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Development and characterization of glipizide loaded transdermal matrix patches with penetration enhancers

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Abstract

We aimed to develop matrix type transdermal delivery system of glipizide for its delivery via skin using penetration enhancers. Glipizide loaded transdermal matrix system containing different amounts of ethyl cellulose (EC) and polyvinylpyrrolidone K-30 (PVP) were prepared by the solvent evaporation technique. The combined effect of oleic acid (OA) and propylene glycol (PG) as penetration enhancers on the percutaneous permeation of drug was studied. The patches containing 5% OA+1% PG exhibited highest release rate and highest drug permeation at the end of 24 h. Moreover, *in vivo* studies with glipizide showed significant hypoglycemic activity.

Keywords: Transdermal permeation, ethyl cellulose, polyvinylpyrrolidone K-30, penetration enhancers, human cadaver skin

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency and is often combined with insulin resistance (Mutalik and Udupa 2006). Glipizide has been in extensive use to treat non-insulin dependent diabetes mellitus (NIDDM) and acts by increasing the release of endogenous insulin, but it is associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heart burn and anorexia after oral therapy in the normal doses (Davis and Granner 1996). Since this drug is usually intended to be taken for a long period, patient compliance is also very important (Takahshi et al. 1997).

A controlled release dosage form may be advantageous over conventional oral dosage forms. Transdermal drug delivery systems offer many advantages as compared to their corresponding classical oral, injectable and inhaler systems (Murthya and Shobharani 2004). This route allows for controlled release of the drug at rate approaching zero order simulating those provided by IV infusion. It also delivers the medication as a continuous input, which is important for drugs

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that have short biological half-lives and low therapeutic indices. Once the drug is absorbed through the skin, the first pass intestinal and hepatic metabolism is bypassed. As a consequence, patient compliance is improved (Ayman et al. 2000, Chien 1987).

Glipizide has no skin metabolism and the properties like low molecular weight (445.5), its partition coefficient (log octanol/buffer: 0.39±0.05), plasma half life (3-5 h), and small amount of daily dose (5-20 mg) makes it suitable for transdermal drug delivery (Mutalik and Udupa 2006).

The flux and diffusion coefficient can be increased with transdermal permeation enhancers due to their ability to change the structure of lipophilic and keratinized domain in *Stratum corneum* (Ramesh et al. 2007). Oleic acid as chemical penetration enhancer may be a useful tool to diminish the barrier function of skin (Edurne et al. 2001). Oleic acid can interact with *Stratum corneum* lipids and disrupt structure, increasing the fluidity and consequently increasing their flux (Barry 1995, Ongpipattanakul et al. 1995). Propylene glycol may increase the solubility of drug in vehicle and can alter the structure of skin and modify percutaneous absorption (Williams and Barry 2004). Use of PG in combination with OA may offer a synergistic enhancement. One possible explanation for this synergistic effect may be the facilitated incorporation of OA into the stratum corneum lipid alkyl domain by the interaction of PG at the polar head group region (Oh et al. 1998).

The objective of present work was to develop and characterize the glipizide loaded transdermal matrix patches and how to with a patches were expected to reduce frequent dosing, improve the efficacy and diminish associated side effects.

Materials and Methods

Materials

Glipizide was kindly gifted by Wockhardt, Pvt. Ltd., India. Ethyl cellulose (with an ethoxy content of 47.5-53.55 % w/w and viscosity of 14 cps in a 5% w/w, 80:20 toluene: ethanol solution at 25°C) and Polyvinylpyrrolidone-K30 were purchased from CDH (P) Ltd., India. Chloroform, propylene glycol, oleic acid, methanol and acetone were procured from Ranchem, India. Sodium hydroxide pellets and potassium dihydrogen phosphate were purchased from Qualigens Fine Chem., India. Formaldehyde solution 4% w/w LR (formalin) was procured from SD Fine Chem Ltd., India. Triethyl citrate was purchased from Sigma-Aldrich, USA. Human cadaver skin was procured from Lala Lajpat Rai Medical College, Meerut and cellophen membrane was procured from SD Fine Ltd., India. Other chemicals were of analytical grade.

Determination of partition coefficient

The partition coefficient was determined in n-octanol and isotonic phosphate buffer pH 7.4 system. Both the phases and quilibrium concentration of glipizide was measured by UV spectroscopy. The partition coefficient was calculated by formula 1 (Krisztina and Alex 1996).

$$P = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}}$$

Drug-polymers compatibility study

The physicochemical compatibility between drug and polymers used in the patches was studied by using differential scanning calorimetry (DSC, Perkin-Elmer-Pyris 6 DSC, USA) and fourier transform infrared (FTIR, Perkin Elmer, Spectrum BX, USA) spectroscopy. In DSC analysis, the samples were weighed (5 mg), sealed in flat bottom aluminum pans, and heated over a temperature range of 50-250°C at scanning rate 10°C/min. in an atmosphere of nitrogen. The thermograms obtained for drug, polymers and physical mixture of drug with polymers were compared.

The Infrared (IR) spectra were recorded by KBr pellet method and spectra were recorded in wavelength region 14000 to 400 cm^{-1}. The spectra obtained for drug, polymers and physical mixture of drug with polymers was compared (Serajuddin et al. 1999).

Preparation of transdermal drug delivery patches

The matrix-type transdermal patches containing glipizide were prepared by solvent evaporation technique, using different ratios of ethyl cellulose and polyvinylpyrrolidone K-30. The polymers (total weight 300: mg) and drug (15 mg) were weighed in requisite ratios (EC: PVP, 4.5:0.5, 4.0:1.0, 3.0:2.0, 2.5:2.5, 2.0:3.0) and dissolved in 5 mL chloroform. Triethyl citrate (10% w/w) was used as a plasticizer. The solution was poured on the mercury surface (25 cm^2), covered with inverted funnel for uniform evaporation and dried at room temperature in a dust-free environment. After 24 h, the patches were cut into required size and stored in desiccator. Formulation F6, F7, F8, F9 and F10 contained different concentration of oleic acid and propylene glycol (as permeation enhancers) (Mutalik and Udupa 2006, Gannu et al. 2007). The composition of various patches is given in Table 1.

Table 1. Composition of transdermal patches

Constituents	Formulation codes									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Glipizide (mg)	15	15	15	15	15	15	15	15	15	15
Ethyl Cellulose (mg)	270	240	180	150	120	180	180	180	180	180
PolyvinylpyrrolidoneK-30 (mg)	30	60	120	150	180	120	120	120	120	120
Chloroform(mL)	5	5	5	5	5	5	5	5	5	5
Tri-ethylcitrate (%) w/w	10	10	10	10	10	10	10	10	10	10
Propylene glycol (%) w/w	-	-	-	-	-	5	-	-	1	1
Oleic acid (%) w/w	-	-	-	-	-	-	5	10	8	5

Physicochemical characterization of patches

Weight Variation and Thickness

The weight variation test was performed by weighing 4 patches individually and collectively. This determination was carried out for each formulation. Thickness of the patches was determined using screw gauge (with least count of 0.01mm) at various positions and the average thickness was computed (Udhumansha et al. 2007).

Folding endurance

Folding endurance is directly related to fragility of the patches. Folding endurance was determined by folding the film at the same place repeatedly until it broke and the number of times of folding was recorded as the folding endurance value (Tanwar et al. 2007).

Flatness

Flatness is the measure of percent constrict of the patches. Flatness of transdermal patches was measured by cutting three longitudinal strips from a patch, one from the centre, one from left side, and one from

right side. The length from each strip and variation in length were measured by determining percent constriction. Flatness was calculated by measuring constriction of strips using the formula:

% constriction = $(l_1-l_2/ l_2) \times 100$

where l_1= initial length, l_2= cut film length

Patches with zero percent constriction were taken equivalent to 100% flatness (Arora and Mukherjee 2002).

Percentage moisture content

The patches were weighed individually and kept in desiccator containing activated silica, at room temperature for 24 h. The process was repeated regularly until the patches exhibited a constant weight. The percent moisture content was calculated as the difference between the initial and final weight with respect to final weight (Udhumansha et al. 2007).

Percentage moisture uptake

The patches were weighed individually and kept in desiccator at room temperature and exposed to 84% RH (a saturated solution of aluminum chloride) for 24 h. The percentage of moisture uptake was measured by calculating as the difference between the initial and final weight with respect to initial weight (Udhumansha et al. 2007).

Estimation of drug content

Drug content of the patches was assessed by dissolving the patches in 100 mL methanol and filtered using Whatman filter paper no.1. The drug content was determined spectrophotometrically (Mutalik and Udupa 2006).

In vitro release studies

Cellophane membrane was boiled in distilled water for 30 min and then in phosphate buffer pH 7.4 for 6 h prior to the experiment (Ansari et al. 2006).

The drug release from the patch through treated cellophane membrane was determined using modified diffusion cell, with surface area 1.75 cm^2 and receptor volume 20 mL. The receptor medium was phosphate buffer saline (PBS) pH 7.4. The membrane soaked in receptor medium for 8 h was used as barrier membrane and mounted between donor and receptor compartment. Glipizide loaded patch was placed on the membrane surface which was sealed with paraffin. The donor compartment was then placed in position such that surface of membrane just touches the receptor fluid surface. The whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was continuously stirred using a magnetic bead. During the experiments, the solution in the receptor side was kept at 37±1°C. The samples were withdrawn at different time intervals up to 24 h and analyzed for drug content. Receptor phase was replenished with an equal volume of buffer solution at each time interval (Edurne et al. 2001).

In vitro permeation studies

Human cadaver skin obtained was dermatomed carefully, and washed with deionized distilled water. The skin was then treated with a 5% w/v solution of EDTA for 8 hours. The epidermis was removed carefully from the dermis with forceps. The epidermal side was again washed with distilled water and spread on a cellophane sheet. The skin was then stored in a freezer until further use. Before the experiment, the skin was taken out and was soaked in PBS pH 7.4 for 1 h. It was gently blotted dry with filter paper (Panigrahi et al. 2005).

Modified Franz diffusion cell with a surface area 1.75 cm^2 and receptor volume 20 mL was used for in vitro permeation studies. The human cadaver skin was mounted between donor and receptor compartment. Glipizide loaded patch with area 1 cm^2 was placed on the membrane surface which was sealed from the atmosphere with paraffin. The donor compartment was then placed in position such that the dermis side of the skin just touches the receptor fluid surface. The whole assembly was kept on a magnetic stirrer and solution (PBS pH 7.4) in the receiver compartment was continuously stirred using a magnetic bead. During the experiments, the solution in the receptor side was kept at 37±1°C. The samples were withdrawn at different time intervals up to 24 h and analyzed for drug content. Receptor phase was replenished with an equal volume of PBS pH 7.4 at each time interval (Panigrahi et al. 2001).

In vivo evaluation of transdermal patches

Animals

For *in vivo* experiment 6-8 weeks old Swiss albino mice of either sex (weighing 25-30 gm) from the animal house, Pranveer Singh Institute of Technology, Kanpur were used. The animals were placed in standard laboratory diet (Lipton Feed, Mumbai, India) and water. They were kept at 25±1°C and 45-55% relative humidity with a 12 h light/dark cycle. The *in vivo* experimental protocol was approved by the institutional animal ethical committee, Pranveer Singh Institute of Technology, Kanpur.

Hypoglycemic activity in normal mice

The hair on the backside of the mice was removed with hair clipper on the previous day of the experiment. Overnight fasted mice were divided into 4 groups (n=6) and treated as follows:

Group I (control): 0.2 mL of 0.5% w/v sodium carboxymethyl cellulose (CMC); p.o.

Group II: glipizide (5mg/kg; p.o.)

Group III: formulation F3 glipizide patch (2.5 cm2; 1.5 mg of drug)

Group IV: formulation F10 glipizide patch (2.5 cm2; 1.5 mg of drug)

The study blood was collected from orbital sinuses at different time intervals over 2-24 h after treatment and blood glucose levels were determined using glucometer (Mutalik and Udupa 2006).

Hypoglycemic activity in diabetic mice

Diabetes was induced by injecting streptozotocin (150mg/kg, i.p.) dissolved in citrate buffer (3mM, pH 4.5) to overnight fasted mice. Seven days later mice with blood glucose levels between 300-400 mg/dLwere selected for the study. Hypoglycemic activity of the transdermal patches was evaluated in overnight fasted diabetic mice as described in the earlier section (Mutalik and Udupa 2006).

Skin irritation test

The mice whose hair was removed on previous day were divided into six groups and treated as follows (Mutalik and Udupa 2006).

Group I: no treatment

Group II: control (applied with USP adhesive tape)

Group III: formulation F3 glipizide patch (2.5 cm^2; 1.5 mg of drug)

Group IV: formulation F10 glipizide patch (2.5 cm^2, 1.5 mg of drug)

Group V: formalin (a standard irritant 0.8 % v/v)

Drug analysis

The amount of glipizide in the receptor phase was assayed spectrophotometrically (UV Schimadzu, Japan) at 276 nm. Assay performance of the present method was assessed by linearity, accuracy, precision and applicability in the percutaneous permeation studies. The assays exhibited linearity between the response (y) and the corresponding concentration of the drug (x), over the range 4-32 µg/mL (typical equation: y= 0.026x-0.016, R2=0.998) (Krull and Swartz 1999).

Analysis of data

The data obtained from *in vitro* study can be analyzed by various parameters like release rate, flux (Jss), permeability coefficient (kp), enhancement ratio (E.R.), lag time (h) and cumulative amount of drug release after 24 h (Q24). Release rate was calculated using the formula, $Q/A = k \times t_{1/2}$, where Q is the amount of drug released, A is the area of the diffusion membrane, t is the time (h) and k is the release rate constant ($mg/cm^{-2}\ h^{-1/2}$) which was determined from the slope of the curve obtained between the amount of drug released per unit area and square root of time. Flux (Jss) was determined from the slope of the stead state portion of the amount of drug permeated divided by area and time. The steady state permeability coefficient (Kp) was calculated using the equation $KP = Jss / C_0$, where Jss is the flux and C_0 is the concentration of the drug in the patch added to the skin in the donor compartment. The lag time (hour) was determined by the x-intercept of the linear portion of curve. Enhancement ratio is the ratio of the flux value obtained with and without enhancers. It was calculated by the following formula (Sadashivaiah et al. 2008).

$$ER = \frac{\text{Jss of drug at steady state in the presence of enhancers}}{\text{Jss of drug at steady state in the absence of enhancer}}$$

Evaluation of release kinetics

Data obtained from *in vitro* release studies was fitted in various kinetic equations to establish the mechanism of drug release. The kinetic models used were zero order, first order, Higuchi and Peppas model. The curves were plotted between; Q_t and t (zero order kinetic model), log (Q_0-Q_t) and t (first order kinetic model), Q_t and square root of time (Higuchi model), $Q = k \times t_n$ (Peppas model) (Siepmann and Siepmann 2008). Where Q_t is the amount of drug released at time t and Q_0 is the initial amount of drug present in patch, Q is the amount of drug released, k is constant incorporating structural and geometrical characteristic of the release device and n is the diffusional exponent indicative of the mechanism of release. Plots were subjected to regression analysis to find out the regression coefficient and hence the order of release (Paulo and Manuel 2001).

Stability studies

Transdermal patches (F3, F9, F10) were exposed to 45 °C/75% RH for a period of 4 weeks. The desired area of patches was cut from each formulation and was evaluated for various physicochemical parameters and *in vitro* permeation study. The readings were taken in triplicate (Panigrahi et al. 2005).

Statistical analyses

The statistical significances of the differences between the formulations was analyzed by student t-test using Graph Pad InStat 3 Software the value $p<0.05$ was considered statistically significant. Stability study data were also subjected to statistically treatment (Magnusson et al. 1997).

Results

Glipizide showed partition coefficients (log octanol/buffer: 0.38±0.18). The IR spectral analysis of glipizide showed 1689.59, 1650.9, 1583.7, 1444.49, 1160.11, 1034.22 and 903.62 cm^{-1} wave numbers as major peaks. There were no changes in the major peaks of glipizide in the presence of EC and PVP. The DSC analysis of glipizide alone showed a sharp endothermic peak at 206.58 °C corresponding to its melting point. The DSC analysis of physical mixture of drug and polymers demonstrated negligible change in the melting point of glipizide in the presence of any polymer mixture studied (205.11°C for glipizide+EC+PVP) as shown in Fig. 1.

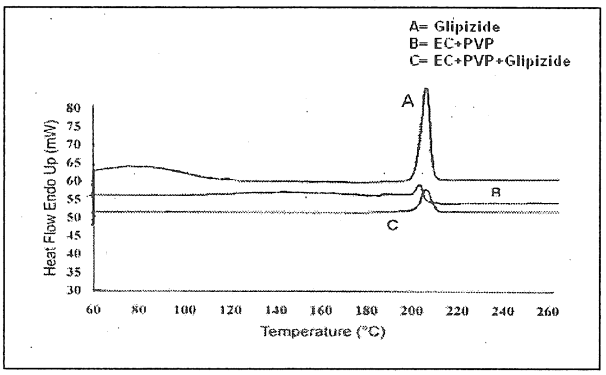

Figure 1. DSC thermograms of glipizide, polymers and physical mixture

Physicochemical characterization of patches

The results of the physicochemical characterization of the patches are shown in Table 2. The average weight ranged between 327.1-359.7 mg. Weights of formulations F6-F10 were found comparatively more due to presence of penetration enhancers. Thickness of patches was determined and found uniform in different batches of patches.

Table 2. Evaluation of Physicochemical properties of patches

Formulation code	Weight variation (mg)	Thickness (mm)	Folding endurance	Flatness (%)	Moisture content (%)	Moisture uptake (%)	Drug content (%)
F1	330.6±1.12	0.180±0.005	58±2.16	100±0	2.78±0.66	1.09±0.46	90.59±2.48
F2	329.9±1.38	0.173±0.006	60±3.59	98.02±0.01	3.31±0.57	1.29±0.10	87.22±0.59
F3	327.1±1.22	0.166±0.004	67±2.44	100±0	3.64±0.46	1.83±0.54	92.39±0.34
F4	331.4±1.50	0.168±0.014	40±3.26	98.04±0.05	4.24±0.73	1.98±0.46	81.52±0.67
F5	333.7±2.19	0.158±0.006	35±5.25	96±0	4.97±0.811	2.20±0.53	71.33±0.89
F6	347.9±3.23	0.168±0.003	82.3±2.51	98.09±0.02	1.32±0.82	2.50±0.60	93.12±1.23
F7	342.1±1.80	0.166±0.004	93.3±4.16	100± 0	1.24±0.219	1.63±0.77	94.28±0.60
F8	359.7±0.85	0.169±0.003	124±3.60	100±0	1.15±0.170	1.66±0.72	93.74±0.61
F9	358.7±1.00	0.168±0.002	121±1.00	100±0	1.32±0.72	2.16±0.15	94.28±0.59
F10	355.2±1.22	0.167±0.002	106.6 ±7.57	100±0	1.25±0.23	2.20±0.42	94.15±1.34

*Results are expressed as mean ± SD ($n = 6$)

The average thickness of patches was found to be between 0.158-0.180 mm. The average folding endurance of patches was found to be 35-124. Flatness study showed that most of the formulations had the same strip length before and after the cuts, indicating 100% flatness. No constriction was observed and most of the patches had a flat and smooth surface. Drug content

of the patches was found to be between 71.33-94.28 %. The results indicated that the process employed to prepare patches was capable of producing patches with uniform drug content and minimal variability.

In vitro release study

Effect of different ratio of EC and PVP

Glipizide patches with EC: PVP (3:2) (formulation F3) exhibited high cumulative amount of drug release (540.9±0.64 μg/cm^2) and higher release rate (122.4±0.12 μg/cm^2 h$^{-1/2}$) as shown in Table 3 and Fig. 2.

Table 3. Release rate of different patches containing different ratio of EC: PVP

Formulation code	Release rate (μg/cm^2 h$^{-1/2}$)	Coefficient of Correlation
F1	108.5±0.34	0.957±0.012
F2	111.6±0.84	0.964±0.034
F3	122.4±0.12	0.987±0.046

*Results are expressed as mean ± SD (n = 6)

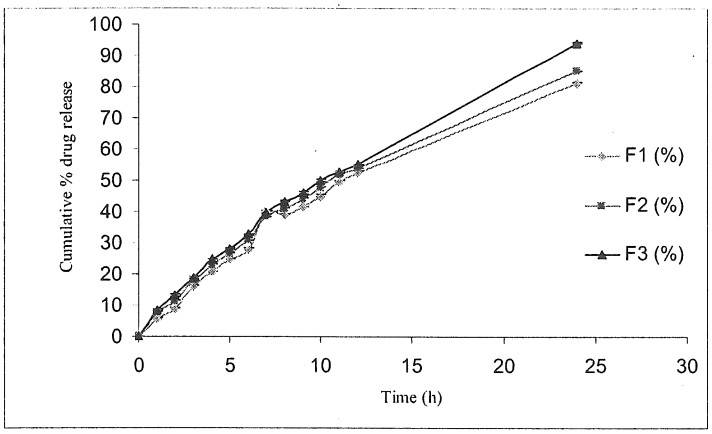

Figure 2. Cumulative percentage of drug release across cellophane membrane (n=6)

Effect of penetration enhancers

The effect of penetration enhancers (OA and PG) was observed with formulation F6-F10. Diffusion of drug from transdermal patches containing optimized amount of enhancers as 5% and 10% w/w OA, 5% w/w PG, combination as 1%+5% w/w PG+OA and 1%+8% w/w PG+OA, were studied through treated cellophane membrane. The release rate increased with increasing oleic acid concentration and reached a maximum (118.7±0.56 μg/cm^2 h$^{-1/2}$) with 5% w/w, however, the value at 10% w/w (115.7±0.34 μg/cm^2 h$^{-1/2}$) was less than at 5% w/w (118.7±0.56 μg/cm^2 h$^{-1/2}$) as shown in Table 4 and Fig. 3.

Table 4. Release rate of different formulations containing permeation enhancer through cellophane membrane

Formulation code	Release rate ($\mu g/cm^2\ h^{1/2}$)	Coefficient of correlation
F3	114.4±0.98	0.967±0.34
F6	118.3±0.67	0.971±0.67
F7	118.7±0.56	0.974±0.32
F8	115.7±0.34	0.973±0.26
F9	119.4±0.56	0.979±0.78
F10	121.8±0.12	0.983±0.58

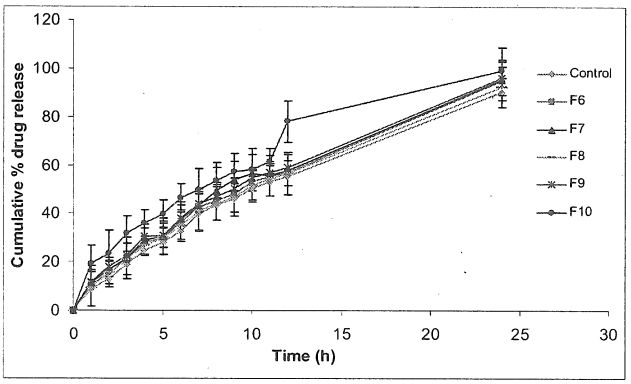

Figure 3. Comparative percentage of drug release from patches containing different concentration of permeation enhancers across cellophane membrane (n =6).

Table 5. Permeation rate of different formulations containing permeation enhancer through human cadaver skin

Formulation code	Permeation rate ($\mu g/cm^2\ h^{-1/2}$)	Coefficient of correlation
F3	6.98±0.89	0.956±0.32
F6	9.85±0.56	0.979±0.07
F7	10.67±0.32	0.942±0.69
F8	9.44±0.34	0.968±0.13
F9	12.13±0.21	0.959±0.58
F10	14.66±0.16	0.963±0.41

*Results are expressed as mean ± SD ($n = 6$)

Drug permeation study through human cadaver skin

The permeation studies resulted that the formulation without penetration enhancers i.e. F3 showed less permeation as compared to the formulations with penetration enhancers i.e. F6 to F10. Among the formulation F6 to F10, formulation F8 containing 10% OA showed least drug permeation (228.81±0.96 $\mu g/cm^2$). Formulation F10 containing 1% PG + 5% OA showed the highest drug permeation (367.19±0.82 $\mu g/cm^2$) and higher permeation rate (14.66±0.16 $\mu g/cm^2\ h^{-1/2}$) as shown in Table 5. The amount permeated were found to be in the order F10>F9>F7>F6>F8>F3 as shown in Fig. 4.

Hypoglycemic activity

The results of reduction in blood glucose levels with transdermal patches in comparison to oral administration of glipizide (5mg/kg) in mice (both normal and diabetic) are presented in Table 6.

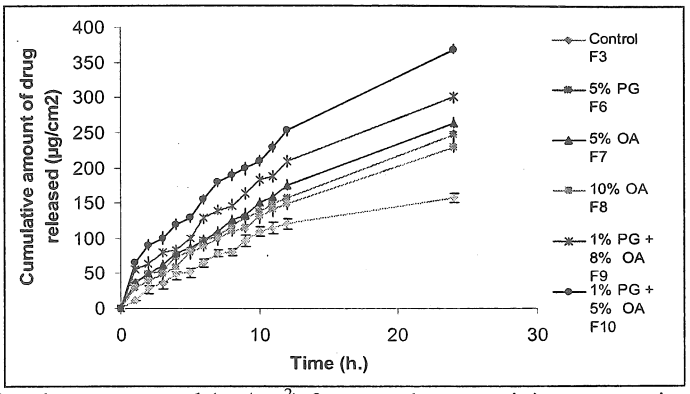

Figure 4. Cumulative drug permeated (µg/cm^2) from patches containing permeation enhancer across human cadaver skin

The hypoglycemic effect showed by oral and transdermal device was significant ($p< 0.05$; compared to control) upto 10 and 24 h, respectively. Glipizide (oral) produced a decrease in blood glucose levels upto 5.32±0.11mmol/L (32.75±0.67%) (normal mice; $p< 0.05$) and 16.45±0.87 mmol/L (37.25±6.12%) (Diabetic mice; $p<0.05$) at 2 h. In case of transdermal patches, the hypoglycemic response was gradual. A maximum hypoglycaemic response was observed after 6 h and thereafter remained stable upto 24 h. The untreated group did not show any hypoglycaemia. Formulation F10 showed greater hypoglycemic effect with higher reduction of blood glucose level at each time interval than formulation F3 due to presence of penetration enhancers, which enhanced the diffusion of drug through skin.

Table 6. Reduction in blood glucose levels after oral and transdermal treatment of glipizide in normal and diabetic mice (acute study)

Group	Treatment	Absolute blood glucose level(mmol/L)	Reduction in blood glucose level (mmol/L) (Percentage reduction in blood glucose level)				
			2 h	4h	6h	10h	24h
Normal mice	Control (0.2 mL CMC)	8.92±0.34	8.68±0.32 (2.59±1.23)	8.53±0.13 (4.38±0.56)	8.44±0.24 (4.44±1.02)	8.51±0.63 (4.96±0.98)	8.57±0.56 (3.93±1.11)
	Formulation F3	8.13±0.32	7.67±0.99 (5.66±0.75)*#	6.45±0.63 (20.67±0.48)*	6.31±0.47 (22.39±1.09)*	5.86±0.43 (27.93±0.27)*	6.02±0.31 (25.96±0.88)*#
	Formulation F10	7.89±0.27	6.45±0.64 (18.26±1.26)*#	6.11±0.21 (22.57±0.99)*	5.76±0.85 (27±0.12)*	5.35±0.82 (32.2±0.78)*	5.83±0.55 (26.11±0.32)*#
	GPZ (5mg/kg)	7.91±0.54	5.32±0.11 (32.75±0.67)*	4.12±0.56 (47.98±0.46)	4.03±0.65 (49.06±0.21)*	3.99±0.95 (49.56±0.78)*	7.13±0.87 (9.87±0.95)
Diabetic mice	DC (0.2 mL CMC)	17.98±0.21	17.34±0.88 (3.56±0.37)	17.02±0.21 (5.34±0.91)	16.98±0.78 (5.57±0.44)	17.23±0.53 (4.18±0.58)	17.42±0.76 (3.12±0.92)
	Formulation F3	18.23±0.78	17.01±0.11 (6.7±0.39)^	16.12±0.54 (11.58±1.12)^	15.31±0.45 (16.02±0.73)^	14.31±0.42 (21.51±0.81)^	14.98±0.76 (17.83±0.89)^+
	Formulation F10	18.96±0.55	16.67±0.54 (12.08±0.84)^	15.53±0.33 (18.1±0.76)^	14.13±0.57 (25.48±0.24)^	13.95±0.13 (26.43±0.84)^	14.08±0.62 (25.74±0.48)^+
	GPZ(5mg/kg)	19.01±0.11	16.45±0.87 (13.47±0.16)^	13.11±0.46 (31.04±0.47)^	11.54±0.21 (39.3±0.41)^	11.12±0.23 (41.51±0.65)^	18.37±0.46 (3.37±0.57)

All values are expressed as Mean±SE, n=6; CMC=Sodium carboxymethyl cellulose; TP=Transdermal patch; GPZ=Glipizide; *significant compared to control ($p<0.05$); #significant compared to GPZ ($p<0.05$); ^significant compared to DC (Diabetic control) ($p<0.05$); + significant compared to GPZ in diabetic mice ($p<0.05$).

Skin irritation test

The result of skin irritation tests of the developed patches in comparison with control and formalin (0.8%) are presented in Table 7.

Table 7. Results of skin irritation test

Rat no	Control		Formulation F3		Formulation F10		Formalin	
	Erythema*	Edema^	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	1	1	3	2
2	0	0	1	1	2	1	2	1
3	0	0	1	0	1	0	2	2
4	0	0	1	1	0	0	3	3
5	0	0	1	2	1	1	3	2
6	0	0	2	1	2	1	2	3
Average	0	0	1±0.31*+	0.66±0.231*+	1.17±0.33*+	0.67±0.33*+	2.5±0.23	2.1±0.30

Observation values are expressed as Mean±SE, n=6; *Erythema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation; ^ Edema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, severe; *+ significant compared to formalin ($p<0.05$)

Analysis of data

A comparison between the rate of skin permeation and the rate of release (Table 8) suggested that under sink conditions the system delivered the drug at a rate greater than its rate of permeation across the skin (Fig. 5).

Table 8. Comparison of penetration data of drug from formulations (F3, F6, F7, F8, F9 and F10) through cellophane membrane and human cadaver skin

Formulations code	Release rate ($\mu g/cm^2/h^{-1/2}$)	Permeation rate ($\mu g/cm^2/h$)	*Q_{24}	
			$\mu g/cm^2$	$\mu g/cm^2$
	Cellophane membrane	Human cadaver skin	Cellophane membrane	Human cadaver skin
F3	114.4±0.23	6.98±0.89	540.9±0.32	156.95±0.04
F6	118.3±0.12	9.85±0.56	569.7±0.76	246.19±0.13
F7	118.7±0.29	10.67±0.32	572.67±0.45	263.32±0.75
F8	115.7±0.26	9.44±0.34	553.72±0.86	228.81±0.71
F9	119.4±0.16	12.13±0.21	577.12±0.52	300.91±0.32
F10	121.8±0.67	14.66±0.16	587.99±0.91	367.19±0.34

*cumulative amount of drug released or permeated after 24 h; each value was an average of three replicate experiments

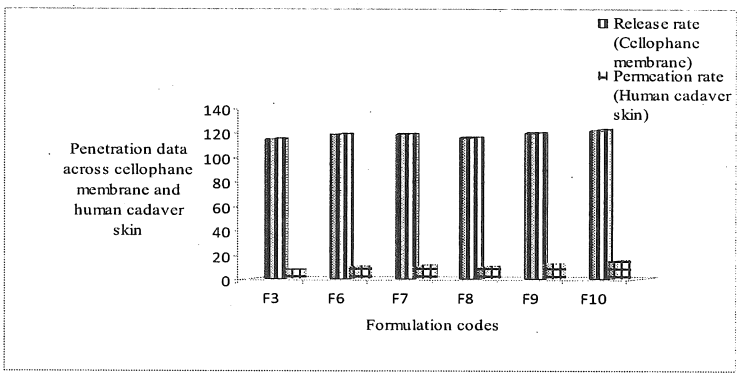

Figure 5. Comparison of penetration data across cellophane membrane and human cadaver skin

The penetration parameters of glipizide from different formulations (F3, F6, F7, F8, F9, and F10) across human cadaver skin were compared. Glipizide flux value at steady state from control (F3, without enhancers) was found 6.98 $\mu g/cm^2/h$ and with addition of enhancers, flux value of formulations F6, F7, F8, F9, F10 were found 9.85 $\mu g/cm^2/h$, 10.67 $\mu g/cm^2/h$, 9.44 $\mu g/cm^2/h$, 12.13 $\mu g/cm^2/h$ and 14.66 $\mu g/cm^2/h$ respectively. The enhancement ratio with formulation F10 was 2.10 fold higher than the control formulation. The permeability coefficient was found from 1.16 to 2.44 (x 10^{-3} cm/h). The lag time range was found to be between from

0.91 h.-3.60 h for formulations F3, F6, F7, F8, F9 and F10. All the penetration parameters of drug from different formulations are listed in Table 9 and shown in Fig. 6.

Table 9. The penetration data of glipizide from different formulations cross human cadaver skin

Formulation code	Flux ($\mu g/cm^2/h$)	Enhancement ratio	Q_{24} ($\mu g/cm^2$)	Lag time (h)	Permeability coefficient (K_P) (x 10^{-3} cm/h)
F3	6.98±0.041	1	156.95±0.46	0.91	1.16±0.01
F6	9.85±0.067	1.41	246.19±0.63	1.56	1.64±0.34
F7	10.67±0.048	1.54	263.32±0.27	0.96	1.77±0.27
F8	9.44±0.074	0.79	228.81±0.94	1.86	1.57±0.39
F9	12.13±0.043	1.78	300.91±0.28	1.56	2.02±0.38
F10	14.66±0.083	2.1	367.19±0.19	3.73	2.44±0.38

* Each value was an average of three replicate experiments

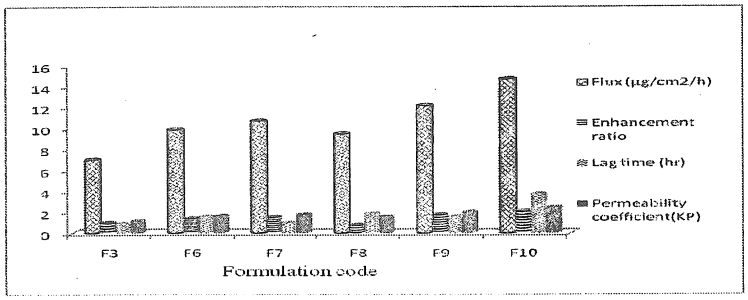

Figure 6. The penetration parameters of drug from different formulations across human cadaver skin

Kinetics of drug release

In order to obtain meaningful information for release models, the drug release profiles were fitted to various kinetic models. Regression coefficients for different release models for formulations F3, F6, F7, F8, F9 and F10 are summarized in Table 10. On treating the data as per Korsmeyer's equation the slope (n) values were found ($0.311 < n > 0.373$).

Table 10. Mathematical models used to describe drug release profile on the basis of regression coefficient

Formulation code		Zero order	First order	Higuchi model	Korsmeyer-Peppas
		R	R	R	N
F3	H	0.942±0.051	0.950±0.062	0.978±0.098	0.341±0.028
	C	0.979±0.039	0.963±0.091	0.983±0.084	0.373±0.084
F6	H	0.979±0.041	0.955±0.076	0.986±0.098	0.352±0.078
	C	0.980±0.032	0.965±0.049	0.985±0.034	0.337±0.064
F7	H	0.981±0.045	0.963±0.035	0.985±0.023	0.353±0.043
	C	0.978±0.036	0.964±0.054	0.987±0.64	0.323±0.053
F8	H	0.979±0.076	0.975±0.064	0.984±0.037	0.387±0.063
	C	0.980±0.012	0.972±0.046	0.994±0.075	0.349±0.076
F9	H	0.970±0.015	0.982±0.065	0.985±0.037	0.318±0.098
	C	0.977±0.097	0.958±0.064	0.994±0.075	0.311±0.056
F10	H	0.969±0.076	0.989±0.045	0.990±0.028	0.313±0.043
	C	0.972±0.065	0.942±0.027	0.991±0.037	0.329±0.023

*H and C means human cadaver skin and cellophane membrane respectively Each value was an average of three replicate experiment

Stability studies

Patches were observed for various parameters including change in color, appearance, flexibility, drug content and *in vitro* drug permeation study for a month. Patches were found to be stable at

45 °C/75%RH with respect to their physicochemical parameters; drug permeation and flux value. Observations are summarized in Table 11-13 and Fig. 7.

Table 11. Physicochemical properties of transdermal patches (stability studies data)

Formulation code	Weight variation (mg)	Thickness (mm)	Folding endurance	Flatness (%)	%Moisture uptake	%Moisture Content	% drug content
F3	329.9±1.23	0.163±0.003	98.3±2.02	100±0	3.31±0.34	1.45±0.45	95.67±0.35
F9	358.8±1.54	0.168±0.003	106.6±1.89	100±0	3.54±0.87	1.23±0.65	97.77±0.85
F10	354.6±1.85	0.167±0.002	113.7±1.98	100±0	2.54±0.38	1.67±0.86	98.02±0.36

Table 12. Cumulative amount of drug permeated (μg/cm^2) from glipizide patches through human cadaver skin

Time (h)	Control F3	1% PG + 8% OA (F9)	1% PG + 5% OA (F10)
0	0	0	0
1	16.89±0.62	60.27±0.32	63.26±0.33
2	31.69±0.25	74.34±0.76	86.92±0.19
3	39.86±0.4	89.64±0.43	94.97±0.27
4	52.74±0.14	92.23±0.54	117.52±0.23
5	59.57±0.61	109.45±0.28	124.79±0.78
6	68.32±0.55	125.54±0.28	158.37±0.37
7	79.15±0.36	136.97±0.34	174.79±0.53
8	80.57±0.74	142.75±0.23	183.86±0.18
9	105.69±0.23	168.73±0.46	198.28±0.29
10	116.85±0.33	185.93±0.45	207.13±0.74
11	124.75±0.84	198.24±0.19	223.12±0.67
12	131.79±0.54	213.17±0.23	256.58±0.84
24	172.34±0.88	301.16±0.47	366.98±0.58

Table 13. Penetration data of drug from formulations (F3, F9 and F10) through human cadaver skin

Formulations code	Permeation rate (μg/cm^2/h)	*Q_{24} μg/cm^2
	Human cadaver skin	Human cadaver skin
F3	7.573±0.54	172.34±0.88
F9	11.93±0.91	301.16±0.47
F10	14.53±0.48	366.98±0.58

*cumulative amount of drug permeated after 24 h.

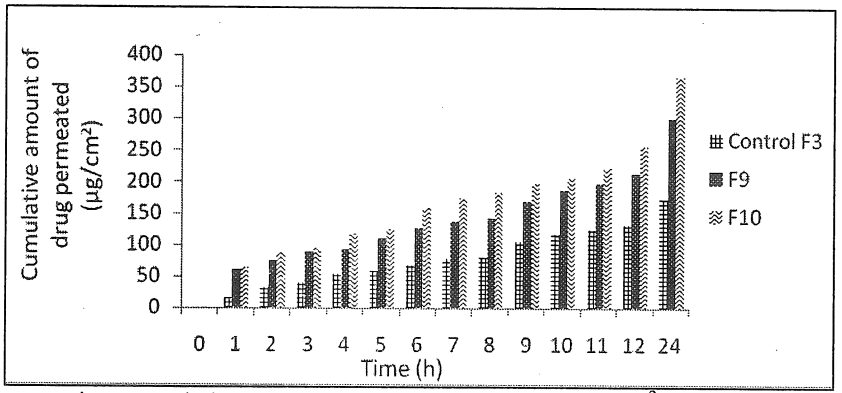

Figure 7. Comparative cumulative amount of drug permeated (μg/cm^2) from patches across human cadaver skin (stability studies).

Statistical analysis

As per statistical analysis of data, the cumulative amount of drug permeated and the flux value of formulation F10 was significantly ($p<0.05$) different from the formulation F3. Results of

statistical analysis of stability data showed that there was no significant difference ($p<0.05$) in drug permeated and flux values after exposure to the stability condition.

Discussion

Glipizide showed favorable partition coefficient (log octanol/buffer: 0.38±0.18) and negligible skin degradation. Hence, matrix patches of glipizide containing different concentration of EC/PVP were formulated to overcome the side effects associated with oral administration of the drug. There were no distinct differences in the IR peaks in the presence of polymers used in the study which indicates that the polymers do not interact with the drug (Wade and Weller 1994).

Physicochemical characterization of patches indicated that the weight of different patches was relatively similar. Observations of folding endurance test indicated that the patch would be able to maintain their integrity during use. Moisture content and moisture uptake studies showed that increase in the concentration of hydrophilic polymer increased the moisture content and moisture uptake of the patches. The moisture content of the formulations was found to be low, which could contribute to the stability and reduction in brittleness during long term storage. The moisture uptake of the formulation was also low, which could protect the formulations from microbial contamination and reduce bulkiness (Mutalik and Udupa 2004). The results indicated that the process employed to prepare patches was capable of producing patches with uniform drug content and minimal variability.

The *in vitro* studies are important tool that predicts the *in vivo* performance of a drug (Katayose and Kataoka 1997). *In vitro* drug release study of formulations F1-F3 was carried out through treated cellophane membrane. The results showed that the drug release was increased on increasing the amount of PVP, which may be due to the high solubility of these hydrophilic polymers leading to pore formation on the film surface resulting in higher release rate (Mutalik and Udupa 2006).

The permeation study revealed that enhancers increase flux value of the drug through skin. The absorption process of drug is conditioned by the nature of vehicle and other components of formulation. OA interact and modify the intercellular lipid domain of the *Stratum corneum.* It induces phase separation and reduces the diffusional resistance of the skin by interacting with the lipid matrix. PG has property to increase the release rate of drug. It increases the solubility of drug in vehicle (Squillante et al. 1998). It was observed that the enhancing effect of OA was affected not only by its concentration but also by the presence of PG. When combination of both enhancers was used, flux value was found to be increased. The combination 1% PG + 5% OA showed higher release rate of drug than the 1% PG + 8% OA. This could be due to increasing lipophilicity of formulation with higher content of OA. That reduced the partition coefficient of drug between vehicle and skin (Squillante et al. 1998). It was concluded that system consisting of PG and fatty acids was more effective as enhancers than either PG or fatty acid alone. Such an enhancement was considered to be the result of two different mechanisms in which PG enhanced intracellular drug mobility by solvating alpha keratin in corneocytes and allowing OA to act on lipid barrier (Squillante et al. 1998, Murakami et al. 1998).

In hypoglycemic studies it was found that the blood glucose reduction was prolonged by transdermal patches for 24 h. The formulation F3 (without enhancers) and F10 (with enhancers) were selected for *in vivo* study. The formulation F10 provided high cumulative amount of drug permeation at the end of 24 h. The slow and sustained hypoglycemic response could be due to slow permeation rate of drug from patches. Transdermal patch, with enhancers showed better control of blood glucose in comparison to patch without enhancers (F3). The reason may be the penetration of drug through skin which was improved by enhancers. In orally treated group, the hypoglycemic effect was decreased after 6 h, which could be due to its short biological half life. The oral route produced greater decrease of blood sugar in the initial hours. The blood glucose reduction observed with transdermal system was persistent up to end of 24 h in a controlled manner. So, the transdermal patches of glipizide with enhancers showed better *in vivo* effectiveness in mice as compared to oral administration. This could be due to slow and continuous supply of drug at a desirable rate to systemic circulation. The result of skin irritation tests showed that the transdermal systems produced negligible erythema and edema, the values of which were significantly less compared to those of formalin (a standard irritant) which produced severe erythema and edema (Draize et al. 1944, Mutalik and Udupa 2006). The comparison of penetration data of the patches across cellophane membrane and human cadaver skin showed that human cadaver skin exhibited better barrier property due to the barrier nature of the skin (epidermis, specially the *Stratum corneum*). The barrier properties are based on the specific content and composition of the *Stratum corneum* lipids particularly, the structural arrangement of the intercellular lipid matrix and the lipid envelope surrounding the cells (Mutalik and Udupa 2006).

The skin permeation parameters of the drug from different formulations across human cadaver skin showed that combination of OA and PG led to a significant increase in glipizide flux compared to control formulation, while formulations F6, F7 and F8 slightly increased the flux. The enhancement ratio with formulation F10 was 2.10 fold higher than the control formulation. The permeability coefficient was found to be in the range of permeability coefficient for human skin ($\sim 10^{-6}$ to 10^{-2} cm/h) and it was observed that as the flux increased, permeability coefficient also increased. The highest lag time was noted for formulations F10. This may be attributed to the fact that drug required more time to diffuse from the vehicle and to penetrate through the skin from formulations F10 (Santoyo et al. 1995).

In release kinetics study, models with higher regression coefficient are judged to be more appropriate model for release data (Siepmann and Siepmann 2008). In this experiment, the *in vitro* permeation profiles of all formulations did not fit in to zero order behavior completely, but they almost seemed to fit into Higuchi kinetics, while considering higher coefficient of correlation value (0.974-0.991). It indicated a slow and controlled release of drug. Thus Higuchi model supported the formulation suitable for transdermal use, where the rate controlling step was the process of diffusion through the matrix patch. Hence the formulations controlled the release of drug (Paulo and Manuel 2001, Siepmann and Siepmann 2008). The slope (n) values ($0.311 < n > 0.373$) obtained by Korsmeyer's equation, indicated that the amount of drug released

by all formulations followed Fickian diffusion (Paulo and Manuel 2001, Chandak and Verma 2008, Siepmann and Siepmann 2008).

Conclusion

The glipizide transdermal patches exhibited greater efficacy with synergistic effect of oleic acid and propylene glycol and could be practicable option for effective and controlled management of diabetes. However, their potential to improve glipizide bioavailability in human needs to be investigated further.

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