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Studies on the Development of Transdermal Patches of Nebivolol hydrochloride: *In Vitro, Ex Vivo* and *In vivo* Evaluation

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ABSTRACT

An attempt was made to formulate and evaluate the Nebivolol HCl transdermal drug delivery system. The matrix type films were prepared by using solvent casting technique with polymers HPMC 15cps,PVP K-30,Eudragit RL100 and Methyl cellulose 15cps.Propylene glycol was used as plasticizer. The prepared films were evaluated for physicochemical characteristics such as thickness, weight variation, folding endurance, % moisture uptake and % moisture loss. The drug excipient compatibility was determined by Fourier Transform Infra red Spectroscopy. The results revealed that there were no interaction between drug and selected polymers. *In vitro* permeation studies were performed in Franz diffusion cell using commercial semi permeable membrane. *Ex vivo studies* were performed using skin of albino rats. Biological studies such as skin irritation test and % drug diffusion studies were carried out by using rabbits. Drug content varied from 73.72 ± 0.1to 95.4±0.15%. Moisture content and moisture uptake were increased for patches containing higher amount of HPMC due to its hydrophilic nature. The batch F4 (HPMC 1.5%, PVP 0.5%) had shown drug release for 24 h to the extent of 86.87% and drug release followed zero order release kinetics which was of non-Fickaian type of diffusion. The results of *ex vivo* and *in vivo* studies were well correlated with *in vitro* diffusion studies

Keywords: Nebivolol, HPMC, PVP, Eudragit RL100, MC, Transdermal matrix films

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INTRODUCTION

Tremendous efforts have been focused on the development of new drug delivery systems[1]. Transdermal drug delivery system was introduced to overcome difficulties faced by the oral drug delivery systems such as absorption variability, differential metabolism, drug degradation due to enzymes present in GIT and high hepatic first pass effect of the drugs. Transdermal patches are mainly used to deliver a specific dose of drug through the skin in to blood stream and this was first approved by FDA in 1981.Transdermal drug delivery (TDD) provides controlled release of drug, avoids first pass metabolism and useful for drugs with short half life[2]. TDD also improves patient compliance and interruption or termination of treatment when necessary [3]. Nebivolol is a selective β1-receptor antagonist and approved for the management of hypertension and has a protective effect on left ventricular function, reduces pre load, after load and increases stroke volume. Nebivolol undergoes extensive first pass metabolism through the enzyme cytochrome P450 leading to poor bioavailability. Nebivolol possesses ideal characteristics to suit TDD such as low Molecular weight (441.90 g/mol), partition coefficient of (Log *P* (octanol/water): 3.23; 4.03 (pH 11.8, 23°C). and negligible skin degradation. The plasma half life is about 8-10 h which make frequent dosing necessary to maintain the therapeutic blood levels of drug for a long term treatment. Thus the objective of present study is an attempt to develop Nebivolol transdermal system with optimum physico-chemical characteristics and good stability [4,5,6,14]

MATERIALS AND METHODS

Nebivolol was obtained as generous gift from MSN Labs Pvt. Ltd. (Hyderabad, India).Eudragit RL100, Hydroxy propyl Methyl cellulose (HPMC) 15cps, Poly vinyl Pyrrolidone (PVP)K-30 and Methyl cellulose (MC)15cps were purchased from S.D Fine Chemical company, Hyderabad India.All other chemicals and reagents were of analytical grade. Double distilled water was used throughout the study

Experimental Methods

Preparation of the Transdermal films

Nebivolol loaded matrix type patches were prepared by solvent casting method. The transdermal films containing Eudragit RL100, HPMC, PVP and Methyl cellulose with 10mg of drug per patch size of 5 cm^2 of Nebivolol w/w with plasticizer (Propylene glycol) were prepared. HPMC was dissolved in 50:50 mixtures of chloroform and methanol and similarly MC, Eudragit and PVP were dissolved. Drug was added to the polymer solution and dissolved completely and were then mixed and stirred on a cyclomixer to get homogenous mixture. To this plasticizer propylene

glycol30%w/w was added and mixed thoroughly. The resulting solution was poured over petriplate and to prevent non uniform evaporation of solvent an inverted funnel was placed over it. After the period of 24h the dried medicated transdermal films were placed in an aluminum foil and stored in dessicator[13,15,17]

Table1: Composition of Transdermal Patch

Formulation Code	Nebivolol (mg)	HPMC	PVP	Eudragit RL100	MC
F1	10	2%	-	-	-
F2	10	-	2%	-	-
F3	10	1%	1%	-	-
F4	10	1.5%	0.5%	-	-
F5	10	0.5%	1.5%	-	-
F6	10	-	-	2%	-
F7	10	1%	-	1%	-
F8	10	1.5%	-	0.5%	-
F9	10	0.5%	-	1.5%	-
F10	10	-	-	-	2%
F11	10	-	1%	-	1%
F12	10	-	1.5%	-	0.5%
F13	10	-	0.5%	-	1.5%

RESULTS AND DISCUSSION

Evaluation of physicochemical properties

Thickness and Weight variation Thickness of the patches was checked at 6 different points of the film using a micrometer. For weight variation 3 films of each of 1 cm^2 from each batch were weighed individually and the average weight was calculated.

Folding Endurance

A strip of the film was cut and repeatedly folded at the same place until the film breaks. The more thin the film the more will be the flexibility of the film.

Percentage of Moisture Loss [25]

The dried films were weighed individually and kept in a desiccator containing activated silica. The films were weighed regularly until a constant weight was obtained. The percentage of moisture loss was calculated as a difference between initial and final weight with respect to the final weight.

Percentage Moisture Uptake[25]

The dried films were weighed and placed in a desiccator containing 200ml of saturated solution of potassium chloride (84% relative humidity) at room temperature. The percentage moisture uptake of the films were calculated as the difference between final and initial weight with respect

to the initial weight.

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

Drug Content[13]

The drug content was determined by taking a patch ($5cm^2$) cut it into small pieces and added to a beaker containing 100ml of PBS (pH 7.4). The film was completely dissolved by cyclomixer. The contents were filtered using Whattman filter paper and the drug content in the filtrate was estimated by using UV spectrophotometer at 281nm using the reference solution consisting of placebo film.

Drug Polymer Compatibility Studies

The Fourier Transforms Infrared (FTIR) Spectroscopy was used to know the compatibility between the drug and the polymers. All the excipients and pure drug individually and physical mixture of drug and excipients, were mixed with IR grade KBr in the ratio of 1:100 and pellets were prepared by applying 15000 lb of pressure. The pellets were scanned in an inert atmosphere over a wave number range of 4000-400 cm^{-1} in Magna IR 750 series II (Nicolet, USA) FTIR instrument. The spectrums obtained were studied for compatibility.

Scanning Electron Microscopy (SEM)determination

SEM photographs were taken to determine surface morphology of placebo patch and nebivolol matrix. This study was carried out to understand how the morphology of patch changes after the drug is completely released from the matrix.

***In-vitro* drug release studies**[22]

In-vitro drug release studies were performed by using a Franz diffusion apparatus. The dialysis membrane was mounted between the donor and receptor compartment of the diffusion cell. The prepared transdermal film of $5cm^2$ was placed on the membrane which was previously soaked in phosphate buffer. The receptor compartment was filled with PBS pH 7.4. The total assembly was on a hot plate magnetic stirrer and stirred by maintaining the temperature at 32 ± 0.5 ^oC. 5 ml of the sample was withdrawn at regular interval and replaced with an equal volume of PBS. The drug content was estimated spectrophotometrically at 281nm.

***Ex-vivo* Skin permeation studies**

The studies were performed by taking the rat skin as the membrane. Young albino rats weighing between 200 to 250g were taken and sacrificed by CO_2inhalation. The abdominal hair was removed and the abdominal skin was carefully separated from the body with the dermis part remaining intact. Subcutaneous tissue was surgically removed. The inner part of the skin was washed with distilled water thoroughly to separate the adhering fat. The full thickness of the skin

obtained was placed in normal saline solution and stored at 4 ± 1^{o}C until used for the experiment. The drug permeation from the transdermal patch through the skin was determined using Franz diffusion cell where the contents of donor and receptor compartment was separated by placing the excised skin and the transdermal patch of 5 cm^2 containing the stratum corneum side of the skin. The Franz diffusion cell was placed on a magnetic stirrer and stirred by placing Teflon coated bead at 500 rpm. Samples were withdrawn periodically and replaced with PBS to maintain sink condition. The drug content was estimated spectrophotometrically at a wavelength of 281nm.

***In vivo* studies on Rabbits**

Skin irritation test using rabbits:

Albino Rabbits of sexes, each weighing 1.5-2 kg and 24 months of age were used in this study (n=5 in each group). The dorsal surface of rabbits was cleared and the hair was removed by shaving. The skin was cleared with rectified spirit. The patches were applied to the shaved skin of rabbits and secured using adhesive tape USP (Leucoplast TM). On one side, a control patch (without any drug, group I) and on the other side an experimental patch (group II)were secured. The animals were observed for any sign of erythema or edema for period of 7days.

Biological studies

Selection of animals

Rabbits (Crytoagus cuniculus) of male sex 10-12 weeks old weighing 1.5kg were selected from animal housing facility, Department of Pharmacology, Malla Reddy College of Pharmacy, Secunderabad (An approved and registered facility under CPCSEA 2010).

The experimental protocol for the all biological studies was approved by Institutional Animal Ethical Committee, which is an approved body under CPCSEA 2010 (Registration number CPCSEA/MRCP/1207).They were kept with husk bedding and fed with standard rodent pellet diet and water. Light and dark cycles with 12 h light and 12 h dark were maintained. The temperature and relative humidity were 28°C±2% and 60±15% respectively.

Method

A set of healthy rabbits were selected. They were checked to ensure, they were free from disease. The dorsal surface of the rabbits was cleaned and hair was removed. The dose of nebivolol was calculated according to the body weight. The patch F4(HPMC 0.75%,PVP0.25%) was placed on the dorsal surface. At specific intervals of time, the films were removed carefully and analyzed for the remaining drug content.

Drug release at any time interval = Initial drug content(Before placing the film)- drug content (After removal of the film)

The drug content was subtracted from the initial content in the film. The value obtained denotes the amount of drug release in the rabbits.

***In vitro in vivo* correlation**

In-vitro and *in-vivo* correlation was carried out for the therapeutic efficacy of a pharmaceutical formulation. It is governed by the factors related to both *in-vitro* and *in-vivo* characteristics of the drug. Percent *in vitro* release on x-axis was plotted against *in-vivo* drug release on y-axis for the same period of time.

RESULTS AND DISCUSSION

In the present study, efforts have been made to prepare Nebivolol transdermal patches using solvent evaporation technique. The films were prepared by using different hydrophilic polymers such as HPMC, PVP, Eudragit RL 100 and MC (Table 1). In this study the polymers used were kept at concentration of 2% and for combination of polymers, the total amount of polymers in formulation was2% but the proportions of different polymers were varied, in order to achieve zero order release pattern.

Drug excipient compatibility studies by FTIR revealed that the drug and excipients are compatible Figure 3 to 6. Physico-chemical evaluation data of table 2 indicates that the formulation F11 (MC 1%, PVP 0.25%) had shown highest maximum absorption than the other formulation. This might be due to the presence of high hydrophilicity of MC and PVP. The Batch F3 (HPMC 1.5%, PVP 0.5%) has shown the least percent moisture absorption. The batch F3 (HPMC 1%,PVP 1%) had shown high percent moisture loss when compared to other formulations. This could be attributed to the low moisture retaining capacity of the HPMC and PVP. The thickness of the films varied from 0.21 to 0.30 mm. The minimum standard deviation values assured that the process used for preparing the delivery system is capable of giving reproducible results. In order to evaluate the flexibility, the films were subjected to folding endurance studies. The various batches of films of were able to be intact till 71 to 89 n number of repeated folding. This revealed that the prepared films were having capability to withstand the mechanical pressure along with good flexibility (Table 2).Average weight was varied from 214 to 312mg.The drug content was found to vary from 7.37 to 9.54%.This might be either due to improper solubility of drug in polymer solution or uneven distribution of drug in transdermal patch. The formulation F4 showed maximum drug content 9.54% (Table 2).

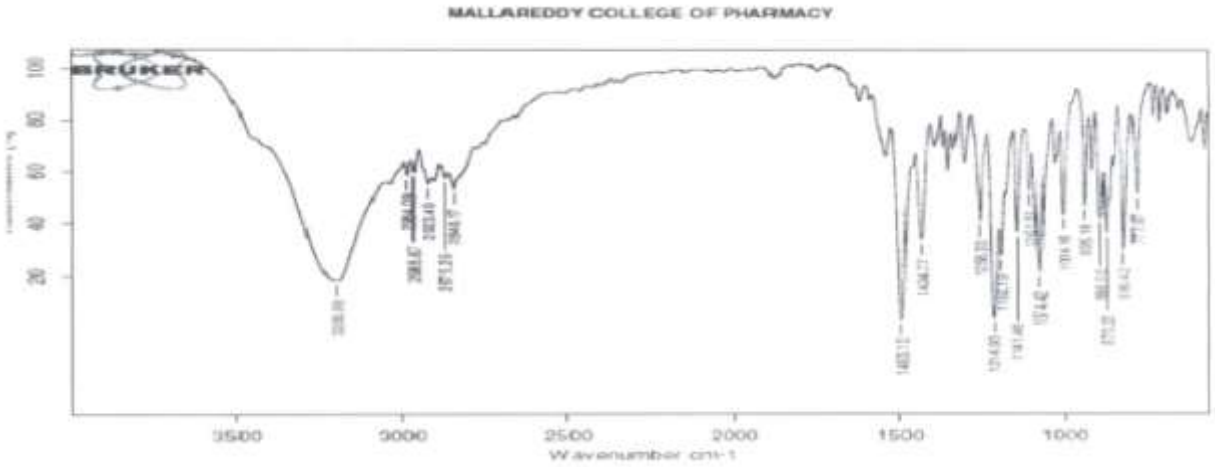


Figure 3: FTIR spectra of drug

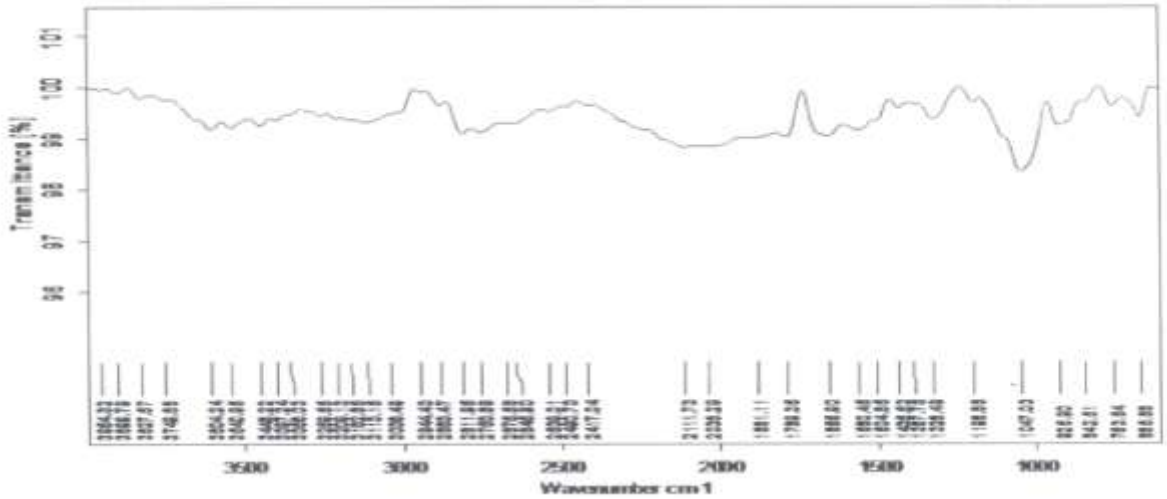


Figure 4:FTIR spectra of HPMC

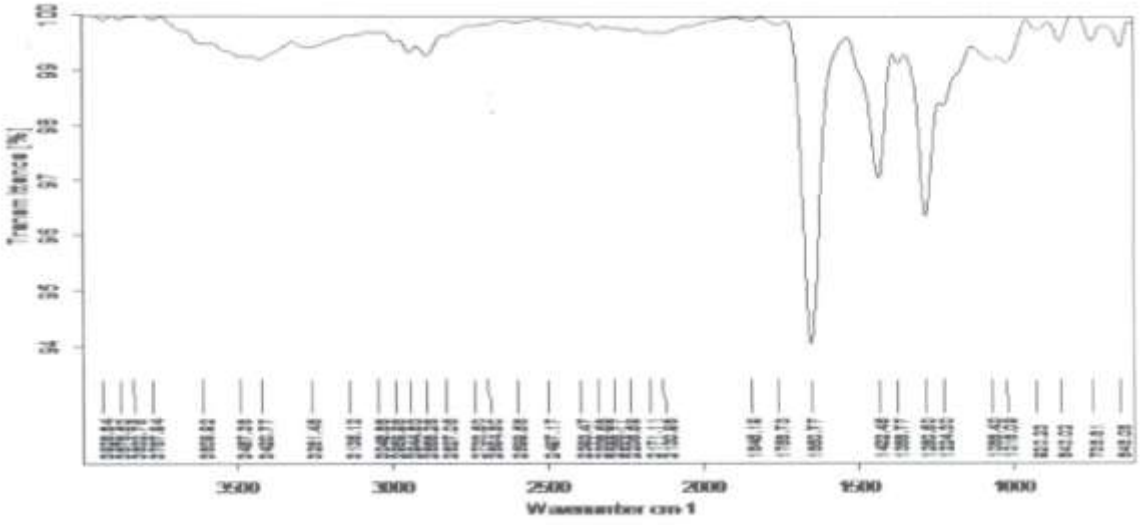


Figure 5:FTIR spectra of PVP

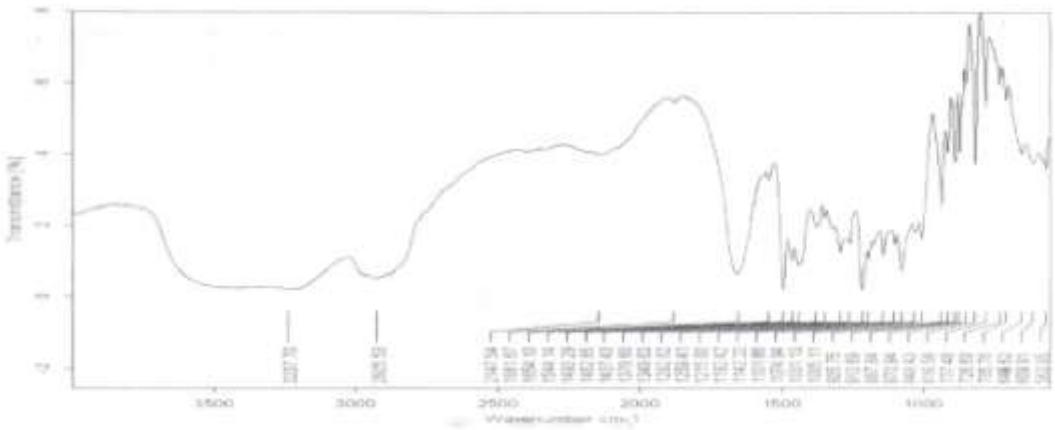


Figure 6: FTIR spectra of Drug and polymers mixture for the batch F4

Table 2: Physicochemical Evaluation of transdermal patches

Formulation Code	Weight variation (mg)±SD	%Moisture absorption ±SD	%Moisture loss ±SD	Thickness (mm)±SD	Drug content (mg)±SD	Folding endurance ±SD
F1	312±2.6	9.23±0.12	6.73±0.02	0.22±0.008	8.62±0.02	79±4
F2	303±3.5	9.76±0.04	5.71±0.01	0.24±0.012	8.89±0.05	72±2.6
F3	248±4.6	11.33±0.02	9.34±0.01	0.3±0.012	8.86±0.1	85±5
F4	263±5.4	8.12±0.27	7.22±0.06	0.26±0.008	9.54±0.15	89±3.6
F5	235±2.7	12.65±0.07	5.91±0.06	0.25±0.004	8.88±0.15	77±2.3
F6	223±2.3	10.73±0.82	5.21±0.08	0.21±0.008	9.37±0.05	80±3.2
F7	268±3.2	8.33±0.03	5.17±0.01	0.24±0.016	8.51±0.1	82±3.5
F8	261±2.5	9.32±0.01	6.02±0.02	0.26±0.008	7.37±0.1	81±1.2
F9	240±3.3	11.25±0.06	4.82±0.01	0.21±0.008	8.87±0.02	71±2.3
F10	214±4.1	9.25±0.13	5.15±0.04	0.22±0.008	9.21±0.05	77±1.7
F11	288±4.4	16.78±0.02	8.82±0.08	0.26±0.012	9.32±0.1	76±2.7
F12	216±3.6	12.00±1.54	5.48±0.01	0.27±0.008	8.72±0.15	73±2.3
F13	249±2.2	13.22±0.22	4.77±0.02	0.29±0.004	9.41±0.1	79±3.2

SEM was done to study the surface morphology of the patches before and after the release of the drug from the patches. Figure 7 showed the homogenous distribution of drug clusters in the matrix, before applying on skin, figure 8 is the SEM micrograph of patch after 24h of drug permeation. It showed the number of holes present in the patch after the release of drug clusters.

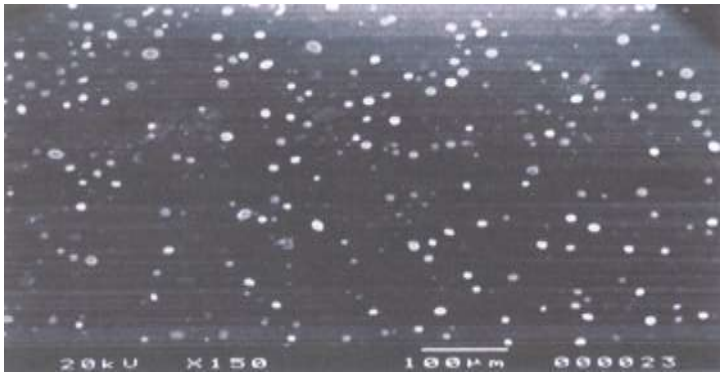


Figure 7: SEM image of the patch F4 before application

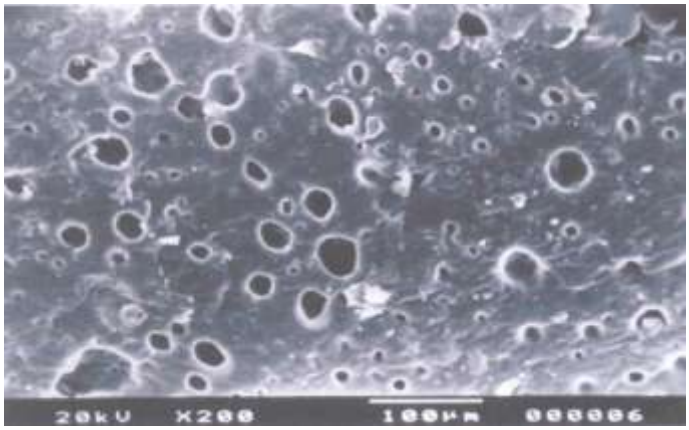


Figure 8: SEM image of the patch F4 after 24h of application

The batch having HPMC 2% alone (F1) had shown the release of loaded drug for 24h to the extent of 76.25%. The *in vitro* drug release plot had shown that the drug release followed zero order kinetics which was evidenced from the regression value of the above mentioned plot. The Higuch's plot had shown the regression value of 0.944, which indicated that diffusion, might be one of the prominent mechanisms influencing the drug release, in order to confirm this fact Peppa's plot was drawn, which had shown the slope value of 1.024, which confirmed that the diffusion mechanism involved in the drug release was of non-Fickian diffusion type(Table 3). The batch F2 (PVP 2%) and F3 (HPMC 1% , PVP1%) had shown the drug release of 61.87% and 80.15% respectively. In these formulations also it is proved that diffusion is one of the mechanisms and that is of non Fickian type. The batch F4 (HPMC 1.5%, PVP 0.5%) had shown drug release for 24 h to the extent of 86.87% following zero order release kinetics with the mechanism of non-Fickian type of diffusion(Table 3).The rate of release of the loaded drug from the batch F4 increased to the extent of 3.616 %/h when compared to the release rate of 2.56%/h in batch F2 formulated with PVP alone. This substantial difference may be attributed to the slow dissolving nature of HPMC. The formulations prepared using Eudragit alone and in combination with HPMC to the polymer content of 2% had shown the % release of 78.43, 67.96, 82.34, 74.53 (F6-F9). The formulations prepared using MC alone and in combination with PVP to the polymer content of 2% had shown the % release of 83.9, 71.56, 64.68, 77.64 (F10-F13). Almost all formulations followed zero order non fickanian release kinetics (Figure 1 and 2) (Table 3).

Table 3:Release kinetics of different formulation

Formulation Code	Zero Order (r^2)	First Order (r^2)	Higuchi (r^2)	Peppas (n)
F1	0.992	0.794	0.990	0.815
F2	0.981	0.893	0.949	0.806

F3	0.944	0.882	0.951	0.947
F4	0.996	0.882	0.951	0.809
F5	0.985	0.789	0.987	0.872
F6	0.983	0.715	0.986	0.979
F7	0.995	0.821	0.962	0.857
F8	0.989	0.782	0.988	0.846
F9	0.991	0.820	0.965	0.875
F10	0.993	0.884	0.961	0.878
F11	0.984	0.775	0.979	0.948
F12	0.995	0.852	0.964	0.856
F13	0.998	0.835	0.978	0.884

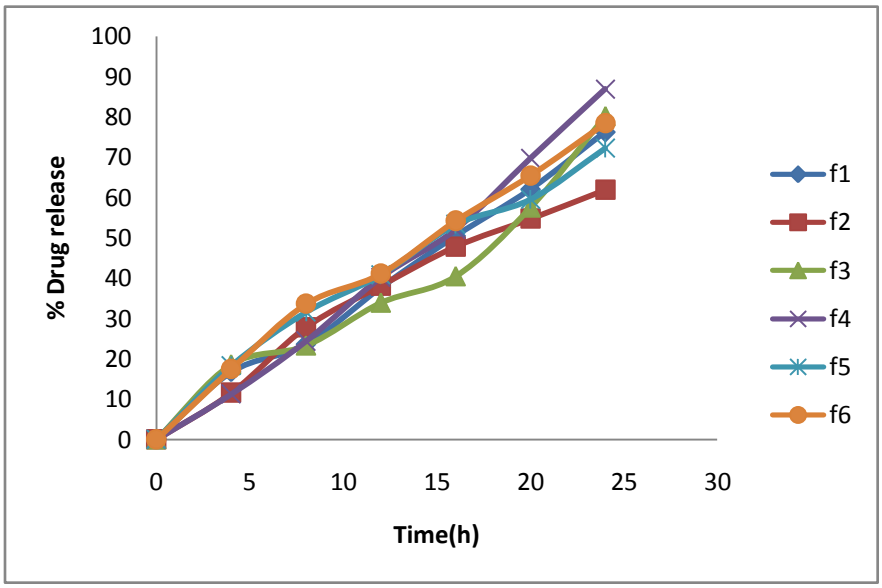


Figure 1: *In vitro* drug release profile of batches from F1-F6

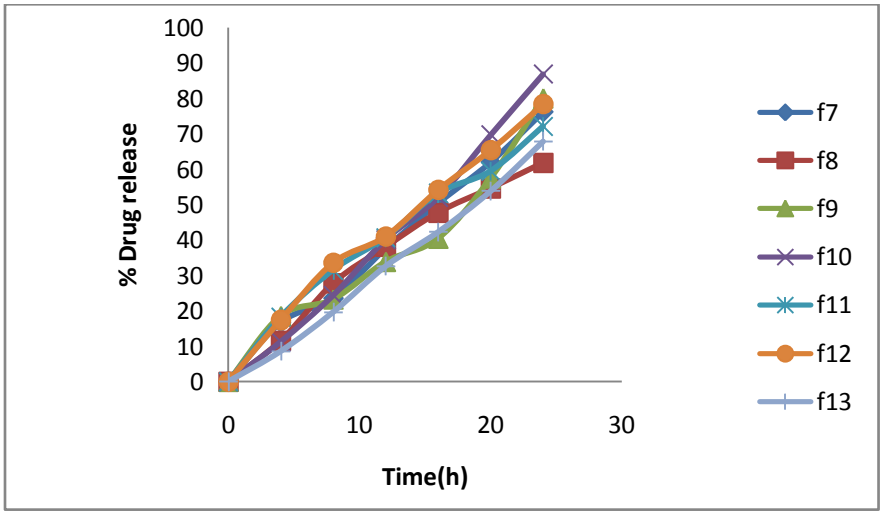


Figure 2: *In vitro* drug release profile of batches from F7-F13

After carrying out the *in vitro* diffusion studies for all the formulations the batch F4 was selected for *ex vivo* permeation studies using rat skin. (Figure 9 and figure10). Release was found to be 81% in 24h.To ensure the correlation between the *in vitro* and ex-*vivo*, release pattern analysis was carried out. They were well correlated and it was found that the release pattern followed the predicted zero order kinetics. The matrix F4 was reproducible even in biological environment. Due to the problems encountered in withdrawing samples each hour, sampling intervals and volumes were adjusted accordingly. The *in vivo* drug release showed 79.82% (Table 4). To ensure the correlation between *in vitro-in vivo* release patterns, regression analysis was carried out(Table 4); Figure 11 and figure 12. They were found to well correlate and confirmed that the release pattern had followed zero order kinetics. Skin irritation studies revealed that the batch F4 (HPMC1.5%, PVP 0.5%) had no erythema and edema (Figure 13).

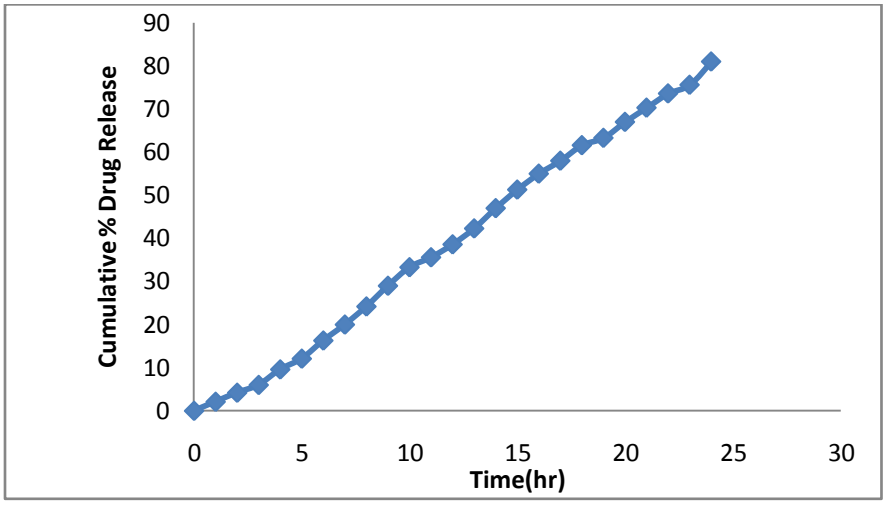


Figure 9: Ex-vivo permeation studies using rat skin for patch F4

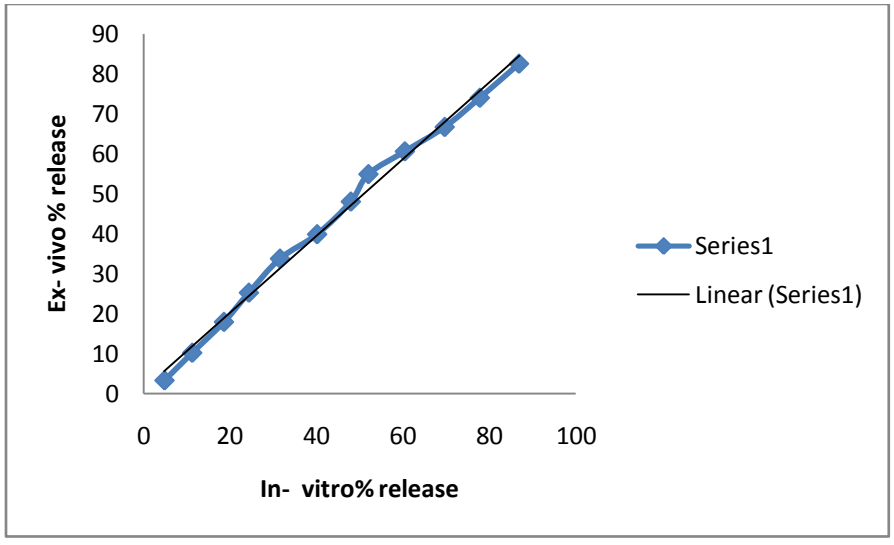


Figure 10*: In -vitro - Ex- vivo* correlation

Table 4: *In -vivo* drug release data for F4

Time in hours	Amount of drug remaining	Amount of drug released	%drug release
2	2.38	0.08	3.25
4	2.21	0.25	10.16
6	2.05	0.41	17.88
8	1.84	0.62	25.2
10	1.63	0.83	33.73
12	1,48	0.98	39.83
14	1.26	1.2	48
16	1.11	1.35	54.87
18	0.97	1.49	60.57
20	0.82	1.64	66.67
22	0.64	1.82	73.98
24	0.43	2.03	79.82

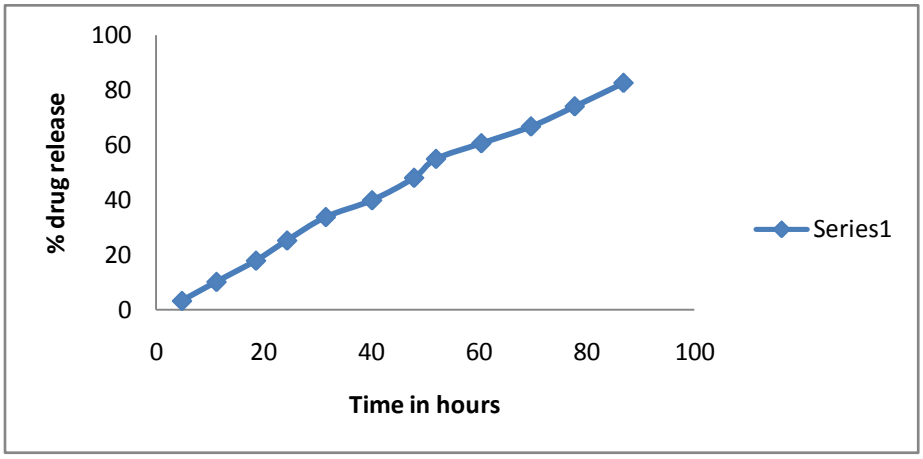


Figure 11: *In – vivo* drug release profile for F4

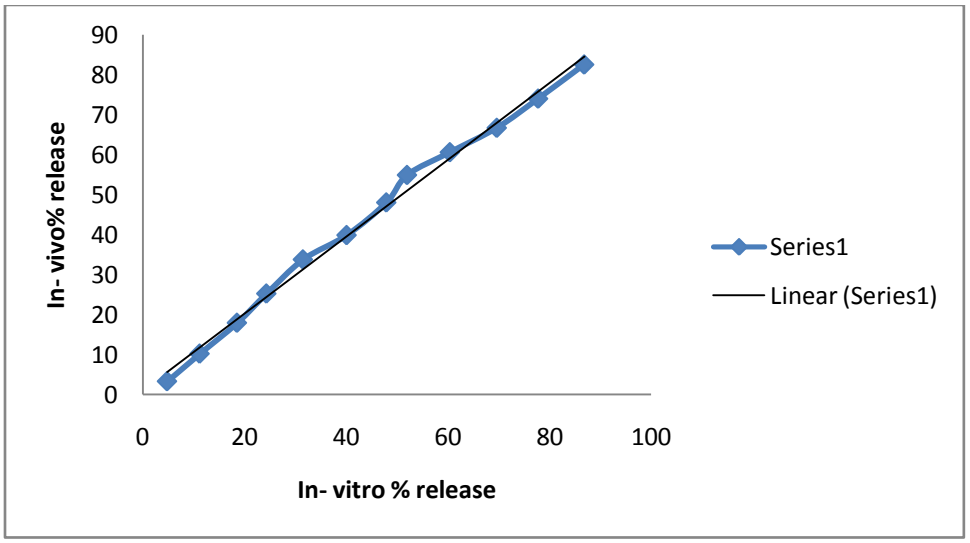
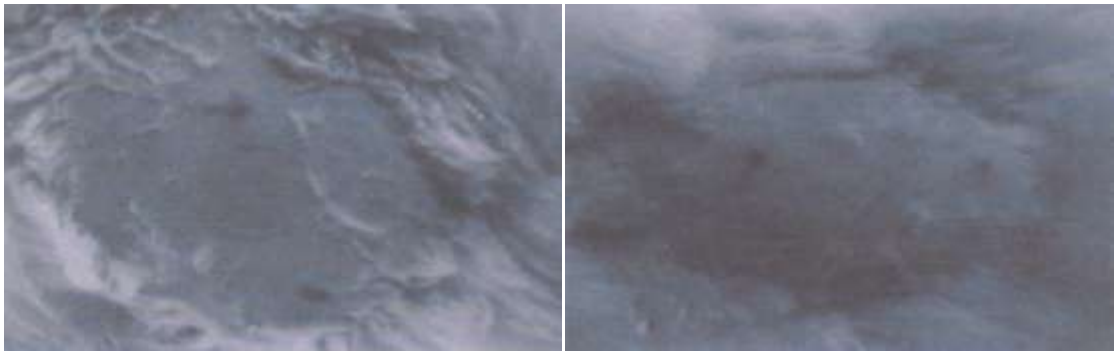


Figure 12: *In vitro – in vivo* correlation for F4



Application of patch as control Results showing no sign of erythema or edema

Photographs showing no signs of redness or edema after application of F4 formulation patch

Figure13:Skin irritation studies on rabbit

CONCLUSION

Formulation F4(HPMC 1.5%,PVP 0.5%) was found to be the best among all batches because of its consistent release rate for 24h and the release found to be well correlated *ex vivo* and *in vivo*. The selected formulation F4 could achieve the objectives of controlled release and hence reduced frequency of administration, avoids the first pass effect and thus improve patience compliance.

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