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Design, development, *In vitro* and *In vivo* evaluation of transdermal patches of glimepiride

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Abstract
Transdermal drug delivery system topically administered owns discrete dosage forms in the form of patches that are capable of drug delivery for systemic effects at a predetermined and controlled rate to the systemic circulation. Transdermal drug delivery system provides continuous input of drugs with short biological half lives and eliminates pulsed entry into systemic circulation which often causes undesirable side effects. Glimepiride, a second generation sulfonylureas anti diabetic drug is effective for the treatment of Type II diabetes mellitus and acts by stimulating pancreatic β- cells to produce more insulin and lower the blood glucose level. Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is used to produce experimental diabetes (due to the selective destruction of the insulin producing pancreatic beta islets) in animals such as rabbits, rats, mice and dogs. Using Alloxan it is possible to induce different grades of severity of diabetes by changing its doses.

Keywords: transdermal drug delivery system, glimepiride, type II diabetes mellitus, alloxan

Introduction

Diabetes mellitus: Diabetes is a Pathophysiological situation of human body in which the body's ability to produce or to recognize the hormone insulin is impaired. It results in abnormal metabolism of carbohydrates leading to elevation in levels of glucose in the blood.

Diabetes is an important cause of prolonged ill health and premature mortality, and claims more death per year than HIV-AIDS with nearly 1 death every 10 seconds [1].

Diabetes mellitus is a metabolic disorder with an increasing global prevalence and incidence. High blood glucose levels are the symptoms of diabetes mellitus which causes an inadequate pancreatic insulin secretion by β- cells. Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins originating from defective insulin secretion, insulin action, or both.

To counter the hyperglycemic situation, the body tries to arrest it by withdrawing water from the cells and sending it into the bloodstream. The excess sugar is excreted in the urine. This is why diabetics present with constant thirst, drinking large amounts of water and Polyurea as the cells try to get rid of the extra glucose.

Glimepiride, a second generation sulfonylureas anti diabetic drug is effective for the treatment of Type II diabetes mellitus and acts by stimulating pancreatic β- cells to produce more insulin and lower the blood glucose level. It has shorter half life of 5-7 hrs, low bioavailability and extensive first pass metabolism. However, the drawback for the use of Glimepiride as oral dosage form is attributable to its low aqueous solubility and slow dissolution rate, which lead to low oral bioavailability.

Due to its short elimination half life (5-7 hrs) repeated doses are required which may cause different side effects such as headache and gastro intestinal disorders. It is required to maintain the therapeutic level, so it was thought to select Transdermal Drug Delivery System. Sustained delivery of Glimepiride through a transdermal route will also help to avoid toxicity due to sudden high blood concentration.

Hence seeing the importance of Glimepiride in treating Type II diabetes mellitus, it was thought worthable to develop and formulate into transdermal patches so as to release the drug in systemic circulation. It was further thought that such a study, if comes with fruitful results, might be able to improve the patient compliance.

Goal of any drug delivery system is to provide a therapeutic amount of drug to proper site in the body and maintained the desired drug concentration [2]. The most rapid advancement area of transdermal drug delivery is represented by Transdermal drug delivery system [2]. Transdermal drug delivery system topically administered owns discrete dosage forms in the form of patches that delivered drugs for systemic effects at a predetermined rate and a controlled rate to the systemic circulation. Transdermal drug delivery system provides continuous input of drugs with short biological half lives and eliminates pulsed entry into systemic circulation which often causes undesirable side effects. Transdermal drug delivery system also provides controlled and constant administration of drugs. To decrease and avoid the limitation allied for oral and parenteral administration of drugs; is alternatively appealed by Transdermal drug delivery system. As a form of controlled drug delivery, these patches are extremely commodious, user friendly and provide easy administration. Transdermal drug delivery along with sustain drug release providence reduces the intensity of action and also reduces the side effects of oral therapy [3]. TDD is one of the non-invasive methods for drug administration. Applying the formulation transdermal on skin shows an improved and continuous, sustained release of drug achieved and patient compliance is improved.

Conventional system of medication which requires multi dose therapy are full of problems. The medications may not be absorbed if it is released too slowly. If it is delivered too rapidly, the patient may suffer untoward effects and its desired effects may not last as long as needed. If patient is expected to take medicine more than two times a day, compliance will be adversely affected. The market for transdermal product has been in a significant upward trend that is likely to continue for the foreseeable future. The market share of TDDS is gaining momentum day by day and the number of API'S has also increased tremendously.

Experimental Section

Materials and Methods

Pure Glimepiride was obtained as a gift sample from Aristo Private Limited, Andheri Mumbai. The other chemicals were obtained from authenticated manufacturers i.e. HPMC K4M, HPMC K 15M, HPMC K 100M (Leo Chem. Banglore), Cellulose acetate (Loba Chemie Pvt ltd., Mumbai), Polyethylene glycol-200 (Loba Chemie Pvt ltd., Mumbai), Dimethyl sulphoxide (Thomas Baker (chemicals) ltd., Mumbai), Ethanol (Changshu Yangyuan Chemical., China), Chloroform (Ramkem laboratories, New Delhi), Dichloromethane (M/s Oxford Laboratory., Thane) Franz diffusion cell (Bharat instruments and chemicals, Hisar), Alloxan (Loba Chemie Pvt ltd., Mumbai), Blood glucose kit [Glucose reagent. Glucose standard, Glucose diluent, (Transia Bio medicals Ltd, Solan)]. 42 Male Wistar rats (100g-175g) were purchased from DFSAH (Disease Free Small Aniaml House), LUVAS (Lala Lajpat Rai University of Veterinary and Aniaml Sciences). Animals were housed in animal house. The animals were maintained on standard pellet chow diet and water *ad libitum*. The rats were exposed to 12 hr light and 12 hr dark cycle and were acclimatized to the laboratory conditions prior to the behavioral study.

Formulation of Transdermal Patches

For the formulation of transdermal films, mercury was used as the backing membrane and spread uniformly on a glass petri dish which was kept on a table with smooth horizontal surface.

A glass bangle as a mould was placed in a petri dish over mercury surface and about 10 mL of the solution was poured on the mercury (34.19 cm^2area). The rate of evaporation was controlled by inverting the funnel over the mould. This procedure was repeated for making each film.

After 24 hours, the dried patches were cut into 2 cm diameter, wrapped in aluminium foil and stored over fused calcium chloride in desiccators at room temperature for further use.

Rate controlling membranes were prepared by dissolving Cellulose acetate: HPMC K4M, Cellulose acetate: HPMC K 15M, Cellulose acetate: HPMC K100 M (total weight of polymer was kept 400 mg) in 10 ml solvent (Casting solvent chloroform, DCM ethanol).

PEG-200 (30%w/w) was used as plasticizer and Tween-80 (5%w/v) was used as a permeation enhancer [4].

Care was taken to keep a uniform environment so that variations based on such conditions could be omitted. All films were prepared batch wise so as to avoid any confusion the compositions of polymeric films are shown in table 1

Table 1: Composition of drug loaded film

Batch code	Polymers	Drug (mg)	Polymer ratio*	Plasticizer PEG 200 %(w/w)**	Permeation enhancer Tween 80 % w/w***	Casting Solvent 2 : 2 : 1
C_1K_4	CA : HPMC K4 M	90	2 : 1	30	5	Chloroform : DCM : Ethanol
C_2K_4	CA : HPMC K4 M	90	1 : 1	30	5	Chloroform : DCM : Ethanol
C_3K_4	CA : HPMC K4 M	90	1 : 2	30	5	Chloroform : DCM : Ethanol
C_1K_{15}	CA : HPMC K15 M	90	2 : 1	30	5	Chloroform : DCM : Ethanol
C_2K_{15}	CA : HPMC K15 M	90	1 : 1	30	5	Chloroform : DCM : Ethanol
C_3K_{15}	CA : HPMC K15 M	90	1 : 2	30	5	Chloroform : DCM : Ethanol
C_1K_{100}	CA : HPMC K100 M	90	2 : 1	30	5	Chloroform : DCM : Ethanol
C_2K_{100}	CA : HPMC K100 M	90	1 : 1	30	5	Chloroform : DCM : Ethanol
C_3K_{100}	CA : HPMC K100 M	90	1 : 2	30	5	Chloroform : DCM : Ethanol

*Total polymeric weight = 400mg

**Density of PEG 200 =1.124g/ml therefore, Amount used 0.106 ml.

Solubility analysis

The equilibrium solubility studies or saturation solubility of Glimepiride were carried out in phosphate buffer saline pH 7.4, methanol, chloroform. An excess amount of Glimepiride was added to each 10 mL solvent (PBS 7.4, ethanol, chloroform and DCM) taken in 50 mL conical flasks separately and then placed in a mechanical shaker at room temperature for 24 h. At the end of 24 h, samples were filtered through the whatman filter paper and amount of Glimepiride dissolved in each solvent was determined spectrophotometrically [5].

Solubility of drug in each solvent was calculated by using equation: -

$$\text{Solubility} = \frac{\text{Sample abs.} \times \text{dilution factor}}{\text{Slope of std. curve.} \times 1000}$$

Determination of partition coefficient

Partition coefficient of Glimepiride was determined by taking 10mL of n-octanol which was saturated with aqueous phase (7.4 PBS) by stirring with magnetic stirrer and kept undisturbed for half an hour. After that, 10 mg of drug was added to this solution and was shaken on mechanical stirrer. Two layers were separated through separating funnel and filtered through whatman filter paper 0.45µ. Using U.V spectrophotometer at wavelength 228nm, partition coefficient of Glimepiride was determined. The experiment was performed in triplicate [6].

The partition coefficient of drug ($K_{O/W}$) was calculated using equation.

$$K_{O/W} = \frac{\text{Concentration of drug in n- octanol}}{\text{Concentration of drug in PBS}}$$

Drug excipients compatibility studies

The pure drug Glimepiride and mixture of drug with polymers HPMC K4M, HPMC K15M, HPMC K100M and cellulose acetate were mixed separately with IR grade KBr and corresponding pellets were prepared by applying pressure in hydraulic press. The pellets were scanned over a wave number range of 4000-400 cm^{-1} [7, 8].

Evaluation of Transdermal patches

Transdermal patches have been developed to improve clinical efficacy of the drug and to enhance patient compliance by delivering smaller amount of drug at a predetermined rate. This makes evaluation studies more important in order to ensure their desired performance and reproducibility under the specified environmental conditions.

Evaluation of drug free films

The composition of Transdermal patches has a profound influence on the physical and mechanical properties as well as permeability of drugs. Transdermal patches of 3.14 cm^2 were taken out from each casted film after complete drying and evaluated for the following physiochemical properties.

- **Folding endurance**

This test was carried out to check the efficiency of plasticizer and strength of the prepared patches. Folding endurance of the film was determined repeatedly by folding a small strip (2cm x 2cm) at the same place till it breaks. The number of times the film can be folded at the same place without breaking gives the value of folding endurance.

- **Percentage moisture absorption**

The films were weighed accurately and placed in the desiccator containing 100mL of saturated solution of aluminium chloride, which maintains 84 % RH. After 3 days, the films were taken out and reweighed. The percentage moisture absorption was calculated by: -

$$\text{Moisture absorption (\%)} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

- **Percentage moisture loss**

The films were weighed accurately and kept in a desiccator containing anhydrous calcium chloride. After 3 days the films were taken out and reweighed. The moisture loss was calculated by: -

$$\text{Moisture loss (\%)} = \frac{\text{Initial weight- Final weight}}{\text{Final weight}} \times 100$$

- **Swellability**

The films (3.14cm^2) were weighed and placed in a petri dish containing 10 mL of double distilled water and was allowed to imbibe. Increase in weight of patch was determined at present time intervals, until a constant weighed was observed. The degree of swelling (S) was calculated by: -

$$S\% = \frac{W_t - W_0}{W_o}$$

Where

S% is percent swelling

W_t is the weight of patch at time t

W_o is the weight of patch at time zero

Evaluation of drug loaded films

- **Drug content uniformity**

The film was dissolved in pH 7.4 phosphate buffer (upto 1 mL) and stirred for 30 minutes. The resulting solutions were further diluted with ph 7.4 phosphate buffer and filtered. A blank was prepared in the same manner using drug free patches to neglect the absorption of formulation components. After filteration, the drug content was determined spectrophotometrically at 228 nm [9, 10].

- ***In-vitro* permeation studies**

The *in-vitro* release profile is an important tool that regulates the predictions in advance *Viz.* how a drug might respond *in-vivo. In-vitro* studies were performed using a Franz Diffusion cell with a receptor compartment capacity of 25 mL. The receptor compartment was filled with phosphate buffer saline pH 7.4 and egg membrane was mounted between the donor and receptor compartment of the diffusion cell. The prepared Transdermal film was placed on egg membrane. The whole assembly was kept on a magnetic stirrer and the solution was stirred continuously at 600 rpm using a magnetic bead at 37$\pm$2 ºC. The 0.2 mL of sample was withdrawn at different time interval and replaced with equal volume of diffusion medium. Sample was analyzed using spectrophotometer at 228 nm for the determination of Glimepiride [11].

Skin irritation study [12].

The selected formulation (C_3K_4) was tested for its potential to cause skin irritation or sensitization in rats.

The procedure used for skin irritation study was as follows-

Procedure

a. The rats were divided into three groups (each group having 6 rats).
b. On the previous day of experiment, the hair of abdominal portion of the rat was removed physically with the help of hair removal cream and the skin was cleared with rectified spirit.
c. The animals of the group I served as the control and received no prior treatment.
d. The animals of group IV served as the test group and treated with transdermal patches of Glimepiride.
e. The animals of group VI served as the Alloxan + Glimepiride and treated with transdermal patches of Glimepiride after injecting Alloxan.
f. After 24 hrs of exposure, each patch was removed with the help of alcohol swab and the test site was rinsed with tap water.
g. After 24 hrs of application, the application sites were examined and scored for signs of erythema according to the Draize dermal scoring criteria.
h. The erythema scores were given from 0-4 depending upon the degree of erythema according to Table 2

Table 2: Draize scoring criteria

S. No	Erythema formation	Score assigned
1	No erythema	0
2	Very slight erythema	1
3	Well defined erythema	2
4	Moderate to severe erythema	3
5	Severe erythema	4

Pharmacodynamic activity

Alloxan induced diabetes mellitus in rats

The anti-diabetic effect of selected transdermal formulation was evaluated by Alloxan induced diabetes in rats [13].

Alloxan Model

Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is used to produce experimental diabetes (due to the selective destruction of the insulin producing pancreatic beta islets) in animals such as rabbits, rats, mice and dogs. With this agent it is possible to produce different grades of severity of the disease by varying the dose of Alloxan used [15]. After overnight (12 hrs.) fasting a single dose of Alloxan (150 mg/kg) *i.p* is administered as a 5% w/v in distilled water. After administration of drug, the animals were allowed to free access on food and water for rest 12 days period [14, 15].

Procedure

1. The rats were divided into four groups. (Each group having 6 rats).
2. On the previous day of the experiment, the hair of the abdominal portion of the rats was removed physically with the help of hair removal cream and the skin was cleared with rectified spirit.
3. The animals of group I served as the control and received no treatment.
4. The animals of group II served as the Alloxan *per se.* Alloxan was dissolved in distilled water and injected intraperitoneally (150 mg/kg) after 12 hours of fasting to induce hyperglycemia in rats.
5. The animals of group VI were administered with Alloxan followed by application of Glimepiride transdermal patch (C_3K_4) on 12th day of experiment
6. The animals of group VII were administered with Alloxan followed by application of Glipizide transdermal patch on 12th day of experiment

Results

Solubility analysis

The solubility of Glimepiride found in different solvents showed that Glimepiride was more soluble in chloroform than that of other solvents *viz.* PBS 7.4, DCM and ethanol. Comparative solubility profile of drug in various solvents is shown in fig.1.

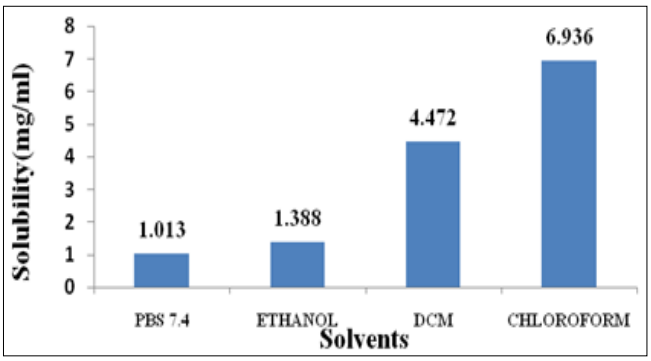

Fig 1: Histogram of solubility (mg/mL) of Glimepiride

Determination of partition coefficient

Partition coefficient study of Glimepiride was done by utilising the shake flask method. Partition coefficient value of Glimepiride was found to be 1.365±0.007 which is in the range of ideal properties of drug candidate for transdermal patch.

Drug- excipients compatibility studies

The drug Glimepiride along with the physical mixture of HPMC K4M, HPMC K15M, HPMC K100M and cellulose acetate was kept at different environmental conditions to observe the physical compatibility of the drug with excipients. A comparison of the initial sample, control sample and samples kept at different environmental conditions for physical changes were observed with respect to color, odour, and lump formation. The result obtained from physical compatibility studies were confirmed by FTIR studies figure 2 (a, b) compares the FTIR spectra of pure drug and its mixture with HPMC K4M, HPMC K15M, HPMC K100M and cellulose acetate.

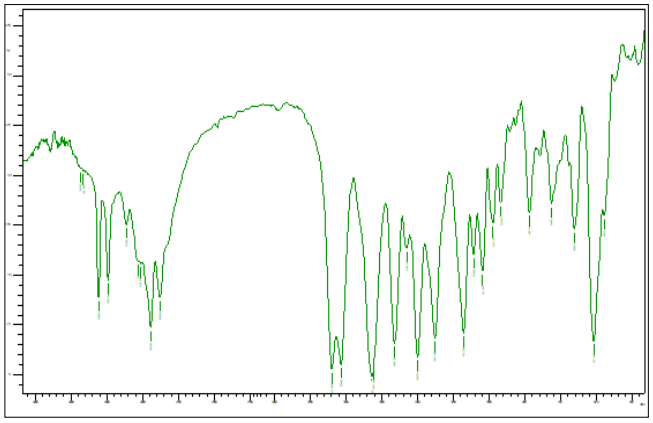

Fig 2(a): FTIR spectrum of pure Glimepiride

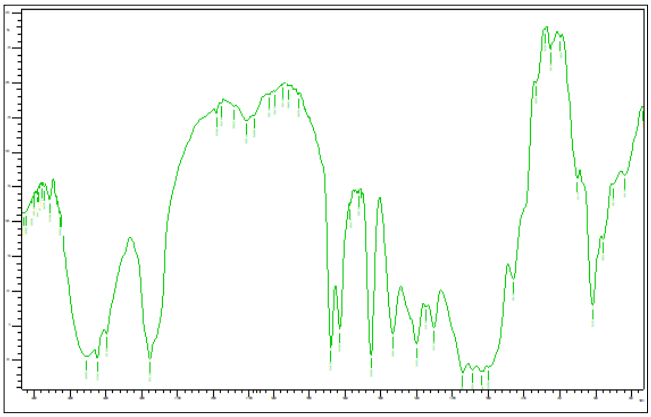

Fig 2(b): FTIR spectrum of physical mixture of Glimepiride with HPMC K4M, HPMC K15M, HPMC K100M and Cellulose acetate.

Evaluation of transdermal patches

Evaluation of drug free transdermal patches

- **Folding endurance**

Folding endurance was measured manually and its results were summarized in Table 3. Folding endurance test results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

- **Percentage moisture absorption**

The results of the percentage moisture absorption studies for different formulations are shown in Table 3. Results indicated that increase in concentration of hydrophilic polymer (HPMC) was directly proportional to the increase in moisture absorption of the patches and it fulfills the acceptance criteria for the transdermal patches.

- **Percentage moisture loss**

The results of the percentage moisture loss are shown in Table 3. The results were found that there are low values of moisture loss, that may help the formulations to remain stable and from being completely dried and reduce the brittleness of the film during long storage. Results also revealed that high concentration of hydrophilic polymer (HPMC) have more moisture loss than the hydrophobic polymer (Cellulose Acetate).

- **Swellability index**

Table 3 shows the present Swellability index values of various Glimepiride transdermal formulations. Swellability varies with the nature and composition of the patches. It is evident from the results that increase in concentration of hydrophilic polymer increased the surface swellability and consequently increased the water penetration within the matrix and it is in well accordance with the criteria for transdermal patches [4].

Table 3: Physicochemical parameters for drug free films

#	Batch code	%Moisture absorption	%Moisture loss	Folding endurance	Swellability index (%)
1	C_1K_4	1.60±0.385	1.728±0.127	347±3.78	29.831±0.023
2	C_2K_4	1.566±0.869	2.696±0.284	309±6.11	37.423±0.015
3	C_3K_4	4.544±0.149	4.207±0.236	421±4.58	43.351±0.013
4	C_1K_{15}	2.853±0.492	3.777±0.371	413±11.23	41.078±0.031
5	C_2K_{15}	2.811±0.170	3.699±0.751	390±22.59	45.009±0.031
6	C_3K_{15}	5.996±3.24	4.476±0.195	314±11.15	48.147±0.161
7	C_1K_{100}	4.23±0.652	3.669±1.292	316±9.64	29.192±0.004
8	C_2K_{100}	4.111±0.337	3.926±0.279	278±11.0	40.391±0.025
9	C_3K_{100}	4.72±0.839	5.233±0.672	255±26.83	40.722±0.032

Evaluation of drug loaded films

- **Drug content uniformity**

The drug content uniformity of formulations (C_1K_4- C_3K_4, C_1K_{15} – C_3K_{15} and C_1K_{100} – C_3K_{100}) was determined by UV spectrophotometric method. The results of drug content varies between 95.03±.001to 98.17±.002 as shown in Table 4 which indicate that the process employed to prepare patches in the study was capable of producing patches with uniform drug content and minimal patch variability. Comparative drug content of various formulations in the form of histogram are shown in figure 3.

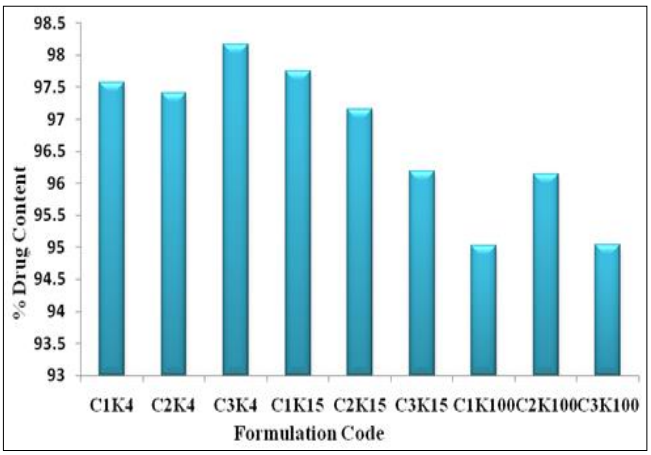

Fig 3: Histogram showing percentage drug content transdermal patches of (C_1K_4- C_3K_4, C_1K_{15} – C_3K_{15} and C_1K_{100} – C_3K_{100})

- ***In vitro* skin permeation studies**

The *In vitro* skin permeation studies were conducted to investigate the effect of polymer ratio on the release rate of Glimepiride patches. Release of a drug delivery system mainly involves diffusion factor and drug polymer affinity that control release of drug from formulation. In this study different grades of hydrophilic polymers (HPMC K4M, HPMC K15M and HPMC K100M) and hydrophobic polymer (Cellulose acetate) are used to formulate the Glimepiride transdermal patches. Formulation C_3K_4 exhibited the greatest 78.42±0.56 % of drug release value at 16 hrs and formulation C_1K_{100} exhibited the lowest 58.63±0.57% value of drug release at 16 hrs.

The cumulative percentage of drug permeated from formulations containing hydrophilic polymer was found to be at faster rate than the formulations containing hydrophobic polymer as hydrophobic polymer helps to retain the drug in the matrix system by reducing the penetration of solvent molecule into the patch.

In addition to the nature of the polymer, concentration of the polymer also affects the drug release, as the concentration of hydrophilic polymer increased, drug release also increased. The viscosity grade of hydrophilic polymers also affects the drug release, as the viscosity of hydrophilic polymer (HPMC) decreased from high viscosity grade to low viscosity grade, drug permeation increased.

The results indicated that the permeation of Glimepiride was affected by varying the proportions and viscosity grade of hydrophilic polymers and it was found that from all the formulations C_3K_4 appears to be the best selection of matrix transdermal patch of Glimepiride. Hence, it was selected as the best formulation by virtue of its maximum release and skin permeation. Drug release profile from various formulations are shown in fig. 4 and comparative drug permeation profile of all the formulations is shown in fig. 5.

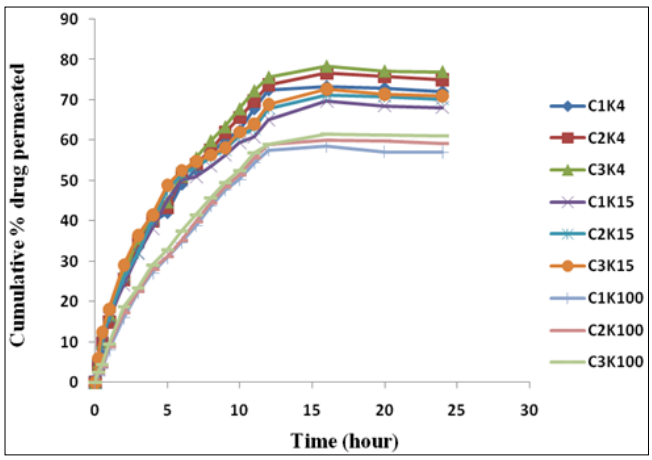

Fig 4: Drug permeation profile of formulations C_1K_4 to C_3K_{100}

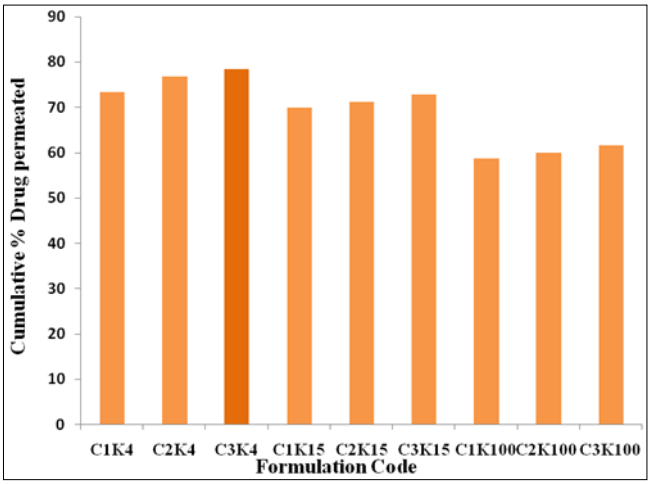

Fig 5: Histogram of cumulative % drug release of formulation C_1K_4- C_3K_{100}

Skin irritation study

Results of skin irritation study of formulation C_3K_4 is shown in Table 4, which revealed that animals containing Glimepiride patch showed no signs of erythema throughout the period of 24 hr. Hence, the formulated transdermal patches were declared to be free from skin irritation and compatible with the studied rat skin. Figure 6 (a) depicts rat skin after removal of hair, (b) rats having transdermal patches of formulation C_3K_4 on skin and (c) rat skin after removal of patch.

Table 4: Skin irritation scores following application of transdermal patch of selected formulation (C_3K_4)

Animal code	Group I (Control)	Group IV (Test "a'' group)	Group VI (Test "b'' group)
	Erythema	Erythema	Erythema
I	0	0	0
II	0	0	1
III	0	0	0
IV	0	1	0
V	0	0	0
VI	0	0	0

a) Erythema scale: - 0- No erythema; 1- very slight; 2- well defined; 3- moderate to severe; 4- severe erythema

Group I: (Normal Control), Group IV: (Test "a'' group i.e Glimepiride *per se*), Group VI: (Test "b'' group i.e Alloxan+ Glimepiride)

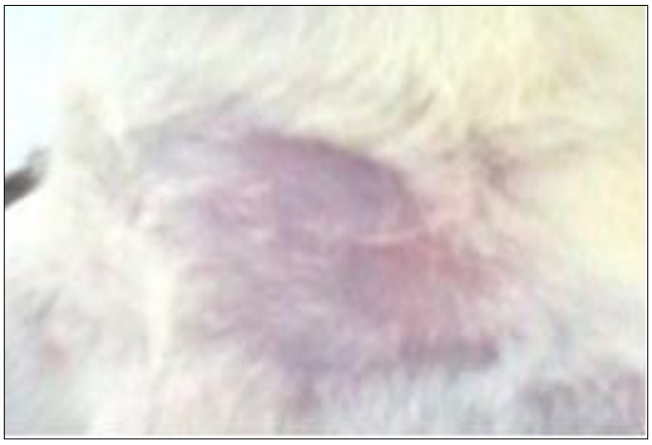

Fig 6(a): rat skin after removal of hair

Fig 6(b): Rat skin having transdermal patch (C_3K_4)

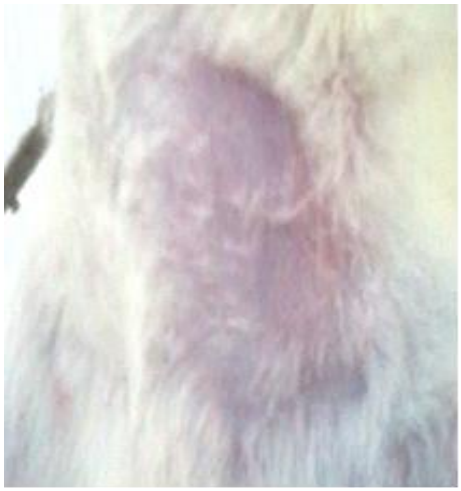

Fig 6(c): rat skin after removal of patch.

Pharmacodynamic activity of Glimepiride

The results of diabetic anti diabetic activity of selected formulations of Glimepiride are shown in table 5 and 6

Table 5: Blood glucose levels of Normal control, Alloxan *per se* and DMSO *per se*

Group	Mean ± S.D (mg/dl)				
	Day 0	Day 3	Day 6	Day 9	Day 12
I	92.3±1.46	94.3± 1.32	96.5± 1.52	90.6± 1.37	88.4± 1.46
II	112.6±1.45	215.6± 1.30	309.7± 2.77	356.3± 1.47	295.3± 1.60
III	92.3± 1.44	94.2± 1.32	96.4± 1.53	90.6± 1.37	88.4± 1.46

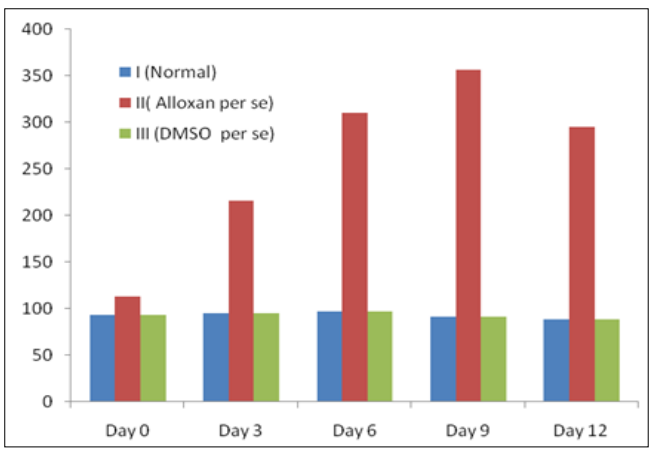

Fig 7: Histogram of Blood glucose levels of Normal control, Alloxan *per se* and DMSO *per se*

Table 6: Effect of Glimepiride and glipizide patch on blood glucose level in Alloxan induced diabetic rats

Group	Mean ± S.D (mg/dl)							
	0 hrs	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	24 hrs
IV	87.3 ± 2.18	89.1 ± 2.22	91.2 ± 2.22	86.2 ± 1.50	83.4 ± 1.58	82.5 ± 1.46	82.7 ± 1.48	83.2 ± 1.50
V	88.1 ± 1.70	89.1 ± 1.72	90.8 ± 2.23	85.3 ± 1.44	81.0 ± 1.43	82.3 ± 1.55	82.6 ± 1.36	83.1 ± 1.52
VI	285.3 ± 1.46	205.1 ± 1.10	181.5 ± 1.31	158.3 ± 1.44	102.4 ± 1.47	110.4 ± 1.33	109.5 ± 1.44	109.3 ± 1.59
VII	282.0 ± 1.94	198.3 ± 2.17	176.7 ± 3.46	150.3 ± 2.08	99.5 ± 2.20	107.4 ± 1.21	105.9 ± 1.10	105.2 ± 1.68

References

1. Kaul K, Tarr J, Ahmad SI. Kohner EM, Chibber R. Introduction to diabetes mellitus. Advances in Experimental Medicines and Biology. Research Gate, 2012, 1-12.
2. Ramteke KH, Dhole SN, Patil SV. Transdermal Drug Delivery System: An Overview. J of Advanced Sci Res. 2012; 3(1):22-35.
3. Debjit B, Chiranji B, Marget C, Jayakar B, Sampath K. Recent Advances in Transdermal Drug Delivery System. Int J of Pharm Tech Res. 2010; 2(1): 68-77.
4. Swetha T, Ahad A, Reddy K, Kumar A. Formulation and *In vitro* permeation studies of ketoprofen Ficus Reticulata fruit mucilage Transdermal patches. Scholor Res Library. 2010; 2(6):190-199.
5. Bharkatiya M, Nema RK, Bhatnagar M. Development and characterization of transdermal patches of metoprolol tartrate. Asian J Pharmaceutics and clinical Res. 2010; 3(2):130-134.
6. Asija R, Gupta A, Maheshwari B. Formulation and evaluation of transdermal patches of torasemide. Int J advances Sci Res. 2015; 1(01):38-44.
7. Pathak K, Ganju E. Formulation and evaluation of transdermal patch of Perchlorperazine Maleate fir Hypermesis. Int J Res Pharm and Chemistry. 2011; 1(4):1115-1118.
8. Jha KA. Impacts of ketorolac tromethamine in Transdermal drug delivery system. Int J Pharm Sci Res. 2010; 1(12):56-61.
9. Pavia DL, Lampman GM, Kriz GS. Introduction to spectroscopy. 3rd edition. Chapter 2. Infrared spectroscopy. Samat printers. Kundli, 2007, 30-62.
10. Solunke SR, Chaudhari PD. Formulation and evaluation of Glimepiride patches for Transdermal drug delivery. J biomedical and Pharm Res. 2016; 5:108-120.
11. Patel RP, Patel G, Baria A. Formulation and evaluation of transdermal patch of accelofenac. Int J Drug Delivery. 2009; 1:41-51.
12. Pachisia N, Agrawal SS. Formulation, development and evaluation of transdermal drug delivery system of Glimepiride. Int J Pharm and Pharmaceutical Sci Res. 2012; 2(1):1-8.
13. Mutalik S, Udupa N. Formulation, development, *In vitro*, *In vivo* evaluation of membrane controlled Transdermal systems of glibenclamide. J Pharm Sci. 2005; 8(1):26-38.
14. Rohilla A, Ali S. Alloxan induced diabetes: mechanisms and effects. Int J res Pharm Biomedical Sci. 2002; 3(2):819-823.
15. Etuk EU. Animals models for studying diabetes mellitus. Agriculture and Biology J North America. 2010; 1(2):130-134.
16. Nagy MA, Amin KA. Biochemical amd histopathological analysis of cystoseira myrica aqueous extract on Alloxan induced diabetic rats. Biochemistry: an Indian J. 2015; 9(3):081-091.