International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 4, 2023

Original Article

DRUG IN ADHESIVE TRANSDERMAL SYSTEM OF FUROSEMIDE: *IN VITRO IN VIVO* EVALUATION

INDIRA MUZIB YALLAMALLI[1*], REVATHI MANNAM[1], RAMJIAMBEDKAR YELLAMELLI[2]

[1]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-517502, India. [2]College of Veterinary Science, Sri Venkateswara Veterinary Univesity, Garividi, Vizayanagaram-535101, Andhra Pradesh, India
[*]Corresponding author: Indira Muzib Yallamalli; [*]Email: yindira1415@gmail.com

Received: 25 Feb 2023, Revised and Accepted: 05 May 2023

ABSTRACT

Objective: The objective of the present work aims to check the effect of penetration enhancers on release kinetics in a novel drug in the adhesive transdermal system of furosemide.

Methods: The adhesive systems were evaluated for pharmacotechnical properties and *in vitro* permeation of the drug through the excised rat epidermis. Among the DURO-TAKs screened for *in vitro* permeation studies. The results were quantified using RP-HPLC. The optimized drug in the adhesive system was subjected to *in vivo* kinetic studies using New Zealand male rabbits. The adhesive systems were further evaluated for tack properties and skin irritation studies.

Results: DURO-TAK 2510 demonstrated a best permeation profile than DURO-TAK 2852. A combination of penetration enhancers was proved to more be efficient than alone, in the case of F9 (IPM: PG 7.5:2.5) maximum permeation of the drug (314.45±5.97 µg/cm^2) was observed by the end of the study with a flux of 9.2052±0.33 µg/cm^2/h. The optimized drug in the adhesive system was subjected to *in vivo* kinetic studies using New Zealand male rabbits. The studies confirmed that controlled release of the drug for a prolonged duration with an extended AUC and MRT decreased Cmax in adhesive systems compared to the oral route, which can provide a promising pharmacological effect the in the DIA system compared to the oral route.

Conclusion: The findings in the present study confirmed that drugs in adhesive systems can provide promising results and can enhance both bioavailability and patient compliance with a combination of penetration enhancers in the development of DIA systems.

Keywords: Furosemide, Penetration enhancers, Adhesive systems, *In vitro* permeation, *In vivo* kinetics

DOI: https://dx.doi.org/10.22159/ijap.2023v15i4.47681. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a group of diseases that share a common feature like progressive, obstructive pathological changes of the pulmonary microcirculation that lead to an increase in pulmonary vascular resistance. Pulmonary arterial hypertension is most often diagnosed in its advanced stages because of the nonspecific nature of early symptoms and signs [1, 2]. Symptoms include chest pain, weakness, shortness of breath, and fatigue. Echocardiography is a key screening tool in the diagnostic algorithm, where it provides an estimate of pulmonary artery pressure, either at rest or during exercise [3]. The goal of treatment is to control the symptoms, although the disease usually develops into heart failure.

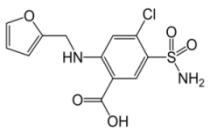

Fig. 1: Chemical structure of furosemide

Furosemide (fig. 1) 4-chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoyl benzoic acid, (M. Wt 330.7 Daltons, pKa-2.29) [4] is one of the potent diuretic agents used as a therapeutic agent for pulmonary arterial hypertension. Furosemide causes diuresis by blocking the absorption of sodium, chloride, and water from the filtered fluids in kidney tubules. Considering the clinical pharmacokinetics of furosemide it was evident that 65% of the drug is eliminated unchanged through urine with an approximate elimination half-life of 1h and bioavailability of 50% [5].

The poor pharmacokinetics may be due to the less absorption window of furosemide which is due to its extensive protein binding. The major setback for the oral therapy of furosemide is losing its natriuretic effect for diuresis between the dosages due to osmoregulation and volume regulation [6, 7]. It was clinically proven that intravenous infusion can improve the natriuretic and diuretic effects of furosemide [8]. The potency of the natriuretic and diuretic effects of furosemide can be enhanced with continuous infusion of the drug.

Controlled release mechanisms like transdermal drug delivery systems are the non-invasive mechanism where a continuous infusion of drug can be administered with the added advantage of minimizing the drug-related side effects [9, 10]. A continuous infusion mechanism can relieve this kind of disorder where effective therapeutic levels can be maintained in the plasma. This can improve the clinical pharmacokinetics of drug molecules. The very peculiar advantage of transdermal systems is that the medication can be terminated at any given time and these systems are proven to be patient-friendly.

Pressure-sensitive adhesive polymers (acrylic polymers) suitable for many drugs to solubilize, to load more amount of a drug and to get satisfactory drug flux. Drug-in adhesive (DIA) transdermal systems are very popular modern-day l systems where we can go from a 1d patch to 7d patch. A lot of advancements in adhesive technology, which are hypoallergenic and drug compatible, advanced the progress in DIA systems [11]. Functional-based Pressure sensitive adhesives patches shows excellent permeation through the skin, but recrystallization and degradation of the drug may be possible with internal and external conditions of the drug and components present in DIA matrix [12]. Variability and enhancement in flux can be achieved by using different combinations of penetration enhancers [13]. Many fatty acids also used to enhance the solubility and permeation of drugs through the skin [14, 15]. Hence in the present work study was carried to select suitable pressure sensitive adhesive and investigated the effect of penetration enhancers Further, they are subjected to *in vivo* kinetic studies.

MATERIALS AND METHODS

Materials

Furosemide is a gift sample received from IPCA laboratories, Mumbai, DURO-TAK 2852, DURO-TAK 2510were gift samples

received from Henkel Corporation, Germany, propylene glycol (PG), Isopropyl myristate (IPM), Oleic acid, Polyvinyl alcohol (PVA), Ethyl acetate, HPLC grade Acetonitrile and Methanol were purchased from S D Fine chemicals, Mumbai. All the reagent and chemicals used were of analytical grade.

Methods

HPLC method

A modified HPLC method from previous studies was established for the determination of furosemide [16]. Waters 2695 HPLC with class Empower-2 software with a high-speed autosampler and 2996 PDA detector with dual-wavelength at 210 nm were used. Inertsil ODS column C18 of 250X4.6 mm dimensions and 5 µl capacity was used. A mobile phase of phosphate buffer and acetonitrile in the ratio of 30:70 %v/v was used and pH was adjusted to 4.6 with triethanolamine. Diclofenac was used as an internal standard with a retention time of 4.02 min and for furosemide, the retention time was found to be 4.89 min.

Extraction of furosemide from plasma

250 µl of plasma and 50 µl of internal standard, 10 µl of furosemide was taken into a centrifuging tube and 2 ml of Acetonitrile was added. Cyclomixing was done for 15 sec and then vortexed for 2 min and finally centrifuged at 3200 rpm speed for 2 min. After the centrifugation organic layer was separated, filtered, and evaporated to dryness at 40 °C. The residue was dissolved in 20 µl of mobile phase and was injected into HPLC at a flow rate of 1 ml/min with a run time of 10 min.

Fabrication of DIA patches of furosemide

Table 1: Composition of the drug in an adhesive transdermal patch of furosemide

Formulation code	Drug (%)	Duro tak (5%w/w)	PE*	Ratio of PE used (%w/w)
F1	5	D-2852	-	-
F2	5	D-2510	-	-
F3	5	D-2852	PG	5
F4	5	D-2852	IPM	5
F5	5	D-2852	Oleic acid	5
F6	5	D-2510	PG	5
F7	5	D-2510	IPM	5
F8	5	D-2510	Oleic acid	5
F9	5	D-2510	IPM: PG	7.5:2.5
F10	5	D-2510	IPM: Oleic acid	7.5:2.5
F11	5	D-2510	IPM: PG	5:5
F12	5	D-2510	IPM	10

All the quantities are mentioned in % w/w, *Penetration Enhancers was mentioned as PE

DIA patches were prepared according to the formulation given in table 1. Furosemide is dissolved in 0.5 ml ethanol, then added to 10 ml of ethyl acetate solution and mixed well. The ethyl acetate drug mixture was added to the adhesive solution (5% w/w) along with the penetration enhancers. The adhesive mixture was stirred on a magnetic stirrer until a homogenous mixture is obtained for about 1h. The adhesive solution was then coated on the previously prepared PVA (6%w/v) backing membranes and the films were allowed to dry at room temperature. Dried adhesive patches were then stored in an aluminum membrane [17].

Evaluation of DIA patches of furosemide

After complete drying, DIA patches were evaluated for pharmacotechnical properties. Weight uniformity was calculated by taking the average weight of three transdermal patches and the results were reported in triplicate. The thickness of the film was measured three times with the help of a digital micrometer by excluding the thickness of the backing membrane and release liner and the mean observation was noted. Flatness was calculated by measuring the length of the strip of a patch after drying.

Drug content

The DIA patch with an area of 3.14 cm^2 was measured and added to phosphate buffer saline pH 7.4 (PBS) and kept stirring overnight to solubilize the drug completely. The drug solution was filtered to remove the un-dissolved contents. The drug content was determined using a UV-Visible spectrophotometer at 271 nm after appropriate dilutions.

***In vitro* permeation studies**

In vitro permeation studies were carried out on the excised skin of male albino rats using the modified Franz diffusion cell [18]. The release media used was pH 7.4 (PBS) as the drug is having complete solubility in the media. Before mounting onto the diffusion apparatus the skin was kept hydrated in PBS overnight. The receptor compartment contains the PBS of 30 ml and the donor compartment contains DIA patch of area 3.14 cm^2. The DIA patch was mounted on the excised epidermis and the whole assembly was kept on a magnetic stirrer with a fixed speed of 50 rpm, 37±0.5 °C temperature and sink conditions were maintained. A Sample of 3 ml was withdrawn at time intervals and replaced with the same volume of fresh solution to maintain the sink conditions. The drug release was estimated with HPLC. Samples withdrawn were centrifuged and an aliquot of 20µL along with internal standard was injected into the column to determine the drug content. All the results were reported in triplicate.

Drug release kinetics

The flux was calculated from the slope, lag time, and diffusion coefficient were calculated from the drug release plot (fig. 2). The enhancement ratio was calculated by using the below-given equation to assess the potentiality of penetration enhancers in enhancing the flux.

$$\text{Enhancement ratio} = \frac{\text{Permeability coefficient of drug with PE}}{\text{Permeability coefficient without PE}}$$

Where, PE = Penetration enhancer

The data obtained from permeation studies are fitted to various kinetic models to find the penetration of drug patterns.

Evaluation of adhesion properties

Probe tack test

The Probe tack test is done to know the adhesive property of DIA patches using probe tack apparatus (PT, 559, USA) [19]. A Sample of diameter 2.5 cm is attached to the stainless steel plate. A 0.5 cm diameter steel probe was attached to the sample with a force of 5N and kept in touch for 2 sec. Then the probe was removed at a rate of 300 mm/min, maximum force was recorded. An experiment repeated three times and the average value was noted. Tack force was calculated from the given formula.

Tack force = maximum force/contact area

Peel strength

A Peel adhesion test at 180° was carried out to measure the peel strength using adhesion apparatus (Chemi instruments, AR 1000, USA) according to American standard test methods (ASTM) [19]. A DIA patch of size 2.5x1.5 cm^2 is applied to the adherent plate, which is made of the bakelite. The DIA patch was smoothened with a 2-kg roller and pulled from the adherent plate at an angle of 180 ° at a constant rate 300 mm/min. The experiment was repeated three times and the average force was recorded in Newton. Peel strength was calculated from the below-given formula.

Peel strength = peel force/width of the patch

Stability studies

Following ICH guidelines at accelerated conditions, i.e. at 40±20C/75%RH stability, studies were carried out for an optimized DIA transdermal system. All the results were reported in triplicate.

Skin irritation studies

Skin irritation studies were conducted using male albino rats (180-200 gm). Rats were divided into three groups (n=6). Group I, II, and III are noted as the control group, where no patch has adhered to the skin, the medicated DIA patch and the formalin were applied (0.8%v/v), respectively. The applied areas were evaluated for erythema and edema by visual observation. The test was carried out for 7 days and on each day the erythema and edema scores noted and scored according to Draize's scale [20].

***In vivo* pharmacokinetic studies**

After taking approval from the Institutional Animal Ethics Committee 1677/PO/Re/S/2012/CPCSEA), pharmacokinetic studies were conducted by using male New Zealand rabbits (1.3-1.5 kg). They stabilized and kept fasting for 24h before commencing the standard diet given to rabbits. They were divided into three groups (n=5). Groups I, II, and III received the vehicle, furosemide (5 mg/kg) by oral route and furosemide DIA patch, respectively. Hair on the vertebral side of the rabbit was removed and the skin was cleaned with warm water and alcohol subsequently. Skin was patted to dry and the patch was applied to the skin with pressure. 0.5 ml of blood sample was collected into heparinized tubes from the left marginal ear vein at time intervals for the transdermal route. Blood samples were centrifuged immediately at 4000 rpm for 5 min and the plasma was collected and stored at-20 °C until analysis.

Pharmacokinetic analysis

Pharmacokinetic parameters like AUC, Cmax, and Tmax can be read directly from the plots (fig. 6, 7) and are calculated by non-compartmental analysis using PK solver, an add-in program for pharmacokinetic analysis in Microsoft Excel 2010.

Statistical analysis

All the results were represented as mean±SD. Statistical analysis of the data was carried out by using paired t-tests using Microsoft Excel 2010 software. A Significant difference was considered at $p \leq 0.05$ and at $p \leq 0.001$.

RESULTS AND DISCUSSION

Fabrication and characterization of DIA patches

Table 2: Pharmacotechnical properties of DIA transdermal patches of furosemide

Formulation code	Weight variation (%)	Thickness (µm)	Drug content (mg/cm^2)
F1	1.24±0.21	100±3.15	1.21±0.23
F2	1.09±0.22	101±1.25	0.99±0.03
F3	1.30±0.29	99±2.65	0.95±0.04
F4	1.07±0.31	102±2.18	0.97±0.15
F5	1.34±0.08	101±1.15	1.02±0.18
F6	1.06±0.11	98±1.15	1.08±0.24
F7	1.07±0.16	102±3.15	0.99±0.08
F8	1.22±0.18	102±1.55	1.14±0.09
F9	1.08±0.24	101±1.52	1.05±0.09
F10	1.22±0.16	100±1.15	1.06±0.15
F11	1.06±0.11	100±2.61	1.05±0.23
F12	1.17±0.30	100±1.58	1.01±0.23

All the values were expressed as mean±SD, n=3

Different DIA patches were prepared by using pressure-sensitive adhesives DT-2510 and DT-2852 by varying the concentrations of penetration enhancers. The concentration of adhesive was optimized to avoid the crystallization of the drug. DIA patches formulated with an optimized concentration of the adhesive were evaluated for pharmacotechnical properties and the results were reported in table 2. The drug content in all the patches was maintained at 1±1.0 mg/cm^2. Formulated DIA patches were found to have 100% flatness.

***In vitro* permeation studies**

The diffusion coefficient of a drug in a Pressure-sensitive adhesive (PSA) DIA patches is influenced by functional groups present in the drug. In the present study, Durotak-2510(D-2510) with hydroxyl functional group and Durotak-2852(D-2582) with carboxyl functional group were selected (Henkel Corporation). Furosemide was loaded at a concentration of 5% w/w of adhesive weight. The cumulative drug release profile of DIA patches of D-2510 and D-2852 without penetration enhancers, i.e., F1 and F2 was plotted (Fig. 2) and the results were reported in table 3. The release studies revealed a 2.1 times higher flux for D-2510 when compared with D-2852 [21]. The low penetration of the drug from D-2852 was due to carboxyl group interaction with the functional group present in the drug. The highest permeation of galantine was observed with hydroxyl-containing (Duro-Tak-2510) [22]. Previous studies reports that the selection of functional and non-functional pressure-sensitive adhesive depends on the functional groups present in the drug as they influences the drug release from DIA matrix [23, 24]. Drugs with secondary amino groups markedly interact with the PSA carboxyl group [25]. Miyajima *et al.* (1999) reported that the stronger the drug-polymer interactions the greater the reducing the drug diffusion rate [26]. In the current investigation also the drug release rate was found to be less from PSAs containing a carboxyl group (D-2852) than PSAs containing no functional group or a hydroxyl group (D-2510). Hence the pressure-sensitive adhesive D-2510 was selected for further studies.

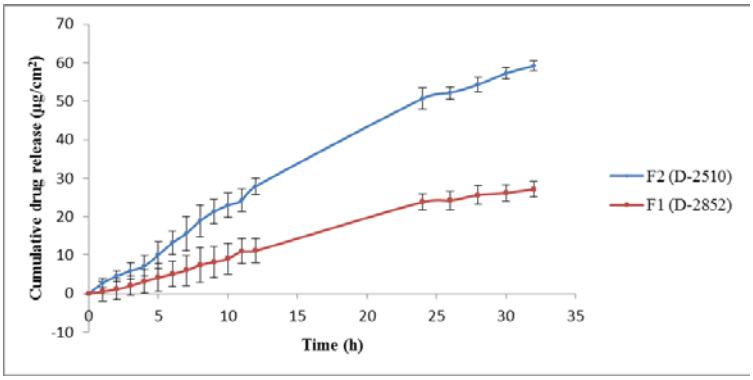

Fig. 2: *In vitro* permeation profile of furosemide from DIA patches using different adhesives (mean±SD, n=3)

Table 3: *In vitro* release studies of DIA transdermal patch of furosemide

Formulation code	Q (µg/cm²)	Flux (µg/cm²/h)	Enhancement ratio	Lag time (h)	Diffusion coefficient (cm²/h)
F1	27.11±3.17	0.9094±0.21	-	0.147±0.04	0.024±0.001
F2	59.16±4.22	1.9018±0.14	-	0.971±0.01	0.161±0.001
F3	145.07±5.61	4.4101±0.18	4.849±0.21	3.91±0.14	0.651±0.001
F4	175.09±5.11	5.4504±0.34	5.993±0.04	3.26±0.15	0.543±0.001
F5	136.19±4.21	4.1762±0.41	4.592±0.15	3.65±0.16	0.608±0.001
F6	185.21±4.89	5.8068±0.59	3.053±0.12	3.55±0.05	0.591±0.001
F7	223.08±5.18	6.9946±0.48	3.677±0.20	3.02±0.07	0.503±0.001
F8	206.18±6.21	6.3879±0.39	3.358±0.16	3.32±0.04	0.553±0.001
F9	*314.45±5.97	9.2052±0.33	4.840±0.13	5.76±0.05	0.960±0.001
F10	257.18±4.91	8.0220±0.45	4.218±0.11	3.55±0.09	0.591±0.001
F11	274.15±4.57	8.4449±0.51	4.440±0.18	3.63±0.05	0.605±0.001
F12	263.09±5.11	8.1855±0.46	4.305±0.19	3.51±0.05	0.583±0.001

Q is cumulative drug release in micrograms per cm² in DIA patch. All the values were expressed as mean±SD, n=3 (*p≤0.05)

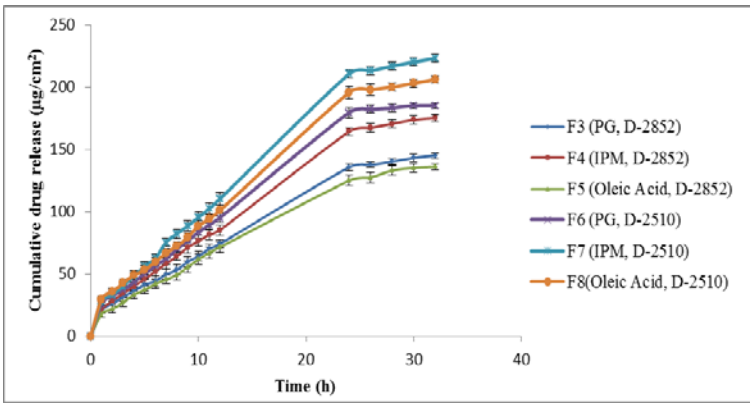

Fig. 3: *In vitro* permeation profiles of furosemide from DIA patches using different penetration enhancers (mean±SD, n=3)

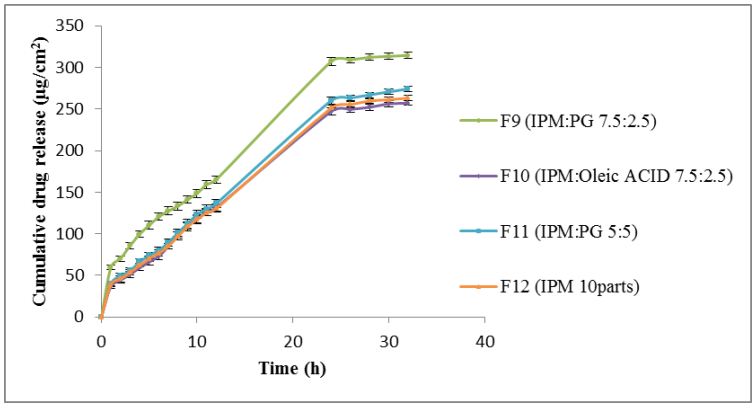

Fig. 4: *In vitro* permeation profiles of furosemide from DIA system using D-2510 and a combination of penetration enhancers (mean±SD, n=3)

Effect of chemical penetration enhancers on drug release

Generally, lipid-rich penetration enhancers are used for enhancing the drug permeation as they can easily solubilize and penetrate into the stratum corneum. Penetration enhancers like PG, IPM, and Oleic acid were selected and their effect on drug release kinetics on pressure-sensitive adhesives D-2510 and D-2852 was observed. Initially, the concentration of penetration enhancers was fixed at 5%. In DIA patches of D-2852 (F3-F5) (fig. 3), maximum cumulative drug release was observed with IPM and the minimum was obtained with oleic acid and the difference was less significant [27]. Whereas in case of DIA patches of D-2510 (F6-F8) (fig. 3), maximum cumulative drug release was observed with IPM [28] and minimum with oleic acid and the difference was found to be highly significant ($p \leq 0.05$). The cumulative drug release data and flux values were reported in table 3. The results depicted that IPM has maximum penetration enhancement than oleic acid.

Pressure-sensitive adhesive D-2510 and penetration enhancer IPM were optimized based on the above results. Further studies were done to enhance the drug permeation by doubling the penetration enhancer concentration i. e, 10%, and combinations of penetration enhancers in various concentrations were also tried (F9-F12) (Fig.4). Cumulative drug release results were reported in table 3. Formulation F9 with (IPM: PG 7.5:2.5) has produced exceptional results with a drastic increase in the amount of drug permeated when compared with F10, F11, and F12; the difference was found to be statistically significant ($p \leq 0.05$). As reported in table 3, enhancement in flux was observed with the addition of penetration enhancers and maximum flux was observed in DIA patches containing IPM, as a penetration enhancer. In F7 with IPM, at 5% w/w flux of 6.9946±0.48 µg/cm^2/h was observed and is the maximum flux achieved in the case of a single penetration enhancer, whereas the combination of penetration enhancers, maximum flux of 9.2052±0.33 µg/cm^2/h was achieved in F9 (IPM: PG 7.5:2.5) and the results were reported in table 3. The mechanism involves the combined action of penetration enhancer i. e, by altering the lipid properties of stratum corneum and by changing the thermodynamic activity of drug molecule [29]. For further confirming that a combination of penetration enhancers as beneficial in DIA patch using D-2510 was formulated by taking IPM alone at 10% (F12). The cumulative drug release was observed to be less when compared with the combination of penetration enhancers (F9-F11). The drug release in all the pressure-sensitive systems was controlled up to 32h and in the case of F9 maximum amount of drug was released.

The *in vitro* kinetic data summarizes that the drug release behavior all formulations except F1 followed the mixed order Higuchi kinetic model and the kind of diffusion is non-fiction which is confirmed by the 'n' values of Korsmeyer-Peppas.

Table 4: *In vitro* kinetics of DIA transdermal patches of furosemide

Formulation code	Zero-order	First order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	N
F1	0.9920	0.9953	0.9762	0.9921	1.1389
F2	0.9906	0.9828	0.9839	0.9898	0.9463
F3	0.9804	0.9891	0.9762	0.9777	0.6286
F4	0.9824	0.9896	0.9765	0.9864	0.6754
F5	0.9818	0.9915	0.9815	0.9828	0.6580
F6	0.9770	0.9838	0.9722	0.9727	0.6486
F7	0.9821	0.9876	0.9784	0.9765	0.6851
F8	0.9813	0.9848	0.9663	0.9628	0.6386
F9	0.9644	0.9592	0.9709	0.9691	0.5361
F10	0.9772	0.9828	0.9735	0.9676	0.6488
F11	0.9798	0.9810	0.9712	0.9668	0.6323
F12	0.9794	0.9803	0.9697	0.9640	0.6373

Adhesion properties of DIA patch of furosemide

Table 5: Adhesion properties of DIA transdermal patches of furosemide

Formulation code	Tack force (N/cm^2)	Peel strength (N/cm)
F1	3.51±0.21	1.39±0.15
F2	4.01±0.35	1.76±0.16
F3	3.22±0.28	1.06±0.11
F4	3.38±0.27	1.17±0.21
F5	3.04±0.28	0.55±0.22
F6	3.87±0.33	1.15±0.15
F7	3.98±0.37	1.29±0.18
F8	3.64±0.38	1.08±0.16
F9	3.91±0.25	1.66±0.21
F10	3.53±0.36	1.37±0.27
F11	3.87±0.33	1.44±0.23
F12	3.77±0.34	1.54±0.24

All the values were expressed as mean±SD, n=3

Peel strength and probe tack test results were reported in table 5. Stainless steel and Bakelite were used as substrates, as they were widely accepted and the Bakelite resembles the skin. In all the formulations, the amount of pressure-sensitive adhesive was kept constant and the probe tack test and peel strength exhibited no significant difference. The addition of penetration enhancers IPM, PG, and oleic acid have varied the adhesive strength and the variation was found to be less significant.

ICH guidelines were followed to conduct the stability studies for optimized formulation. Results showed that no significant difference in drug content, *in vitro* studies and tack properties. Pharmacotechnical properties showed a slight variation but were observed to be non-significant. It indicates the drug in the adhesive patch was found to be stable at accelerated conditions ($p \leq 0.05$). All the results were reported in table 6.

Skin irritation studies of DIA patch of furosemide

Table 7 summarizes the skin irritation studies of DIA transdermal patch of furosemide. By visual observation, edema and erythema were scored according to Draize's scale. After seven days of

treatment with the sample, slight swelling was observed in the control patch and medicated patch and is negligible when compared with the formalin-treated group. A Significant difference between the standard irritant and the DIA transdermal patch was observed (**p≤0.001). It indicates the drug-embedded patch was safe to use during treatment.

Table 6: Stability studies of optimized F9 DIA transdermal patch of furosemide

Parameters	0 Mo	1st Month	3rd Month	6th Month
Drug Content (mg/cm²)	1.05±0.09	1.04±0.06	1.03±0.21	1.03±0.06
In vitro permeation studies (µg/cm²)	314.45±5.97	305.69±6.91	304.08±5.21	300.5±4.08
Tack force (N/cm²)	3.91±0.25	3.54±0.28	3.57±0.48	3.41±0.27
Peel strength (N/cm)	1.66±0.21	1.59±0.24	1.57±0.28	1.54±0.58
Physical appearance	Acceptable	No change	No change	No change

All the values were expressed as mean±SD, n=3

Table 7: Skin irritation studies of F9 transdermal patch of furosemide

S. No.	Formulation code	Erythema	Edema
1	Control patch	0±0	0±0
2	Medicated patch (F9)	0.07±0.184**	0.19±0.134**
3	Formalin-induced group (0.8%v/v)	4.21±0.045	4.75±0.141

Data was expressed as mean±SD, n=6. Erythema scale: 0, none; 1, slight; 2, well defined; 3, moderate; 4, scar formation. Edema scale: 0, none; 1, slight; 2, well defined; 3, moderate; 4, severe (**p≤0.001).

Pharmacokinetics of DIA patch of furosemide

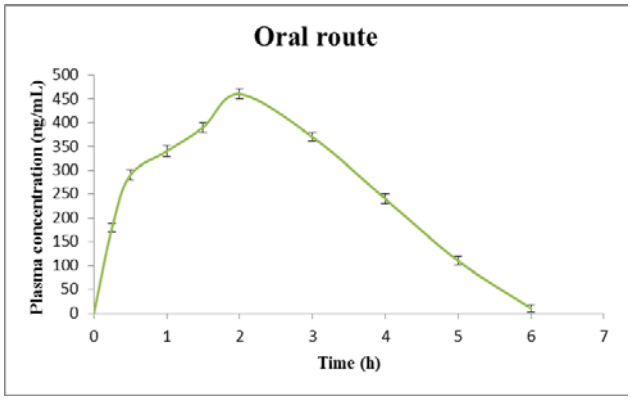

Fig. 5: Mean plasma concentration-time profile of oral route of furosemide (F9) in Newzealand male rabbits (mean±SD, n=5)

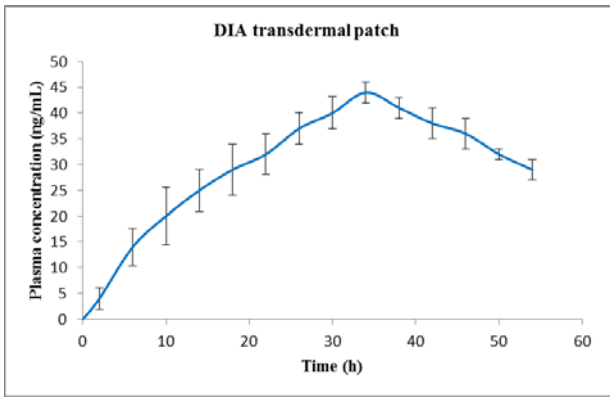

Fig. 6: Mean plasma concentration-time profile of DIA transdermal patch of furosemide (F9) in New Zealand male rabbits (mean±SD, n=5)

Since F9 has maximum cumulative drug release, it was selected for further *in vivo* studies. The mean plasma concentration-time profiles after oral administration of furosemide and transdermal administration of DIA patch of furosemide were plotted (fig. 5, 6) and the kinetic data was reported in table 8. Five rabbits were used in each group and the blood samples were taken at pre-determined time intervals. After oral administration of furosemide rapid absorption of the drug was observed with a half-life of 0.44±0.015h. C_{max} of 460±14.112 ng/ml was achieved early at T_{max} of 2±8.241h. Whereas in the case of DIA patches, steady release of the drug was maintained for a longer duration and it took 34±0.657h (T_{max}) to reach the maximum concentration of

44±10.151ng/ml (C_{max}), eventually C_{max} was reduced in case of DIA patch. The time required to reduce the half of the given drug concentration i.e., the half-life, was extended up to 25.6±9.125h in DIA patches. In case of oral route MRT of furosemide is 2.45±1.015h, whereas in case of the DIA patch MRT has increased exponentially up to 49.43±2.054h [24, 25]. This shows the presence of the drug for a longer duration (2days) in plasma possible with PSA-based DIA transdermal patches and it can be viewed from the extended plasma time profile as enhanced $t_{1/2}$ and MRT are common features for transdermal drug delivery systems. All the results had shown a statistically significant difference at p≤0.05. In our previous study also it was proved that solid lipid nanoparticles of furosemide loaded transdermal patches lowers the Cmax, enhances AUC and, extends MRT values [30].

Table 8: *In vivo* kinetics of oral route and optimized DIA transdermal patch of furosemide

Pharmacokinetic units parameters	Oral route	DIA transdermal patch (F9)
C_{max} ng/ml	460±14.112	44±10.151*
T_{max} h	2±0.241	34±8.657*
Half-life ($t_{1/2}$) h	0.44±0.015	25.6±9.125*
$AUC_{0-\infty}$ ng/ml/h	1562.14±34.142	6692.72±79.048*
AUMC ng/ml/h^2	3969.42±120.619	330824.19±251.615*
MRT h	2.45±1.015	49.43±2.054*

All the values were expressed as mean±SD, n=5 (*p≤0.05)

CONCLUSION

The Furosemide transdermal patches were prepared with different pressure-sensitive adhesives using a single and combination of penetration enhancers. As the penetration enhancers also has the adhesive property, all the patches shows good adhesive, tack and peel strength properties. *In vitro* release studies confirmed that PSA D-2510 along with a combination of PEs IPM: PG 7.5:2.5 shows more drug release, flux value and influenced on a positive note for further studies. *In vivo* studies also proved that low Cmax, prolonged MRT and extended AUC of DIA transdermal patch compared with the oral route can minimize multiple administrations of drug and can minimize drug-related side effects. Finally, it was concluded that DIA transdermal patches is superior over oral administration and also best option for the treatment of pulmonary hypertension. But their efficacy at the clinical level need to be studied further.

ACKNOWLEDGEMENT

Authors would like to thank DST-CURIE, DST-FIST for their technical support in Modern Instrumentation facilities, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visva Vidyalayam for providing the advanced lab facilities.

FUNDING

The research work was financially supported by UGC-BSR-RFSMS (Ref no. F. 7-346/2011).

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The author declares no conflicts of interest. The author is alone responsible for the content in the paper.

REFERENCES

1. Pietra GG, Edwards WD, Kay JM, Rich S, Kernis J, Schloo B. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. Circulation. 1989;80(5):1198-206. doi: 10.1161/01.cir.80.5.1198, PMID 2805258.
2. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G. Clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2004;43(12 Suppl):5S-12S. doi: 10.1016/j.jacc.2004.02.037, PMID 15194173.
3. Badesch DB, Abman SH, Ahearn GS, Barst RJ, McCrory DC, Simonneau G. Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest. 2004 Jul 1;126(1 Suppl):35S-62S. doi: 10.1378/chest.126.1_suppl.35S, PMID 15249494.
4. National Center for Biotechnology Information. PubChem compound database; CID=340; 2017. Available from: https://pubchem.ncbi.nlm.nih.gov/compind/3340. [Last accessed on 20 Jun 2017]
5. Jackson EK. Diuretics. In: Hardman JG, Limbird LE. editors. Goodman and Gilman's, the pharmacological basis of therapeutics. 10th ed. New York: Mcgraw-Hill; 2001. p. 757-87.
6. Klausner EA, Lavy E, Stepensky D, Cserepes E, Barta M, Friedman M. Furosemide pharmacokinetics and pharmacodynamics following gastroretentive dosage form administration to healthy volunteers. J Clin Pharmacol. 2003 Jul;43(7):711-20. doi: 10.1177/0091270003254575, PMID 12856384.
7. Antes LM, Fernandez PC. Principles of diuretic therapy. Disease-A-Month. 1998 Jun 1;44(6):254-68. doi: 10.1016/s0011-5029(98)90003-7, PMID 9679501.
8. Lee MG, Li T, Chiou WL. Effect of intravenous infusion time on the pharmacokinetics and pharmacodynamics of the same total dose of furosemide. Biopharm Drug Dispos. 1986 Nov;7(6):537-47. doi: 10.1002/bdd.2510070603, PMID 3828484.
9. Thomas BJ, Finnin BC. The transdermal revolution. Drug Discov Today. 2004;9(16):697-703. doi: 10.1016/S1359-6446(04)03180-0, PMID 15341783.
10. Allen LV, Popovich NG, Ansel HC. Ansel's pharmaceutical dosage forms and drug delivery systems. 9th ed. Philadelphia: Wolters Kluwer and Lippincott Williams & Wilkins; 2005. p. 298-315.
11. Banerjee S, Chattopadhyay P, Ghosh A, Datta P, Veer V. Aspect of adhesives in transdermal drug delivery systems. Int J Adhes Adhes. 2014 Apr 1;50:70-84. doi: 10.1016/j.ijadhadh.2014.01.001.
12. Weng W, Quan P, Liu C, Zhao H, Fang L. Design of a drug-in-adhesive transdermal patch for risperidone: effect of drug-additive interactions on the crystallization inhibition and *in vitro/in vivo* correlation study. J Pharm Sci. 2016 Oct 1;105(10):3153-61. doi: 10.1016/j.xphs.2016.07.003, PMID 27522527.
13. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta. 2009 Nov 1;1788(11):2362-73. doi: 10.1016/j.bbamem.2009.08.015, PMID 19733150.
14. Gwak HS, Chun IK. *In vitro* percutaneous absorption of tenoxicam from pressure-sensitive adhesive matrices across the hairless mouse skin. Arch Pharm Res. 2001 Dec;24(6):578-83. doi: 10.1007/BF02975169, PMID 11794539.
15. Arellano A, Santoyo S, Martín C, Ygartua P. Influence of propylene glycol and isopropyl myristate on the *in vitro* percutaneous penetration of diclofenac sodium from Carbopol gels. Eur J Pharm Sci. 1999 Jan;7(2):129-35. doi: 10.1016/s0928-0987(98)00010-4, PMID 9845796.
16. Mannam R, Yallamalli IM. RP-HPLC method for estimation of furosemide in rabbit plasma. J Chem Pharm Res. 2018;10:1-5.
17. Liu C, Fang L. Drug in an adhesive patch of zolmitriptan: formulation and *in vitro* /*in vivo* correlation. AAPS

PharmSciTech. 2015;16(6):1245-53. doi: 10.1208/s12249-015-0303-3. PMID 25771739.

18. 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. doi: 10.1016/s0168-3659(99)00171-6, PMID 10528073.
19. Hu Y, Wu YY, Xia XJ, Wu Z, Liang WQ, Gao JQ. Development of drug-in-adhesive transdermal patch for α-asarone and *in vivo* pharmacokinetics and efficacy evaluation. Drug Deliv. 2011 Jan 1;18(1):84-9. doi: 10.3109/10717544.2010.520350, PMID 20939674.
20. Draize JH. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther. 1944;82:377-90.
21. Nair AB, Kumria R, Al-Dhubiab BE, Attimarad M, Harsha S. Development of the transdermal delivery system of vildagliptin and its comparison with oral therapy. Indian J Pharm Educ Res. 2016 Jan 1;50(1):130-7. doi: 10.5530/ijper.50.1.17.
22. Hossain MK, Subedi RK, Chun MK, Kim EJ, Moon HS, Choi HK. Formulation and *in vitro* evaluation of transdermal drug delivery system for galantamine. J Pharm Investig. 2011;41(1):1-7. doi: 10.4333/KPS.2011.41.1.001.
23. Lei Y, Yang G, Du F, Yi J, Quan L, Liu H. Formulation and evaluation of a drug-in-adhesive patch for transdermal delivery of colchicine. Pharmaceutics. 2022 Oct 21;14(10):2245. doi: 10.3390/pharmaceutics14102245, PMID 36297680.
24. Pu T, Li X, Sun Y, Ding X, Pan Y, Wang Q. Development of a prolonged-release pramipexole transdermal patch: *in vitro* and *in vivo* evaluation. AAPS PharmSciTech. 2017 Apr;18(3):738-48. doi: 10.1208/s12249-016-0555-6, PMID 27245330.
25. Lal N, Verma N. Development and evaluation of transdermal patches containing carvedilol and effect of penetration enhancer on drug release. Res J Pharm Technol. 2018;11(2):745-52. doi: 10.5958/0974-360X.2018.00140.3.
26. Miyajima M, Koshika A, Okada J, Ikeda M. Effect of polymer/basic drug interactions on the two-stage diffusion-controlled release from a poly(l-lactic acid) matrix. J Control Release. 1999;61(3):295-304. doi: 10.1016/s0168-3659(99)00149-2, PMID 10477802.
27. Tank CJ, Kapse GK, Sarvaiya JI. Transdermal drug delivery of fluvastatin sodium: effect of permeation enhancers and pressure sensitive adhesive. J Pharm Res. 2013 May 1;6(5):573-8. doi: 10.1016/j.jopr.2013.03.029.
28. Jung E, Lee EY, Choi HK, Ban SJ, Choi SH, Kim JS. Development of drug-in-adhesive patch formulations for transdermal delivery of fluoxetine: *in vitro* and *in vivo* evaluations. Int J Pharm. 2015 Jun 20;487(1-2):49-55. doi: 10.1016/j.ijpharm.2015.04.012, PMID 25863117.
29. Taghizadeh SM, Soroushnia A, Mohamadnia F. Preparation and *in vitro* evaluation of a new fentanyl patch based on functional and non-functional pressure sensitive adhesives. AAPS PharmSciTech. 2010;11(1):278-84. doi: 10.1208/s12249-009-9366-3, PMID 20182829.
30. Mannam R, Yallamalli IM. Enhanced both in vitro and in vivo kinetics by SLNs induced transdermal system of furosemide: a novel approach. Recent Pat Drug Deliv Formul. 2017 Dec 1;11(3):187-97. doi: 10.2174/1872211311666171129115441, PMID 29189185.