International Journal of Life science & Pharma Research

PREPARATION OF AMITRIPTYLINE HYDROCHLORIDE FILMS USING EUDRAGIT RL 100 AND HYDROXY PROPYL METHYL CELLULOSE POLYMERS AND THEIR *IN-VITRO* EVALUATION FOR EFFECTIVE TRANSDERMAL DELIVERY

R.VIJAYA*, S.SURESHKUMAR, S.UMAMAHESWARI, M.PRAKASH, T. SENBAGAPRIYA AND A.UMAMAHESWARI

Department of Pharmaceutical Technology, Anna University of Technology Tiruchirappalli, Tiruchirappalli – 620 024.

ABSTRACT

Objective: The aim of the present study was to prepare a transdermal film for the antidepressant Amitriptyline hydrochloride to improve the treatment efficacy. **Method:** The matrix type transdermal film was prepared by solvent evaporation method using two different polymers Eudragit RL 100 & hydroxy propyl methyl cellulose (HPMC) in different ratios using solvents ethyl alcohol and dichloromethane. Dibutyl phthalate was used as a plasticizer. The films were evaluated for physical properties such as thickness, percentage moisture absorption, percentage moisture loss, drug content, folding endurance and flatness. The *in-vitro* release studies were performed using USP dissolution apparatus. The optimized film was further evaluated for skin permeation, stability and skin irritation studies. **Results:** The data obtained shows that the transdermal film F4 (Eudragit RL 100 & HPMC at ratio of 2:1) had produced a highest drug release of 98 ±1.03% up to 24 hrs. When the release study was conducted for F4 on an animal skin there was a drastic decrease (61.56 ± 0.14%) in the percentage of drug release. The kinetics of drug release followed higuchi model with diffusion controlled mechanism. Skin irritation studies showed no sign of edema and erythema. There was no interaction found between the drug and polymer as proved by FTIR studies. The film F4 was found to be stable with respect to drug content. **Conclusion:** The prepared film of Amitriptyline HCl is found to provide a controlled release and permeation which is suitable for transdermal delivery.

Key words: Transdermal Delivery, Amitriptyline Hydrochloride, Hydroxy Propyl Methyl Cellulose, Eudragit RL 100, *In-vitro* Evaluation, Skin Permeation.

1. INTRODUCTION

Transdermal drug delivery is the non-invasive delivery of drug from the surface of the skin through its layers, to the circulatory system. Generally the transdermal film comprises a polymer to control the rate of delivery of the drug that can pass through the skin and into the bloodstream over a period of several hours to days. It offers various advantages like maintenance of plasma levels, reduction in dosing frequency, improved patient compliance and simple application. In the modern era, depression is one of a major disease affecting human kind and is treated pharmacologically using antidepressant. One such agent is Amitriptyline HCl, a tricyclic anti depressant that has been prescribed more often in severe cases of major depression. Amitriptyline HCl acts by inhibiting the reuptake of serotonin-norepinephrine in the central nervous system neurons. The drug upon oral administration produces adverse effects of nausea, dry mouth, diarrhea, constipation, insomnia, anxiety, restlessness, decreased sex drive, dizziness, weight

gain, tremors, sweating, sleepiness or fatigue and headaches. These effects are mainly due to the dose and fluctuation in plasma drug concentration. The side effects could be reduced by controlled administration of drug using transdermal films so as to improve the treatment efficacy. The main objective of this work was to prepare transdermal films of Amitriptyline hydrochloride using two different polymers Eudragit RL 100 and hydroxy propyl methyl cellulose and to evaluate the film for physicochemical characteristics, *in vitro* drug release and skin permeation behavior. The chosen polymer combination has been used successfully for the transdermal administration of several therapeutic agents (Kevin C et al. 2010; Vijayan V, 2010).

2. MATERIALS AND METHODS

2.1 Materials

Eudragit RL 100 received as a gift sample from evonik industries (Mumbai, India). Hydroxy propyl methyl 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). Potassium chloride, potassium dihydrogen phosphate and sodium hydroxide were purchased from Qualigens Fine Chemicals (Mumbai, India). Double-distilled water was used throughout the study.

2.2 Experiment

2.2.1 Compatibility Study: The infrared spectra of drug and physical mixture of drug and polymer were studied by potassium bromide disc method using FTIR (Perkin Elmer Spectrum RXI) (Jamakandi VG et al. 2009) to identify any possible physical, chemical interaction.

2.2.2 Preparation of Transdermal Film

A transdermal film was prepared by solvent casting technique employing mercury as a substrate. The casting solutions were prepared by dissolving the appropriate polymers and plasticizer in a suitable solvent ethanol, using magnetic stirrer for 20 min to get uniform dispersion. The concentration of plasticizer was kept at 30 % w/w of dry polymers. The solution was then transferred quantitatively to a glass ring placed on the surface of mercury in a petridish. Controlled solvent evaporation was achieved by placing an inverted funnel over the petridish. This was then left undisturbed at room temperature for 48hrs. The films were retrieved intact by slowly lifting the rings from the mercury substrate and kept in the dessicator until used. The ratio of the drug and the polymer is given in table1. Each formulation was added with 10mg of the drug (Jamakandi VG et al. 2009; Chien YW et al.1987).

Table 1:Polymer Composition of Transdermal Films Containing Amitriptyline Hydrochloride

S.No	Formulation	Polymer ratio		Film Formation
		Eudragit RL 100	HPMC	
1	F1	1	0	FF
2	F2	1	1	FNF
3	F3	1	2	FNF
4	F4	2	1	FF
5	F5	1	3	FNF
6	F6	3	1	FF
7	F7	1	4	FNF
8	F8	4	1	FF
9	F9	2	3	FNF
10	F10	3	2	FF
11	F11	1	5	FNF
12	F12	5	1	FF

FF-Film Formed, FNF- Film Not Formed

2.2.3 Thickness of the Film

The thickness of the drug loaded film was measured in different points using a digital micrometer (Mitutoyo co; Japan) and determined the average thickness and standard deviation for the same to ensure the thickness uniformity of the prepared films **(**Umesh D, 2009).

2.2.4 Percentage Moisture Absorption

The films were weighed accurately and placed in the desiccators containing 100 ml of saturated solution of potassium chloride, which maintains 79.50% RH. After 3 days, the films were taken out and weighed. The study was performed at room temperature. The percentage moisture absorption was calculated using the formula **(**Umesh D, 2009**).**

$$\% \text{ Moisture Uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

2.2.5 Percentage Moisture Loss

The films were weighed accurately and kept in a desiccators containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture loss was calculated using the formula **(**Umesh D, 2009**).**

$$\% \text{ Moisture Loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.2.6 Folding Endurance

This was determined by repeatedly folding the film at the same place until it broke. The maximum number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance (Venkateswara Rao J, 2010).

2.2.7 Flatness Test

Three longitudinal strips were cut from each film at different portion like one from the center, other one from the left side and another one from the right side. 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 (Jamakandi VG et al. 2009).

$$\% \text{ Constriction} = (I_1 - I_2)/ I_1 \times 100$$

I_2 = Final length of each strip

I_1 = Initial length of each strip

2.2.8 Weight Uniformity

The prepared films were dried at 60°C for 4hrs before testing. The films were weighed in a digital balance (Sartorius, Germany). The average weight and the standard deviation values were calculated for the individual film weight (Shinde Anil kumar J et al. 2009).

2.2.9 Drug Content

A film was cut into 4 quadrants and put in 100ml buffer (pH 6.8).This was then shaken in a mechanical shaker for 24 hrs to get a homogeneous solution and then filtered. The drug content was determined spectrophotometrically at 240nm after suitable dilution (Shinde Anil kumar J et al. 2009).

2.2.10 *In vitro* Drug Release Study

The paddle over disc method (USP apparatus V) was employed for the assessment of drug release from the prepared films. Dry films of known thickness and drug content was cut into definite shape and fixed over a glass plate containing a mesh. The glass plate was then placed at the bottom of a dissolution basket holding 500ml of the dissolution medium of phosphate buffer (pH 7.4). The medium was equilibrated to 32 ± 0.5°C. The paddle was then fixed at a distance of 2.5 cm from the glass plate and rotated at a speed of 50 rpm. Samples of 5ml aliquots were withdrawn at appropriate time intervals up to 24 hrs and analyzed by UV spectrophotometer at λmax of 240nm. The experiment was conducted for six times and the mean and standard deviation value was calculated for each determination (Sadhashivaivah R et al. 2008; Vijayan V, 2010).

2.2.11 Release Kinetics

The analysis of a drug release from a transdermal film F4 (optimized film) was performed to identify the contribution to overall kinetics. The *in vitro* release data of film F4 was treated with different release kinetics equations such as zero order, first order, higuchi, korsmeyer peppas and hixon- crowell. From these plots the regression coefficient values were calculated. Ritger and peppas (Ritger PL and Peppas NA, 1987) introduced the power law equation $M_t/M_\infty = Kt^n$ to characterize the controlled-release behavior of a drug from polymer matrices. Where Mt / M∞ is fraction of drug released at time t, K is the rate constant and n is the release exponent. 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 (Harris Shoaib M et al. 2006).

2.2.12 *In vitro* Skin Permeation Study

The *in vitro* permeation study was carried out using keshary chien type diffusion cell. Full thickness abdominal skin of male wistar rats weighing 200 to 250g was used. Hair from the abdominal region was removed carefully using a hair removal cream (Veet, Reckitt benckiser, India). The dermal side of the skin was thoroughly cleaned with isoporopyl alcohol to remove any adhering tissues or blood vessels, equilibrated for an hour in receptor medium (phosphate buffer pH 7.4) before starting the experiment and was placed on a magnetic stirrer (Remi, India) with a small magnetic bead for uniform distribution of the diffusant. The temperature of the diffusion cell was maintained at 37 ± 0.5°C with the help of a hot plate in the magnetic stirrer. The isolated rat skin piece was clamped between the donor and receptor compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Film F4 of suitable area was placed over the stratum corneum of the skin. Sample of definite volume was removed from the receptor compartment at regular time intervals and replaced every time with an equal volume of fresh receptor medium. Samples were filtered and analyzed spectrophotometrically for drug content (Sadhashivaivah R et al. 2008).

2.2.13 Skin Irritation Test

Skin irritation studies were performed on male healthy rabbits. The dorsal surface of the rabbits was cleaned and the hairs were removed by shaving. The skin was cleansed with rectified spirit. The rabbits were divided into two groups (n = 3). Group I received transdermal film F4 and Group II received 0.8% v/v aqueous solution of formalin as a standard irritant. At 24 and 72 hrs after test article application, the test sites were examined for dermal reactions of erythema/edema and scored in accordance with the Draize scoring criteria (Draize JH et al. 1944) and the primary dermal irritation index was calculated (Shinde Anil kumar J et al. 2009).

2.2.14 Statistical Analysis

Data were expressed as mean ± SD. 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.

3. RESULTS AND DISCUSSION

3.1 Compatibility Study

The existence of principal peaks of the drug in the spectra of the mixture of drug and polymer (spectra not shown) showed no physical and chemical interaction between them at the concentration used in the film.

3.2 Evaluation of Transdermal Film

The physiochemical properties of Amitriptyline hydrochloride films are recorded in Table 2. The percentage moisture absorption (%MA) and percentage moisture loss (%ML) was found to increase with increasing HPMC concentration, which might be attributed to the hydrophilic nature of the HPMC. The thickness of the films varied from 0.16±0.01mm to 0.22 ±0.04mm. The folding endurance was found to be high in films containing higher amount of the Eudragit RL 100. The value of the folding endurance measures the ability of the film to withstand rupture. The 100% flatness observed in the flatness study of all the films indicates 0% constriction which helps in uniform dispersion of the polymer throughout the films. (Kusum Devi V et al. 2003). The weight of the films varied between 416±0.36mg to 425±0.42mg. This is due to the preparation technique which has yielded films of uniform weight for all the film composition despite the composition of the polymer.

TABLE 2: Physiochemical Properties of Amitriptyline Hydrochloride Transdermal Films

Formulation	%MA±SD	%ML ±SD	Thickness±SD (mm)	Folding endurance (No of folds)	Flatness	Weight (mg)
F1	1.72±0.02	1.13±0.03	0.16±0.01	40	100	420±0.57
F4	5.94±0.01	3.95±0.01	0.21±0.02	80	100	418±0.36
F6	4.46±0.04	2.47±0.02	0.22 ±0.04	40	100	421±0.44
F8	3.17±0.02	2.74±0.04	0.15±0.01	40	100	422±0.41
F10	6.63±0.05	4.98±0.02	0.17±0.02	30	100	416±0.36
F12	1.46±0.03	1.83±0.01	0.21±0.01	60	100	425±0.42

Values are expressed as mean ± SD (n=3)

3.3 Drug Content Determination

The drug content in the film ranged from 9.85±0.098mg to 9.94±0.108mg. It was determined using the standard graph plotted between the concentration range of 0 to 12 µg/ml with the regression coefficient value of 0.9992. The results are tabulated in table 3. The results showed uniform drug content in all the film formulations (Jamakandi VG et al. 2009).

Table 3: Drug Content of Amitriptyline Hydrochloride Transdermal Films

S.No	Formulation	Drug Content (mg)
1	F1	9.85±0.098
2	F2	9.92±0.142
3	F3	9.90±0.089
4	F4	9.94±0.108
5	F5	9.91±0.132
6	F6	9.89±0.088

Values are expressed as mean ± SD, (n=3)

3.4 *In Vitro* Drug Release

The results of the in vitro release are shown in figure 1. The film F4 has released a maximum of 98 ± 1.03% of Amitriptyline hydrochloride release up to 24 hrs. The cumulative percentage of drug released from film formulation F4 was significantly high when compared with other film formulations. The addition of hydroxy propyl methyl cellulose with the Eudragit RL 100 resulted in more drug release than the Eudragit RL 100 alone i.e. in case of film F1. It was revealed from the release results that an increase in concentration of Eudragit RL 100 in the film tends to decrease the Amitriptyline hydrochloride release, A burst release followed by a slow release was observed for all the formulations. This burst release might be due to the presence of water soluble polymer HPMC which gets dissolved rapidly when exposed to the dissolution medium. The water permeable polymer Eudragit RL 100 may be responsible for the slow and constant release of drug in the later stages. This may be due to the polymeric network which restricts the diffusion of the drug molecules out of the film (Sadhashivaivah R et al. 2008; Vijayan V, 2010).

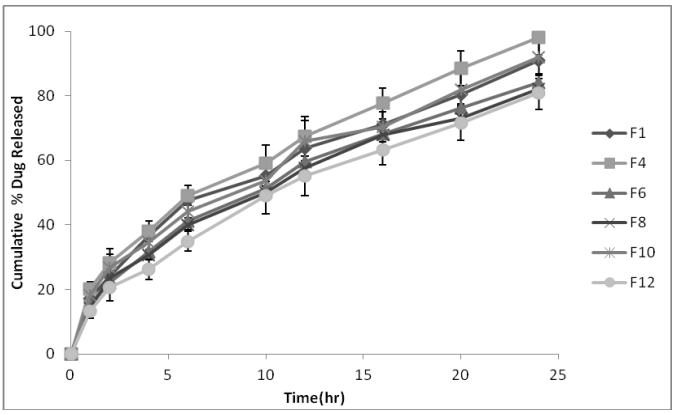

Figure 1: Comparison of In Vitro Release of Amitriptyline Hydrochloride from Transdermal Films

3.5 Release Kinetics

The data obtained from the release kinetic study of film F4 is shown in table 4. The *n* value obtained from the korsmeyer peppas equation was 0.4938. The R^2 values of the kinetic models are given in table 4. The release process can be represented by a fickian mechanism ($0.45<n< 0.85$). (Harris Shoaib M et al. 2006). It can be concluded that the drug release from the matrix films followed higuchi model and the mechanism of the drug release was diffusion mediated.

TABLE 4: The R^2 Value for the Various Kinetic Models for the Transdermal Film F4

KINETIC MODEL	R^2 VALUE
Zero Order	0.936
First Order	0.8847
Higuchi Model	0.998
Hixon-Crowell	0.9696
Korsmeyer-Peppas	0.9977

3.6 *In vitro* Skin Permeation

The film F4 was selected for the skin permeation study based on the higher drug release obtained in the *in vitro* release data. The skin permeation of Amitriptyline hydrochloride film F4 was 61.56 ± 0.14% over a period of 24 hrs. The drastic decrease in the amount of drug release was due to the barrier nature of skin for the transport of small molecules. A result of the skin permeation study is given in figure 2. A lag time of 30min was observed. The permeation was slow and sustained and the transdermal flux of 26µg/cm^2/hr was obtained for the film without the addition of any penetration enhancer (Sadhashivaivah R et al. 2008).

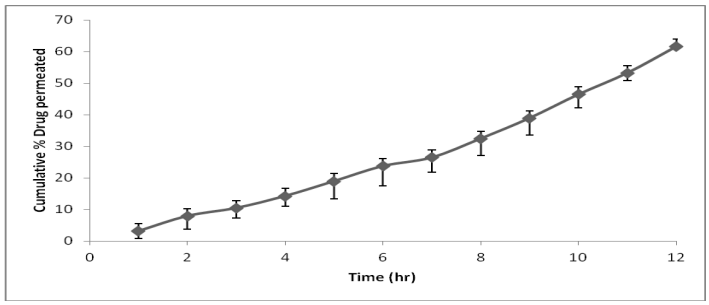

Figure 2: In Vitro Skin Permeation of Amitriptyline Hydrochloride (F4)

3.7 Skin Irritation Study

Skin irritation studies carried out on rabbits revealed that the formulation F4 of Amitriptyline hydrochloride showed no erythema and edema. The photographs taken are shown in figure 3, 4, 5 & 6. Figure 3 show the rabbit skin treated with standard irritant. The score assigned to the skin reactions at various time points are given in table 5. There was no irritation produced by the film formulation as found from the primary dermal irritation index value of 0.25. The results showed that the prepared film is suitable for application on skin (Shinde Anil kumar J et al. 2009; Shinde Anil kumar J et al. 2010).

Table 5: Skin Reaction Score Assigned to Transdermal Film F4 during Skin Irritation Test

Animal No	Sex	After 1hr	After 24hrs	After 48hrs	After 72hrs
1	Male	0/0	0/0	0/0	0/0
2		0/0	1/0	0/0	0/0
3		0/0	0/0	0/0	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

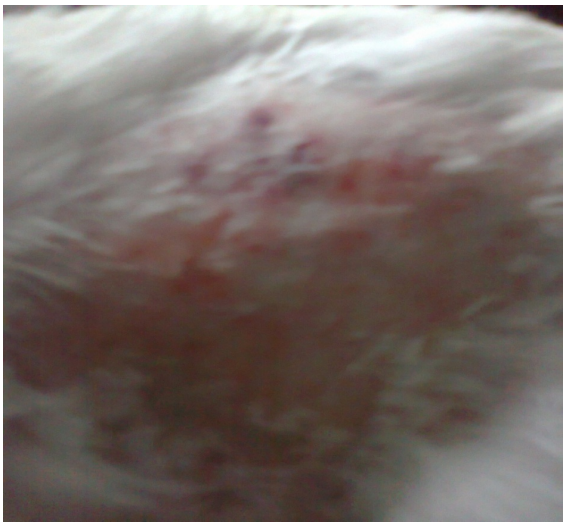

Figure 3: Rabbit Treated With Standard Irritant

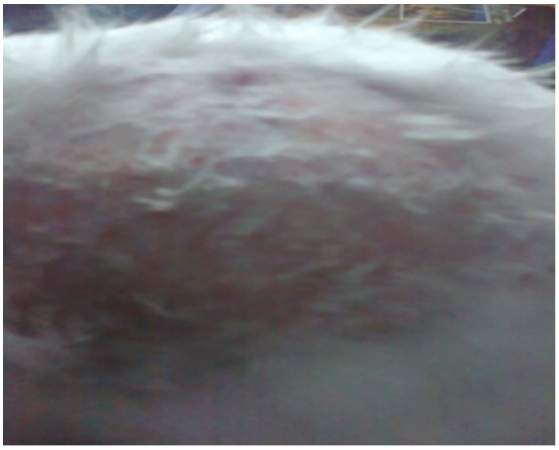

Figure 4: Rabbit Skin after Shaving the Hair

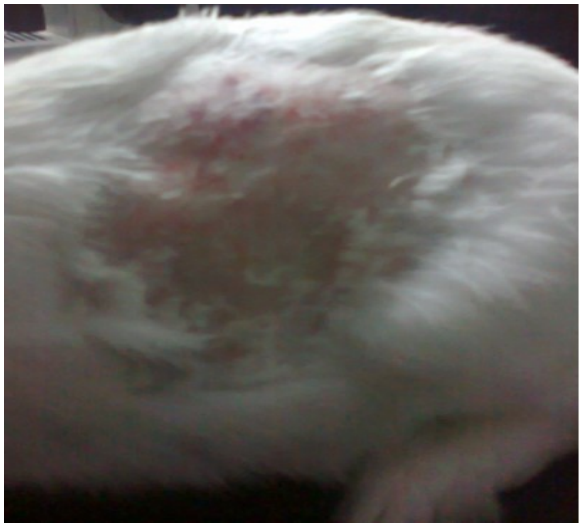

Figure 5: Rabbit Skin after 24hrs of Removal of the Transdermal Film F4

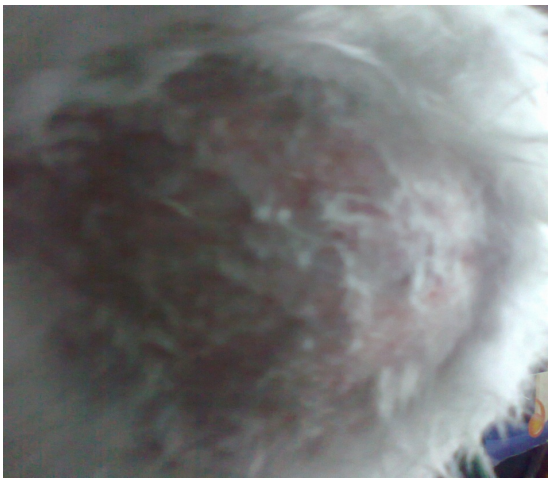

Figure 6: Rabbit Skin after 72hrs of Removal of the Transdermal Film F4

3.8 Stability Study

Stability study of Amitriptyline hydrochloride film F4 formulation was conducted using the air tight container. The containers were kept at room temperature (30 ± 2^0C) for 45 days. The samples were withdrawn at 10, 20, 30, 40 and 45 days and analyzed for drug content using UV spectrophotometer at λmax 240 nm. The result illustrate no significant (P>0.05) change in drug content after 45 days (Sanjay Dey and Ananya Malgope, 2010).

Table 7: Drug content of Amitriptyline HCl film F4 after 45 days

S.No	Period (Days)	Drug Content(mg) ± SD
1	0	9.93±0.05
2	10	9.91±0.14
3	20	9.89±0.09
4	30	9.89±0.12
5	40	9.85±0.06
6	45	9.84±0.13

Values are mean ± SD, n=3

4. CONCLUSION

It can be concluded that the composition of the polymer influences the film formation and the addition of HPMC had influenced the drug release from the film. The film F4 (Eudragit RL 100 and HPMC at a ratio of 2:1) revealed a 98% of amitriptyline hydrochloride release up to 24 hrs. Both the release and permeation were exhibited a controlled release of drug from the film. The drug release was found to follow higuchi model and the mechanism of the drug release was diffusion mediated. There was no interaction found between the drug and polymer as proved by FTIR studies. Skin irritation study on rabbits proved no erythema and edema formation. The prepared film of Amitriptyline HCl is suitable for the transdermal route of delivery in the treatment of depression. However the *in vivo* studies need to be conducted in future to confirm the therapeutic efficacy of the developed film.

5. REFERENCES

1. Chien YW, Bova D, Ahmuty VN, Cirrito RL, O'toole holmes K, Kasper K, Laprade C. Product development and technology transfer for transdermal Therapeutic Systems In. Transdermal Controlled systemic Medications, Marcel Dekker Inc Publishers.1987:379-396.
2. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to

the skin and mucous membrane. J Pharm Exp.1944; 82:377-390.
3. Harris Shoaib M, Jaweria tazeen, Hamid A. Merchant and Rabia Ismail yousuf. Evaluation of drug release kinetics from Ibuprofen matrix tablets using HPMC. Pak J Pharm Sci. 2006;19(2):119-124.
4. Jamakandi VG, Smulla J, Vinay BL, Shivakumar HN. Formulation, characterization and evaluation of matrix-type transdermal patches as a model Antihypertensive drug. Asian J Pharm. 2009;3:59-65.
5. Kevin C, Garala, Pratik H, Shah. Influence of Crosslinking Agent on the Release of Drug from the Matrix Transdermal Patches of HPMC/Eudragit RL 100 Polymer Blends. J Macro Sc. 2010; 47(3):273-281.
6. Kusum Devi V, Saisivam S, Maria GR, Deepti PU. Design and evaluation of matrix diffusion controlled transdermal patches of verapamil Hydrochloride. Drug Dev In Pharm. 2003;29:495-503.
7. Ritger PL, Peppas NA. Simple equation for solute release. Part 1.fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or disks. J Cont Rel. 1987;5:37-42.
8. Sadhashivaivah R, Dinesh BM, Uma apatil Desai, Raghu KS. Design and *in vitro* evaluation of haloperidol lactate Transdermal patches containing ethyl cellulose- povidone as film former. Asian J Pharm. 2008;2:43-49.
9. Sanjay Dey, Ananya Malgope. Preparation of Carvediol transdermal patch and the effect of propylene glycol on permeation. In J Pharm Pharmaceu Sci. 2010;2(1):137-143.
10. Shinde Anil kumar J, Amit L, Shinde Harinath N. Design and evaluation of transdermal drug delivery system of gliclazide. Asian J Pharm. 2010; 4(2):121-129.
11. Shinde Anil kumar J, Garala Kevin C, More Harinath N. Formulation and evaluation of transdermal patches of metoprolol tartarate. Bazaz Publications. New Delhi. 2009;8:69-74.
12. Umesh D. Shivhare. Effect of Polymeric Compositions on pharmacotechnical properties of carvedilol transdermal film. In J Pharmaceu Sci Nano. 2009;2(1):457-464.
13. Venkataeswaran Rao J, Mamatha T, Mukkanthi, Ramash G. Development of matrix type transdermal patches of lercanidipine hydrochloride physicochemical and in-vitro characterization. 2010;18(1):9-16.
14. Vijayan V. development and physiochemical, in-vitro evaluation of antihypertensive transdermal patches. J Pharm Sci Res. 2010; 2(3):171-177.