Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Reserch Article

# FORMULATION AND EVALUATION OF TRANSDERMAL FILMS OF ANTI DEPRESSANT DRUG AMITRIPTYLINE HYDROCHLORIDE USING EUDRAGIT E100, HPC AND PVP POLYMERS

**VIJAYA R*, MANJUNATH M. N AND UMAMAHESWARI S.**

**Department of Pharmaceutical Technology, Anna University of Technology Tiruchirappalli, Tiruchirappalli-620024, Tamilnadu, India. Email: vrssvrs@gmail. com**

*Received: 21 Dec 2011, Revised and Accepted: 11 Mar 2012*

**ABSTRACT**

Transdermal drug delivery system (TDDS) delivers the drug through topical route for systemic effect at a predetermined and controlled rate. The drug safety, therapeutic efficacy and patient compliance can be gained by reducing both the size and number of doses which can ultimately be achieved through TDDS. In this study, transdermal films of Amitriptyline HCl have been formulated by solvent evaporation technique. Matrix type of film was prepared using polymers of Eudragit E100, hydroxy propyl cellulose (HPC) and polyvinyl pyrolidone (PVP) in different compositions incorporating dibutyl phthalate as plasticizer. The films were evaluated for physicochemical properties, *in vitro* release, kinetics of drug release, skin permeation and skin irritation studies. Six translucent films (F1, F2, F3, F4, F5, F6) containing Amitriptyline HCl having uniform thickness, drug content and flexibility were prepared. Formulation F5 (Eudragit E100 & PVP at a ratio of 7:3) was selected as the optimized film considering the highest drug release of 96. 45 ± 1. 24% in 24hrs. The release followed first order kinetics with diffusion based mechanism. The addition of PVP has resulted in much higher drug release than HPC. Formulation F5 showed maximum skin permeation of 49. 27 ± 0. 62% over period of 24hrs. The value of primary dermal irritation index was found to be zero. Formulation F5 had produced controlled release and permeation of Amitriptyline HCl up to 24hrs and thus suitable for transdermal drug delivery.

**Keywords** Amitriptyline HCl, Eudragit E100, Transdermal delivery, *In vitro* release, Skin permeation, Skin irritation.

## INTRODUCTION

Transdermal drug delivery is one of the most promising methods for systemic administration of drugs via the skin for variety of clinical indication. The adhesive dispersion type TDDS combi-patch®, fempatch®, climara patch® [1] have received wide commercial acceptance. These systems deliver therapeutically effective concentration of the drug across the patient's skin at a preprogrammed rate for prolonged period [2]. TDDS offers advantages such as avoidance of gastro-intestinal disturbances, hepatic first pass metabolism, and pulsed entry into the systemic circulation, pain with injections, frequent dosing and rapid termination of drug input [3].

Amitriptyline HCl is a tricyclic antidepressant used to treat depression. The drug is often used to manage nerve pain resulting from cancer treatment and also chronic migraines. It undergoes first pass effect and produces side effects of weight gain, dry mouth, changes in appetite, drowsiness, muscle stiffness, nausea, constipation, nervousness, dizziness, blurred vision, urinary retention, insomnia and changes in sexual function. The adult dose for pain management is 10 mg to 150 mg[4]. Longer duration of therapy and withdrawal due to side effects are the major concerns with existing oral route of delivery.

Eudragit classes of polymers have been widely used in pharmaceutical formulations [5]. The polymers are well tolerated by the skin and have a high capacity for loading drugs. Eudragit® E 100 is an adhesive cationic copolymer based on dimethyl aminoethyl methacrylate and neutral methacrylic esters [6]. PVP is used as a binder, complexing agent, as an aid for increasing the solubility of drugs in liquid and semi-liquid dosage forms (syrups, soft gelatin capsules) and as an inhibitor of recrystallisation [7]. Eudragit E 100 transdermal films have been prepared using either HPC or PVP, whereas the combination of PVP and HPC with eudragit E 100 has not yet been reported [8-12]. Hence the present study was aimed to study the effect of PVP and HPC combination on eudragit E 100 film. This article summarizes the formulation of Amitriptyline HCl transdermal films and emphasizes the physicochemical, release, permeation and irritation properties of polymeric films.

## MATERIALS AND METHODS

Amitriptyline HCl was obtained as a gift sample from Star Drugs Pharmaceutical Industries, Hosur (India). Eutragit E100 was obtained from Rohm Pharma (Darmstadt, Germany) Polyvinyl pyrrolidone, Hydroxy propyl cellulose were purchased from SD fine chemicals Ltd, Mumbai (India).

### Method of preparation of transdermal films [13]

Amitriptyline hydrochloride films were prepared by solvent evaporation technique according to the formula given in Table 1. Weighed quantity of eudragit E100 was added to 5 ml ethanol and dissolved completely using mechanical stirrer (Remi, Mumbai). To the above solution, drug (10mg), PVP, HPC were added and mixed thoroughly followed by plasticizer (dibutyl phthalate) addition and mixed. The solution was poured on the thick aluminum foil (five cm$^2$ area) and covered by a funnel for 24 hrs for the solvent to evaporate. Total 6 formulations were prepared and stored for evaluation.

**Table 1: Formulation composition of Amitriptyline HCl transdermal films**

| Formulation Code | Ingredient(mg) | | | |
|---|---|---|---|---|
| | Amitriptyline HCl | Eudragit E100 | HPC | PVP |
| $F_1$ | 10 | 210 | 45 | 45 |
| $F_2$ | 10 | 210 | 60 | 30 |
| $F_3$ | 10 | 210 | 30 | 60 |
| $F_4$ | 10 | 210 | 90 | …. . . |
| $F_5$ | 10 | 210 | …… | 90 |
| $F_6$ | 10 | 300 | …… | …… |

### Physicochemical evaluation[14-16]

The films were evaluated for the following physiochemical properties.

### Thickness

The thickness of transdermal film was determined at different points in the film using screw gauge (Syracuse, Newyork).

### Uniformity of weight

10 randomly selected films were weighed individually and their average weight was calculated. The standard deviation for each film weight was noted to check the uniformity of weight.

**Drug content determination**

Entire film was cut into pieces and dissolved in 100ml of water. The solution was stirred continuously for 24 hrs in a shaker incubator (Daihan Labtech Pvt Ltd, India) maintained at room temperature. The solutions were filtered and absorbance was measured at 239nm in a UV-Visible spectrophotometer (Shimadzu, Japan).

**Percentage moisture absorption**

The films were weighed accurately and placed in the desiccator containing 100ml of saturated solution of aluminum chloride, which maintains 79. 50% relative humidity (RH). After three days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula.

$$\text{Percentage moisture absorption} = \frac{\text{Final weight- Initial weight}}{\text{Initial Weight}} \text{ ---(1)}$$

**Percentage moisture loss**

The films were weighed accurately and kept in the desiccator containing one gram of anhydrous calcium chloride. After three days, the films were taken out and weighed. The moisture loss was calculated using the formula

$$\text{Percentage moisture loss} = \frac{\text{Initial weight-Final weight}}{\text{Initial Weight}} \text{ ---------- (2)}$$

**Water vapor transmission rate (WVTR)** [17]

For the determination of WVTR, one gram of calcium chloride was weighed and placed in dried empty vials. The polymer films were pasted over the brim with the help of silicon adhesive grease and allowed to set for 5 minutes. The vials were weighed and placed in a humidity chamber (Bionik Innovation, India) maintained at 68 % RH. The vials were taken out and weighed at the end of every 1st day, 2nd day, 3rd day and up to 7 consecutive days. The increase in vial weight was considered as a quantitative measure of moisture transmitted through the film.

WVTR = W/ ST --------------(3)

W is the increase in weight in 24 hrs; S is the area of the film exposed ($cm^2$); T is the exposure time (hrs)

***In Vitro* drug release** [9, 18]

The release study was conducted using the USP dissolution apparatus V (Veego Instruments Pvt. Ltd, India). The film was supported with circular glass plate and covered by a mesh. This was then placed inside the dissolution basket that contains 500 ml of phosphate buffer (PH 7. 4). The paddle was rotated at 100 rpm and the temperature was maintained at 32 ± 50C. The samples were withdrawn at a regular time intervals and analysed using spectrophotometer at 239 nm. The dissolution medium was replaced with an equal volume of phosphate buffer at each sample withdrawl.

**Kinetics of drug release** [19]

The release kinetics of formulation (F5) was determined by fitting the drug release data with zero order kinetics (Eq. 1), first order kinetics (Eq. 2), higuchi equation (Eq. 3), korsmeyer-peppas equation (Eq. 4) and hixson crowell equation.

Qt = K0t ----------------------- 1

ln Qt = ln Q0 – K1t ---------------------- 2

$Qt = K\eta t^{1/2}$ ----------------------- 3

The following plots were made: Qt vs. t (zero order kinetic ), ln(Q0 –Qt) vs. t (first order kinetic model) and Qt vs. $t_{1/2}$ (higuchi model), where Qt is the percentage of drug released at time t, Q0 is the initial amount of drug present in the formulation and K0, K1 and Kη are equation constants. . The mechanism of drug release was found using the korsmeyer–peppas model.

$M_t/M_\infty = K_p t^n$ ------------------------- 4

Where $M_t/M_\infty$ is the fraction of released drug at time t, Kp is the rate constant and "n" is the release exponent.

The value of "n" was calculated from the slope of the curve plotted between log fraction of drug released ($M_t/M_\infty$) vs. log time.

***In-vitro* skin permeation study** [20, 21]

The permeation study was carried out for the film F5 using a locally fabricated keshary-chien type diffusion cell. Full thickness skin of rat was used for the study. Hair on the abdominal skin was removed and animal was sacrificed by cervical dislocation. The skin was cut by surgical procedure using scalpel and the subcutaneous tissue attached to the skin was peeled off. The film was applied to the stratum corneum side of the skin and then mounted in the diffusion cell with the dermal side in contact with the receptor fluid. The receptor compartment was maintained at a temperature of 32 ± 5°C and was continuously stirred at a constant rate of 500rpm using a small magnetic bead rotated with the help of a magnetic stirrer (Remi, India). The receptor compartment was filled with 50ml of saline phosphate buffer (pH 7. 4). The samples were withdrawn from the receptor compartment at different time intervals and equal volume of receptor fluid was replaced at each sample withdrawal. The samples were diluted and then analyzed by UV – visible spectrophotometer at 239nm.

**Skin irritation study** [22]

Skin irritation test was performed for the film F5 to identify the presence of skin reactions erythema and edema. The test was carried out on healthy male rabbits. The dorsal surface of the skin was cleaned well and the hair was removed by shaving. The shaved skin was washed with rectified spirit and left overnight for any untoward reactions of shaving. The film F5 was fixed over the shaved area and observed for skin reactions after 1hr, 24 hrs, 48 hrs and 72 hrs. Scores were assigned according to the Draize scoring method. The scores assigned to the skin reactions and Primary Dermal Irritation Index (PDI) values are tabulated in Table 2 and Table 3.

**Table 2: Dermal reactions and the score assigned in accordance with the Draize scoring criteria**

| S. No | Erythema | Edema | Score |
|---|---|---|---|
| 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 to slight Eschar formation | Severe Edema | 4 |

**Table 3: Classification of irritation according to Primary dermal irritation index**

| PDI | Classification |
|---|---|
| Less than 0. 5 | No irritation |
| 0. 5-2. 0 | Irritation barely perceptible |
| 2. 0-5. 0 | Moderate Irritation |
| More than 0. 5 | Severe Irritation |

**Statistical analysis of data**

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.

**RESULTS**

The physicochemical properties of the matrix type transdermal films of Amitriptyline HCl are recorded in Table 4.

**Drug content determination**

The drug content of 2 $cm^2$ film of F1 to F6 was found to be in the range of 9. 87±0. 31mg to 9. 95±0. 24mg. The results of the drug content analysis are given in Table 5.

**Table 4: Physicochemical properties of amitriptyline HCl transdermal films**

| Formulation Code | [1]WVTR | [2]% MA | [3]% ML | Thickness | Weight (mg) |
|---|---|---|---|---|---|
| F1 | 0.049±0.002 | 4.58±1.12 | 1.68±0.51 | 0.57±0.042 | 356±1.56 |
| F2 | 0.012±0.003 | 3.21±1.59 | 0.96±0.41 | 0.51±0.06 | 342±1.65 |
| F3 | 0.01±0.002 | 4.15±0.98 | 0.86±0.42 | 0.55±0.047 | 345±2.58 |
| F4 | 0.014±0.004 | 3.56±1.42 | 1.21±0.52 | 0.51±0.008 | 341±1.11 |
| F5 | 0.017±0.002 | 6.41±1.98 | 2.31±0.61 | 0.54±0.008 | 351±1.62 |
| F6 | 0.011±0.003 | 3.06±1.32 | 0.75±0.43 | 0.52±0.007 | 358±2.31 |

Values are expressed as mean ± SD (n=6); [1] Water vapour transmission rate;

[2] Percentage moisture absorption; [3] Percentage moisture loss.

**Table 5: Drug content of amitriptyline HCl films**

| S. No | Formulation code | Drug content (mg) |
|---|---|---|
| 1 | F1 | 9. 95±0. 24 |
| 2 | F2 | 9. 87±0. 31 |
| 3 | F3 | 9. 86±0. 48 |
| 4 | F4 | 9. 91±0. 26 |
| 5 | F5 | 9. 94±0. 31 |
| 6 | F6 | 9. 88±0. 26 |

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

### *In- vitro* drug release study

The results obtained from the *In vitro* release study are shown in the Figure 1. The cumulative percent of drug released from F5 (96. 45%±1. 24% in 24 hrs) was significantly (p<0. 05) high when compared with other formulations. Film F6 exhibited a lowest drug release of 78. 87% ±1. 32%. All the formulations showed an initial burst release followed by controlled release that has been extended for 24 hours. Highest Regression-coefficient ($R^2$) value was obtained for the zero order kinetic model for F5 and the results are given in table 6. The calculated “n” value from the slope of korsmeyer-peppas equation was 0. 441(i. e. n<0. 5).

### *In vitro* skin permeation study

The formulation F5 was selected for the permeation study based on its highest *in vitro* release. The drug permeation from the film F5 and aqueous solution of the drug were 49. 27%±0. 62% in 24hrs and 94. 30%±0. 58% in 14 hrs respectively. *In vitro* skin permeation of film F5 is shown in Figure 2.

### Skin irritation study

The results of the skin irritation studies are shown in Table 7. The calculated primary dermal irritation index value was zero.

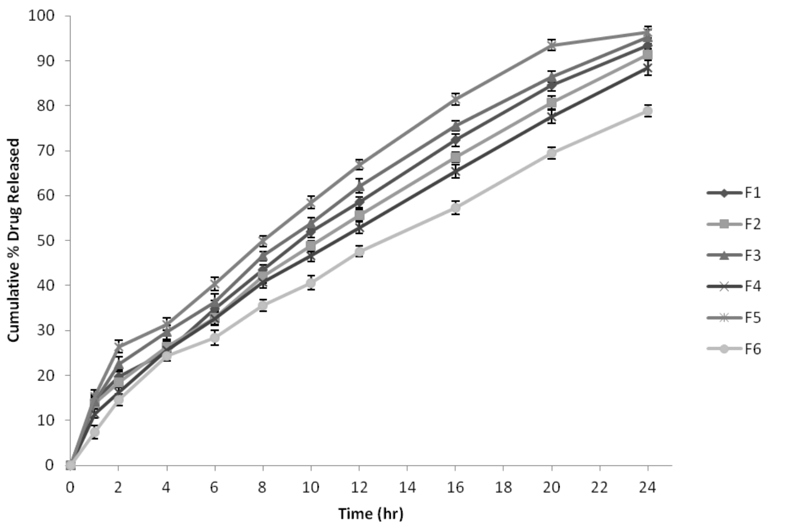


**Fig. 1: *In vitro* release of Amitriptyline HCl transdermal films**

**Table 6: Regression-coefficient ($R^2$) values of release kinetic models for F5**

| S. No | Kinetics model | Regression-Coefficient ($R^2$) |
|---|---|---|
| 1 | Zero order kinetics | 0. 982 |
| 2 | First order kinetics | 0. 912 |
| 3 | Higuchi model | 0. 955 |
| 4 | Korsmeyer - peppa’s model | 0. 972 |
| 5 | Hixson crowell | 0. 942 |

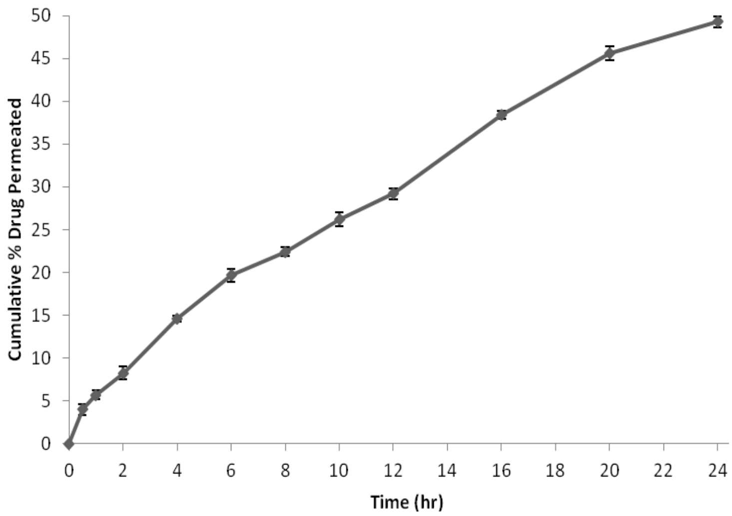


**Fig. 2: *In vitro* skin permeation of Amitriptyline HCl film (F5)**

**Table 7: Score obtained in the skin irritation test for F5**

| Animal no | Sex | Score obtained after a period of | | | |
|---|---|---|---|---|---|
| | | 1hr | 24hrs | 48hrs | 72hrs |
| 1 | Male | 0/0 | 0/0 | 0/0 | 0/0 |
| 2 | Male | 0/0 | 0/0 | 0/0 | 0/0 |
| 3 | Male | 0/0 | 0/0 | 0/0 | 0/0 |

## DISCUSSION

Amitriptyline HCl transdermal film was designed using an adhesive polymer matrix of eudragit E 100 for the controlled release of the drug. Total of six different formulations were prepared by varying the composition of HPC and PVP. The eudragit E 100 (hydrophobic polymer) composition was fixed so as to study the effect of HPC and PVP (hydrophilic polymers) on drug release. The added plasticizer dibutyl phthalate at a concentration of 30% w/w of polymer, helps increasing the drug release in addition to impart flexibility to the film[8]. Plasticizer act by binding with the polymer chains and thus may relax the polymeric network enabling rapid diffusion of the drug across the matrix[23]. The physicochemical properties of the films indicated that there is no much difference among the formulations F1 to F6. Thickness, weight of all the formulations remained uniform and the WVTR, % MA, % ML were found to increase with increase in PVP concentration. This might be due to the more hydrophilic property of PVP when compared to HPC. PVP allows water to diffuse more easily into the film which results in higher uptake of water[24]. The film F6 showed least WVTR, % MA, % ML which might be attributed to the hydrophobic nature of eudragit E 100. However, a small percentage of moisture present in the film may help in retaining the flexibility, reduces brittleness during their storage. The uniform drug content in all the formulations showed the homogenous dispersion of the drug in the entire film. The miscibility of drug, polymer and the plasticizer in the chosen solvents may be responsible for the homogenous dispersion of the drug in the film.

The results of the *in vitro* drug release clearly indicates that the hydrophobic polymer Eudragit E100 resists the release of hydrophilic drug Amitriptyline HCl ,whereas the addition of hydrophilic polymers PVP and HPC increases the release of Amitriptyline HCl from the transdermal films. Initial burst release followed by slow release was observed for the formulations containing PVP and HPC. The burst release might be due to the hydrophilic polymers which do not have interaction with the hydrophilic drug. Whereas the eudragit E 100 that account for the slow release of drug may be due to the interaction of this hydrophobic polymer with the hydrophilic drug [10]. The burst release obtained may also be due to the migration of the drug towards the surface of the polymeric matrix when it comes into contact with the dissolution medium. Here the crystallization of drug substances might have been prevented by the addition of PVP [11]. The initial burst release helps to achieve minimum effective plasma drug concentration quickly. However PVP shows higher drug release effect than HPC. This may be due to the raise in solubility of the drug by PVP. It has been suggested that the PVP might have interrupted with the polymer chain continuity greatly than the HPC, hence offering much matrix relaxation for rapid diffusion of the drug. It has been observed that the increase in concentration of PVP increases the drug release rate [25]. The drug release from the transdermal film F5 followed the fickian (diffusion controlled) transport mechanism. There was no lag time observed with respect to skin permeation of the drug. The steady state flux was attained after a time period of three hrs and was maintained up to 24hrs. The drastic decrease in skin permeation of film F5 was due to the presence of stratum corneum of skin that acts as a barrier for the transport of drug across the skin. The drug permeation from the film was slower than its permeation from solution. It could be said that the process of drug release was controlled by the polymeric film. Further the combination of eudragit E 100/PVP has been reported as the best for diclofenac diethanolamine and chlopheniramine transdermal patch with respect to drug release and skin permeation[9, 8]. The PDI of skin irritation study was found to be zero and hence the prepared film does not produce skin irritation which is suitable for transdermal drug delivery.

## CONCLUSION

Amitriptyline HCl adhesive matrix type transdermal film prepared from blends of HPC, PVP and Eudragit E 100 using dibutyl phthalate as plasticizer was thin, flexible and smooth. The film showed satisfactory physicochemical performance. From the *in vitro* release and skin permeation results it can be concluded that the controlled release of Amitriptyline HCl across the skin could be achieved for prolonged period. No skin irritation was observed for the optimized formulation F5. The result clearly indicates that hydrophobic eudragit E100 resists the release of hydrophilic drug Amitriptyline HCl whereas the addition of hydrophilic polymers PVP and HPC increases the drug release. Amitriptyline HCl transdermal matrix film containing eudragit E100 and PVP has shown promising results than the film containing eudragit E 100 and HPC.

## REFERENCES

1. Kanikkannam N, Andega S, Burton S, Babu RJ, Mandip Singh. Formulation and In Vitro Evaluation of Transdermal Patches of Melatonin. Drug Dev Ind Pharm 2004; 30: 205–212.
2. Divyesh patel, Nirav patel, Meghal patel, Navpreet kaur. Transdermal Drug Delivery System:Review. Int J Biopharm Toxicol Res 2011; 1: 61-64.
3. Anil L Shinde, Kelvin C Garala, Harinath N More. Development and characterization of transdermal therapeutics system of tramadol hydrochloride. Asian. J. Pharm 2008; 4: 265-269.
4. Martindale, The complete Drug Reference. 36th edition, Edited by Sean C Sweetmen, published by the Pharmaceutical Press 2009; 1: 376-377.
5. Ritschel WA, Udeshi. Drug release mechanisms from matrix and barrier coated tablets prepared with acrylic resin, with and without addition of chanelling agent. Pharm Ind 1987; 49:734-739.
6. Rohm Gmbh co-KG, Darmstadt, Germany, Evonik, 2004-2009.
7. Fischer Frank, Bauer Stephan. Polyvinylpyrrolidone. Ein Tausendsassa in der Chemie. Chemie in unserer Zeit 2009*;* 43: 376–383.
8. Rajan rajabalaya, sheba rani nakka david, jasmine khanam, arunabha nanda. Studies on the effect of plasticizer on in vitro release and ex vivo permeation from eudragit e 100 based chlorpheniramine maleate matrix type transdermal delivery system. J excipients and food chem. 2010; 1: 3-12.
9. Yu Liu, Liang Fang, Haifa Zheng, Ligang Zhao, Xinyu Ge, Zhonggui He. Development and in vitro evaluation of a topical use patch containing diclofenac diethanolamine salt. Asian. J. Pharm. Sci 2007; 2: 106-113.
10. Prashant D Sawant, Dewilt Luu, Rose Ye, Richard Buchta. Drug release from hydroethanolic gels. effect of drug's lipophilicity (logP), polymer-drug interactions and solvent lipophilicity. Int. J. Pharm 2010; 396: 45-52.
11. Gao Yanli, Jinying Liang, Jianping Liu, Yan Xiao. Double-layer weekly sustained release transdermal patch containing gestodene and ethinylestradiol. Int. J. Pharm 2009; 377: 128-134.
12. Aqil M, Bhavna Choudhary I, Sultana Talegaonkar S, Ahmad F. J, Ali M. M. Transdermal therapeutic system of enalapril maleate using piperidine as penetration enhancer. Current Drug Delivery 2008; 5: 148-152.
13. Biswajit Mukherjee, Sushmita Mahapatra, Ritu Gupta, Balaram Patra, Amit Tiwari, Priyanka Arora. A comparison between povidone-ethyl cellulose and povidone-eudragit transdermal dexamethasone matrix patches based on in vitro skin permeation. Eur J pharm. Biopharm 2005; 59: 475-483.
14. Ubaidulla U, Reddy VS, Ruckmani S. Transdermal therapeutic system of carvedilol: effect of hydrophilic and hydrophobic matrix on in vitro and in vivo characteristics. AAPS PharmSciTech 2007; 8: E1-E8.
15. Kevin C Garala, Anil J Shinde, Pratik H Shah. Formulation and in vitro characterization of monolithic matrix transdermal systems using HPMC/Eudragit S 100 polymer blends. Int J Pharm Pharm Sci 2009; 1: 108 – 120.
16. Sanjay Dey, Ananya Malgope. Preparation of carvedilol transdermal patch and the effect of propylene glycol on permeation. Int J Pharm Pharm Sci 2010; 2: 137 – 143.
17. Tipre DN, Vavia PR. Acrylate-based transdermal therapeutic system of nitrendipine. Drug Dev Ind Pharm 2003; 29:71-78.
18. Yanli Gao, Jinying Liang, Jianping Liu, Yan Xiao. Double layer weekly sustained release trandermal patch containing gestodene and ethinyl estradiol. Int. J. Pharm 2009; 377: 128-134.
19. Yuveraj Singh. Tanwar, Chetan Singh Chauhan, Anshu Sharma. Development and evaluation of carvedilol transdermal patches. Acta. Pharm 2007; 57: 151–159.
20. Nicoli S, Penna E, Padula C, Colombo P, santi P. New transdermal bioadhesive film containing oxybutynin : In vitro permeation across rabbit ear skin. Int. J. Pharm 2006; 325: 2-7.
21. Babu R. J, Pandit J. K. Effect of penetration enhancers on the release and skin permeation of bupranolol from reservoir type transdermal delivery system. Int. J. Pharm 2005; 288: 325-334.
22. Draize J. H, Woodard G, Calvery H. O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membrane. J. Pharm. Exp. Ther. 1944; 82: 377-390.
23. Manning SC, Moore RB. Reactive compatibilization of polypropylene and polyamide – 6, 6 with carboxylated and maleated polypropylene. Polymer engineering and science 1999; 39: 1921-1926.
24. Rajan rajabalaya, Jasmine khanam, Arunabha nanda. Design of a matrix patch formulation for long acting permeation of diclofenac potassium. A. J. Pharm Sci 2008; 3: 30-39.
25. Shankar M. S, Suresh V Kulkarni, Sandeep H. N, Ranjith Kumar P, Someshwara Rao B, Ashok Kumar P. Development and evaluation of aceclofenac transdermal patches using hydrophilic and hydrophobic polymers. J Global. Pharm. Technol 2010; 2:102-109.