Formulation Optimization and Study on Effect of Penetration Enhancers on Reservoir Transdermal Therapeutic Systems of Hydralazine Hydrochloride

Revathi Mannam, Indira Muzib Yallamalli*

Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-517502, Andhra Pradesh, INDIA.

ABSTRACT

Background: Hydralazine hydrochloride (HZH) is a drug candidate used to treat pulmonary arterial hypertension. Extreme variability in oral dosing, bioavailability of 31%, variable half-life of 3 to 7 h and molecular weight of 196.6 Daltons, made the drug molecule suitable for transdermal system. **Objective:** The objective of the present investigation is to study the effect of penetration enhancers on release kinetics of hydralazine hydrochloride both *in vitro* and *in vivo* through reservoir based transdermal systems. **Materials and Methods:** Rate controlling membranes were formulated by using HPMC, Eudragit RLPO and Eudragit RSPO. Reservoir was formulated by using 16% poloxamer 407 gel by cold method as it is having sol-gel temperature at 37°C. **Results and Discussion:** The drug release followed zero order non-fickian super case-II diffusion transport mechanism. Enhancement ratio was more with IPM as penetration enhancer. Formulation F7 [ERLPO: HPMC 5:5 and IPM 15%] had maximum *in vitro* release of 95.68 ± 1.16% with flux value of 64.168±0.071 $\mu g/h/cm^2$, no significant difference in *ex vivo* permeation study was observed. The fabricated reservoir system was found to be safe for skin. *In vivo* kinetic studies projected a controlled release pattern with extended AUC of 25.760 ± 9.124 $\mu g/mL/h$ with MRT 17.7 ± 0.37 h in transdermal route compared to oral route where AUC and MRT are 4.409 ± 2.015 $\mu g/mL/h$ and 2.98 ± 1.24 h respectively. **Conclusion:** The drug reservoir transdermal system of HZH had varied *in vitro* release profile with varying the concentrations of penetration enhancer. The optimized transdermal system had extended AUC and MRT values compared to oral route and can enhance the bioavailability.

Key words: Hydralazine hydrochloride, Isopropyl myristate, *in vitro* kinetics, *in vivo* kinetics, Poloxamer 407.

Correspondence :

Indira Muzib Yallamalli, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University) Tirupati-517502, Andhra Pradesh, INDIA.

Phone: +919441593292

Email: yindira1415@gmail.com

INTRODUCTION

Pulmonary arterial hypertension is a progressive disease which is due to constriction of pulmonary artery by excessive production of endothelien receptors. Hydralazine hydrochloride (HZH) is a drug candidate used to treat pulmonary arterial hypertension and it acts as a vasodilator by relaxing smooth muscles of pulmonary artery and the treatment lasts for long time.[1,2]

Extreme variability in oral dosing of HZH, bioavailability of 31% and variable half-life of 3 to 7 h has made the dosage regimen complicated for oral usage.[3] Metabolism variations depending on the phenotype and food interactions had much narrowed its oral usage. Low bioavailability, variable and short half-life may not be suitable to meet the therapeutic needs of the patient where the treatment is for longer duration. There is a need for alternative to oral route where hepatic first pass metabolism can be excluded and which can improve the therapeutic activity and quality of life of patient. As the drug has of low molecular weight 196.6 Daltons[4] transdermal delivery is a better alternative to oral route, where a continuous infusion of drug can be provided and patient compliance can be improved.

Transdermal systems with a rate control membrane can provide a relatively constant rate of infusion of drug when the drug was maintained in required concentrations in reservoir system where the release rate will depend upon permeation through rate controlling membrane.[5] Hence, in the present work gel drug reservoir of HZH was formulated and the release rate was controlled by using rate controlling membrane and by varying penetration enhancers.

Gel reservoir was formulated by using poloxamer a temperature sensitive hydrogel which is very stable, biocompatible and biodegradable polymer.[6,7] Poloxamer solution forms a clear solution at 4-5°C and converts to gel at room temperature.[8] Rate controlling membranes were formulated by using E RLPO and E RSPO, the effect of penetration enhancer on release kinetics was evaluated. Pharmacotechnical properties, *in vitro, ex vivo* studies, stability studies and *in vivo* pharmacokinetic studies were evaluated.

MATERIALS AND METHODS

Materials

Hydralazine Hydrochloride was a gift sample received from Hetero drugs Pvt. Ltd, Hyderabad. Poloxamer 407 was provided as gift sample by Hi-Media Laboratories, Mumbai. Eudragit RLPO (E RLPO) and Eudragit RSPO (E RSPO) were gift samples provided by Zhaveri Pharma Chemicals., Mumbai. Release liner (poly iso butylene tape) was purchased from 3M. Hydroxy Propyl Methyl Cellulose (HPMC) and Poly Vinyl Acetate (PVA) were purchased from Hi-Media Laboratories, Mumbai. Azone (AZ), isopropyl myristate (IPM) and menthol (MT) were purchased from S.D Fine chemicals, Mumbai, India. All the solvents and reagents used were of analytical grade.

Measurement of sol-gel transition temperature

Sol-gel transition temperature was done to determine the poloxamer 407 concentration which retains its gel formation properties at room temperature. Poloxamer 407 solution with different concentrations

(15-20 % w/v) were prepared by cold method, where poloxamer 407 was added to distilled water at 4°C and kept for stirring until a clear solution was formed. Then the solution was kept for 12h at 4°C for complete dispersion of poloxamer, in a transparent beaker solution was taken where initial temperature will be 4-5°C and kept for stirring on a magnetic stirrer by increasing the temperature at a rate of 1°C per min up to 45°C. The temperature where magnetic bead stopped rotating was noted and is considered as sol-gel transition temperature.

Formulation of reservoir gel of hydralazine hydrochloride

Reservoir gel was prepared by cold method by using poloxamer 407 (16% w/v) based on sol-gel transition temperature values, where poloxamer was added to distilled water at 4°C and kept for stirring on a magnetic stirrer. Then the solution was kept for 12 h at 4°C for complete dispersion of poloxamer. Then HZH (2% w/v) along with various penetration enhancers (PE) were incorporated and kept for stirring until it reaches room temperature as mentioned in Table 1. Propyl paraben (0.002%w/v) was added as a preservative. Stirring was continued until the magnetic bead stopped rotating.

Formulation of rate controlling membranes

As discussed in Table 1 rate controlling membranes of E RLPO and E RSPO along with HPMC were prepared by casting method using solvent mixture of 10mL (dichloromethane and methanol in 3:2). Polymeric mixture was kept for stirring on a magnetic stirrer for 2 h for complete mixing. Di butyl phthalate 1.2 mL (30% w/v of polymer concentration) was added as penetration enhancer and kept for stirring for 30 min. Then the polymeric solution was poured in to petri dish and covered with a funnel for complete and uniform drying for 24 h. Films dried were packed and stored in an air sealed pack for further use.

Preparation of PVA backing membranes

A 6% w/v PVA solution was prepared by heating at 60°C until it completely dissolves in distilled water. The solution was continuously stirred and care was taken that no air bubbles were formed. Then the PVA solution was poured on a petri dish and air dried for 24 h by covering with a funnel. The dried films were stored in an air sealed pack until further use.

Preparation of transdermal reservoir system of HZH

Poloxamer 407 gel containing hydralazine hydrochloride of weight 0.25 gm was placed on the rate controlling membrane with a diameter of 2 cm and covered by PVA backing membrane by heat sealing to avoid leaks. A release liner was applied to the rate controlling membrane (Poly iso butylene Tape 3M).

Table 1: Formulation of poloxamer gel reservoir transdermal system of hydralazine hydrochloride.

Code	Rate controlling membrane (%w/v)			Reservoir gel
	HPMC	E RLPO	E RSPO	PE* (% w/v)
F1	2.5	7.5	-	-
F2	5	5	-	-
F3	5	-	5	-
F4	5	5	-	AZ (10%)
F5	5	5	-	IPM (10%)
F6	5	5	-	MT (10%)
F7	5	5	-	IPM (15%)
F8	5	5	-	IPM (20%)

*PE is Penetration enhancer

Measurement of pH of gel

pH of gel was measured by using calibrated pH meter and the experiment was done in triplicate, average of the readings was noted.

Determination of viscosity of gel

Viscosity of the poloxamer gel was determined by using Brookfield viscometer with spindle no 62 at 100 rpm. Viscosity was measured at 37°C.

Drug content determination in reservoir system

Drug content determination studies were done by taking reservoir system in to a beaker containing 100 mL of phosphate buffer saline (PBS) (pH 7.4) and kept for stirring for 24 h. The solution was filtered and the required dilutions were prepared, the amount of drug present was determined by using UV spectrophotometer at 262 nm.

Weight variation in transdermal reservoir systems

Weight variation was carried out by weighing the transdermal reservoir system for three times and average weight was noted.

Percentage moisture content

The moisture content studies were done by transferring formulated reservoir system, initially weighed (W_i) in to a desiccator containing silica gel and the experiment was continued till there is no change in the weight of reservoir system (W_c).[9] The values were then substituted in the following Equation (1).

$$\text{Moisture content (\%)} = [(W_i\text{-}W_c)/W_c] \times 100 \quad (1)$$

Percentage moisture absorption

Percentage moisture content studies were done by keeping the initially weighed (W_i) reservoir system in to a desiccator containing 100 mL of saturated NaCl (RH 75%) and after 3 days patches were taken out and weighed (W_f).[1e] Percentage of moisture uptake was calculated according to Equation (2).

$$\text{Moisture absorbed (\%)} = [(W_f - W_i)/W_i] \times 100 \quad (2)$$

Drug-polymer compatibility studies

DSC studies (Mettler Toledo, 821[e] DSC module) were done by scanning at a rate of 10°C per min to know any polymorphic change in the drug molecule after the formulation.

In vitro drug release studies of reservoir system of hydralazine hydrochloride

In vitro drug release studies were carried out by using modified Franz diffusion cell with an effective area of 3.14 cm[2].[11] Diffusion cell receiver compartment was filled with PBS (30 mL) (pH 7.4) and the donor compartment contains reservoir system placed on dialysis membrane 150. Entire system was kept on magnetic stirrer and stirred at a speed of 50 rpm at a temperature of 37°C throughout the study. Sample of 3mL was taken at pre-determined time intervals and drug release was estimated in UV Spectrophotometer at 262 nm.

Ex vivo permeation studies of reservoir system of hydralazine hydrochloride

Ex vivo permeation studies for the reservoir system was carried out by using rat skin on modified Franz diffusion cell. Hair was removed on the abdominal side and full thickness of skin was separated from the male albino rat anesthetized by chloroform. Adhering fat was removed and skin was cleaned with isopropyl alcohol. Stratum corneum was separated

by hot method where the skin was kept in hot water at 60°C for 1 min. Epidermis was removed carefully with scalpels and preserved in PBS 24h before the study.[12] The reservoir system was placed on the stratum corneum side where the dermal side was facing the receptor compartment. The receptor compartment contains PBS (30 mL) (pH 7.4), stirred at a speed of 50 rpm and the temperature was maintained at 37°C. Sample of 3 mL was taken at pre-determined time intervals and the drug release was determined at 262 nm using UV Spectrophotometer.

Tensile strength

Tensile strength for the optimized formulation was carried out using TX.TA plus texture analyzer (Stable Micro Systems, UK) at preset conditions of test speed 2 mm/sec, pre-test speed 1 mm/sec, pro-test speed 10 mm/sec, force 100 g and 5 kg load cell.[13] Film strip of dimensions 4 cm² was held between the clamps and pulled by the upper clamp at the speed of pre-set conditions. The force required to break the film and the elongation length were measured. The tensile strength and elongation at break were calculated according to Equation (3) and (4).

Tensile strength (kg/mm²) = Force at break (kg) / Initial cross-sectional area (mm²) Eq. (3)

Elongation at break (%) = [Increase in length at breaking point (mm)/ initial length (mm)] x 100 Eq. (4)

Skin irritation studies of reservoir system of hydralazine hydrochloride

Skin irritation studies were carried out by scoring the edema and erythema up to 7 days by visual observation using Draize's scale and the average reading was taken. Male Albino rats were divided in to three groups (n=6) where Group I am control, Group II (medicated group) reservoir system with drug and for Group III a standard irritant i.e., formalin (0.8% v/v) was applied.[14] Results were considered significant at p≤0.0001 using student t-test.

Stability studies of reservoir system of hydralazine hydrochloride

Stability studies were mainly conducted to assess whether the formulation is retaining its pharmacotechnical properties and release kinetics after subjecting to varied climatic conditions. Here the reservoir systems were subjected to accelerated conditions for 6 months using REMI stability chamber by maintaining temperature at 40±2°C and a relative humidity of 75±5% RH. Transdermal reservoir systems were evaluated for drug content and *in vitro* drug release.

In vivo Studies
HPLC Analytical conditions

Waters 2695 HPLC with class Empower-2 software with high speed auto sampler and 2996 PDA detector with dual wavelength at 228nm were used. Inertsil ODS column C18 of 250X4.6 mm and 5μm size was used. Mobile phase used was 0.1% orthophosphate buffer and acetonitrile in the ratio 65:35 v/v% and pH was adjusted to 4.6 with triethanolamine. The retention time for hydralazine hydrochloride is 4.8 mins and 6.2 mins for metaprolol.

Extraction of hydralazine hydrochloride from plasma

250μL of plasma and 50μL of metoprolol as internal standard, 10μL of hydralazine hydrochloride was taken in to a centrifuging tube and 2 mL of Acetonitrile was added. Cyclomixing was done for 15 sec and then vortexed for 2 min finally centrifuged at 3200 rpm speed for 2 min. After

the centrifugation organic layer was separated, filtered and 20 μL was injected into HPLC at a flow rate of 1 mL/min with a run time of 10 min.

Estimation of plasma concentration in rabbits

The whole experiment conducted was approved by the animal ethics committee guidelines (CPCSEA NO. 1677/PO/Re/S/2012/CPCSEA). *In vivo* pharmacokinetic studies were done on male New Zealand rabbits (n=5) of weight 1.3-1.5 kg. Pharmacokinetic studies were carried out between pure drug and reservoir system. Animals were divided in to three groups Group I serves as control, Group II received hydralazine hydrochloride orally at 1.76 mg/kg, Group III received HZH reservoir transdermal system of area 3.14 cm². All the animals were kept for fasting for 24 h before conducting the study. Blood volume of 0.5 mL was collected at pre-noted time intervals 0 min, 15 min, 30 min, 1, 2, 3, 4 and 6 h for oral route and 0min, 15 min, 30 min, 1, 2, 4, 6, 10, 15, 20, 25, 30, 35 and 40 h for transdermal route from the left marginal ear vein; plasma was separated immediately by centrifuging at 5000rpm and stored in heparinized tubes at -25°C. The kinetic parameters were estimated using in built PK solver program in Microsoft Excel 2010.

Statistical analysis

Statistical studies were done by using Microsoft Excel 2010. The results were analyzed by student t-test, p≤0.001 and p≤0.05 was considered as statistically significant difference.

RESULTS AND DISCUSSION

A 16% concentration of poloxamer 407 gel was chosen as it is having sol-gel transition temperature at 37 ± 0.5°C. pH of 16% gel was found to be at 7.36 ± 0.11 with viscosity of 1566.66 ± 57.7 cps.

Drug content in the reservoir systems was found to be in the range of 97.6 ± 0.6 % to 99.3 ± 0.9%. Weight variation was found to be in the range of 1.2 ± 0.21% to 1.8 ± 1.28% and the results were reported in Table 2.

The results of moisture content and moisture absorption studies revealed that the moisture content increased with the increase in HPMC concentration i.e., from 2.44 ± 0.43 to 3.77 ± 0.69% in F1- F8 and moisture absorption was found to be 2.47 ± 1.21 to 3.67 ± 1.02% in F1-F8. The results were reported in Table 2.

Drug-polymer compatibility studies

In the DSC spectra of HZH a characteristic endothermic peak was observed at 285.4°C as shown in Figure 1(A) and in Figure 1(C) endothermic peak of poloxamer 407 was observed at 53.6°C, where as in case of HZH gel endothermic peak was observed at 284.4°C as shown in Figure 1(B) indicating no polymorphic change and its compatibility with the drug molecule.

In vitro release studies of transdermal reservoir systems of hydralazine hydrochloride

In vitro release studies were performed using modified Franz-diffusion cell using dialysis membrane 150 as a barrier and PBS as media. Cumulative percentage drug release *vs* time was plotted and represented in Figure 2. Rate controlling polymers like E RLPO and E RSPO has eased out in achieving the control release patters in reservoir systems. The drug release was extended up to 24ch in all the formulations and in formulation F7 maximum of 95.68 ± 1.16% drug release was achieved by the end of 24 h. As discussed in Table 3 the effect of E RLPO over E RSPO was clearly observed. The release rate was more in E RLPO and this may be due to more hydrophilicity compared to E RSPO, where E RLPO tend

to release the drug through the pores by wetting of the polymer.[15] Addition of HPMC has substantially enhanced the drug release by further increasing the wetting property of the release retarding layer.[16] A significant difference in percentage cumulative drug release was observed between F1, F2 and F3 as depicted in Figure 2, $p \leq 0.05$ was found to be statistically significant difference. The effect of penetration enhancers on the drug release was studied and the enhancement effect was found to be in the order IPM>MT>AZ. The cumulative drug release effect with different concentrations of IPM was observed and the flux value increased with increase in concentration of IPM from 10% w/v (F5- 58.732 ± 0.028 $\mu g/h/cm^2$) to 15% w/v (F7- 64.168 ± 0.071 $\mu g/h/cm^2$). Further increasing the concentration of IPM to 20%w/v (F8- 61.334 ± 0.015 $\mu g/h/cm^2$) not much variation in flux was observed and didn't show much effect on the penetration enhancement as discussed in Table 3. In formulation F7 maximum of $95.68 \pm 1.16\%$ drug release was achieved by the end of 24 h (IPM 15% w/v) and was optimized for further studies. It was observed that in all the reservoir systems the drug release mechanism depended on the combined action of swelling of poloxamer gel, wetting property of HPMC, penetration enhancers and rate controlling by eudragits.

The dissolution kinetics description was done by using various models like zero order,[17] first order,[18] Huguchi and Korsmeyer-Peppas.

$$\frac{Mt}{M\alpha} = K.t^n$$

Where Mt is the amount released at time t and Mα is the amount of drug present in the transdermal system t is the time taken to release K is the kinetic constant and n is the diffusional release exponent.[19]

Higuchi kinetic model is well-established to explain the diffusion model where it follows the Fick's first law and the drug release depends on the square root model.[2]

$$Q = \sqrt{t}$$

Where Q is the cumulative percentage drug release and t is the time. The regression values of F1-F7 for zero order (0.9945 ± 0.024-0.9972 ± 0.022) are very much linear compared to first order (0.9051 ± 0.034-0.9938 ± 0.018) indicating that the release followed zero order kinetics. Regression values of Higuchi equation (0.9079 ± 0.017-0.9627 ± 0.024) indicate that the diffusion is the dominant mechanism. The n values for Korsmeyer-Peppas equation are more than 1 (1.016 ± 0.025-1.227 ± 0.034) indicating that the drug release is by non-fickian diffusion super-case II transport mechanism.

Ex vivo permeation studies of reservoir systems of hydralazine hydrochloride

Based on the in vitro release and enhancement ratio formulation F7 was optimized for the further studies. Ex vivo permeation studies for F7 were carried out on modified Franz diffusion cell using epidermis of rat skin as a barrier. Figure 3 represents the cumulative percentage drug release vs time plot of ex vivo release and the release was found to be $92.96 \pm 1.2\%$ by the end of 24 h. The flux of ex vivo release was found to be $60.487 \pm 0.047 \mu g/cm^2/h$ with a lag time of 0.61 ± 0.021 h. No significant difference in drug release profile was observed between ex vivo and in vitro results ($p \leq 0.05$).

Tensile strength of the optimized formulation

Tensile strength of the rate controlling membrane of the optimized formulation was found to be 0.35 ± 1.22 kg/mm^2 with elongation at
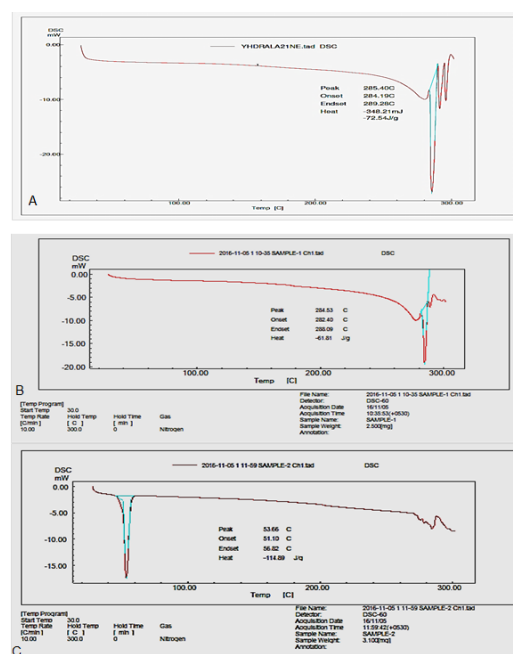

Figure 1: (A) DSC spectra of hydralazine hydrochloride, (B) DSC spectra of formulation, (C) DSC spectra of poloxamer 407. (Column width).
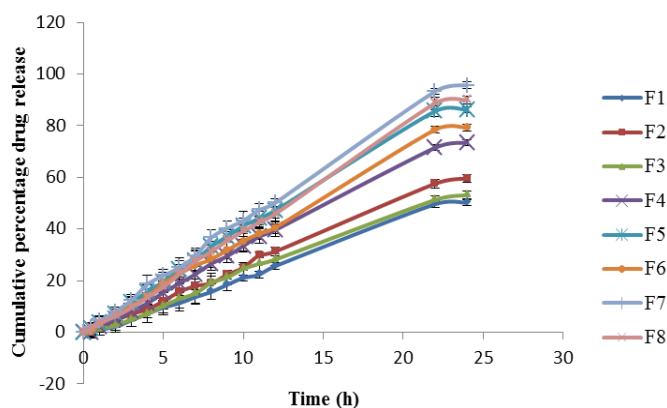

Figure 2: In vitro release profile of HZH from reservoir transdermal system in phosphate buffer saline media (Mean±SD, n=3) (Full page width).

Table 2: Pharmacotechnical properties of HZH loaded poloxamer gel reservoir transdermal system.

Code	%Drug content	%Weight variation	% Moisture content	% Moisture absorption
F1	98.7±0.2	1.4±0.15	2.51±0.01	2.47±1.21
F2	97.6±0.4	1.8±0.28	3.10±1.64	3.21±1.18
F3	98.8±0.3	1.4±0.34	3.45±1.89	3.67±1.02
F4	98.9±0.1	1.2±0.21	3.77±0.69	3.18±1.17
F5	98.5±0.2	1.5±0.45	2.99±0.41	3.41±1.94
F6	98.9±0.4	1.5±0.34	2.44±0.43	2.98±1.01
F7	99.3±0.9	1.2±0.11	2.70±1.99	2.99±0.94
F8	99.1±0.1	1.6±0.19	2.55±1.08	3.24±0.98

All values are expressed as Mean±SD, n=3

Table 3: *In vitro* release studies of HZH loaded poloxamer gel reservoir transdermal system.

Code	Q_{24}	Flux (µg/cm²/h)	Lag time (h)	Enhancement ratio	Diffusion coefficient (cm²/h)	Permeability coefficient (cm²/h 10²)
F1	50.16±1.09	34.691±0.105	0.60±0.014	-	0.100±0.001	6.938±0.074
F2	59.60±1.46	40.378±0.051	0.14±0.024	-	0.023±0.001	8.075±0.051
F3	53.12±1.55	36.665±0.067	0.29±0.016	-	0.048±0.001	7.333±0.06
F4	73.48±1.19	50.324±0.109	0.03±0.035	1.246±0.147	0.004±0.001	10.064±0.021
F5	86.12±1.11	58.732±0.028	0.41±0.039	1.454±0.218	0.068±0.002	11.746±0.041
F6	79.22±1.22	53.627±0.046	0.27±0.015	1.328±0.156	0.045±0.001	10.725±0.049
F7	*95.68±1.16	64.168±0.071	0.35±0.041	1.589±0.144	0.058±0.001	12.833±0.027
F8	89.91±1.37	61.334±0.015	0.14±0.021	1.518±0.158	0.023±0.001	12.266±0.028

Where Q_{24} is cumulative percentage drug release at 24h, All the values were expressed as mean±SD, n=3. *$p \leq 0.05$ was found to statistically significant.
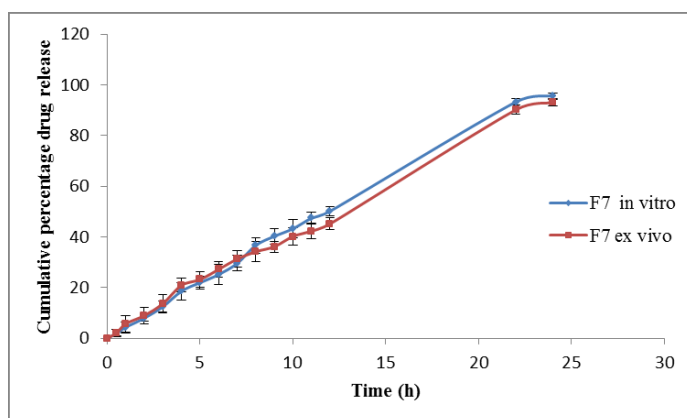

Figure 3: *In vitro* and *ex vivo* release profile of HZH (F7) from reservoir transdermal system in phosphate buffer saline media (Mean±SD, n=3). (Column width).
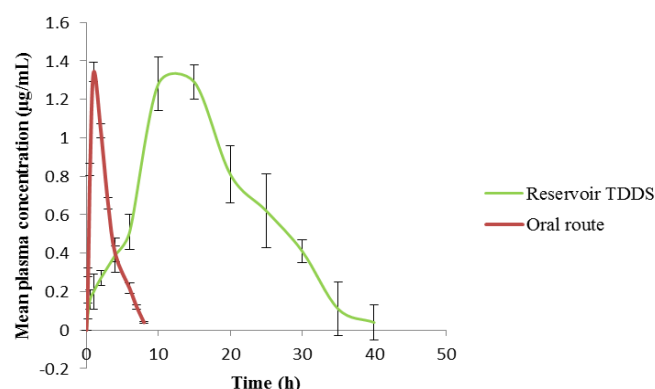

Figure 4: Mean plasma concentration time profile of HZH after oral and transdermal administration (F7) in male New Zealand rabbits (Mean±SD, n=5). (Column width).

break value 9.81 ± 1.28 kg/mm². The films were found to have moderate tensile strength and high elongation at break values.

Skin irritation studies of reservoir system of hydralazine hydrochloride

Skin irritation studies were conducted according to the Draize's method by scaling. Male albino rats were divided in to three groups (n=6). Group I was considered as control where no irritant was applied. Group II (medicated group) transdermal reservoir system (F7) was applied by moistening the rate controlling membrane. Erythema and edema was found to be 1.09 ± 0.312 and 1.04 ± 0.018 respectively. Group III standard irritant formalin was applied (0.8% v/v), erythema and edema was found to be 4.21 ± 0.045 and 4.75 ± 0.141 respectively. Irritation and edema was found to be negligible in medicated group when compared with formalin treated group and a significant difference was observed at $p \leq 0.05$. The reservoir system was found to be safe to apply to the skin.

Stability studies of reservoir system of hydralazine hydrochloride

Stability studies were conducted for formulation F7 according to ICH guidelines for 6 months and evaluated for drug content and *in vitro* drug release. Drug content in initial stages was found to be 99.3 ± 0.021% and after stability studies drug content was found to be 93.59 ± 0.421%. Cumulative drug release studies were conducted by maintaining the same conditions. The drug release in initial stage was found to be 95.68 ± 1.16% and after the accelerated conditions by the end of 6th month the drug release was found to be 91.08 ± 1.21%. No significant difference in drug release and drug content was observed between initial studies and after stability studies. The reservoir system was found to be stable and acceptable.

In vivo pharmacokinetic studies

The *in vivo* kinetic studies were conducted using male New Zealand rabbits and the results were quantified using HPLC. *In vivo* kinetic parameters for oral route and transdermal route were assessed from the mean plasma concentration *vs* time profile depicted in Figure 4. The *in vivo* absorption of drug in oral route was very fast and T_{max} was reached within 1 ± 0.09h with C_{max} of 1.34 ± 0.45 µg/mL and the MRT was 2.98 ± 1.24h, where as in case of transdermal route T_{max} was reached at 15 ± 0.09h with a C_{max} of 1.29 ± 0.24 µg/mL and the MRT was extended to 17.7 ± 0.37h as reported in Table 4. The extended plasma concentration profile curve in transdermal route depicted increased AUC value (25.760 ± 9.124 µg/mL/h) compared to oral route (4.409 ± 2.015 µg/mL/h) and the results reported the same, this can enhance the bioavailability of drug. The kinetic parameters clearly projected the extended release pattern of drug which can enhance the *in vivo* absorption of drug and clinical pharmacokinetics of HZH.

Table 4: *In vivo* pharmacokinetic parameters after oral and transdermal administration of HZH in male New Zealand rabbits.

Pharmacokinetic parameters	Oral	Transdermal route (F7)
C_{max} (µg/mL)	1.34±0.45	1.29±0.24
T_{max} (h)	1±0.09	15±0.09*
Elimination rate constant (h^{-1})	0.4±0.02	0.11±0.01
Half-life (h)	1.74±0.27	6.183±0.28*
$AUC_{(0-\infty)}$ (µg/mL/h)	4.409±2.015	25.760±9.124*
AUMC (µg/mL/h^2)	13.1535±6.121	413.991±12.694*
MRT (h)	2.98±1.24	17.7±0.37*

All the values were expressed as Mean±SD, n=5, *p≤0.05 was found to statistically significant

CONCLUSION

The thermo sensitive poloxamer gel drug reservoir transdermal system of HZH was successfully prepared. Poloxamer gel of 16% prepared by cold method remained maintained its texture at room temperature. The gel reservoir was translucent with slight yellow color of drug and had neutralized pH value. The *in vitro* release studies confirmed that the polymer concentration of rate controlling membrane, type of penetration enhancer and its concentration can affect the drug release profile. Combination of HPMC and E RLPO with IPM (15%) as penetration enhancer had maximum *in vitro* release and the *ex vivo* release was found to be not statistically significant. The *in vivo* release studies demonstrated that the reservoir transdermal system of HZH can provide extended drug release and can enhance the bioavailability with high AUC and MRT values.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest

REFERENCES

1. Nazzareno G, Marius MH, Marc H, Adam T, Jean-LV, Joan AB, *et al.* Guidelines for the diagnosis and treatment of pulmonary hypertension. Euro Heart J. 2009;30(20):2493-537.
2. Simonneau G, Galie N, Rubin L, Langleben D, Seeger W, Domenighetti G, *et al.* Clinical classification of pulmonary hypertension. J Am Coll Cardiology. 2004;43(suppl 1):S5-S12.
3. Kirsten R, Nelson K, Kirsten D, Heintz B. Clinical pharmacokinetics of vasodilators. Part II. Clinical Pharmacokinetics. 1998;35:9-36. (1998;34(6):457-82).
4. Chester EO, Norris GN, Raymond DD. Hydralazine hydrochloride. In: Klaus F, editor. Profile of drug substances, excipients and related methodology. New York: academic press Inc. 1979;8.pp.284.
5. Sarath-Chandra RG. A thesis entitled the preparation and characterization of poloxamer-based temperature-sensitive hydrogels for topical drug delivery. M. Pharm. Thesis, Faculty of pharmacy, The University of Toledo, Ohio, United States. 2013.
6. Pereira GG, Dimer FA, Guterres SS, Kechinski CP, Granda JE, Cardozo NSM. Formulation and characterization of poloxamer 407®: thermoreversible gel containing polymeric microparticles and hyaluronic acid. Quim. Nova. 2013;36(8):1121-5.
7. Taís GA, Guilherme MG, Eduardo MR, Victor HS, Osvaldo DF, Renata FVL. A Poloxamer/Chitosan *in situ* Forming Gel with Prolonged Retention Time for Ocular Delivery. Euro J Pharm Biopharma. 2010;75(2):186-93.
8. Mutalik S, Udupa N. Formulation development *in vitro* and *in vivo* evaluation of membrane controlled transdermal System of glibenclamide. J Pharm and Pharm Sci. 2008;8(1):26-38.
9. Arora P, Mukherjee B. Design, development, physicochemical, and *in vitro* and *in vivo* evaluation of transdermal patches containing diclofenac diethyl ammonium salt. J Pharm Sci. 2002;91(9):2076-89.
10. Amnuaikit C, Ikeuchi I, Ogawara K, Higaki K, Kimura T. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. Inter J Pharm. 2005;289(1):167-78.
11. Paranjyothy KL, Thampi PP. Development of transdermal patches of verapamil hydrochloride using carboxymethyl guar as a monolithic polymeric matrix and their *in vitro* release studies. Indian J Pharm Sci. 1997;59(2):49-54.
12. Zhao K, Singh J. *In vitro* percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release. 1999;62(3):359-66.
13. Peh KK, Choy FW. Polymeric films as vehicle for buccal delivery: swelling, mechanical and bio adhesive properties. J Pharm Pharma Sci. 1999;2(2):53-61.
14. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther. 1944;82(3):377-90.
15. Verma PRP, Ashok RC. Development of matrix controlled transdermal delivery systems of pentazocine: *In vitro / in vivo* performance. Acta Pharma. 2009;59(2):171-86.
16. Ubaidulla U, Reddy MVS, Ruckmani K, Ahmad FJ, Khar RK. Transdermal therapeutic system of carvedilol: Effect of hydrophilic and hydrophobic matrix on *in vitro* and *in vivo* characteristics. American Asco Phar Sci Pharm Sci Tech. 2007;8(1):E1-E8. (2007;8(1):E13-20)
17. Donbrow M, Samuelov Y. Zero order drug delivery from double-layered porous films: release rate profiles from ethyl cellulose, hydroxypropyl cellulose and polyethylene glycol mixture. J Pharm Pharmacol. 1980;32(1):463-70.
18. Wagner JG. Interpretation of percent dissolved-time plots derived from *in vitro* testing of conventional tablets and capsule. J Pharm Sci. 1969;58(10):1253-7.
19. Peppas NA. Analysis of fickian and non-fickian drug release from polymers. Pharm. Acta Helv. 1985;60:110-1.
20. Higuchi T. Mechanism of sustained action medication theoretical analysis of rate of release of solid drug dispersed in solid matrices. J Pharm Sci. 1963;52(12):1145-9.