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Design and Development of Transdermal Patches for Perindopril.

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ABSTRACT

Perindopril is an antihypertensive agent which undergoes extensive first pass metabolism making it a possible candidate for transdermal delivery. Patches were prepared by solvent casting technique. The results of FTIR and DSC revealed no interaction between drug and polymers. The loss of moisture and uptake of moisture were within the limits. The formulations showed an extended release of the drug upto period of 24 hours during *in vitro* permeation studies and showed non Fickian drug release. Stability of the optimized formulation was investigated as per ICH guidelines and was found to be stable with respect to drug content and *in vitro* permeation.

Keywords: *In vitro* permeation, Franz diffusion cell, moisture uptake, non Fickian diffusion, perindopril.

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INTRODUCTION

Transdermal administration refers to continuous drug infusion through an intact skin surface to control the delivery of drug [1]. Transdermal drug delivery is a non-invasive delivery of medicament from the surface of the skin [2]. This has advantages over the oral route of administration, in patient compliance and bypassing first pass metabolism. Several drugs were explored for the possible use in transdermal drug delivery for treating hypertension. A few of these are: nifedipine [3], metaprolol [4] and isradipine [5].

Perindopril is an angiotensin converting enzyme inhibitor, orally active and undergoes substantial first-pass metabolism by cytochrome P450 enzymes. The terminal half-life of perindopril is about 0.8 to 1 hr [6]. Following oral administration, perindopril is well absorbed and undergoes substantial first-pass metabolism; the systemic bioavailability of perindopril is about 20% [7]. In view of these facts, this drug can be considered as a suitable candidate for trandermal delivery.

MATERIALS AND METHODS

Perindopril was a gift from Hetero Drugs Ltd., (Hyderabad, India). HPMC, SCMC and eudragit was generous gift from Colorcon Asia PVT Ltd., (Goa, India); Carbopol was gifted by Inventis drug delivery systems PVT Ltd., (Hyderabad, India). All reagents were used of analytical grade.

Animals

Male Wistar rats weighing approximately 200 – 250 g were used for the diffusion studies of perindopril transdermal patches. The animals were suFied with food and water ad libitum. The animal studies were approved by the Institute Animal Ethics Committee (IAEC), Regd no. 1434/PC/a/11/ CPCSEA and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

Investigation of drug–excipient interactions

Fourier transform infrared spectroscopy

Compatibility between drug and the polymers were studied by FTIR spectra. FTIR studies were carried out for drug and its physical mixture (1:1). The sample was dispersed in KBr powder and the pellets were made by aFying 6000 kg/cm^2 pressure and analyzed. FTIR spectra were obtained by diffuse reflectance on a FTIR spectrophotometer type FTIR 8400 (Schimadzu Corporation, Japan). The positions of FTIR bands of important functional groups of drug were identified and were cross checked in obtained spectra [8].

Differential scanning calorimetry (DSC)

DSC studies for drug and its physical mixture (1:1) were carried out using DSC-60 calorimeter (Schimadzu Corporation, Japan). The instrument was calibrated with an indium and zinc standard. The sample was heated from 10 to 300°C at a heating rate of 25°C/min to

remove thermal history. The sample was then immediately cooled to 10°C and reheated from 10 to 300°C under the flow of nitrogen at a heating rate of 10°C/min.

Preparation of patches

Perindopril transdermal patches were prepared by solvent casting technique using hydroxypropylmethylcellulose, eudragit RL 100, sodium carboxymethylcellulose and carbopol 934P as polymers. Propylene glycol and DMSO were used as plasticizer and penetration enhancer respectively. Ethanol, methanol and dichloromethane were used as solvents. Drug was dissolved in little quantity of solvent and polymers were dissolved in remaining solvent/solvent mixture. Drug, polymer solutions along with plasticizer and permeation enhancer were sonicated for 30 min and examined for air entrapment. The solution was poured onto glass moulds of 10 x 5 cm^2 and air dried for overnight at room temperature. An inverted funnel was kept on the mould for controlled evaporation. The dried film of the drug was peeled from the mould and packed in aluminium foil and kept in desiccator till further use.

Table 1: Composition of perindopril patches

Ingredients	Formulation			
	F 1	F 2	F 3	F 4
F (mg)	200	200	200	200
HPMC K15M (mg)	900	600	600	600
SCMC (mg)	--	300	--	--
Eudragit RL 100 (mg)	--	--	300	--
Carbopol 934P (mg)	--	--	--	300
DMSO (ml)	0.2	0.2	0.2	0.2
Propylene glycol (ml)	0.4	0.4	0.4	0.4
Ethanol (ml)	--	5	--	5
Methanol (ml)	7.5	5	7.5	5
DCM (ml)	7.5	5	7.5	5

Thickness

Thickness of patches was measured using a micrometer (Mitutoyo co., Japan) for a pack of 5 films. Mean ± standard deviation was calculated [9].

Weight Variation

Ten patches (1×1 cm^2) were selected and weight variation was evaluated for each formulation [10].

Folding Endurance

Each patch was folded repeatedly several times at the same place until the patch breaks. The first appearance of breaking was observed and then the folding endurance was reported by the number of foldings before it was broken [11].

Loss of Moisture

The patches were initially weighed (W1) individually and placed in a dessicator (containing activated silica) at room temperature (30 ± 0.5 °C). After three days, the films were taken out and weighed (W2). Percent loss of moisture was calculated using formula, given below [12].

$$\% \text{ Loss of Moisture} = W1 - W2/ W2 \times 100$$

Moisture Uptake:

The patches were weighed (W1) and placed in a dessicator (containing 100 ml saturated solution of sodium chloride, 75% RH) at room temperature (30 ± 0.5 °C). After three days, the films were removed and weighed (W2). Percent gain of moisture was calculated using the given formula [13].

$$\% \text{ Gain of moisture} = W1 - W2/ W2 \times 100$$

Drug Content

Drug content of patches was determined by dissolving five patches (1 cm^2) in 100 ml of 7.4 buffer. After suitable dilutions the resultant solution was filtered and analysed for perindopril content spectrophotometrically [6,7].

HPLC analysis

Analysis of samples was performed using a Schimadzu 10 AVP (Japan) HPLC system equipped with UV detector and a waters C-18 column (300 × 4.6 mm i.d) at ambient temperature. The mobile phase was mixture of phosphate buffer pH 3.0 and acetonitrile in 65:35 ratios. The solution was filtered through 0.45 µm filter and degassed by sonication. The flow rate was 1 ml per minute. The detection was carried on at 215 nm wavelength. A calibration curve was plotted for perindopril in the range of 25-150 µg/ml A good linear relationship was observed between the concentration of perindopril and its peak area (r^2 = 0.9981). Precision and accuracy of the HPLC method were estimated [14].

***In vitro* permeation studies using rat skin**

Franz diffusion cell was used for *in vitro* skin permeation studies. The skin of the rat abdominal region was used. The preparation of skin for diffusion study was as follows. Male wistar rats weighing 200-250 g were used. The rats were anaesthetised using chloroform and the abdomen was carefully shaved with scissors and razor [15]. Full thickness skin (i.e., epidermis, subcutaneous and dermis) was excised from the abdominal site [16]. Any skin with damages was rejected. The skin sample was placed in the Franz diffusion cell. Slightly larger skin was taken to help its fixation on the diffusion cell. The patch of area 3.14 cm^2 was used. The receptor compartment was filled with phosphate buffer, pH 7.4. Samples of one ml were withdrawn at predetermined time intervals and one ml was replaced with fresh solution. Required dilutions were made for the sample and the amount of drug that reached receptor compartment was analyzed for drug content using HPLC and the data was

statistically analysed by one way ANOVA followed by turkey post hoc test for multiple comparison using graph pad prism. Differences were considered to be significant at a level of *p<0.05.*

The permeability coefficients (P) were calculated as follows [17]

$$P = (dQ/dt)/ (CA)$$

Where,

dQ/dt - Permeation rate,
C - Concentration of the donor chamber
A - Surface area of diffusion

Steady state fluxes (J_{ss}) were calculated by dividing the slope of cumulative amount permeated Vs time curve by the diffusional area.

Stability studies

Stability studies were conducted according to the ICH Q1A (R2) guidelines. Patches were wrapped in aluminum foil and were kept in stability chamber at a temperature of 40±2ºC and 75±5% RH for 6 months [18]. Samples were withdrawn at the end of 6 months and analyzed for drug content and *in vitro* permeation. Zero time samples were used as control for the study and the results were statistically analyzed by using t-test and *p <0.05* were considered as significant.

RESULTS AND DISCUSSION

Investigation of drug–excipient interactions

FTIR spectral analysis

Perindopril and its physical mixture were subjected to FTIR spectroscopic analysis. The obtained spectra are shown in figure 1.

The FTIR spectra of pure perindopril showed sharp characteristic peaks at 1020 (C–C stretch), 1207 (C-N stretch), 1392 (Carboxylate anion stretch), 1566 (N-H bending), 1745 (C=O stretch), 2870 (C–H stretch), 2929 (O-H stretch) and 3281 cm^{-1} (N–H stretch). All the above characteristic peaks appeared in the spectra of the physical mixture at the same wavenumbers indicating no modification or interaction between the drug and polymers.

Differential scanning calorimetry

DSC studies were carried out for perindopril pure drug and its physical mixture. The DSC thermogram of perindopril showed an endothermic peak at 123.19ºC corresponding to its melting temperature, which was also detected in the thermograms of physical mixture, signifying no interaction between perindopril and the polymers.

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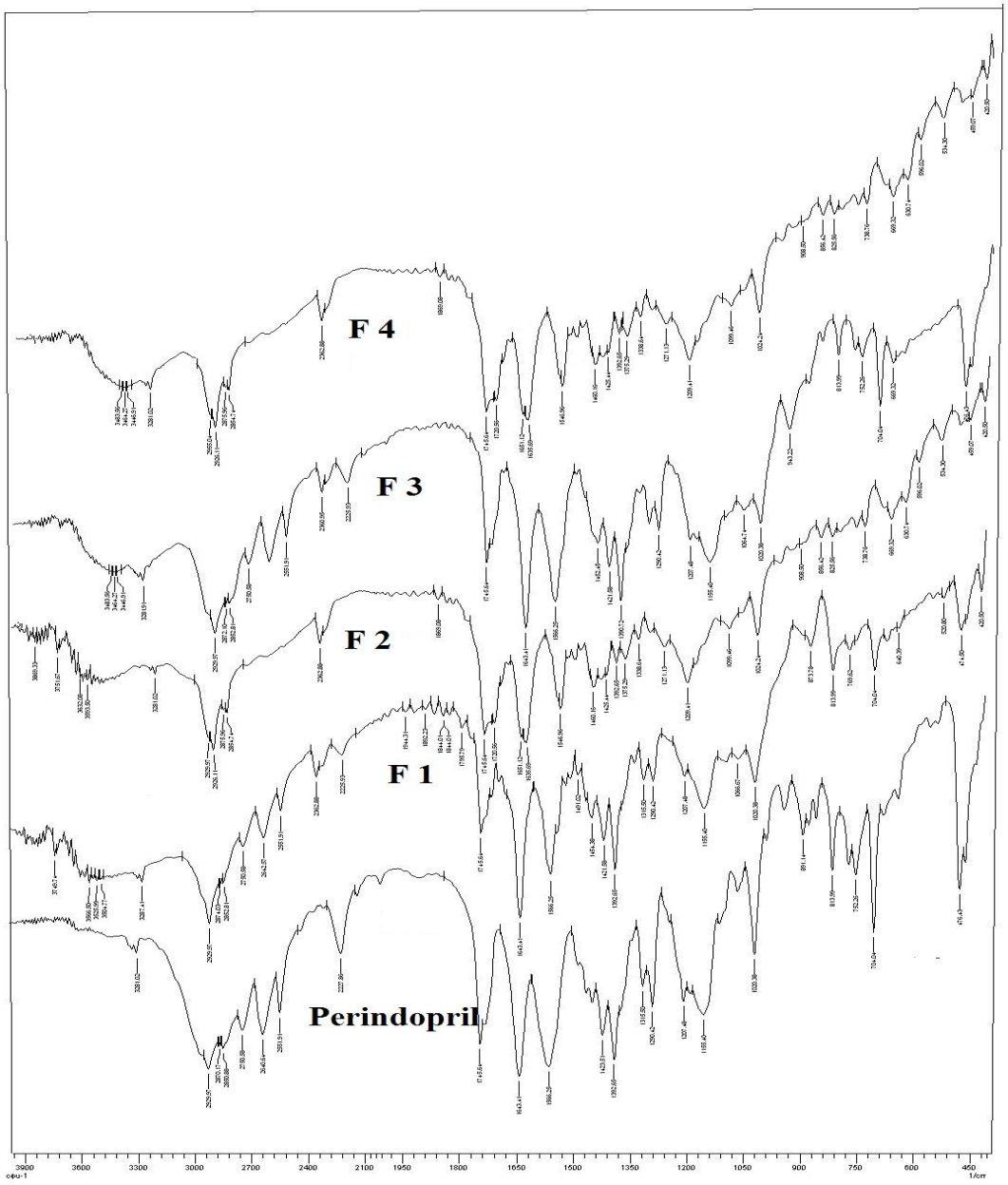

Figure 1: FTIR spectra of perindopril and its physical mixture

Physicochemical evaluation of perindopril patches

Table 2 shows the values of drug content, weight variation, thickness, folding endurance, loss of moisture and moisture uptake. The mean drug content of films was found to be 3.83±0.14 mg, 3.95±0.15 mg, 3.86±0.08 mg and 3.93±0.15 mg for the formulations F 1, F 2, F 3 and F 4 respectively.

The weight of the films was found to be 27.22±0.35 mg, 26.09±0.18 mg, 27.28±0.47 mg and 26.28±0.16 mg for the formulations F 1, F 2, F 3 and F 4 respectively.

The thickness was found to be 291±2.08 µm, 258±1.52 µm, 253±2 µm and 271±2.51 µm for the formulations F 1, F 2, F 3 and F 4 respectively.

The mean folding endurance values were found to be 231±9.16, 243±7.02, 299±2.51 and 239±7.53 for the formulations F 1, F 2, F 3 and F 4 respectively. Folding endurance of the films was in the order F 3>F 2>F 4>F 1. Formulation F 3 showed maximum folding endurance may be due to the presence of eudragit. The folding endurance of all the films was optimum, the films exhibited good physical and mechanical properties.

The overall moisture uptake was low (~10%), which was satisfactory for the patches. Thus the general physical properties are satisfactory.

Table 2: Physical evaluation of perindopril patches

Formulation	Parameter					
	% Drug content	Weight variation (mg)	Thickness (µm)	Folding endurance	Moisture Uptake (%)	Loss of moisture (%)
F 1	3.83±0.14	27.22±0.35	291±2.08	231±9.16	2.75±2.7	2.75±2.7
F 2	3.95±0.15	26.09±0.18	258±1.52	243±7.02	4.71±3.86	4.71±3.86
F 3	3.86±0.08	27.28±0.47	253±2.00	299±2.51	2.98±1.54	2.98±1.54
F 4	3.93±0.15	26.28±0.16	271±2.51	239±7.53	5.37±3.18	5.37±3.18

In vitro permeation studies

In vitro permeation studies for the films were carried out in triplicate. After 24 hours the release was found to be 75.12±6.98, 90.63±6.21, 64.71±6.71 and 81.62±4.81% for the formulations F 1, F 2, F 3 and F 4 respectively (figure 2). The data of *in vitro* permeation was analyzed by one way ANOVA and significant difference was observed between the means.

Formulation containing SCMC (F 2) showed swelling and a gel layer was formed on the surface which may be due to more hydrophilic nature of SCMC. When swelling is prevalent, drug diffusion may occur through the solvent-filled pathways of swollen patch.

Erosion of polymer matrix can also affect the drug release. The loosely bound polymer molecules in these films eroded readily, allowing faster release of verapamil from the films. Though the formulation F 1 showed slow release of the drug which may be due to the high viscous nature of HPMC K15M. In addition, HPMCK 15M forms a thick gel (diffusion path length) that acts as a barrier for drug diffusion and prevents matrix disintegration and additional water penetration.

Formulation F 4 showed faster release, which may be due to presence of carbopol and HPMC. Carbopol having tendency to undergo ionization at pH 6.6 and activates negative charges at the backbone of polymer. Repulsion between these like charges leads to uncoiling of the polymer to produce an extended structure capable of greater uptake of water. Thus owing to generation of pores by HPMC and uncoiling of carbopol at pH 6.6, the system absorbs more water and there by promotes diffusion, which in turn leads to an increase in release of drug. Presence of eudragit in formulation F 3 slows the drug release, which may be due to water insolubility of eudragit, lower dissolution and slower erosion of films.

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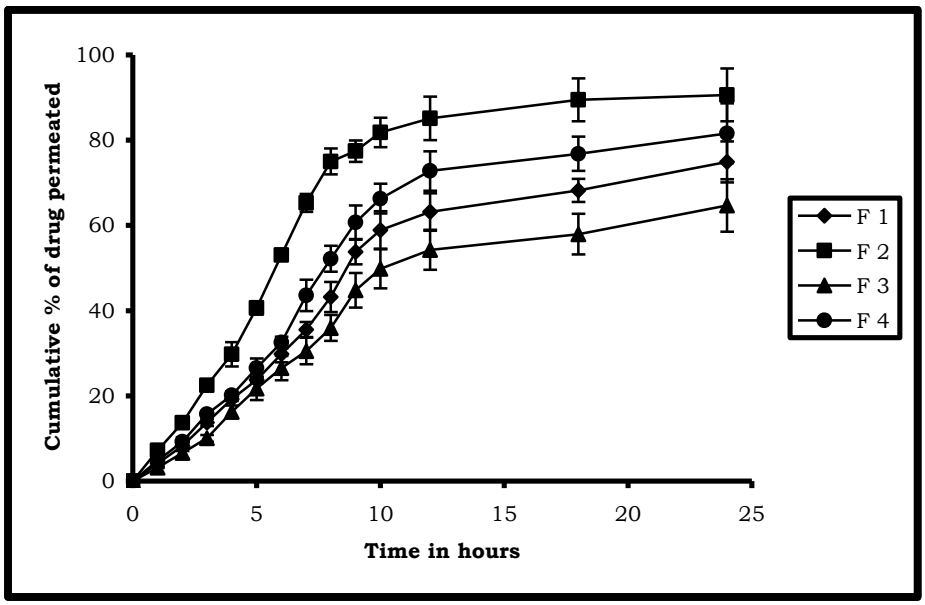

Figure 2: *In vitro* drug permeation profile of perindopril patches

In vitro permeation study of formulation F 2 showed maximum release of drug (90.63±6.21%) in 24 hr, and this formulation was considered as optimized one and used for further study. The order of drug release is F 2>F 4>F 1>F 3.

Table 3: Correlation coefficient (r^2) and rate constant of different kinetic models for perindopril patches

Formulation	n value	Correlation coefficient (r^2)				Drug transport mechanism
		Zero order	First order	Higuchi	Peppas	
F 1	0.8806	0.9208	0.9318	0.958	0.958	Non - Fickian diffusion
F 2	0.6992	0.8651	0.8516	0.9143	0.9934	Non - Fickian diffusion
F 3	0.847	0.9116	0.939	0.9553	0.976	Non - Fickian diffusion
F 4	0.8096	0.9102	0.9135	0.9506	0.9928	Non - Fickian diffusion

The *in vitro* permeation profile of all formulations could be best expressed by Korsmeyer-Peppas model. All the formulations showed a non- Fickian release pattern as it was evidenced from the release exponent (n > 0.5). This indicates coupling of the diffusion and erosion mechanism, called anomalous diffusion and shows that the drug release is controlled by more than one process. So, the suggested drug release mechanism for perindopril patches may be combination of diffusion and erosion of polymer matrix.

The mean steady state flux (J_{ss}) was found to be 0.1338±0.01, 0.2273±0.01, 0.1156±0.009 and 0.148±0.01 mg/cm^2/hr and the permeability coefficient was found to be 0.0335±0.003, 0.057±0.002, 0.0289±0.002 and 0.04±0.003 cm/hr for the formulations F 1, F 2, F 3 and F 4 respectively.

Stability studies

Accelerated stability studies were performed for optimized formulation (F 2) as per ICH Q1A (R2) guidelines at 40±2ºC and 75±5% RH for 6 months. After specified duration, visual examination of the patches did not show any change in morphology. The results of the stability studies revealed that there was significant change in drug content and *in vitro permeation*.

The cumulative percentage of F permeated in 24 h was found to be 84.93±3.56%. Flux and permeability coefficient of F was found to be 0.1481±0.05 mg/cm^2/hr and 0.0370±0.01 cm/hr respectively.

The *in vitro* permeation profile of F 2 after stability study could be best expressed by zero order model, as the plots showed highest linearity (r^2: 0.9298) and the obtained release exponent (n) value, 0.9942, supported non Fickian release and it was observed that there was a change in the best fit model and no change in transport mechanism after stability study.

CONCLUSION

Transdermal patches of perindopril were prepared by solvent evaporation technique. The patches exhibited good physical properties. The *in vitro* permeation patches was attempted using Franz diffusion cell, with and rat skin. Good results were obtained both *in vitro* conditions for patches. The statistical investigation of *in vitro* permeation data showed that the coupling of the diffusion and erosion is the mechanism of drug release. From the present investigation, it can be concluded that transdermal patches of perindopril may provide sustained delivery for prolonged periods in the management of hypertension, which can be a good way to bypass the extensive hepatic first-pass metabolism.

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