drugs were obtained using HPLC equipped with a Prominence diode-array detector. A purospher STAR, merck RP 18C Pre-packed column (5 µm, 250 mm 4 mm i. d) was used as an analytical column. Methanol: water (70:30) combination after optimization was used as the mobile phase at a flow rate oflml/minl ml/min. The analytical column was maintained at 37 °C the same temperature as for drug release and skin permeation studies to minimize the potential drug precipitation in the column. A 20 µl sample volume was injected each time though a manual injector using Hamilton microlitre syringe. The chomatographic peaks for ethinylestradiol and testosterone were well resolved at retention times (RT) at 6.6 and 8.6 min. respectively.

Stability evaluation

Stability studies were performed for 3 mo using optimized formulation ET3. All the stability samples (packed in aluminum foil) were prepared in triplicates and were kept at two stability testing conditions: viz. Accelerated (40±2 °C/75%±5%RH) and Intermediate (30±2 °C/65%±5%RH) as per (ICH) Q1AR2 guidelines [30]. Stability samples were evaluated for drug excipient interaction and *in vitro* permeation through human cadaver epidermis at the following time points; initial, 1st, 2nd and 3rd month [42].

Skin irritation studies

The optimized transdermal patches were evaluated for primary skin irritation studies on rats. All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC). All the experimental procedures were carried out in accordance with the committee for the purpose of control and supervision of experiment on animal guidelines (125/2013/CPCSEA). Wistar rat's hairs were removed by shaving from the dorsal area one day before the test. The rats were divided into 4 groups (n= 6). Group, I served as the control (without any treatment), Group II received optimized medicated transdermal patch, Group III received a blank transdermal patch and Group IV applied with a 0.8% v/v aqueous solution of formalin as a standard irritant [31]. A new patch, or new formalin solution, was applied at Ist, 4th and at 7th day. The patch was secured using an adhesive tape. These patches were covered with an occlusive covering to approximate the condition of use. Finally, the application sites were graded for any sign of erythema or edema according to a visual scoring scale.

RESULTS AND DISCUSSION

Transdermal Therapeutic Systems are self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), though the skin, at a controlled rate to the systemic circulation. Testosterone has been combined with ethinylestradiol since ERT alone leads to a decrease in free testosterone level. TDDS have been designed for controlled drug delivery with the intention of maintaining constant plasma levels.

The physicochemical studies like the percentage moisture absorption, flatness, folding endurance etc. provide information regarding the stability of the formulations. Formulations ET 1-5 contained fixed amount of testosterone (25 mg) and ethinylestradiol (2.5 mg) and polymer (chitosan) concentration was varied from 20-40 mg/ml respectively. The area of the patch was 15 cm^2 (table 1).

The physical appearance of the various formulations in terms of their transparency, smoothness, flexibility, stickiness, homogeneity and opaque properties were recorded, and formulation ET3 was found to be uniform, translucent, slightly sticky and flexible. The percentage of moisture absorption, average thickness, and weight varied to a small extent in all formulations studied.

Table 1: Physicochemical evaluations of formulations

Formulation code	Uniformity of weight (mg)/cm²±SD[a]	Thickness (mm)±SD[a]	Flatness (%)	Folding endurance[a]	Percentage moisture absorption	Average drug content (%)[a]	
						Testosterone	Ethinylestradiol
ET1	21.5±0.36	0.070±0.008	100	372±8	15	92±4.3	96±4.8
ET2	22.6±0.72	0.074±0.007	100	381±9	18	87±4.8	94±5.4
ET3	22.9±0.39	0.076±0.008	100	390±11	16	93±4.2	96±6.2
ET4	23.2±0.45	0.081±0.009	100	367±7	20	88±5.3	95±5.8
ET5	23.2±0.45	0.083±0.005	100	400±9	23	89±5.1	91±3.7

[a]mean±SD, n = 3.

Matrices properties

The weight variations of the patches were in the range of 20.6 mg/cm² to 22.9 mg/cm² for 5 formulations, the difference in weight variation was due to the addition of polymer in different ratios which influence weight of patches. The results are given in table 1.

The thickness of the transdermal patches ET l-ET5 for 5 different polymer ratios varied from 0.070±0.008 mm to 0.083±0.005 mm. The maximum difference between the thicknesses of patches was 0.013 mm, which indicates that all the prepared patches were of nearly uniform thickness. The results are given in table 1.

All the formulations ET1-ET5 showed 100% flatness which indicates 0% constriction of the formulated patches (table 1). Test results indicated that all the patches can withstand to rupture and would maintain their integrity with general folding when used. The folding endurance values lie in between 372 and 400 and were measured manually. The value was found to be high in patches containing a higher amount of the chitosan. The prepared transdermal patches showed good tensile strength and there was no sign of cracking in the prepared transdermal film. Mechanical properties of a polymer matrix were improved by the use of polymers.

Tensile strength lies in between 372 g/cm2 and 400 g/cm2, the difference in values were due to the composition of polymer used. Also, there was an increase in the tensile strength with increasing concentration of chitosan. Highest tensile strength was observed in ET5, and this might be due to the highest concentration of hydrophilic polymer used and as the concentration decreased from ET5 to ET1tensile strength got decreased. The results are given in table 1.

The percentage moisture absorption was calculated which was found to be as 15 to 23% for ET1-ET5 respectively (table 1). There was an increase in moisture absorption with an increase in the amount of hydrophilic polymer, chitosan. Similarly, the thickness and weight of patch were found to be increased accordingly.

The drug content varied due to polymers which were added in different concentrations. As the polymer concentration increases, the bond formation between the drug molecules and polymer molecules increases which will retard the drug release. The drug content of ethinylestradiol in formulations ETl-ET5 varied from 91-96 % (table l) the drug content of testosterone in formulations ET1-ET5 varied from 88-96 %. (table 1). This demonstrates the homogenous distribution of the drugs.

The FTIR and DSC scans of the patch containing drugs and patch without drug were obtained to assess any interaction between drug and excipients. On analysis of the FTIR spectra of the pure drug and the medicated formulation, no major difference was observed in the absorption peak pattern. The DSC of patch gave an endothermic peak of testosterone at l46 °C (pure drug-149 °C), and an endothermic peak of ethinylestradiol at 184 °C (pure drug-185.13 °C) and this confirmed the purity of drug sample used. The DSC data suggested that possibly there was no chemical interaction between drugs with patch forming polymer/materials. The HPLC spectrum showed absorption maxima at 244 nm for testosterone and an absorption maximum at 280 nm for ethinylestradiol with RT-8.6 and 6.6 min respectively (fig. 1 and 2).

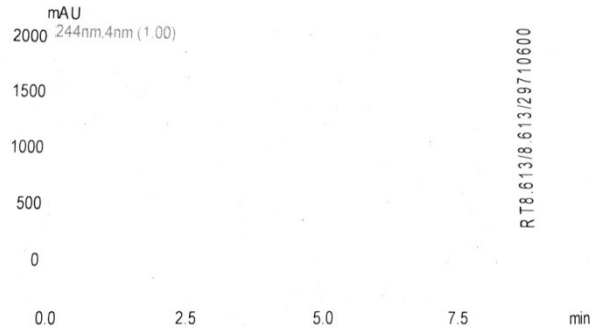

Fig. 1: HPLC chromatogram of testosterone showing retention time (RT) and Area under curve (AUC) at 244 nm

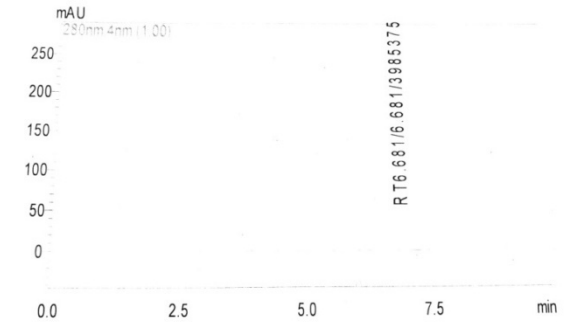

Fig. 2: HPLC chromatogram of ethinylestradiol showing a retention time (RT) and Area under the curve (AUC) at 280 nm

Drug release

The permeability of ingredients was evaluated using modified Franz diffusion Cell. The formulation showed the variable release pattern. The process of drug release in most of the controlled release devices including transdermal patches is governed by diffusion *In vitro* release profile of ethinylestradiol and testosterone across rat skin was maximum f'rom the formulation ET3, with 92.936 % of ethinylestradiol at the end of' 60 h (fig. 3) and 95.033 % of testosterone at the end of 60 h (fig. 4) with flux of 2.088 for ethinylestradiol and 21.398 µg/(cm² h) for testosterone. The *in vitro* skin permeation studies using cadaver skin as rate limiting membrane for formulation ET3 showed a maximum release of 80.456±1.64 % ethinylestradiol in 60 h with a flux of 1.938±10.52 µg/(cm²h) and

79.51±1.05% testosterone in 60 h with a flux of 18.673±0.8l µg/(cm²h) (table 2). The studies indicated that the overall permeation rate in rat skin is higher than that in the cadaver skin.

This showed that the formulations with increased concentrations of chitosan resulted in sustained release of the drug up to a certain level and furthermore decreased due to excess polymer concentration which resulted in high encapsulation of drug. Formulations ET1, ET 2, ET 4 and ET5) with varied polymer concentration, showed a constant increase and then decrease in the cumulative drug release due to drug degradation by the excess moisture content and uptake.

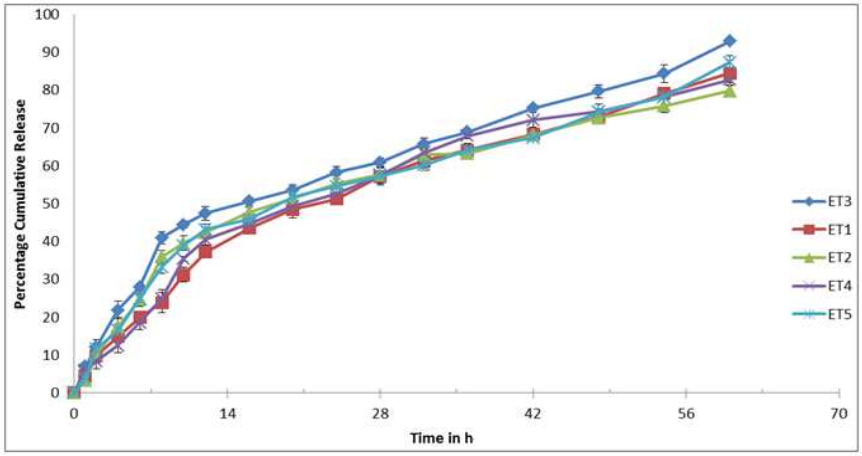

Fig. 3: Comparative permeation profiles of Ethinylestradiol from different patch formulations across rat skin. mean±SD, n = 3

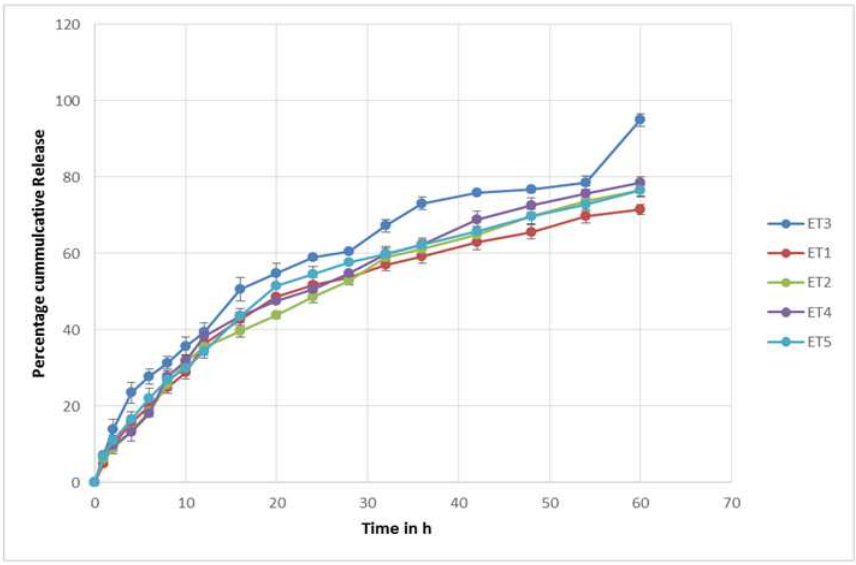

Fig. 4: Comparative permeation profiles of testosterone from different patch formulations across rat skin, mean±SD, n = 3

Table 2: Release kinetics

Formulation	Zero order R^{2*}		First order R^{2*}		Higuchian plot R^{2*}	
	Ethinylestradiol	Testosterone	Ethinylestradiol	Testosterone	Ethinylestradiol	Testosterone
ET1	0.881	0.937	0.852	0.894	0.992	0.998
ET2	0.943	0.905	0.847	0.868	0.997	0.986
ET3	0.903	0.922	0.943	0.879	0.9767	0.985
ET4	0.974	0.903	0.921	0.864	0.984	0.979
ET5	0.956	0.887	0.936	0.898	0.978	0.996

*R^2 values are determination coefficients

On the basis of physicochemical parameters and drug release content out of five formulations made, ET3 (150 mg chitosan) was selected for further studies and was considered as optimized formulation. Comparison of R^2 values was made in order to assess the nature of the release profile of the formulations. The respective R^2 values for formulation ET I to ET5 was found to be 0.881, 0.943, 0.903, 0.974and 0.956 respectively for zero order release plot of ethinylestradiol and 0.937, 0.905, 0.922, 0.903 and 0.887 respectively for zero order release plot of testosterone. (table 2) High R^2 values with zero order plots indicating a zero order release

pattern from the formulations. Optimized formulation (ET3) was further subjected to first order and Higuchian regression parameters to find a best-fit model for the formulation. The regression coefficient value of the first-order plot for formulation ET3 was 0.94 ethinylestradiol and 0.8795 testosterone. The regression coefficient for Higuchian plot was 0.9767 ethinylestradiol and 0.9851 testosterone. Linear curves were obtained on plotting the graphs of cumulative percentage drug released versus square root of time suggesting Higuchian matrix diffusion mechanism of drug release from the TDDS formulation. The results are depicted in table 2. The drug release kinetics studies showed that all formulations were governed Higuchian model and the release was non-Fickianmediated. Regression analysis of the *in vitro* permeation curves was carried out. The slope of the curve obtained after plotting the mean cumulative amount released per patch versus time was taken as the *in vitro* release for ethinylestradiol and medroxyprogesterone acetate.

Skin irritation studies

The optimized transdermal formulation ET3 was evaluated for skin irritation studies on rats. The patches were removed after 48 h, and the area was examined for any signs of skin sensitivity or irritation and the fresh patches were secured at the same site at 1st,4th and 7th day which is a modification over the method of Draize *et al.*, 1946 [38]. No signs of erythema and edema were observed in case of patch group. There was a significant difference (P<0.01) observed between the patch and the formalin treated group (table 3).

Table 3: Visual scores of skin irritation amongst various groups

Group	I	II	III	IV
	Control (No treatment)	Blank (Blank patch)	Medicated (Medicated patch)	Formalin (0.8% aq. Solution)
Eythema	0.00±0.00	1.167±0.408**	1.50±0.548**	3.333±0.516
Edema	0.00±0.00	1.167±0.753**	1.333±0.816**	3.167±0.753

All values are reported as (mean value±SEM) (n=6), **represents P<0.01 (very significant) in comparison to group IV

Stability

The optimized ET3 patches were properly packed in aluminum foil and kept for stability studies as per ICH guidelines. Transdermal patch was studied for three months at intermediate and accelerated conditions (table 3). The physicochemical analysis, *in vitro* permeation across cadaver skin, FTIR and DSC analysis at the end of each month were conducted to assess the stability of formulations during intermediate and accelerated conditions.

Table 3: Permeation profiles (Mean % cumulative release±SD)[a] of patch ET3 during accelerated stability study across cadaver skin

Time	Zero month		First month		Second month		Third month	
(h)	E	T	E	T	E	T	E	T
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	6.97±*1.02*	6.48±1.79	6.87±0.91	6.47±0.62	6.84±0.87	6.47±2.35	6.84±1.58	6.46±1.64
2	10.19±0.34	11.25±1.36	10.02±0.96	11.25±0.50	10.00±0.88	11.25±2.51	10.00±1.76	11.25±1.48
4	19.84±0.41	18.53±0.62	19.78±1.07	18.51±0.99	19.77±0.94	18.50±1.29	19.75±1.63	18.50±1.69
6	23.99±0.55	23.18±1.57	22.95±1.08	23.12±1.82	21.94±1.57	23.11±1.48	21.91±2.57	23.10±1.34
8	30.95±0.48	22.36±*2.99*	30.95±0.25	22.35±0.95	30.78±2.84	22.34±1.64	30.71±2.18	22.33±1.82
10	37.44±1.12	30.47±3.05	37.44±0.39	30.46±3.78	37.31±1.28	30.46±1.98	37.31±2.65	30.44±2.15
12	42.15±0.89	34.31±3.24	42.15±0.44	34.32±2.45	42.02±1.36	34.30±0.65	42.00±2.95	34.30±2.65
16	43.35±0.67	40.99±2.58	42.98±0.70	40.99±3.48	42.24±1.59	40.97±0.48	42.23±1.52	40.96±2.94
20	49.57±0.78	44.71±2.46	49.57±0.69	44.70±2.51	49.45±1.24	44.68±0.95	49.45±1.87	44.65±3.54
24	53.22±1.10	48.24±0.73	53.22±0.61	48.21±1.68	53.22±2.65	48.20±0.69	53.21±1.94	48.20±0.65
28	52.54±1.58	50.25±0.88	53.01±0.54	50.23±1.45	53.02±2.58	50.23±1.25	53.00±1.68	50.23±0.97
32	59.49±1.91	55.36±066	59.43±0.81	55.34±1.82	59.42±1.92	55.32±1.59	5942±1.81	55.30±0.65
36	63.96±2.27	62.16±1.83	63.96±0.70	62.15±1.24	63.95±1.43	62.13±1.86	63.93±1.37	62.11±1.57
42	71.44±2.59	65.72±1.08	71.44±2.10	65.72±1.76	71.43±1.53	65.70±1.67	71.43±1.52	65.70±1.83
48	73.12±2.63	66.83±0.79	73.12±2.24	66.83±1.84	73.11±2.64	66.81±1.23	73.10±0.69	66.80-±1.94
54	77.54±1.55	68.51±0.40	77.54±0.62	68.50±0.62	77.53±2.84	68.49±1.51	77.53±0.94	68.481.64±
60	80.45±0.67	79.51±1.64	80.45±1.36	79.50±1.36	80.05±2.41	79±2.59	80.00±1.51	78.80±1.83
66	78.21±1.56	72.54±2.11	78.21±0.99	72.52±0.64	78.20±1.58	72.50±1.94	78.18±1.67	72.50±1.24
72	74.24±0.98	66.28±1.97	74.24±0.98	66.28±1.47	74.21±2.02	66.25±1.85	74.20±1.59	66.23±1.06

[a]mean±SD, n = 3.

CONCLUSION

Testosterone has been combined with ethinylestradiol as ERT (Estrogen Replacement Therapy) alone leads to a decrease in free for testosterone level. Total testosterone production decreases by around 25% after menopause. Since ethinylestradiol and testosterone both play a significant role in regulating female sexual function and hence, been successfully combined in a single patch. Amongst the five formulations studied, formulation ET3 showed release profile of above 90% at the end of 60 h with 92.93 % of ethinylestradiol and 95.03% of testosterone across rat skin and 80.45% of ethinylestradiol and 79.51 % of testosterone across cadaver skin. The optimized patch was physicochemical stable and had non-irritating nature when applied on rat skin. Further, it can be reasonably concluded that chitosan polymer in 30 mg/ml concentration is better suited over other concentrations used for the development of TDDS of ethinylestradiol and testosterone for HSDD in postmenopausal women. The net result of this study is the formulation of a stable, non-irritating transdermal patch of ethinylestradiol and testosterone, with good bioavailability and the optimized formulation (ET3) may be used for further pharmacodynamics studies in suitable animal models.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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