In vitro and In vivo characterization of the transdermal delivery of sertraline hydrochloride Films

Vijaya R., *Ruckmani K.

Department of Pharmaceutical Technology, Anna University of Technology, Tiruchirappalli India

ABSTRACT

Background and the purpose of the study: Sertraline hydrochloride is a selective serotonin reuptake inhibitor principally used in the treatment of major depressive disorder. To maintain the therapeutic plasma drug concentration of the drug for prolonged period, the transdermal drug delivery has been chosen as an alternative route of drug delivery. The pharmacokinetic properties of sertraline hydrochloride make it suitable for transdermal delivery. The purpose of the study was to investigate the effect of polymers and penetration enhancers on the transdermal delivery of the drug in order to improve its therapeutic efficacy.

Methods: In the preparation of films, Eudragit RL 100, Eudragit RS 100, hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose were used as polymers. The films were characterized for thickness, tensile strength, drug content, moisture uptake, moisture content, water vapor transmission rate and drug release. The films exhibiting higher rates of drug release were subjected to study the effect of oleic acid and propylene glycol as penetration enhancers on skin permeation of sertraline hydrochloride. In vivo and skin irritation studies were performed for the optimized film.

Results: Films containing Eudragit RL 100, Eudragit RL 100 and HPMC showed the highest drug release of 94.34% and 96.90% respectively in a period of 42 hrs. The release data fitted into kinetic equations, yielded zero-order and fickian mechanism of drug release. There was a two-fold increase in skin permeation of sertraline hydrochloride in the presence of penetration enhancers in the film. The physical evaluation indicated the formation of smooth, flexible and translucent films. No skin irritation occurred on rabbit skin and the infrared studies showed the compatibility of the drug with the formulation excipients. The in vivo study revealed a constant plasma concentration of drug for long periods and the films containing penetration enhancers had achieved adequate plasma levels of the drug.

Conclusions: The obtained results indicated the feasibility for transdermal delivery of sertraline hydrochloride using eudragit RL 100 and HPMC.

Keywords: Antidepressant, Eudragit, Hydroxy Propyl Methyl Cellulose, Ethyl Cellulose, In-Vitro and In-Vivo Skin Permeation.

INTRODUCTION

Sertraline hydrochloride is a selective serotonin reuptake inhibitor administered orally which undergoes extensive first pass metabolism. The drug produces gastrointestinal disturbances such as nausea, dry mouth, constipation, diarrhea, decreased appetite, etc. The long-term administration and fluctuation in plasma concentration of the drug causes severe side effects (1). A transdermal delivery has been identified to overcome the difficulties of oral administration (2). This route provides several advantages of controlled delivery, improved patient compliance, gradual dose reduction, prevention of overdose and decreased side effects. The effectiveness of transdermal delivery has been proved for some antidepressants (3, 4).

Present study was aimed to demonstrate the transdermal delivery of sertraline hydrochloride using polymethacrylate polymers to improve its therapeutic efficacy. The drug was chosen because of its suitable physicochemical properties such as low molecular weight (342.73) and relatively low dose (25 to 200 mg) (1). The polymethacrylate polymers such as Eudragit RL100 (ERL 100) and Eudragit RS100 (ERS 100) were selected, because they are stable, possess good film making characters and act as a crystallization inhibitor. The polymers have been used successfully in the design of various transdermal patches (5, 6). In addition to these polymers, hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose (EC) polymers were also used. In the previous study, eudragit E 100 was used as

Correspondence: rmani48@gmail.com

an adhesive polymer matrix for the transdermal delivery of sertraline hydrochloride (7). In the present investigation, the non- adhesive type of polymeric films were prepared and characterized for physicochemical properties, in vitro drug release and kinetics, in vitro and in vivo skin permeation and skin irritation studies. The effect of combined propylene glycol (PG) and oleic acid (OA) on permeation of the drug was also evaluated.

MATERIAL AND METHODS

Materials
Sertraline hydrochloride and paroxetine hydrochloride were gift samples from Orchid Pharmaceuticals, (Chennai, India). ERL 100 and ERS 100 were gift samples from Evonik Degussa Industries (Mumbai, India). HPMC, EC, and dibutylphthalate were purchased from Loba Chemie Pvt. Ltd., (India). All other chemicals and reagents used were of laboratory or analytical grade.

Methods
Compatibility study
Sertraline hydrochloride and the polymers ERL 100, ERS 100, HPMC, and EC were mixed separately and corresponding pellets were prepared. The FTIR spectra (NICOLET 6700 FTIR, USA) was taken and analysed for any interaction between the drug and the polymers.

Preparation of transdermal films
The films were casted by glass rings on a mercury surface contained in a petri plate. The rings had a diameter of 5.8 cm with 5 ml capacity. The polymers composition in the transdermal film (total polymer weight kept at 300 mg) is given in table 1. The polymeric solutions were prepared using ethanol as solvent except for films containing HPMC and EC where the mixture of dichloromethane and ethanol were used as solvents at a ratio of 1:1. The plasticizer dibutylphthalate (DBT) (30 % w/w of the polymer) and 10 mg of sertraline hydrochloride were added and stirred well to get a homogenous solution. The volume was made up to 5 ml and pipetted onto a mercury surface. The solvent was then allowed to evaporate overnight by inverting a funnel over the petri plate. The dried films were stored in desiccators for evaluation. A combination of oleic acid (8) (1% w/w) and propylene glycol (9) (30% w/w) were added to the film formulation for skin permeation studies.

Characterization of transdermal films
Weight variation and thickness
The film weight variation was determined by weighing 6 films individually on an electronic balance (Sartorius, Germany) and calculating the mean and standard deviation. The thicknesses of the films were measured at three different points using a screw gauge (Syracuse, Newyork) and the average thickness was recorded.

Folding endurance
Folding endurance was determined manually by folding a 4 x 3 cm strip of the film repeatedly at the same place until it broke. The number of times that the film could be folded at the same place without breaking or cracking gave the value of folding endurance (10).

Percentage of moisture uptake
The films were weighed accurately and placed in a desiccator containing 100ml of saturated solution of aluminum chloride (84% relative humidity (RH)). After 3 days, the films were taken out and weighed until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight (10).

Percentage of moisture content
The films were weighed accurately and kept in a desiccator containing one gram of anhydrous calcium chloride. After 3 days, the films were taken out and weighed repeatedly until it showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to initial weight (10).

Water vapour transmission rate (WVTR)
Glass vials of equal diameter were used as transmission cells. One gram of anhydrous calcium chloride was placed in the cells and the polymer film was fixed over the brim. The cells were accurately weighed and kept in a closed dessicator containing saturated solution of aluminium chloride (84% RH). The cells were taken out and weighed after 6, 12, 36, 48 & 72 hrs. The WVTR was found using the formula. (10).

$$\text{Water vapour transmission rate} = \frac{\text{Final weight- Initial weight}}{\text{Exposure Time X Area of film}} \times 100$$

Tensile strength
Two ribbon shaped cuttings (4 × 3 cm) from the film were prepared and fitted in a tensile strength apparatus (Instron 3369.UTM, Japan). The force and elongation were measured when the film broke. The tensile strength was calculated using the following equation (11).

Tensile strength= F/a×b (1+L/l)

F - The force required to break, a - Width of the film, b - Thickness of the film, L - Length of the film, l - Elongation of the film at break point.

Table 1. Polymer ratios of transdermal films containing sertraline hydrochloride (10 mg in each formulation) and their formulation codes.

Polymers	F1	F2	F3*	F4*	F5*	F6*	F7*	F8*
Eudragit RL 100	10	8	8	8	8	8	8	8
Eudragit RS 100	-	2		-	1	-	1	0.66
Hydroxy propyl methyl cellulose	-	-	2	-	1	1	-	0.66
Ethyl cellulose	-	-		2	-	1	1	0.66

*HPMC and EC are insoluble in ethanol, but completely soluble in a 1:1 mixture of dichloromethane and ethanol.

Drug content determination

The entire film was cut into small pieces and allowed to dissolve in 100 ml of sodium acetate buffer (pH 4.5). The solution was filtered and the absorbance was determined using a UV/visible spectrophotometer (UV-1700, Shimadzu, Japan) at a wavelength of 273 nm after suitable dilution.

In vitro drug release studies

A modified stainless steel disc assembly (USP dissolution apparatus 5, paddle over disc), was used for the study (12). A circular film with a diameter of 5.8 cm containing 10 mg of drug was used for the study. The apparatus was equilibrated at a temperature of 32±0.5°C and operated at 100 rpm using phosphate buffer saline (PBS of pH 7.4) as dissolution medium. The samples were withdrawn at appropriate time intervals and the absorbance was read at 273nm. Cumulative percent released drug was calculated and plotted against time.

For determination of the kinetics and mechanism of drug release, the release data were analysed using zero order, first order, Higuchi and peppas kinetic models. Coefficient of correlation values were calculated from the linear curves obtained for the above plots (9).

In vitro skin permeation studies

An abdominal skin from 4-17 week-old female mice was used for the study (13). Hair was removed and the area washed with distilled water. Mice were euthanized by cervical dislocation, abdominal skin was excised and the fatty material attached to the dermis was peeled off. The skin was sandwiched between the receptor and donor compartment of the Keshary-Chien type diffusion cell (surface area of 4.9062 cm^2). The receptor compartment contained PBS (pH 7.4) and was maintained at 37±0.5°C with stirring at 500 rpm using a magnetic stirrer (Remi, Mumbai, India). The transdermal under study tested was placed on the skin. Samples of 2.0 ml were withdrawn at different times and replaced immediately with an equal volume of PBS. The permeation of drug in the presence and absence of penetration enhancers was compared. The absorbance of the sample was measured at 273 nm. The percentage of drug permeated was calculated and plotted against time. Flux was determined directly from the slope of the curve which was constructed by the steady state values of the cumulative amount of drug permeated (mg/cm^2) vs time. Permeation coefficients (cm/hr) were deduced by dividing the flux by initial drug loading (mg/cm^2) (14).

In vivo skin permeation studies

Rabbits weighing between 1.8-2.0 kg were divided into three groups of three animals each. Group 1 was given an oral suspension of the drug. Group 2 received film F3 and Group 3 received film F3 containing penetration enhancers. The dose of the drug was calculated according to the body weight of the animal. The animals were fasted overnight. The film was affixed on the shaved dorsal surface of the skin and the blood samples were collected from the marginal ear vein. The animals were fed with rabbit diet during the study. The samples were centrifuged at 4000 rpm and the plasma was separated and refrigerated until analyzed by HPLC (Shimadzu, Japan). For the HPLC analysis of the drug, a mixture of methanol and sodium acetate buffer (pH of 3.8) in the ratio of 25:75 was used as a mobile phase at a flow rate of 1 ml/min with the run time of 15min. Paroxetine hydrochloride was used as an internal standard and the wavelength was set at 239nm. The plasma drug concentrations were analysed using WinNonlin software (version (4.1), US) and the pharmacokinetic parameters C_{max}, T_{max}, AUC and AUMC were derived. Permission was obtained from the institutional animal ethical committee held for these experiments.

Skin irritation studies

The study was performed for the optimized film (F3) employing three healthy rabbits (average weight 3.0 kg). Ethical clearance was obtained from the institutional animal ethical committee held for this purpose. The dorsal surface (50 cm^2) of the rabbits was cleaned and the hair was removed by shaving. The skin was cleaned with 75% ethanol. Representative films were placed on the skin and held in place with an adhesive tape. Films were removed after 24, 48 and 72 hrs and the skin was examined for erythema and edema and scored according to the method of Draize *et al*. (15). The average of 24-72 hrs scores represents the final score.

Table 2. Physicochemical properties of sertraline hydrochloride transdermal films.

Formulation	WVTR (g/cm²/hr)±SD	% MU ±SD	% MC ±SD	Thickness ±SD (mm)	Folding endurance (No's)	Tensile Strength (MPa)
F1	0.0208±0.001	10.612±1.99	4.221±0.36	0.45±0.06	278±1.41	12.023±2.03
F2	0.0188±0.005	8.234±0.71	4.127±1.51	0.36±0.03	267±1.67	11.061±1.04
F3	0.0442±0.002	14.172±1.82	6.283±0.60	0.51±0.05	298±1.67	12.235±1.56
F4	0.0169±0.004	6.112±1.26	2.225±0.03	0.46±0.04	257±1.89	11.543±1.54
F5	0.0147±0.005	7.258±1.22	3.867±1.27	0.43±0.03	286±2.19	11.64±2.51
F6	0.0206±0.003	9.267±1.42	3.484±0.05	0.46±0.02	282±2.19	11.85±1.98
F7	0.0105±0.004	4.332±1.88	1.753±0.99	0.48±0.02	278±1.47	10.01±1.47
F8	0.0198±0.002	3.710±0.84	0.695±0.0.63	0.42±0.05	292±2.19	09.42±2.21

Values are mean±S.D (n = 6). WVTR, water vapor transmission rate; MU, moisture uptake; MC, moisture content

Statistical analysis of data

Data were expressed as mean±S.D. Statistical evaluation was performed by one-way analysis of variance (ANOVA) at a significance level of $p<0.05$ by Dunnett's multiple comparison test using GraphPad Prism software version 4.03.

RESULTS AND DISCUSSION

Compatibility studies

The FTIR spectra of the samples are shown in figure 1. The principal peaks of the drug observed in all the samples showed no chemical interaction between the drug and the polymers. However, some additional peaks were observed due to the presence of polymers. The polymers employed are commonly used in matrix-type films and are compatible with a number of drugs (16).

Preparation and physicochemical characterization of transdermal films

The film preparation method yielded translucent flexible films that did not become brittle over time. ERL 100 was kept as a parent polymer in high concentration since it has major influence on drug release and permeation (17). The plasticizer DBT was able to produce a flexible film without any influence on the drug release property (18). When the total polymer weight was lower than 0.2 g or higher than 0.4 g, the film lost its folding endurance, becoming thick with insignificant tensile strength. If the concentration of plasticizer was higher than 0.1 ml (i.e. > than 30% of polymer weight), the film lost its flexibility and became stiff. The physicochemical properties of the films are recorded in table 2. The weight and thickness of the films varied from 355±4.2mg to 387±3.8mg and 0.36±0.03mm to 0.48±0.02mm respectively. A low standard deviation (SD) value in the film thickness measurements ensures uniformity of the films. The values of folding endurance were found to vary from 257±1.89 to 298±1.67which indicates good strength and elasticity.

The film F3 showed maximum WVTR, %MU, and %MC, which may be attributed to the hydrophilic nature of HPMC. The substitution of ERL100 with ERS100 and EC decreased these values. The results indicated that the hydrophilicity of the polymers is directly proportional to the WVTR, %MU, and %MC. The order of hydrophilicity of the polymers was HPMC> ERL >ERS>EC. However, the small moisture in the formulations may prevent complete drying and brittleness. Overall, the moisture uptake of the transdermal films was low and thus reduced the bulkiness of the films. No significant difference (p>0.05) in drug content was observed for all the formulations which were 9.82±0.17mg to 9.97±0.21 mg. The results indicated that the film preparation was capable of yielding uniform drug content due to the homogenous dispersion of the drug.

In vitro drug release studies

The results of the in-vitro release are shown in figure 2. Formulation F3 exhibited a maximum drug release of 96.9±0.64%. A significant difference (P<0.001) in drug release after 42 hrs was found for the film F3 for all formulations except F1. An initial burst release of 15 -20 % which was observed for all the formulations, may be due to the drug that was saturated on the film surface during storage. The release was decreased gradually with time providing controlled release up to 42 hrs.

The higher proportion of quaternary ammonium groups in ERL 100 (F1) may be the reason for rapid hydration and drug release. Further, ERL 100 prevents crystallization of the drug and the drug in its amorphous form undergoes rapid solubilization when the solvent penetrated into the matrix. The enhanced release rate of F3 may be due to the dissolution of aqueous soluble fraction of HPMC, a hydrophilic polymer that forms pores and produces higher dissolution rates (19). All the forumations followed zero order release as evidenced from the highest correlation coefficients (r^2= 0.988-0.999). The n value (<0.5) obtained from the slope of log m_t/m_T vs log time curve (korsmeyer peppas) indicated a fickian (diffusion-controlled) transport mechanism.
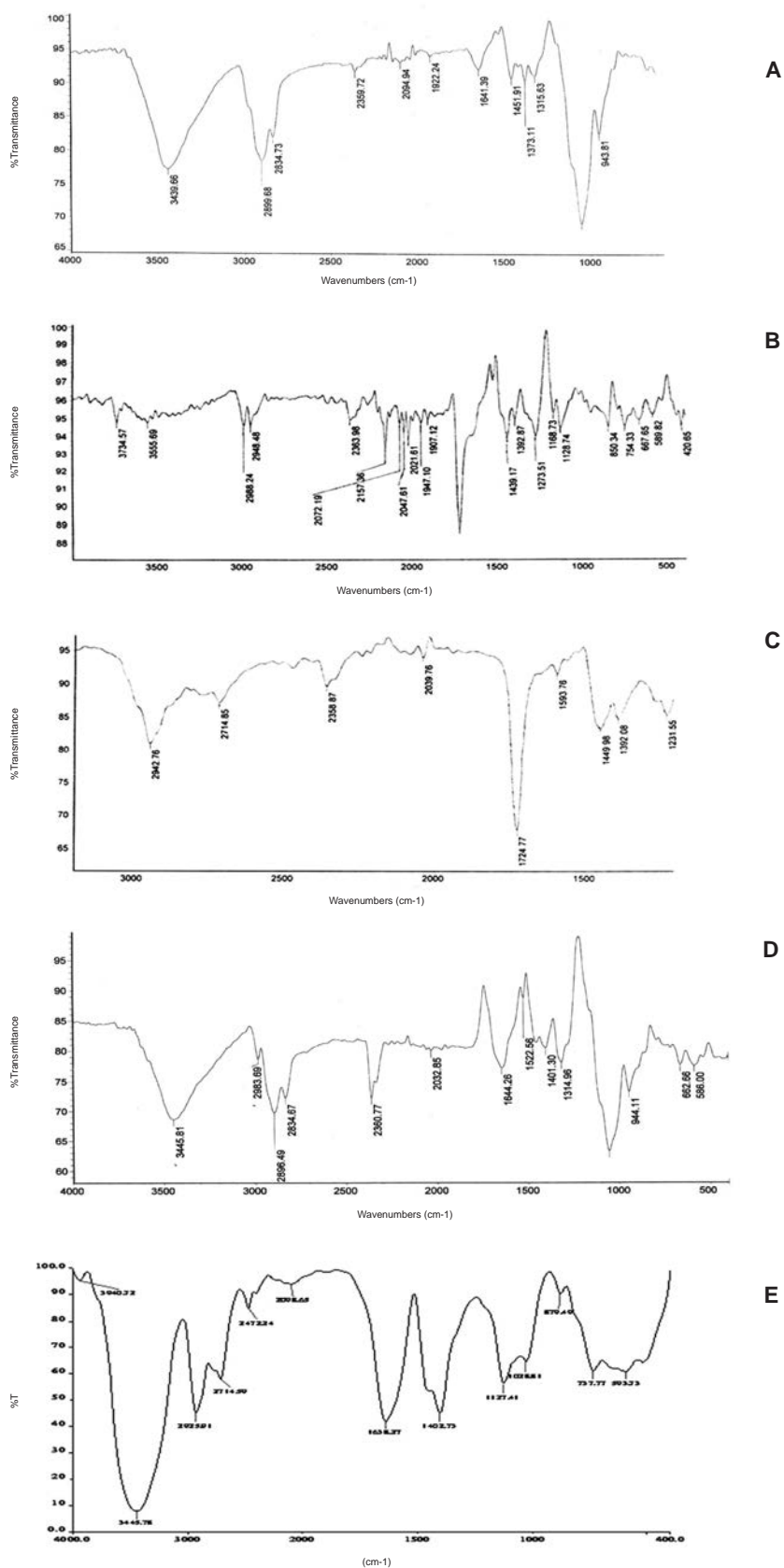
Figure 1. FTIR spectra of the drug (A) , drug and Eudragit RL 100 (B), drug and Eudragit RS 100 (C), drug and HPMC (D) and drug and EC (E).
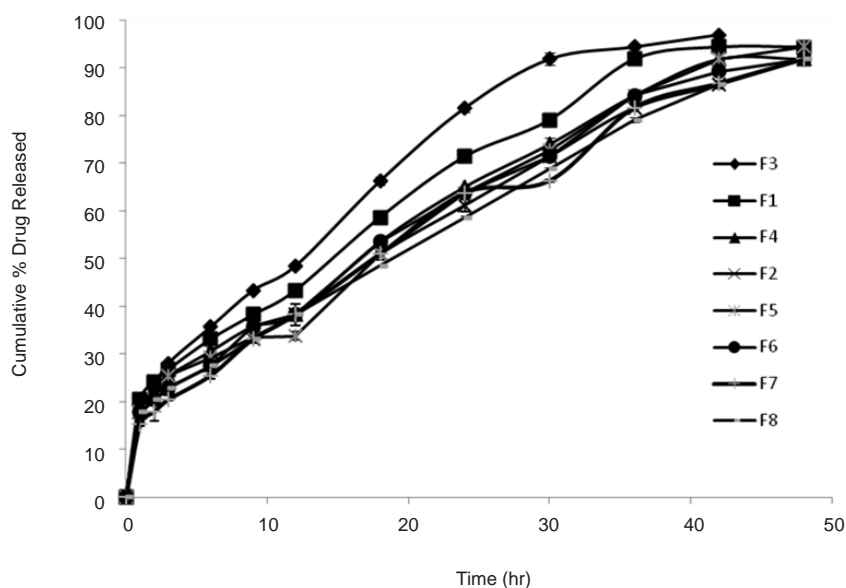
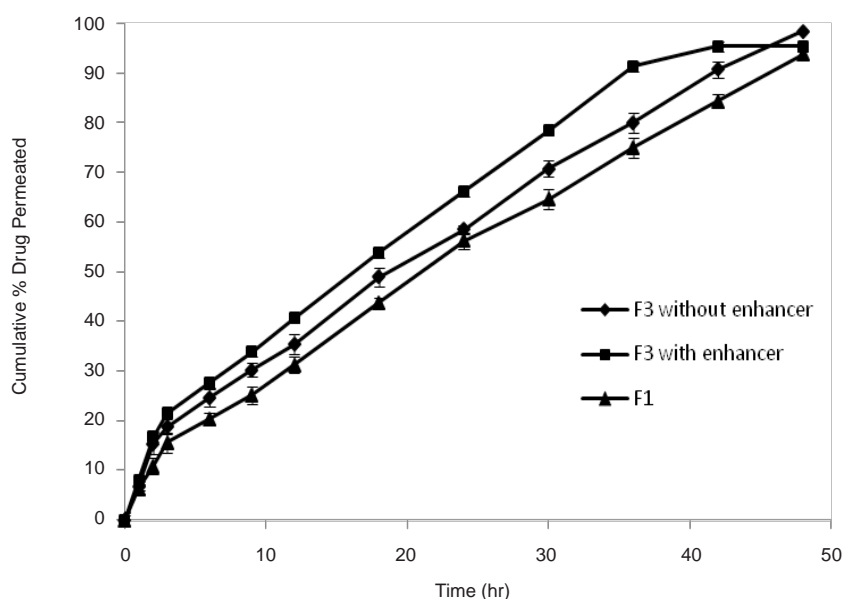**Figure 2.** In vitro drug release of sertraline hydrochloride transdermal films F1-F8.

Figure 3. Comparative in vitro skin permeation profile of sertraline hydrochloride and its transdermal films.

m_t/m_T is the fraction of drug released at time t and 'n' is the diffusional release exponent.

In vitro skin permeation and the effect of penetration enhancers

The formulations F3 and F1 were selected for skin permeation study by virtue of their maximum drug release. The results are shown in figure 3. The skin permeation took place at a faster rate during the initial period and then attained a constant rate. The in vitro burst release which presents more drug on the skin surface may be the reason for rapid skin permeation. A constant and slow skin permeation in later stages might be due to the slow release of drug from the films. It can be said that the film controls the skin permeation of the drug, so that an increase in the release rate from the film can increase the permeation rate of the drug.

The film F3 in the presence of penetration enhancers showed the highest skin permeation of 95.38±1.48% which was significantly ($p<0.05$) different in the absence of penetration enhancers (90.76±2.11%). The effect of penetration enhancer was evaluated by the enhancement ratio, which was found to be 1.98. The transdermal flux and the permeability coefficient values for the film F3 in the presence
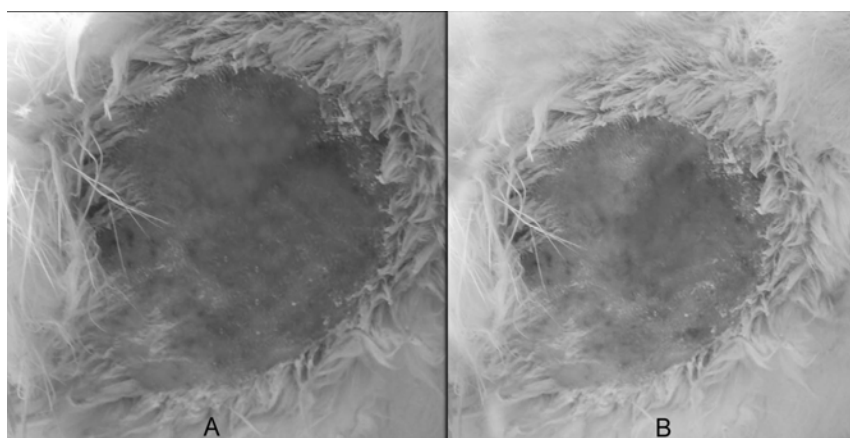
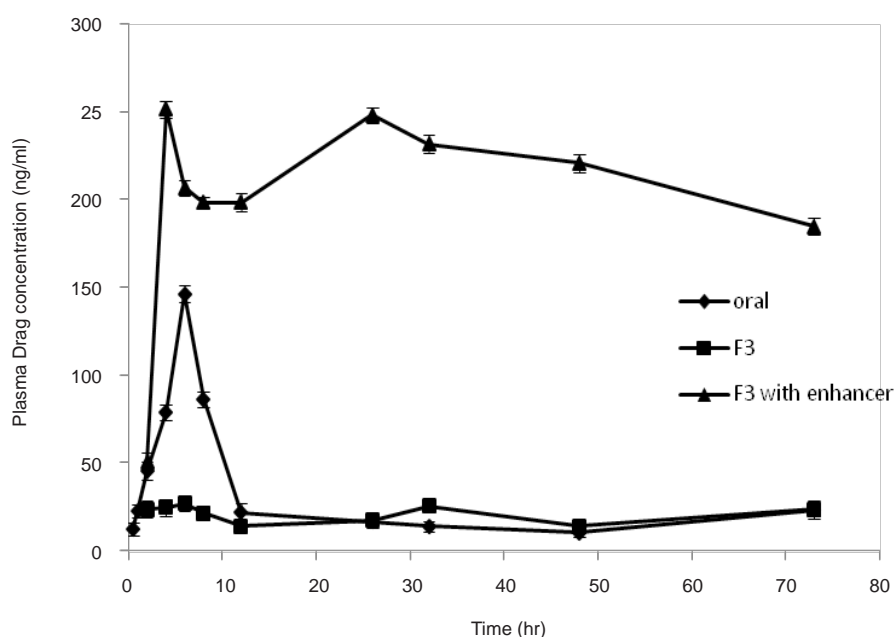**Figure 4.** Rabbit skin before (A) and after (B) skin irritation test.

Figure 5. Plasma drug concentration profile of sertraline hydrochloride after oral and transdermal administration (film F3, film F3 with penetration enhancers).

and absence of penetration enhancer were 0.1922 mg/cm^2/hr, 0.0961 cm/hr, 0.0969 mg/cm^2/hr and 0.0485cm/hr respectively. The penetration enhancing effect of OA and PG (F3 in the presence of enhancer) may be explained by the fact that oleic acid increases the lipid domain fluidity and propylene glycol alters the polarity of the aqueous region and increases its solubilizing ability for lipid-like materials. This favors high drug concentration in the skin that diffuses out into the dermis based on the concentration gradient along with the solvent PG (20).

Skin irritation studies
There was no obvious irritation for the film F3

and the total irritation score was zero in the tested animals. According to draize et al, the film is considered as non irritant and thus it is suitable for transdermal application. The photomicrographs taken before and after the skin irritation studies are shown in figure 4.

In vivo skin permeation studies
The results of the in vivo permeation studies are shown in figure 5. The film F3 in the presence and absence of penetration enhancers indicated the maintenance of constant plasma drug concentration. A maximum drug concentration (C_{max}) of 251.48 ng/ml was exhibited by the film F3 in the presence of penetration enhancers at

Table 3. Pharmacokinetic parameters of sertraline hydrochloride formulations.

Parameters	Oral	Film F3	Film F3 with enhancer
Dose (mg/kg)	4.17	5.6	5.6
Cmax (ng/ml)	146.28	26.72	251.48
Tmax (h)	6	6	4
AUClast (ng/ml*h)	1759.98	1377.35	15244.75
AUCextra (ng/ml*h)	1215.92	NC	31185.59
AUCtot (ng/ml*h)	2975.90	NC	46430.34
%AUCextra	40.86	NC	67.17
T1/2 (h)	36.31	NC	117.03
MRT (h)	67.24	NC	174.63
Clearance (ml/min/kg)	23.35	NC	2.01

NC-Not Calculated

the T_{max} of 4 hrs. This was due to the reduction in skin barrier property by the penetration enhancers. Lower value of T_{max} indicated the rapid permeation of the drug. The C_{max} given by the other two formulations (F3 in the absence of enhancer and oral formulation) was significantly ($p<0.05$) very low. The values of AUC, $T_{1/2}$, MRT and clearance are shown in table 3. The increase in AUC, T1/2, MRT and clearance values of F3 in the presence of penetration enhancer shows the feasibility of maintaining the required plasma drug concentration for prolonged period. Sertraline hydrochloride does not produce any toxic effects at this C_{max} value and the minimum effective concentration in human is 20-50 ng/ml. Hence to achieve therapeutic plasma drug concentration, the size and/or the dose of the film could be reduced to ten times. i.e. dose of 0.16 mg can be incorporated in 0.28 cm^2 area of the film to maintain the C_{max} up to 72 hrs as calculated from the present study. However, addition of penetration enhancer is needed to attain the required concentration of the drug in plasma. Further studies should be carried out on human volunteers to confirm these findings.

CONCLUSIONS

Based on the results of this study, it can be concluded that a well-controlled release and effective skin permeation of the drug was achieved by the film F3 (ERL 100 and HPMC) in the presence of penetration enhancers for extended periods of time. The in vivo study has proved the feasibility of controlled transdermal delivery of sertraline hydrochloride in adequate quantity into the circulation. However, to establish the therapeutic efficacy of this formulation, pharmacokinetic studies in humans needs to be conducted.

ACKNOWLEDGEMENT

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