

Preparation and *in vitro* characterization of the transdermal drug delivery system containing tamoxifen citrate for breast cancer

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A matrix-type transdermal drug delivery system of tamoxifen citrate was developed by using a different ratio of eudragit-RL100, hydroxypropyl methyl cellulose (HPMC-K15), and ethyl cellulose (EC), by the solvent evaporation technique. The effect of the binary mixture of polymers with a penetration enhancer on the physical chemical parameters including, thickness, folding endurance, uniformity of drug content, moisture content, moisture uptake, tensile strength, and *in vitro* drug permeation were evaluated. The *in vitro* drug permeation studies were conducted by using modified Keshary-Chein diffusion cells through female Sprague Dawley rat skin using pH 7.4 phosphate buffer saline (PBS). The selected formulation's stability studies were conducted as per the International Conference on Harmonization (ICH) guidelines, and did not show any degradation of the drug.

Key words: Breast cancer, transdermal, tamoxifen citrate, skin permeation

INTRODUCTION

Transdermal drug delivery systems (TDDS) encompass a wide array of non-invasive or minimally invasive technologies for delivering drugs and vaccines across the skin.^[1-3] Applications of transdermal delivery include easy accessibility of the skin, which aids in high patient compliance, avoidance of the gastrointestinal tract, and the ability to achieve sustained / controlled release.

During the past decade, women had been looking forward to alternatives for oral hormonal chemotherapy. Transdermal drug delivery has been developed for contraception and hormonal therapy. Tamoxifen citrate has been a clinical choice for the treatment of advanced breast cancer and is often an adjuvant therapy after surgical resection. The drug has also been used in treating menopause. However, one of the side effects of the drug is the proliferative effect on the endometrium.^[4] Tamoxifen citrate is a highly lipophilic drug, with poor water solubility.^[5] Furthermore, its oral bioavailability is mainly affected by the first-pass metabolism and

P-glycoprotein (P-gp) pump efflux in the liver and intestine.^[6] Hence, there is a need for the development of a controlled / sustained delivery device, which is desired for successful local hormonal chemotherapy.

MATERIALS AND METHODS

Materials

Tamoxifen citrate was a gift sample from Dabur Pharmaceutical Ltd., Ghaziabad, India. Beta-cyclodextrin, eudragit-RL-100, ethyl cellulose, and hydroxypropyl methylcellulose (K-15) were procured from Lab Care Ltd., Bangalore, India. All other chemicals and solvents were of analytical grade, purchased from Merck Pvt. Ltd., Bangalore, India.

Methods

Preparation of the transdermal drug delivery system

Transdermal films of tamoxifen citrate (5.0 mg / 3.14 cm²) containing a different ratio of, eudragit-RL, hydroxypropyl methyl cellulose (HPMC K-50), and ethyl

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Table 1: Formulation composition of tamoxifen citrate containing matrix transdermal systems

Formulations	F1	F2	F3	F4	F5
Tamoxifen citrate (mg)	50	50	50	50	50
Eudrajit-RL-100 (mg)	150	60	90	96	95
HPMC-E15 (mg)	-	90	60	-	-
EC (mg)	-	-	-	4	5
DMSO (w/w) %	10	10	10	10	10
DBT (w/w) %	30	30	30	20	20
Methanol:DCM (1:2) (ml)	12	12	12	12	12

All the formulation contain 30% DBT except F4 and F5, which contain 20% DBT;
 HPMC: hydroxypropyl methyl cellulose, EC: ethyl cellulose, DMSO: dimethyl sulfoxide,
 IPM: isopropyl myristate, DBT: dibutyl phthalate, DCM: dichloromethane

cellulose were prepared on the mercury surface. The required amount of drug and polymers were dissolved in the methanol-dichloromethane (1 : 1) solvent system. Di-n-butyl phthalate (20 and 30% w/w of polymer) was used as a plasticizer. Isopropyl myristate (IPM) and Dimethyl sulfoxide (DMSO) were added to the polymer drug solution. The resultant homogeneous solution was poured into a circular plane, with a uniform surface, on a mercury substrate. The films were dried for a period of 24 hours, and the rate of evaporation was controlled by inverting a funnel over the petri dish. The dry films were wrapped in aluminum foil and kept in desiccators. Compositions of the prepared formulations are tabulated in Table 1 and photographs of the drug containing patches are shown in Figure1, respectively.

Evaluation of prepared transdermal patches

Thickness

The thickness of the film was determined using a micrometer gauge (Mitoyoto, Japan). The film was measured at different places and the mean value was determined.^[7]

Weight uniformity

The films of different batches were dried at 40°C, for six hours, before testing. Six patches from each batch were accurately weighed on a digital balance.^[8] The average weight and the standard deviation values were calculated from the individual weights.

Drug content analysis

The uniformity of drug distribution in the transdermal films was determined by taking a known area of the films at different places of the film. The films were dissolved in 2 ml of methanol, sonicated for 10 minutes, and subsequently diluted with phosphate buffer saline (PBS), pH 7.4. After appropriate dilution, the solutions were analyzed spectrophotometrically (UV Shimadzu-1700, Japan) for tamoxifen citrate, at 274 nm,^[9] using a solution of films prepared without the drug as a reference, to neglect the absorption of components of the formulation if any.

Moisture content

The prepared films were weighed individually and kept in desiccators containing activated silica at room temperature

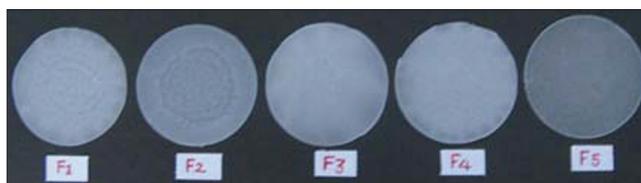


Figure 1: Typical photographs showing drug containing transdermal patches

(30°C) for 24 hours, until a constant weight was attained. The percentage of moisture content was calculated as the difference between the initial and final weight with respect to the final weight.^[7]

Moisture uptake

A weighed film kept in the desiccator at room temperature (30°C), for 24 hours, was taken out and exposed to 84% relative humidity (RH) in a stability chamber (Lab Care, Mumbai, India) until a constant weight of the film was obtained. The percentage moisture uptake was calculated as the difference between the final and initial weights, with respect to the initial weight.^[7]

Folding endurance

A strip of film (2 × 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

Determination of tensile strength

The tensile strength was determined by using a dynamic mechanical analyzer (computerized, EPLEXOR 500 N, IISC, Bangalore). Patches of 2 cm², of all the formulations were subjected and determined.

Determination of flux, diffusion coefficient and permeability coefficient

The flux of drug permeated in case of *in vitro* was calculated from the slope of the steady-state portion of the permeation profile by linear regression analysis.^[10,11] The lag time was calculated from the back extrapolation of the steady-state portion of the graph. The diffusion coefficient (D/h^2) and permeability coefficient (K_p) were also calculated for the *in vitro* studies using the equations mentioned herewith, respectively,

$$D/h^2 = 1/6 \times T_{\text{Lag}},$$

$$K_p = J_{\text{SS}}/CD,$$

Where, T_{Lag} is the lag time, J_{SS} the flux at steady state, CD the concentration in the donor compartment, D the diffusion coefficient, and h the diffusion path length.

In vitro skin permeation study

Female Albino rats weighing 150 – 200 g were selected

for the permeation studies (the study was approved by the Animal Ethical Committee, KLE University, Department of Pharmacology, Belgaum, Karnataka, India). The animals were sacrificed using anesthetic ether. The hair of the test animals was carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by the heat separation technique, which involved soaking of the entire abdominal skin in water at 60°C for 45 seconds, followed by careful removal of the epidermis. The epidermis was washed with water and used for permeability studies.^[10,11] The permeation studies were performed for different formulations across female rat skin in a modified Keshary-Chein diffusion cell at 32±0.5°C. The diameter of the donor compartment cell provided an effective constant area of 3.14 cm². The films with an area of 3.14 cm² were applied to the skin using adhesive tape (cellophane) as the backing layer. The phosphate buffer pH 7.4 (20 ml) was used as the receptor compartment medium, to ensure sink conditions and stability of the drug. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. The samples were withdrawn at different time intervals and replaced with an equal volume of diffusion medium. The samples were analyzed spectrophotometrically at 274 nm. To ascertain whether the components of the skin or other excipients of the film interfered in the drug analysis; a blank experiment (films without drug) was run, using the skin as a barrier membrane, with phosphate buffer saline pH 7.4. When the solution was analyzed at 274 nm for any interfering constituents, the released constituents amounted to an average of 0.04±0.02%.

Stability aspects

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS in a stability chamber at 40±2°C / 75% RH (Thermo Lab., Mumbai, India). The samples were withdrawn at 0, 30, 60, and 90 days, and the physical and the drug content were analyzed by a UV spectrophotometer method.^[7]

RESULTS AND DISCUSSION

The formulations were subjected to physical examination; the films appeared to be slightly translucent suggesting that the drug was not completely solubilized, but rather dispersed /

suspended in the matrix. The studies revealed that addition of di-n-butyl phthalate at 30% w/w was fixed and standardized for formulations F1 – F3 and in the case of F4 and F5, 20% w/w, respectively. All the developed and prepared formulations of the polymer were smooth and uniform, and flexible films were obtained.

Thickness and uniformity of weight

The thickness of films varied between 0.106 and 0.127mm, suggesting that the formulation variables used in the study did not produce any significant effect on the thickness of films. The uniformity of weight varied between 135.7±0.7 and 202.2±0.9, as the eudragit concentration decreased, and decrease in the weight was obtained. The obtained results are shown in Table 2.

Drug content analysis

The drug content of all the formulations [Table.2] was in between 97.15 to 99.62 with a low standard deviation (≤0.61). The results of drug content analysis have shown that the method employed to prepare films in this study was capable of giving films with uniform drug distribution with an insignificant batch variability (p>0.001).

Moisture content and moisture uptake

Moisture content and moisture uptake studies provide information regarding stability of the formulation.^[13] The results revealed that the moisture content and moisture uptake were found to increase with increasing concentration of hydrophilic polymer (HPMC). The presence of penetrations enhancers DMSO and IPM did not show any major changes in moisture content and moisture uptake values. In case of DMSO, slight increment in both parameters was observed. This may be due to the water affinity of DMSO. The small moisture content in the formulation helps them to remain stable and from being a completely dried^[14] and brittle films^[14], and low moisture uptake protects the material from microbial contamination and bulkiness of the films^[3,7,17]. Thus, the results of physicochemical studies conducted on different polymeric films containing tamoxifen favored the combination of these polymers for preparation of transdermal films, the obtained results were shown in Table 2.

Determination of tensile strength and folding endurance

All the formulations showed very good tensile strength, and

Table 2: Evaluation parameters of drug containing transdermal patches

Evaluations	F1	F2	F3	F4	F5
Thickness (mm)	0.11±0.01	0.12±0.02	0.14±0.03	0.12±0.01	0.14±0.02
Uniformity of weight	191.1±0.8	200.06±1.2	200.7±0.4	135.7±0.7	147.5±0.8
Content uniformity (%)	99.62±3.2	99.36±1.51	97.63±1.92	98.93±1.16	98.94±1.39
Moisture content (%)	2.61±0.38	3.06±0.15	3.36±0.06	3.62±0.10	4.93±0.22
Moisture uptake (%)	3.58±0.13	3.74±0.07	4.05±0.11	4.28±0.06	5.39±0.20
Folding endurance	39.50±2.65	39.25±1.26	31.75±0.96	44.50±1.73	46.00±2.16
Tensile strength (gm/cm ²)	12.91±0.15	12.73±0.04	12.82±0.09	12.52±0.05	13.07±0.09

Average of three determinations were reported (±SD, n = 3)

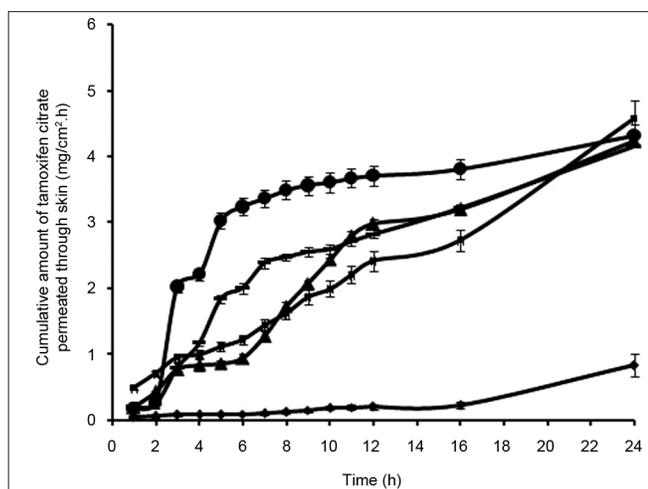


Figure 2: Cumulative amount of tamoxifen citrate permeated through the skin, F1 (♦), F2 (■), F3 (▲), F4 (▼), F5 (●)

the folding endurance values ranged between 12.91 ± 0.15 and 13.07 ± 0.09 kg/cm² and 38.50 ± 1.29 and 46.00 ± 2.16 , respectively, [Table 2].

Determination of flux, diffusion coefficient and permeability coefficient

The *in vitro* release profile is an important tool that predicts in advance how a drug will behave *in vivo*. The results of *in vitro* skin permeation studies of tamoxifen citrate from transdermal patches are shown in Figure 2 and Table 3. The cumulative amount of drug release (area of 3.14 cm²) from formulations F2, F3, F4, and F5 was (4.278, 4.561, 4.224, and 4.665 mg) high when compared to other formulations; this phenomenon was attributed to the amount of the combination, of hydrophilic and hydrophobic polymers,^[14-17] used in the formulations. When the cumulative amount of drug permeated with an area of 3.14 cm² patches through rat skin was plotted against time. The flux, permeation coefficient, and diffusion coefficient were high in formations F2, F3, F4, and F5, when compared to the F1 formulation, due the hydrophilic nature of the polymer, absorption of water, and its swelling nature.^[14-17] From these obtained results it could be revealed that the usual dose of tamoxifen was in the range of 10 – 20 for a single dose and 20 – 40 mg for the daily dose, respectively. However, only 60% of the bioavailability could be predicted, due to its first pass metabolism. Administration through the transdermal route made the drug available directly to the blood stream; hence, a lesser dose was required compared to the oral dose. Application of a patch having a surface area of more than 3.14 cm² or with a suitable large loading dose would provide the effective systemic concentration of tamoxifen. Moreover, once the tamoxifen reached the systemic circulation after transdermal administration; it underwent metabolism by a biological process, to produce hydroxytamoxifen, similar to the one administered orally. This metabolite was also very important in the therapeutic activity of estrogen positive receptor binding, respectively.^[18]

Table 3: Determination of flux, diffusion coefficient, and permeability coefficient

Formulations	(J _{ss})Flux mg/cm ² . hr	Permeation coefficient (cm/h)	Diffusion coefficient (cm/h)
F1	0.009±0.02	0.0017±0.001	0.0009±0.002
F2	0.054±0.04	0.0116±0.003	0.0018±0.004
F3	0.063±0.05	0.0125±0.005	0.0020±0.002
F4	0.052±0.07	0.0102±0.004	0.0020±0.001
F5	0.053±0.06	0.0108±0.003	0.0021±0.002

Average of three determinations were reported (±SD)

Stability aspects

All the samples of formulations when subjected to stability studies, at a periodic interval of days, were observed for changes in color, appearance, flexibility, and drug content. The patches were analyzed at an interval of 30 days for a period of three months. No physical changes were observed, however, a negligible decrease in drug content (2 – 4%) after three months was observed at a temperature of $40 \pm 2^\circ\text{C}/75\% \text{RH}$.

CONCLUSION

The matrix-type of transdermal containing tamoxifen citrate has been successfully formulated, which has brought a new modality delivery system for local chemotherapy, for breast cancer. The model patch formulation defines a positive outcome based on both qualitative observations and quantitative measurements of different parameters. The study demonstrated the possibility of developing an efficacious and acceptable transdermal drug delivery system for tamoxifen citrate, other than the conventional tablets. The study also concluded that the tamoxifen citrate transdermal patch could be a novel drug delivery of choice in the field of local chemotherapy for breast cancer.

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