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Research paper

Monolithic matrix type transdermal drug delivery systems of pinacidil monohydrate: in vitro characterisation

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Abstract

The monolithic matrix type transdermal drug delivery systems of pinacidil monohydrate (PM) were prepared by film casting technique on mercury substrate and characterised in vitro by drug release studies using paddle over disc assembly, skin permeation studies using Keshary and Chein diffusion cell on albino rat skin and drug-excipient interaction analysis. Four formulations were developed which differed in the ratio of matrix forming polymers, Eudragit RL-100 and PVP K-30, i.e. 8:2, 4:6, 2:8 and 6:4 and were coded as B-1, B-2, B-3 and B-4, respectively. All the four formulations carried 20% w/w of PM, 5% w/w of plasticiser, PEG-400 and 5% w/w of DMSO (based on total polymer weight) in isopropyl alcohol: dichloromethane (40:60) solvent system. Cumulative % of drug released in 48 h from the four formulations was 63.96, 55.95, 52.26 and 92.18%. The corresponding values for cumulative amount of drug permeated for the said formulations were 57.28, 50.35, 46.38 and 86.54%, respectively. On the basis of in vitro drug release and skin permeation performance, formulation B-4 was found to be better than the other three formulations and it was selected as the optimised formulation. The interaction studies carried out by comparing the results of assay, ultraviolet, infrared and TLC analyses for the pure drug, medicated and placebo formulations indicated no chemical interaction between the drug and excipients.

Keywords: Transdermal delivery; Matrix system; Pinacidil monohydrate; In vitro release; In vitro permeation

1. Introduction

Transdermal delivery has many advantages over conventional modes of drug administration, especially, it avoids hepatic first pass metabolism and improves patient compliance [1]. Intensive research has showed that transdermal route is a potential mode of delivery of lipophillic drugs in the systemic circulation [2,3]. Matrix based transdermal formulations have been developed for a number of drugs such as nitroglycerine [4] and ephedrine [5].

Pinacidil monohydrate (PM) is a lipophillic drug used for the management of mild to moderate essential hypertension and has very few side effects. It acts by opening the potassium channels leading to hyperpolarisation and peripheral vasodilation [6]. After oral administration, peak plasma concentrations of pinacidil are reached within 0.5–1.0 h [7]. The antihypertensive action requires plasma concentrations in the range of 100–300 ng/ml. There is highly significant correlation between the change in mean blood pressure and the serum concentration of pinacidil of patients with essential hypertension. Oral treatment is usually begun with a dose of 12.5 mg twice daily. If oedema develops, a diuretic may be added. The usual maintenance dose is 12.5–25 mg b.i.d. and can be increased up to 150 mg daily in two divided doses [6]. It possesses low oral bioavailability (57%) due to hepatic first pass metabolism after oral administration and has a short biological half life of 1.6–2.9 h [8] which makes frequent dosing necessary to maintain the drug within the therapeutic blood levels for long periods. Hence, PM is an ideal drug candidate for transdermal drug delivery.

2. Materials

Pinacidil monohydrate (Courtesy, Leo Pharmaceuticals, Denmark), Eudragit-RL 100 (Pharmax, India), PVP K-30 (Dabur, India), Mercury (S.D.S., India), PEG-400 (CDH, India), Dimethyl Sulphoxide (S.D. Fine Chemicals Ltd., India), Isopropyl alcohol and Dichloromethane, (E. Merck India Ltd.). All solvents were of analytical grade.

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3. Methods

3.1. Fabrication of patches

The polymeric solution (10% w/v) was prepared by dissolving Eudragit RL 100 and PVP K-30 (in different ratios, i.e. 8:2, 4:6, 2:8 and 6:4 in formulations B-1, B-2, B-3 and B-4, respectively), along with 20% w/w of PM and 5% w/w of plasticiser, PEG-400 and 5% w/w of penetration enhancer, DMSO (based on total polymer weight) in isopropyl alcohol – dichloromethane (40:60) solvent system for each formulation. The solution was poured into a glass ring of 6.06 cm diameter placed on a mercury substrate. The solvent was allowed to evaporate at ambient conditions (temperature, 32°C and relative humidity, RH, 45%) for 24 h (the solvent got completely evaporated in 24 h whereas PEG 400 and DMSO remained in the drug-polymer matrix). Aluminium foil was used as backing film and wax paper as release liner (which could be removed before application of the patch on the skin). The polymer matrix was found to be self sticking due to the presence of Eudragit polymers along with plasticiser, PEG 400. The patches were cut with a circular metallic die of 2.93 cm internal diameter to give an area of 6.74 cm^2 and stored in air tight container at ambient conditions for 7 days prior to use.

3.2. Evaluation of patches

3.2.1. In vitro drug release studies

A modified stainless steel disc assembly (USP Apparatus 5, paddle over disc assembly), was used for the assessment of the release of the drug from the patches. The transdermal drug delivery system (TDDS) was mounted on the disc and placed at the bottom of the dissolution vessel. The dissolution medium was isotonic phosphate buffer (IPB) of pH 7.4 and the apparatus was equilibrated to 32 ± 0.5°C. The apparatus was operated at 50 rpm and samples were withdrawn at appropriate time intervals up to 48 h and analysed at 257 nm spectrophotometrically. Cumulative % drug released were calculated out (Table 1) and plotted against time (Fig. 1).

3.2.2. In vitro skin permeation studies

A cell fabricated on the lines of Keshary and Chein [9] diffusion cell with a diffusional area of 6.74 cm^2 was used. The skin was removed from the abdominal portion of an albino rat after killing the animal. The hair and fat were removed after treating the skin with 0.32 M ammonia solution for 35 min [10]. The stratum corneum side of the skin was kept in intimate contact with the release surface of the TDDS under test placed between the two halves of the diffusion cell. The receiver phase was 30% v/v isopropanolic IPB of pH 7.4 stirred at 500 rpm on a magnetic stirrer. Isopropanol was used as co-solvent for drug in the buffer. The whole assembly was kept in an oven pre-set at 32 ± 0.5°C.The skin was first stabilised until no ultraviolet (UV) absorbance was observed (to eliminate the possibility of any interference on the permeation of drug through the skin by ammonia pre-treatment and due to other skin contents). The amount of drug permeated was determined by removing 100 μl samples at appropriate time intervals up to 48 h. The volume was replenished with an equal quantity of pre-warmed receiver solution. The absorbances were read at 257 nm spectrophotometrically. Cumulative amounts of drug permeated in mg/cm^2 were calculated and plotted against time (Fig. 2). Flux was determined directly as the slope of the curve between the steady state values of the amount of drug permeated (mg/cm^2) v/s time (hours) [11] and permeability coefficients were deduced by dividing the flux by the initial drug load (mg/cm^2) as shown in Table 1.

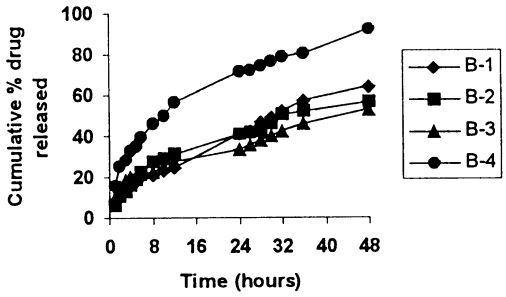

Fig. 1. In vitro release profiles of pinacidil monohyrate from TDDS formulations B-1, B-2, B-3 and B-4 using paddle over disc assembly ($n = 3$, SE did not exceed ±2.35%).

3.2.3. Interaction studies

The interaction studies were conducted on the optimised formulation (B-4) by comparing it with the pure drug and placebo formulation on the basis of assay, UV, infrared (IR) and TLC analyses.

Assay: The TDDS was dissolved in isopropanol and % recovery of the drug was determined by UV analysis.

Table 1
In vitro drug release and skin permeation of the developed transdermal drug delivery systems

In vitro parameter	Formulations			
	B-1	B-2	B-3	B-4
Cumulative % of drug released in 48 h[a]	63.96 (±1.12)	55.95 (±1.03)	52.26 (±2.35)	92.18 (±1.36)
Cumulative % of drug permeated in 48 h[a]	57.28 (±1.74)	50.35 (±1.62)	46.38 (±2.84)	86.54 (±2.05)
Permeability coefficient (cm/h × 10^2)[a]	1.19 (±0.05)	1.18 (±0.07)	0.95 (±0.03)	1.83 (±0.12)

[a] Results are the mean of triplicate observations, SE values are given in parentheses.

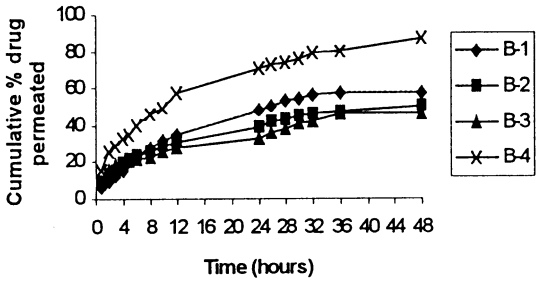

Fig. 2. In vitro skin permeation profiles of pinacidil monohydrate from TDDS formulations B-1, B-2, B-3 and B-4 using Keshary and Chien diffusion cell ($n = 3$, SE did not exceed $\pm 2.84\%$).

UV analysis: The isopropanolic solutions of the pure drug, medicated and placebo formulations were filtered with Whatman filter paper no. 42 and scanned for UV absorption between 200 and 400 nm using Beckman DU-64 spectrophotometer.

IR analysis: The IR absorption spectra of the pure, medicated and placebo formulations were taken in the range of 400–4000 cm^{-1} using potassium bromide disc method.

TLC studies: TLC analysis was conducted according to the method reported in the literature supplied by the drug manufacturer (Leo Pharmaceuticals, Denmark) using silica gel plate with chloroform – methanol – 25% ammonia solution (80:19:1) as mobile phase. Iodine vapours were used as visualising agent.

3.2.4. Stability studies

The stability studies were conducted according to ICH guidelines by storing the replicates of the TDDS at 40 ± 0.5°C and $75 \pm 5\%$ RH for 6 months. The samples were withdrawn at 0, 30, 60, 90 and 180 days and analysed for drug content by a stability indicating high-performance liquid chromatography method.

4. Results and discussions

The monolithic matrix type TDDSs of PM (Formulations B-1, B-2, B-3 and B-4 carrying Eudragit RL-100 and PVP K-30 in a ratio of 8:2, 4:6, 2:8 and 6:4, respectively) were prepared and characterised on the basis of in vitro drug release, skin permeation and interaction studies. The polymers did not interfere with the UV spectrophotometric method used for the analysis as they showed λ_{max} other than that observed for the drug. The cumulative % of drug released in 48 h was found to be the highest (92.18%) from formulation B-4 (Table 1, Fig. 1). The cumulative % of drug permeated was again maximum for formulation B-4 (carrying Eudragit RL-100 and PVP K-30 in 6:4 ratio) with a value of 86.54% (Fig. 2), the permeability coefficient being 0.0183 cm/h (Table 1). Hence formulation B-4 was selected as the optimised formulation by virtue of maximum drug release and skin permeation. Initially rapid release and permeation were observed, gradually approaching to constant values for the rest of the time (Figs. 1 and 2) , thus conforming to the controlled release behaviour of the formulations. The initial quick release (burst effect) would be beneficial as it would help achieve the therapeutic plasma concentration of the drug in minimum time and the constant release later on would then provide a sustained and controlled release of the drug. Burst effect might be due to initial migration of the drug towards the surface of the matrix. Linear curves were obtained on plotting the graphs for cumulative % drug released v/s square root of time suggesting Higuchian matrix diffusion mechanism of drug release from the TDDS formulations. Also, lower coefficients of variation were obtained for zero order release rate constants as compared with first order release rate constants indicating a zero order release pattern from the formulations. In vitro release and permeation results were in conformity to the in vivo pharmacokinetic and pharmacodynamic profile of the TDDS as revealed by the above studies performed using albino rats as furtherance of this work. The permeation rate constant of the drug was found matching the elimination rate constant and the therapeutically effective steady plasma concentration of the drug was achieved for the effective management of hypertension for 48 h.

The interaction studies were carried out to ascertain any kind of interaction of the drug with the excipients used in the preparation of TDDSs. For this the optimised formulation B-4, placebo formulation and the pure drug were subjected to assay, UV, IR and TLC analyses. The TLC values for the pure drug and the medicated formulations were proximal (0.73 and 0.74, respectively). The UV absorption maxima (λ_{max}) for the pure drug and the medicated formulation was found to be at 257 nm. On analysis of the IR spectra of the pure drug and the medicated formulation, no major difference was observed in the absorption peak pattern. Some of the peaks in the spectrum of the formulation got merged. This might be due to physical and not chemical interaction between the drug and the polymers. The UV and IR spectra of the placebo formulation showed absorption profiles exclusive to those for the pure drug and medicated formulation. On performing the assay, a very high percentage (99.12%) of the drug was recovered from the medicated formulation. The results indicated that the drug remained intact in TDDS and there was no chemical interaction between the drug and the excipients therein.

Very low degradation rate constant ($K = 1.72725 \times 10^{-4}$ day^{-1}) was observed on performing the stability studies according to ICH guidelines and a shelf life of 2 years could be assigned to the TDDS.

5. Conclusions

On the basis of the in vitro characterisation, it was concluded that pinacidil monohydrate could be administered transdermally through the matrix type TDDS developed in our laboratory. The drug remained intact and stable

in the TDDS on storage with no apparent chemical interaction between the drug and the excipients. Further work is underway to establish the therapeutic utility of these systems by pharmacokinetic and pharmacodynamic studies on human beings.

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