www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Preparation and evaluation of Transdermal patches of Papaverine hydrochloride

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ABSTRACT

Transdermal patches of Papaverine hydrochloride were prepared by solvent casting method using ethyl cellulose: PVP, PVA: PVP and eudragit RL-100: eudragit RS-100 in different ratios. The physicochemical parameters like flexibility, thickness, smoothness, weight variation, moisture content, hardness and tensile strength were evaluated and found to be flexible, uniform thickness and weight, smooth, good drug content (92 to 96%) and little moisture absorption. The *in-vitro* diffusion studies were carried out using modified Keshery-Chein cell with cellophane as diffusion membrane and the formulation followed Higuchi diffusion mechanism. The formulation containing PVA:PVP as polymers showed faster release rate (hydrophilic polymers) compared to eudragit RL-100:eudragit RS-100 (hydrophobic polymers) or combination of hydrophilic and hydrophobic polymers (Ethyl cellulose and PVP). The stability studies indicated that all the patches maintained good physicochemical properties and drug content after storing the patches in different storage conditions. Compatibility studies indicated that there was no interaction between the drug and polymers. *In vivo* studies showed that papaverine hydrochloride helps in decreasing the effect of isoproterenol induced myocardial necrosis.

Key words: Transdermal patch; Papaverine HCl; *in-vivo* study; ethyl cellulose; eudragit polymers.

INTRODUCTION

The goal of pharmaceutical research is to find drugs with desirable therapeutic and low risk of undesirable side effects. Recent research and development efforts have been channelized into the development of drug delivery systems for controlled drug administration through various routes (or parts) of administration, for example, the skin, to maximize the bioavailability, to optimize the therapeutic efficacy, and/or minimize the side effects of the drug. In this system (transdermal drug delivery), the drug reservoir is encapsulated in a compartment molded from a drug impermeable backing layer and a rate controlling polymeric membrane. In the drug reservoir compartment, the drug particles are either dispersed or suspended in the solid polymer matrix. It is anticipated that transdermal drug delivery system can be designed to input drugs at appropriate rates to maintain a suitable plasma-drug level for therapeutic efficacy, without the periodic sojourns into the plasma concentration that would accompany toxicity or lack of efficacy (Chein YW, 1987). Till date, various drugs have been successfully incorporated into transdermal drug delivery systems for clinical use (Scopolamine, Nitroglycerine, Clonidine, Estradiol, Nicotine, Isosorbide dinitrate, Norethristerone acetate, etc.), which established the dermal route for systemic drug delivery (Udupa N, Shaila lewis, Pandey S, 2006). Papaverine is an alkaloid present in opium. It belongs to the group of medicines called the vasodilator. It has direct relaxant action on smooth muscle, which is attributed in part to its ability to inhibit phosphodiesterases. It has been given in the management of cerebral, peripheral and coronary disorders. The biological half life of papaverine HCl given by oral route is reported to be between 1-2 h. It shows less solubility in intestine pH. Papaverine HCl is rapidly absorbed orally and undergoes extensive first pass metabolism in the gut wall and liver and the bioavailability is as low as 30%. For the prolonged duration of action, sustained formulation is required because of low biological half life (Lloyd E, Matheson Jr., 1979). Hence, to improve its therapeutic efficacy, patient compliance and to reduce the frequency of dosing and side effects, as well as to avoid its extensive first pass metabolism, transdermal drug delivery approach was considered to be better suitable for papaverine hydrochloride. In view of the above facts, in the present investigation, an attempt is made to develop matrix type transdermal patches of papaverine hydrochloride using suitable polymers like polyvinyl alcohol, polyvinylpyrrolidone, ethyl cellulose, eudragit RL-100 and eudragit RS-100.

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Received on: 26-03-2010
Revised on: 09-05-2010
Accepted on: 12-05-2010

Table 1: Composition of Formulations

Formulation Code	PVP (mg)	PVA (mg)	Ethyl Cellulose (mg)	Eudrgit RL-100 (mg)	Eudrgit RS-100 (mg)	Drug (mg)
F1	100	-	400	-	-	150
F2	200	-	300	-	-	150
F3	167	333	-	-	-	150
F4	250	250	-	-	-	150
F5	-	-	-	125	125	150
F6	-	-	-	100	250	150

Table 2: Physicochemical properties of the prepared Transdermal patches

Formulation code	*Hardness (kg)	*%Moisture absorption	*Thickness (mm)	*%Elongation	*Tensile strength (kg/mm)	Percentage drug content in 1 cm^2
F1	0.326 ± 0.024	2.91	0.320 ±. 021	78.10 ± 08.12	0.416 ±0.051	93.24 ± 0.5127
F2	0.306 ± 0.012	3.14	0.328 ±. 016	82.40 ± 10.31	0.439 ±0.047	95.40 ± 0.5714
F3	0.386 ± 0.021	5.26	0.321 ± .028	89.70 ± 09.41	0.493 ±0.078	92.88 ± 0.4015
F4	0.419 ± 0.018	5.17	0.332 ±. 023	85.70 ± 07.85	0.452 ±0.063	95.04 ± 0.7481
F5	0.396 ± 0.028	3.20	0.249 ±. 071	91.20 ± 08.34	0.545 ±0.022	93.02 ± 0.5104
F6	0.418 ± 0.031	3.50	0.260 ±. 062	90.70 ± 10.94	0.520 ±0.035	93.60 ± 0.7345

MATERIALS

Papaverine hydrochloride was gift sample from Biological E.Ltd., Hyderabad. Polyvinyl alcohol (PVA) was obtained from LDH Laboratory Reagents, Mumbai, polyvinyl pyrrolidone (PVP) was procured from Ozone International, Mumbai and ethyl cellulose was procured from Sulab Reagent, Mumbai. The other chemicals used in the study were of AR grade.

ANALYSIS

Samples were analyzed by UV-visible spectrophotometer (Jasco V-530) for the drug content.

METHODS

Formulation of Transdermal Patches

In the present study, matrix type transdermal patches of papaverine HCl were prepared by molding technique. A flat square shaped, aluminium foil coated glass molds having surface area of 25 cm^2 were fabricated for casting the patches.

Preparation of casting solutions

For Ethyl cellulose and PVP (F1 and F2)

The casting solutions were prepared by dissolving weighed quantities (Table 1) of polymers in chloroform. The drug was dissolved in chloroform and added to the above polymer solution along with propylene glycol, propylene glycol (0.1 ml), as plasticizer, and 0.1 ml of DMSO as penetration enhancer which is thoroughly mixed to form a homogeneous mixture. The volume was made up to 10 ml with chloroform. Entrapped air bubbles were removed by applying vacuum.

For PVA and PVP Polymers (F3 and F4)

The casting solutions were prepared by dissolving weighed quantities (Table 1) of polymers in water by heating on water bath. The drug was dissolved in distilled water and added to the above polymer solution along with propylene glycol (0.1 ml), as plasticizer, and 0.1 ml of DMSO as penetration enhancer which is thoroughly mixed to form a homogeneous mixture. The volume was made up to 10 ml with water. Entrapped air bubbles were removed by applying vacuum.

For Eudragit RL-100 and Eudragit RL-100 (F5 and F6)

The casting solutions were prepared by dissolving weighed quantities (Table 1) of polymers in ethanol:acetone (6:4). The drug was dissolved in chloroform and added to the above polymer solution along with propylene glycol, propylene glycol (0.1 ml), as plasticizer, and 0.1 ml of DMSO as penetration enhancer which is thoroughly mixed to form a homogeneous mixture. The volume was made up to 10 ml with ethanol. Entrapped air bubbles were removed by applying vacuum.

Preparation of Transdermal Patches

The casting solution (10 ml) was poured into glass moulds and dried at room temperature for 24 h for solvent evaporation. The patches were removed by peeling and cut into square dimension of 3 cm x 3 cm (9 cm^2). These patches were kept in dessicator for 2 days for further drying and wrapped in aluminium foil, packed in self-sealing covers. Transdermal patches were prepared with different polymer ratio, with constant plasticizer concentration and permeation enhancers (Saxena M *et al.,* 2006).

Evaluation of physicochemical properties

All the transdermal patches were visually inspected for colour, flexibility, homogenecity and smoothness. The thickness of the patches was measured at five different places on a single patch of each formulation using a screw gauge and the mean values were calculated (Prashant M et al., 2005). Weight variation between the formulated patches can lead to a difference in drug content and hence *in vitro* release behavior. A set of three patches from each batch having a diameter of 1 cm^2 were weighed on a digital balance and the mean values were calculated. The folding endurance is expressed as the number of folds (number of times the film is folded at the same place) required to break the specimen or to develop visible cracks. This also gives an indication of brittleness of the film. A strip of 2 cm x 2 cm (4 cm^2) was subjected to folding endurance by folding the patch at the same place repeatedly several times until a visible crack was observed and the values were reported (Das MK *et al.,* 2006).

The mechanical properties (percentage elongation and tensile strength) were evaluated using Instron universal testing instrument (model F. 4026), Instron Ltd., Japan,) with a 5 Kg load cell. Tensile strength is the maximum stress applied to a point at which the film specimen breaks. Film strips in special dimension and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. During measurement, the strips were pulled by the top clamps at a rate of 100 mm/min; the force and elongation were measured when the film broke. Results from film samples, which broke at and not between the clamps, were not included in the calculations. Measurements were run in triplicate for each film.

To determine the hardness of the patches, an apparatus was designed in our laboratory to study the hardness of the films using the literature report. It consists of a wooden stand of 11 cm height and top area of 16 cm x 16 cm. A small pan was fixed horizontally to one end of the 2 mm thick iron rod whose other end is reduced to a sharp point. A hole of 0.2 cm was made at the center of tip area of wooden stand, which was supported on the pan rod. An electric circuit was developed through a 3 volt battery in such a way that the bulb glows only when the circuit is completed through the contact of a metal plate and sharp end of the rod. The film was placed between the metal plate and sharp end of the rod. The weights were gradually added at an interval of 10 sec for the stabilization of the force till the bulb was glow. The final weight was considered as a measure of hardness (Das MK *et al.,* 2006).

Moisture absorption

Films (1cm^2) of each formulation were accurately weighed and exposed to ambient atmospheric conditions of temperature (avg. temp 34 °C) and humidity (75%) for three days. After three days, the films were again weighed and % moisture absorption was calculated (Das MK *et al.,* 2006).

Drug Content Uniformity

Drug content estimation was carried out in triplicates on each formulation. Each patch from different formulations (patch size of 1 cm^2, equivalent to 6 mg of drug)) was transferred into a graduated flask and phosphate buffer pH 6.8 was added up to 100 ml mark for extracting the drug from the patch. The flask was shaken for 4 h in a mechanical shaker. After extraction of the drug, the solution was filtered and diluted suitably with phosphate buffer pH 6.8 and the absorbance was measured at 249 nm, against the placebo patch solution as blank and the drug content was calculated (Murthy SN & Hiremath SR, 2001).

Compatibility Studies

In the present study, compatibility studies were carried out to assess any incompatibility between the drug and polymers. The FT-IR studies were performed to check the compatibility with excipients. Spectra of the pure drug and the formulated patch were taken individually. This is to ensure that there is no incompatibility between the drug and the polymers and other components with plasticizer and penetration enhancer.

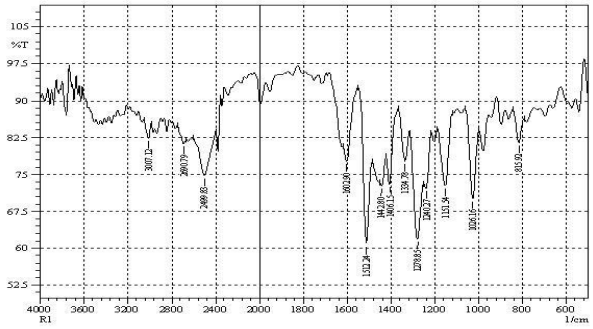

Figure 1: FT-IR spectra of papaverine hydrochloride

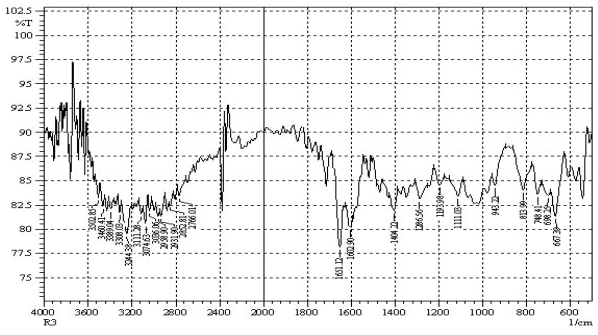

Figure 2: FT-IR spectra of EC: PVP formulation

***In vitro* drug release studies**

In vitro drug release profiles were carried out by using modified Keshery - Chein diffusion cell with cellophane membrane. The cellophane membrane was soaked in 100 ml of phosphate buffer pH 7.4 for overnight and then cut into pieces of 7 cm^2 area. It was mounted on the diffusion cell and equilibrated with re-

ceptor fluid for 15 min and used for the drug release studies. The modified Keshery - Chein diffusion cell designed and fabricated in our laboratory as per the literature (Das MK *et al.,* 2006). The cell consists of two compartments, the donor and the receptor compartment. The donor compartment was in contact with ambient conditions of the atmosphere. The receptor compartment was in contact with a solution in the receptor compartment (phosphate buffer pH 6.8.) and the contents were stirred by a rod-shaped magnetic bead driven by a magnetic stirrer. One patch of 1 cm^2 was placed in the donor compartment of the diffusion cell. The receptor fluid (5 ml) was withdrawn at predetermined time intervals (0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 16 h and 24 h) and replaced immediately with same volume of phosphate buffer pH 6.8. The samples were analyzed for drug content at 249 nm using UV-visible spectrophotometer after suitable dilution with phosphate buffer pH 6.8.

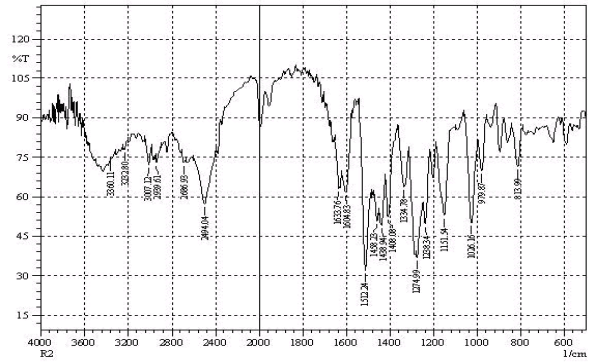

Figure 3: FT-IR spectra of PVA: PVP formulation

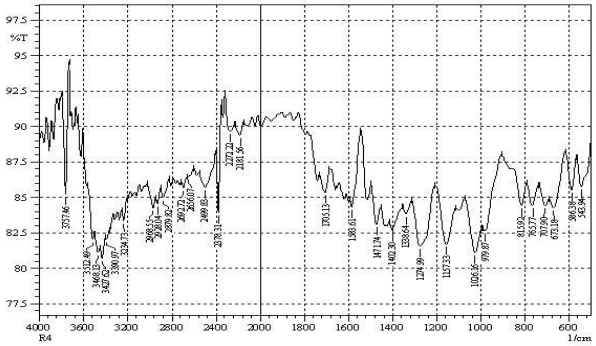

Figure 4: FT-IR spectra of eudragit RL-100: eudragit RS-100formulation

Stability Studies

To any rational design and evaluation of dosage forms, the stability of the active component must be major criterion in determining the acceptance or rejection. The stability studies of the formulated transdermal patches were carried out on prepared films at different temperature and humidity 25-30°C (60%RH) and 45-50°C (75%RH) over a period of 60 days. The patches were wrapped in aluminium foil and stored in stability chamber for stability study. The patches were characterized for drug content and other parameters at regular intervals (0, 15, 30, 45 and 60 days).

Skin Irritancy Studies

Patches were applied to the shaved skin on one side of the back of rabbit and secured using adhesive tape. On other back side of the rabbit, control patch (without drug) was secured in a similar way. The animal was observed for any sign of erythema or oedema for a period of 48 h.

Effect of drug on Isoproterenol induced myocardial necrosis

Male wistar rats (8) weighing 150-200 g were considered for this study. They were divided into three groups (4 in each group). Group 1 was first pretreated with the test drug by applying the transdermal patch of 0.9 cm^2 containing 5.4 mg of drug /200 g of animal (Ghosh MN, 1984). Group 2 were applied transdermal patch containing no drug, and group 3 was normal control did not receive any treatment (for comparison). After 6 h, other than the normal control group, were injected with 8.5 mg/kg isoproterenol by subcutaneous route on two consecutive days. After 48 h of first isoproterenol administration, the rats were sacrificed and autopsied. Blood samples (0.5 ml) were withdrawn on both the days [(day 1, after 24 h of first dose of isoproterenol inj), and day 2, after 48 h of first dose of isoproterenol inj] for Lactate dehydrogenase enzyme estimation by Wroblewski and La Due method (Wroblewski F and La Due JS, 1955). The animal heart was removed from the retro orbital route and weighed, and frontal sections were embedded for histological examination. The histological examination of the hearts was undertaken to study the severity of infarction. Necrosis produced by ischemia reperfusion injury was graded (Vogel HG & Vogel WF, 2002). After microscopic examination, grades were given as follows: grade 0, no change; grade 1, focal interstitial response; grade 2, focal lesions in many sections,consisting of mottled staining and fragmentation of muscle fibres; grade 3, confluent retrogressive lesions with hyaline necrosis and fragmentation of muscle fibres and sequestrating mucoid oedema; grade 4, massive infarct with occasionally acute aneurysm and mural thrombi. This study was conducted after obtaining the Animal ethical clearance from Institutional Animal Ethics Committee (K.S. Hegde Medical Academy).

Curve fitting analysis

In vitro drug release data were fitted to kinetic models such as zero-order (Brazel & Peppas, 2000), first-order (Lapidus & Lordi, 1966), Higuchi equation (Higuchi, 1963). Qt versus t (zero order), log Qt versus t (first order), Qt versus square root of t (Higuchi), where Qt is the amount of drug released at time t. The criteria for selecting the most appropriate model are highest R^2 value as it indicates the linearity of dissolution data (Thakkar *et al.,* 2009).

RESULTS AND DISCUSSION

Formulation of Transdermal Patches

Transdermal patches of Papaverine HCl were prepared by casting method on glass moulds, using PVA, PVP, ethyl cellulose, eudragit RL-100 and eudragit RS-100 as polymers, propylene glycol as plasticizer, DMSO as penetration enhancer. Chloroform was used as solvent for EC: PVP and water was used as solvent for PVA: PVP and mixture of ethanol: acetone (6:4) was used as solvent for eudragit RL-100: eudragit RS-100. Effect of concentration ratio of polymers and nature of polymers was studied by preparing various formulations of transdermal patches. In the preparation, set-up addition of ingredients particularly propylene glycol and DMSO was followed after careful evaluation of patches for physical characteristics. In all these formulations a constant amount of drug (150 mg) was maintained. The casting solution (10 ml) was poured into 25 cm^2 moulds, so that each cm^2 contains approximately 6 mg of drug. Polymers were used in different ratios and the concentration of other ingredients such as plasticizer and penetration enhancers were kept constant.

Evaluation of Transdermal Patches

Transdermal patches of papaverine hydrochloride were formulated and evaluated for various parameters. In the present study total six formulations were prepared by varying polymer ratio, and by using different polymers. These patches were subjected to evaluation of various physicochemical characteristics and drug release studies. Different formulations (F1, F2, F3, F4, F5 and F6) were prepared using PVA, PVP, ethyl cellulose, eudragit RL-100 and eudragit RS-100 to study the effect of polymers at different ratios on the physicochemical properties. Physical appearance of the patches was evaluated. All the patches prepared with different polymer concentration were found to be flexible, smooth, opaque, non sticky and homogeneous. Thickness of the patches in each set was measured. Marginal difference in thickness was observed among each group indicated that more the amount of polymer higher the thickness values (Table 2). All the six patches have showed good folding endurance (75-100) indicated that the patches have good flexibility. Water absorption studies revealed that as the concentration of PVP, PVA, eudragit RS-100 (F2, F3, F6) increased the amount of water absorption also increased. Among the patches, F3 (PVA: PVP ratio 2:1) absorbed higher moisture content. This may be due to the hydrophilic nature of the PVA and PVP. The least percentage of moisture absorption was observed for F-1 patch (EC: PVP) as compared to other patches because of hydrophobic nature of ethyl cellulose. The effect of concentration of polymers

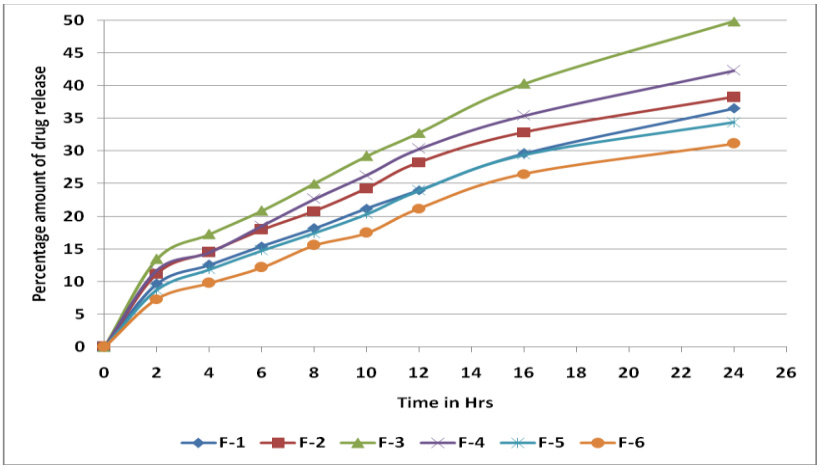

Figure 5: Comparison of *in vitro* release profiles of formulations

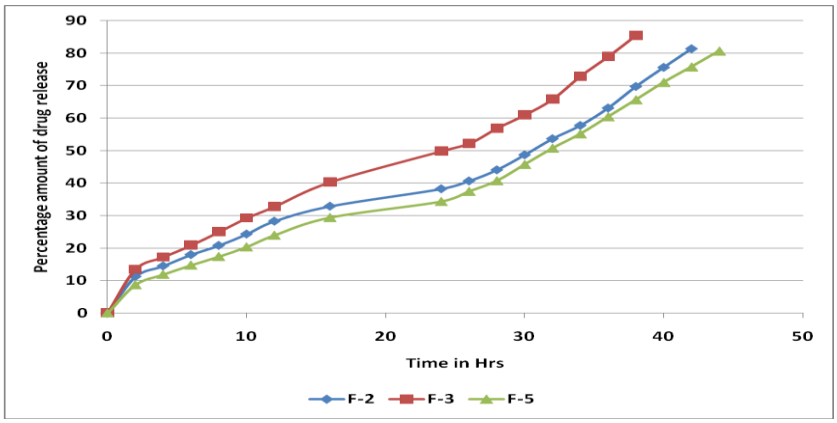

Figure 6: Comparison of prolonged *in vitro* release profiles of F2, F3 and F5

was observed on the percentage elongation and tensile strength. It was found that as the concentration of PVP increased the percentage elongation and tensile strength was also increased within the patches containing the combination of EC and PVP. Eudragit patches showed better tensile strength due to the nature of polymers (Table 2). There was no significant difference in the drug content among the patches indicated content uniformity. All the patches were found to be opaque, smooth, flexible and non-sticky in nature. This may be due to the presence of plasticizer. It was observed that there was no significant difference in the thickness among the patches, indicated that the patches are uniform.

Compatibility Study

The FT-IR spectra of pure drug, polymers and formulations were carried out and represented as in Fig 1-4. The principal peaks of pure drug papaverine hydrochloride were obtained at wave number 1602.90 cm^{-1}, 1512.24 cm^{-1}, 1026.16 cm^{-1}, 1278.85 cm^{-1} which corresponds to the theoretical peaks at wave number 1598 cm^{-1}, 1508 cm^{-1}, 1026 cm^{-1}, 1279 cm^{-1}. The corresponding peaks of pure drug were also present in transdermal formulations. From the spectral studies, it was concluded that there was no interaction between drug and polymers.

***In vitro* drug release studies**

In vitro drug release study was carried out using cellophane membrane and modified keshery-chein diffusion cell. It was observed that from hydrophilic polymers (F3 and F4) the drug release was found to be faster compared to the combination of hydrophilic and hydrophobic polymers (F1 & F2) or only hydrophobic polymers (F5& F6) used in the study (Fig 5). Patches prepared with PVP and EC as polymers, found that more the amount of PVP better the drug release due to the hydrophilic nature of PVP. A significant change in drug release was observed from patches containing more amount of PVA showed highest release (F3 compared to F4). This may be attributed to hydrophilic nature of the polymers which has more affinity for water results in increased thermodynamic activity of the drug in the film. Patches containing eudragit RL-100 and eudragit RS-100 (F5 and F6) showed slower release as the patches contains only hydrophobic polymers, which might have lead to slower release of drug from the patches. Further the drug release study (F2, F3, and F5) was when conducted for 40 h (Fig 6), it was observed that approximately 75-80% of drug was released. Hence transdermal patches can be used for extended period of time. The release profile was correlated with the moisture absorption which further reflected by the nature of polymer.

From the above data, it can be concluded that the release characteristics may be restricted to only *in vitro* release study, as the *in vitro* release model mainly favours the hydrophilicity. However, when theses patches applied to the skin results may differ as the lipophilicity may play a major role for drug transport system.

Curve fitting analysis

To know the mechanism of drug release from these formulations, the data were treated according to first-order, Higuchi's and zero order pattern. The release kinetics of the transdermal patches followed first order (0.9456 – 0.9724) and Higuchi's diffusion kinetics (0.9755 – 0.9992). According to the first order the release of drug is based on the concentration of the drug in the formulation. Further as per Higuchi's release kinetics; the drug release followed diffusion mechanism. Percentage of drug released when plotted against square root of time, the plots showed high linearity. It indicated that release pattern followed Higuchi's diffusion mechanism which states that as the time increases the diffusion path length also increases.

Stability Studies

Stability studies were carried out for 60 days at room temperature, temperature of 25- 30^0 C, 60%RH and 45- $5\,0^0$ C , 75% RH. The patches were observed for physical change and drug content. It was found that, when the patches were stored at 25- 30^0 C, 60%RH, the loss of drug was approximately 2-3% at the end of 60 days. However, the amount of drug loss was found to be much higher (14-18%) when stored at 45- 50°C, 75% RH. Further, the amount of drug loss was found to be more (18%) from hydrophilic polymers (F3 & F4) compared to combination of hydrophilic and hydrophobic polymers or only hydrophobic polymers.

Skin Irritancy Study

Results of skin irritancy study revealed that neither blank patch nor patch containing papaverine hydrochloride caused any noticeable sign of erythema or oedema on rabbit skin throughout the period of 48 h. Hence the patches were found to be compatible with the skin.

Effect of drug on Isoproterenol induced myocardial necrosis

From the in vivo effect of drug on isoproterenol induced myocardial necrosis study, it was found that the LDH (Lactate dehydrogenase) level increased marginally in rats treated with transdermal patch (404 IU/L) on day one (after 24 h), and reduced to normal level of 280 IU/L after 48 h, compared to the group of animals which were not treated with drug containing transdermal patch, where the level of LDH remained very high (717 IU/L) even after 48 h. The normal value for LDH is 100-330 IU/L (Abraham N et al, 2006). Thus the extent of damage was found to be minimum in group of animals treated with transdermal patch containing papaverine HCl compared to the group of animals applied with placebo patch (without drug). The initial (in 24 h) rise in LDH level in animals treated with drug containing transdermal patch was probably due to the slow absorption of the drug into the blood stream and hence sufficient amount of drug was not present in the blood. However, the LDH level reached to normal within 48 h indicates that the

transdermal patches can be used as controlled drug delivery system in the treatment of myocardial necrosis. Further, it was found that there was significant decrease in myocardial necrosis in rats applied with the transdermal patch containing papaverine hydrochloride compared to the group of animals not treated with transdermal patch. When the animals were not treated with drug containing transdermal patch, the myocardial necrosis found to be severe (grade 3-4, Fig 7) compared to the patch containing drug (grade 2-3, Fig 8). Hence papaverine hydrochloride transdermal patches helps in decreasing the effect of isoproterenol induced myocardial necrosis. This suggests that drug absorption through the skin has taken place from the patches.

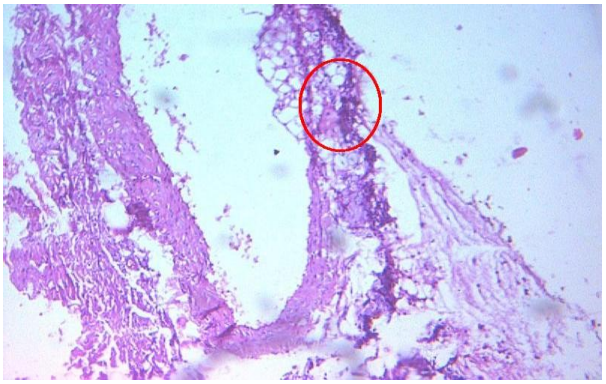

Figure 7: T.S. of rat heart injected with isoproterenol

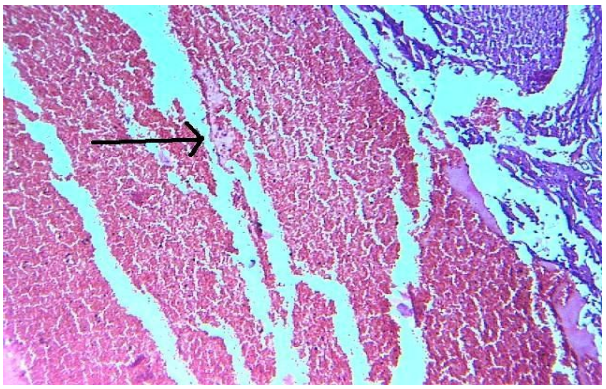

Figure 8: T.S. of rat heart injected with isoproterenol and applied with transdermal patch containing drug

CONCLUSION

Based on the physicochemical parameters and *in vitro* release studies, it was found that formulation containing hydrophilic polymers released faster compared to combination of hydrophilic and hydrophobic polymers or only hydrophobic polymers. Further, *in vivo* study showed that papaverine hydrochloride helps in decreasing the effect of isoproterenol on myocardial necrosis. Results of the present study encouraged that the papaverine hydrochloride transdermal patch can be used as controlled drug delivery system and frequency of administration can be minimized.

ACKNOWLEDGEMENT

The authors are grateful to Nitte University, Mangalore, NITK Suratkal and DR. Pathani Scintific & Industrial Research, Mumbai, for providing the necessary facilities to carry out this study.

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