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Research Article

THE DEVELOPMENT AND EVALUATION OF TRANSDERMAL FILMS OF AMITRIPTYLINE HYDROCHLORIDE

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ABSTRACT:

The matrix type transdermal film of Amitriptyline hydrochloride with and without chemical penetration enhancers was prepared on mercury substrate by solvent evaporation technique. Mixture of polymers Eudragit RL 100, Eudragit RS 100, Hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose were employed in the preparation of films. Dibutyl phthalate (30%w/w of polymer) was added as a plasticizer. The films were evaluated for thickness, weight variation, percentage moisture absorption, percentage moisture loss, water vapor transmission rate, folding endurance and drug content.

The *in vitro* drug release studies were performed using a US Pharmacopoeia paddle-type dissolution apparatus. The data obtained revealed a slow release of drug from all the formulations. A maximum release of 95.0% over a period of 24hrs was obtained with formulation F1 that composed of Eudragit RL 100 and HPMC. When the release study was conducted on female abdomen mouse skin for F1, there was a drastic decrease in the amount of drug release i.e.51.26%. In order to reduce the skin barrier property and to enhance the skin permeation of drug, penetration enhancer's oleic acid and propylene glycol combination at a ratio of 1:30 was incorporated in the polymeric film. The film containing penetration enhancers revealed 83.59% of Amitriptyline hydrochloride release. There was no irritation found on application of film (F1) over rabbit skin. The excipients showed no chemical interaction with the drug as evidenced from the FTIR studies. The Amitriptyline hydrochloride film had produced slow release, which followed zero order kinetics with non-fickian mechanism of drug release.

Keywords: Transdermal delivery, Amitriptyline HCl, Eudragit RL 100, Eudragit RS 100, HPMC, *In-vitro* evaluation.

INTRODUCTION:

The skin as a site of drug delivery has a number of significant advantages over many other routes of drug administration, including the ability to avoid problems of gastric irritation, pH, and emptying rate effects; avoid hepatic first pass metabolism thereby increasing the bioavailability of drug; reduce the risk of systemic side effects by minimizing plasma concentrations compared to oral therapy; provide a sustained release of drug at the site of application; rapid termination of therapy by removal of the device or formulation; the reduction of fluctuations in plasma levels of drugs and avoids pain associated with injections. The transdermal delivery can also eliminate pulsed entry into the systemic circulation, which might often cause undesirable side effects. Transdermal therapeutic systems may produce sustained, constant and controlled levels of drug in the plasma, thereby improving patient compliance, since frequent intake of the drug is not necessary [(1)].

Major Depression has been one of the major diseases affecting the mankind in today's scenario. Amitriptyline Hydrochloride is a widely used anti-depressant, administered as intramuscular depot injection or used orally for suppressing the depression disorder. Amitriptyline Hydrochloride is rapidly absorbed from the gastrointestinal tract (80%), but the oral bioavailability remains low (eg, 23%) because of significant first-pass hepatic metabolism by N-demethylation and bridge hydroxylation [(2)]. Evidence of first pass metabolism, prolonged duration of treatment and low dose required for depression disorder offer the major challenge in its treatment by conventional route. Thus the transdermal route of Amitriptyline hydrochloride delivery would be a better alternative for its improved treatment efficacy.

Polymers are the backbone of a transdermal drug delivery system[(3)]. Reports describing the use of polymers like Eudragit RL (ERL) and Eudragit RS (ERS) in transdermal delivery systems as well as other dosage forms for controlled release of drugs are available[(4)]. ERL is freely permeable to water, whereas ERS is slightly permeable[(5)].

These transdermal delivery systems are neither extremely hydrophobic nor extremely hydrophilic. Therefore, varying the ratio of these polymers in the composition of the films provides control of drug release characteristics[6].

From the above views, the present study was aimed to provide controlled delivery of the drug Amitriptyline HCl across intact skin using polymeric films. The study was planned as given in the following lines (1) to develop different matrix patches of amitriptyline HCl using four different polymers Eudragit RL100 (ERL100), Eudragit RS100 (ERS100), Hydroxy propyl methyl cellulose (HPMC), and ethyl cellulose (EC) in diverse combinations of varying degree of hydrophilicity and hydrophobicity . (2) to find out the effect of the polymers on the technological properties of the films (3) to perform *in-vitro* release and *in vitro* skin permeation studies.and (4) to study the effect of penetration enhancers combinations oleic acid and propylene glycol on skin permeation of the drug.

MATERIALS AND METHODS

Materials

Eudragit RL 100 and Eudragit RS 100 were received as a gift sample from Evonik industries (Mumbai,India). Hydroxy propyl methyl cellulose and Ethyl cellulose, Mercury was procured from Loba chemie pvt. Ltd. (Mumbai, India). Dibutyl phthalate, Dichloro methane, anhydrous calcium chloride and Hydrochloric acid were purchased from Loba chemie pvt. Ltd., (Mumbai,India). Aluminum Chloride, Potassium dihydrogen phosphate and Sodium Hydroxide was purchased from Qualigens Fine chemicals, (Mumbai,India). Double-distilled water was used throughout the study.

Methods

Solubility Determination

The solubility of Amitriptyline hydrochloride in the various vehicles such as acetone, ethanol, phosphate buffer (pH=7.4), distilled water were determined by equilibrating an excess amount of drug with the solvent in a shaking water bath at 37°C. At the end of 24hours, 1ml of aliquots were withdrawn, diluted with the ethanol, and analysed by UV spectrophotometer at 239nm.

Interaction study

The infrared spectra of pure polymers and physical mixtures of polymers and drug were run between 400-4000cm^{-1} to find out any type of interaction between drug and polymers.

Preparation of Transdermal Films: The film was casted on mercury surface contained in a Petri dish using fabricated glass rings. The required amount of drug was dissolved in ethanol and respective polymers were added to it (Table 1). To this, the plasticizers Dibutyl phthalate (30%w/w of the polymer) was added and stirred well to get a homogeneous solution. The volume was made up to 5ml with ethanol and it was pipetted onto the mercury surface contained in a glass ring so that it formed a well. The film was dried for a period of 48hr, and the rate of evaporation was controlled by inverting a funnel over the petri dish. The dried films were stored in a desiccator for a week until further evaluation [(7)].

Preparation Transdermal patch by using Penetration Enhancer

In the preparation of transdermal films, oleic acid and propylene glycol (1:30) penetration enchancers**[(5,7)]** were added and stirred well before pipetting onto the mercury surface and labeled separately to identify the films for further study.

Evaluation of Physicochemical Properties of films[(8,9)]

Thickness of film:

Thickness of films was measured at three different points using digital micrometer screw gauge (Mitutoyo, Japan) and the mean value was calculated.

Weight Variation:

The films were subjected to weight variation by individually weighing ten randomly selected films. Such determinations were carried out for each formulation.

Folding Endurance: Folding endurance of films was determined by repeatedly folding the film at the same place until it broke. Hence the number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.

Percentage Moisture Absorption: The films were weighed accurately and placed in the desiccators containing 100ml of saturated solution of aluminum chloride. After three days, the films were taken out and weighed. The percentage moisture absorption (% MA)was calculated using the formula-

$$\text{Percentage moisture absorption:} \quad \frac{\text{Final weight of film- Initial weight of film}}{\text{Initial weight of film}} \times 100$$

Percentage Moisture Loss:

The films were weighed accurately and placed in the desiccators containing anhydrous calcium chloride. After three days, the films were taken out and weighed. The percentage moisture loss (%ML) was calculated using the formula.

$$\text{Percentage moisture loss:} \quad \frac{\text{Final weight of film- Initial weight of film}}{\text{Initial weight of film}} \times 100$$

Water Vapor Transmission Rate:

Glass vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1g anhydrous calcium chloride was placed in the cells and the respective polymer films were fixed over the brim. The cells were accurately weighed and kept in a closed desiccators containing saturated solution of potassium chloride. The amount of water vapor transmitted was found using the formula.

$$\text{Water vapor transmission rate:} \quad \frac{\text{Final weight of film- Initial weight of film}}{\text{Time X Area}} \times 100$$

Water vapor transmission rate (WVTR) is usually expressed as the number of grams of moisture gained/hr/cm^2.

Drug Content: Films of size 2cm^2 was cut into small pieces and put in a 100ml buffer (pH7.4). This was then shaken in a mechanical shaker for 24hr to get a homogenous solution and filtered. The amount of drug was determined spectroscopically at 239 nm after suitable dilution.

***In vitro* Drug Release Study** [10]**:** The *in vitro* drug release studies were performed using a US Pharmacopeia paddle-type dissolution apparatus using 500 ml of phosphate buffer pH 7.4 as the dissolution medium. The release rate determination is one of the most important studies to be conducted for all controlled-release delivery systems. The dissolution studies of films are crucial because one needs to maintain the drug concentration on the surface of the stratum corneum consistently and keep it substantially higher than the drug concentration in the body, to achieve a constant rate of drug permeation.

A circular film containing 10 mg of drug was used for the study. All dissolution studies were performed at 37 ± 2ºC, at 50 rpm. Samples were withdrawn at different time intervals and analyzed spectrometrically. Cumulative amounts of drug released were plotted against time for all the formulations.

***In Vitro* Skin Permeation:**

To assess the skin permeation, female abdominal mouse skin aged 4-17 weeks was sandwiched between the receptor compartment and donor compartment of Keshary Chien type diffusion cell such that dermal side was batched with the receptor fluid in the receptor compartment and the stratum corneum side faced upward the donor compartment. The receptor compartment contained saline phosphate buffer (pH7.4) and maintained at 37±0.5^{o}C stirring at 500rpm. A unit of TDDS was placed on the skin, with drug releasing surface in close contact with the stratum corneum. Sample of 2.0ml was withdrawn at scheduled time intervals for 24hr and replaced immediately with an equal volume of saline phosphate buffer. The skin permeation of Amitriptyline hydrochloride films were compared with the solution of the drug (1% in water). The samples were assayed for Amitriptyline hydrochloride using UV spectrophotometer at 239nm.

Skin Irritation Study [11]

Skin irritation studies for the films were performed on three healthy rabbits (average weight: 1.43kg). The dorsal surface (50cm^2) of the rabbits was cleaned and was removed by shaving. The skin was cleaned with rectified spirit. Representative films were placed over the skin with the use of adhesive tape and were removed after 24hrs. The scores of skin irritation study are tabulated in Table 2

RESULTS AND DISCUSSION:

Interaction Study

The Infrared spectra of drug and physical mixture of drug and polymer were studied using potassium bromide disc method. No interaction between the drug and polymer confirms no changes in drug molecule.

Evaluation of Physicochemical Properties of Films:

The properties of the Amitriptyline HCl films are recorded in Table 2. The thickness of the films varied from 0.36 to 0.48mm. Moisture content and moisture uptake studies indicated that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture content and moisture uptake of the films. The formulation showed maximum %WVTR, %MA, and %ML, which can be attributed to the hydrophilic nature of Eudragit RL 100. As expected, substitution of Eudragit RL100, with HPMC, Eudragit RS100, and EC decreased the values of %WVTR, %MA with their reducing hydrophilic nature. The film F8 having all the polymers showed the least %WVTR, %MA and %ML. However the small moisture content in the prepared formulations reduce brittleness during long-term storage. Similarly a low moisture uptake of the formulations may protect the formulations from microbial contamination and reduce bulkiness [(12)].

Determination of Drug Content:

The drug content in films was observed using UV spectrophotometer with phosphate buffer as a solvent system for Amitriptyline hydrochloride and the concentration was calculated using the standard graph. Results of content uniformity revealed uniform drug content for all the formulations ranges from 8.945±0.5813 to 7.9±1.1467mg. This indicates the homogenous dispersion of drug during the film preparation.

Skin Irritation Study:

Skin irritation studies carried out on three rabbits revealed that the formulation F1 of Amitriptyline hydrochloride showed no erythema, edema, and ulceration and supposed to be safe for human use. The resulting reaction was evaluated using weight score. The average of 24hrs, 48hrs &72hrs reading was noted and given in Table 4 and Figure 2.

***In vitro* Drug Release Study:** The *In vitro* release results obtained are shown in the table5. The cumulative amount of drug released from formulations F1 was 95.07%, which is high when compared with the release from other formulations F2 to F8. When the cumulative amount of drug released per square centimeter of film through rat skin was plotted against time, the permeation profiles of the drug followed zero-order. However, the release mechanism followed Higuchi's equation (r^2 = 0.9953-0.9979), which indicates that the release of the drug from the film was governed by a diffusion mechanism. Since many release processes can be represented by a coupling of a Fickian and non-Fickian mechanism, Ritger and Peppas introduced the power law equation $M_t/M_\infty = Kt^n$ to characterize the controlled-release behavior of a drug from polymer matrices [13]. The value of *n* can be calculated from the slope of In M_t/M_∞ vs In *t* and can be indicative of the operating release mechanism. The *n* values ($n >$ 0.651) obtained by this equation indicates that the drug was released by Non-Fickian diffusion, predominated with all the formulations. In this context, the results obtained from the Non-Fickian mechanism support the results of Higuchi's equation. The results of *in vitro* study release study are presented in Figure 1.

***In vitro* Skin Permeation Study:**

The saturated solution of Amitriptyline Hydrochloride permeation across the skin was 93.45% for 15hours. Whereas for the Amitriptyline hydrochloride transdermal film was 51.26% within 24hrs. The film F1 was selected for permeation study on the basis of its higher *in vitro* drug release. This drastic decrease in the % of drug release was exactly due to the stratum corneum which acts as a barrier for the transport of drug across the skin. After the addition of penetration enhancers, the permeation was increased upto 83.59% for 24hrs. This enhancement in skin permeation may be explained on the following points Propylene glycol enhances the permeation of drug when applied in a binary mixture [14]. Low molecular weight alcohols have been shown to posses the ability to enhance permeation across the skin. Their mechanism of action seems to be related to stratum corneum lipid extraction, particularly the more polar lipids. The skin permeation study revealed that the controlled release of Amitriptyline hydrochloride from the film, determines its permeation across the skin.

CONCLUSION

The matrix type of Amitriptyline hydrochloride was prepared successfully by using four different polymers by solvent evaporation technique. From the *in vitro* release and skin permeation results it can be concluded that controlled release of Amitriptyline hydrochloride across the skin could be achieved for prolonged period. This would maintain well being in the later stages of antidepressant therapy. Further work is underway to predict the *in-vivo* performance of the formulation.

Table-1: Composition of Transdermal Films Containing Amitriptyline Hydrochloride.

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

*HPMC and EC are insoluble in ethanol and it is completely soluble in the mixture of solvents Dichloromethane & Ethanol in the ratio of 1:1.

Table-2: Skin Reactions and Weight Score.

S.no	Erythema formation	Edema formation	Score assigned
1.	No Erythema	No edema	0
2.	Very slight erythema	Very slight edema	1
3.	Well defined erythema	Slight edema	2
4.	Moderate to severe erythema	Moderate edema	3
5.	Severe erythema	Severe edema	4

Table-3: Physicochemical properties of Amitriptyline hydrochloride transdermal patch.

Formulation	WVTR ($g/cm^2/hr$) ± SD	%MU (Moisture Uptake) ±SD	%MC (Moisture Content) ±SD	Thickness ±SD (mm)	Folding endurance (No's)	Tensile Strength (MPa)
F1	0.0442±0.002	14.172±1.82	6.283±0.603	0.41±0.0529	257±1.89	12.235±1.56
F2	0.0209±0.001	10.612±1.99	4.221±0.3630	0.45±0.0608	298±1.67	12.023±2.03
F3	0.0170±0.004	8.234±0.7127	4.127±1.5105	0.46±0.04	278±1.41	11.543±1.54
F4	0.0188±0.005	6.112±1.2581	2.225±0.0265	0.36±0.03	267±1.67	11.061±1.04
F5	0.0148±0.005	7.258±1.2207	3.867±1.2746	0.43±0.0265	286±2.19	12.64±2.51
F6	0.0206±0.003	9.267±1.4200	3.484±0.0525	0.46±0.0173	282±2.19	12.35±1.98
F7	0.0106±0.004	4.332±1.8773	1.753±0.9982	0.48±0.02	278±1.47	12.68±1.47
F8	0.0198 ±0.002	3.710±0.8427	0.695±0.6326	0.42±0.0529	292±2.19	12.42±2.21

Values are mean ± S.D, (n=3)

Table-4: Weight Score of Skin Irritation Test of Amitriptyline Hydrochloride Transdermal Film F1.

Animal No	Sex	Hours after treatment with patch(F1)			
		1hr	24hrs	48hrs	72hrs
1	Male	1/0	0/0	1/0	0/0
2	Male	1/0	1/0	0/0	0/0
3	Male	0/0	0/0	1/0	0/0

Erythema scale: 0- none; 1- slight; 2-well defined; 3-moderate; and 4- scar formation.

Edema scale: 0-none; 1-slight; 2-well defined; 3-moderate; and 4- severe edema.

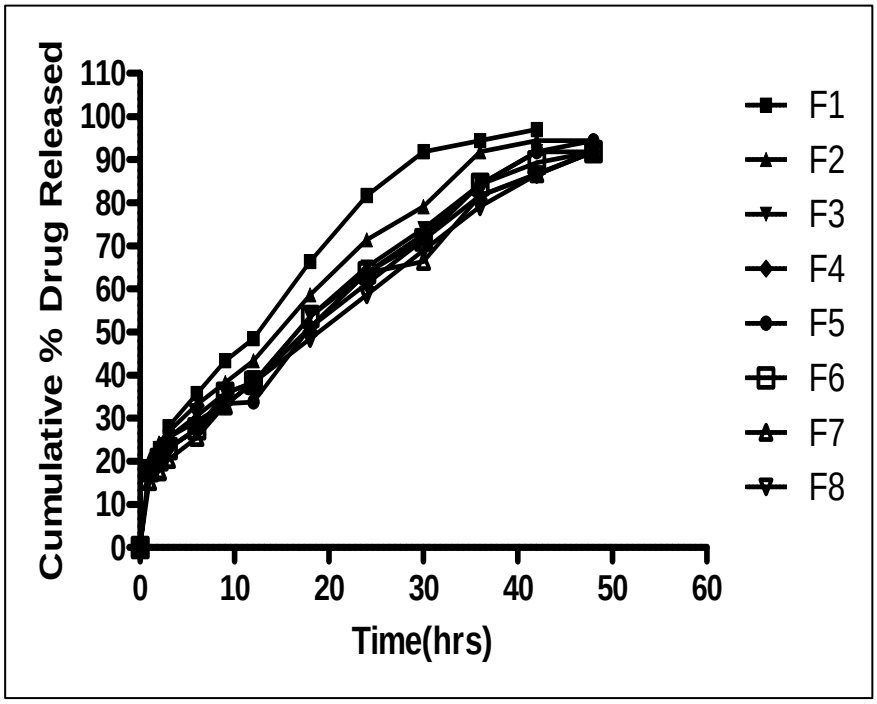

Figure-1: Comparative *In-Vitro* Release Profile of Formulations F1 to F8.

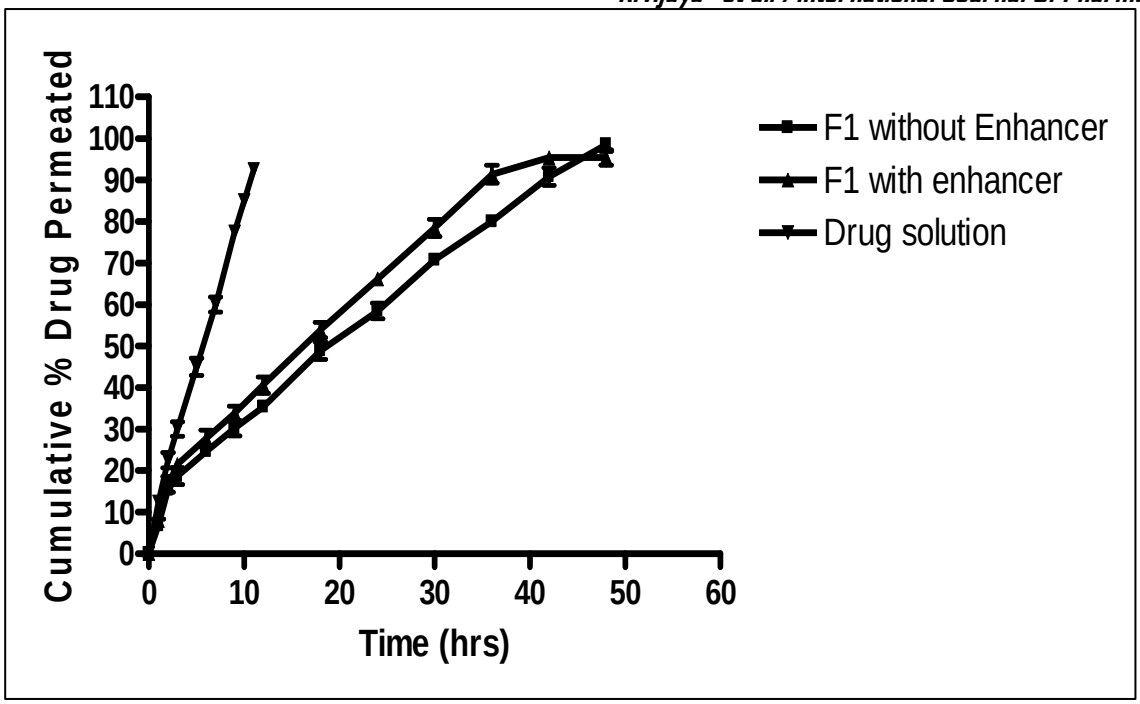

Figure-2: *In Vitro* Skin Permeation Profile of Amitriptyline Hydrochloride.

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