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Matrix type transdermal therapeutic systems of glibenclamide: Formulation, *ex vivo* and *in vivo* characterization

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ABSTRACT: Matrix type transdermal therapeutic systems (TTS) of glibenclamide were formulated using polymers Eudragit RL 100, ethyl cellulose, PVP K-30, and polyvinyl acetate, and citral was used as the penetration enhancer. The polymer films were formulated with Eudragit RL 100 and PVP K-30 in different ratios and subsequently subjected to *ex vivo* studies (drug permeation through rat skin) followed by interaction studies, skin irritation studies, accelerated stability analysis, and *in vivo* studies (determination of blood glucose level in rabbits). The drug content of the formulations was found to be 99.1-99.2%. The cumulative percentages of drug permeated through rat skin from the three selected formulations in 48 h were 95.3%, 98.8%, and 99%, respectively. A plot between cumulative percent of drug permeated and square root of time exhibited linear curves, which suggests the Higuchian matrix mechanism of drug release. The formulation containing Eudragit RL 100 and PVP K-30 showed better improvement in hypoglycemic activity in rabbits (56.2-60.8% reduction in blood glucose level, p < 0.05). There were fewer fluctuations in blood glucose level as compared to oral therapy due to controlled release of the active pharmaceutical ingredient, and no interaction was found between the drug and excipients of the formulation. Accelerated stability analysis showed that the formulation was stable up to 5.5 years, with negligible skin irritation. The formulation precluded severe hypoglycemic reactions (side effect of sulfonylureas) and was effective for management of diabetes mellitus up to 48 h, with a single TTS.

Keywords: Matrix type transdermal therapeutic system, glibenclamide

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1. Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level (fasting plasma glucose > 7.0 mM, or plasma glucose > 11.1 mM 2 h after meal) (*1*). In total diabetic patients more than 90% suffer from type 2 diabetes (*2*). Globally, the burden of type 2 diabetes is rising fast. The global prevalence of diabetes among adults was estimated at 150 million in 1995, and this is projected to rise up to 300 million by 2025 (*3*). Developing countries may experience the largest proportional increase in diabetes (*4*). Type 2 diabetes is caused by insulin deficiency in the body due to partial or incomplete inactivation/destruction of β cells of pancreas and often associated with insulin resistance (*5*,*6*). The blood glucose rises because of impaired hepatic glucose output and reduced uptake of glucose by skeletal muscles. When the renal threshold for glucose reabsorption increases, it leads to glycosurea (glucose in urine) and osmotic diuresis which leads to dehydration, thirst, and increased drinking (*7*). The treatment of type 2 diabetes includes life style changes, drugs that reduce intestinal glucose uptake and hepatic gluconeogenesis, and drugs that increase insulin secretion from the pancreas (*e.g.* sulfonylurea) (*8*).

Glibenclamide, a sulfonylurea, has short term and long term pharmacological actions. During short term treatment it increases insulin secretion from functioning pancreatic islet β cells (pancreatic effect) where as during long term treatment its main action appears to be enhancement of insulin action on peripheral tissues and reduction of glucose output from the liver (extra-pancreatic effect). In short term treatment glibenclamide causes degranulation of the β cells in pancreas (*9*). The evoked release of insulin from the pancreas is very rapid and sulfonylureas appear to stimulate calcium influx into islet cells (*10*,*11*), as glibenclamide is a hydrophobic/lipophilic molecule and acts on sulfonylurea receptors from within the hydrophobic phase of the cell membrane (*12*). The treatment of non-insulin dependent diabetes mellitus (NIDDM) with glibenclamide is associated with severe and some fatal hypoglycemic reactions (*13*,*14*) with symptoms like cold sweats, cool pale skin, tremors, anxious feelings, unusual tiredness or weakness, confusion, difficulty in concentration, excessive hunger, temporary vision changes, headache or nausea (*1*). These side effects occur because of high interindividual variation with glibenclamide therapy. These incidences are related to the potency and duration of action of the active agent, and these incidences mostly occur with chlorpropamide and glibenclamide, so glibenclamide is avoided in the elderly and in patients with renal failure because of the risk of hypoglycemia (*6*). Whereas when glibenclamide is administered through a transdermal therapeutic system (TTS), it provides controlled release of the active pharmaceutical ingredient (API), and thus minimizes plasma fluctuation of the drug, reduces the intensity of action and thus reduces the side effect (hypoglycemia) associated with its oral therapy (*12*,*13*). Patient compliance is also increased because antidiabetic drugs have to be taken for a long period (*15*). The various properties of glibenclamide are very suitable for a TTS, *i.e.*, molecular weight 494 Da (*16*), variable biological half life 0.1-10 h, an effective plasma concentration 30-50 µg/L (*17*), and a reasonable partition coefficient (octanol/water) 4.23 (*18*). Thus transdermal formulation provides many advantages over its oral counterpart, including reduced side effects, easy termination of medication when necessary, improved patient compliance, elimination of first pass metabolism, and controlled drug delivery (*19*). The skin permeation profile of glibenclamide has been previously reported, which suggests that it significantly permeates rat skin and reduces blood glucose level (*20*).

In the present study, we have formulated the matrix type of TTS for glibenclamide delivery; a matrix type system was taken into consideration because of the high dose of the drug. The matrix type system was prepared using the polymer combination of Eudragit® RL 100, PVP K-30, and polyvinyl acetate. PEG-400 was used as plasticizer and citral as a penetration enhancer. The TTS was subjected to *ex vivo* skin permeation studies through rat skin, and *in vivo* studies in rabbit models. The formulation was also tested for drug excipients interaction studies, skin irritation studies, and accelerated stability analysis to determine the shelf life of the formulation.

2. Materials and Methods

2.1. *Materials*

Glibenclamide I.P. was purchased from Hoechst Marion Roussel Ltd., Mumbai, India. Eudragit RL100 and PVP K-30 were from Pharmax India, Mumbai, India. Ethyl cellulose was from Loba Cheme, Mumbai, India. Polyvinyl acetate was from Ranbaxy Laboratories, Gurgaon, India. Eudragit RS PO was obtained from Crossland India Mumbai, India. Eudragit RL 30D was from Rohm Pharma, Darmstadt, Germany. PEG-400 and oleic acid were from CDH, Mumbai, India. All other ingredients were obtained from E. Merck (India) Ltd. (Mumbai, India) and S. D. Fine Chemicals (Mumbai, India) and were of analytical grade.

2.2. *Preparation of polymer films*

Transdermal films were cast on an aluminum surface, and the aluminum pockets were made using two glass rings such that the external diameter of one is equal to the internal diameter of other ring. Aluminium foil was placed between these rings and the internal ring was pressed and then taken out to form a pocket. The internal diameter of the aluminium pocket was 6.4 cm and its depth was 5 mm. The formed aluminium pocket was placed on a Petri dish which was placed in an oven on a flat surface parallel to the horizon. The polymer, plasticizer, drug, and penetration enhancer were accurately weighed and dissolved in different proportions of solvent systems using a magnetic stirrer (Table 1). The resulting solution was poured carefully into the aluminium pocket. The solvent was allowed to evaporate undisturbed in an oven at an elevated temperature (55 ± 5°C) condition. An inverted funnel was placed over the Petri dish to prevent rapid evaporation of the solvent. The film dried up in approximately 2-3 h. A backing film was applied on one side of the film and a release liner on the other side. The resulting films were evaluated for physicochemical properties *viz.*, thickness, weight, folding endurance, and percent elongation at break.

2.3. *Ex vivo skin permeation studies*

For *ex vivo* skin permeation studies, a vertical diffusion cell was used consisting of donor and receiver compartment. The capacity of the receiver cell was 50 mL. The full thickness abdomen skin of an albino rat was taken and treated with depilatory to remove hair and after that the fat were removed with the help of isopropyl alcohol, and the skin was mounted between two compartments with the stratum corneum facing

Table 1. Formulas for optimized TTS formulations of glibenclamide

Ingredients	Formulation code with quantities		
	A	B	C
Eudragit RL 100 (mg)	600	400	900
PVP K-30 (mg)	400	600	–
Polyvinyl acetate (mg)	–	–	100
Glibenclamide (mg)	47.7	47.7	47.7
PEG-400 (%, w/w)	5	5	10
Citral (mL)	5	5	5
Dichloromethane (mL)	5	5	5
Ethanol (mL)	3	3	3

towards the donor compartment whereas the dermis faced the receiver compartment. The donor cell was kept empty while the receptor compartment was filled with 30% (v/v) isopropanolol in isotonic phosphate buffer, pH 7.4 (IPH). The receiver fluid was stirred with a magnetic stirrer at a speed of 500 rpm and the assembled apparatus was placed in a hot air oven preset at 37 ± 2°C. The buffer solution was replaced every 30 min to stabilize the skin (*21*). After applying the transdermal formulation, the samples were withdrawn from the receiver compartment at different time intervals up to 48 h, and an equal volume of permeation medium was added to the receptor compartment to maintain the sink condition. The withdrawn samples were analyzed for drug content.

2.4. *In vivo studies*

Optimized formulations which yielded satisfactory *in vitro* drug release and skin permeation results were subjected to *in vivo* studies. Initial blood glucose values of all rabbits were determined and the TTS was then placed on the back of the rabbit, which was previously cleared of hair with scissors and depilatory, within an area sufficient for application of the transdermal patch (*22*). Blood glucose was estimated at 0, 2, 4, 6, 8, 12, 24, 28, 32, 36, 48, 52, 56, 60, and 72 h.

For these studies, healthy male albino rabbits weighing between 1.5-2 kg were used for better assessment of blood glucose. Rabbits were selected as test animals because the permeability of rabbit skin matches to a great extent that of human skin (*23*). The blood glucose determinations were carried out in 18 normoglycemic rabbits in 3 groups of 6 each for transdermal therapeutic formulations (A, B and C). For the collection of blood, the hair from the area around the marginal ear vein was cut short with scissors to make the vein clear. After applying xylene, a No. 26 disposable needle was used and a prick was given to the marginal ear vein. After discarding the first drop, 20 µL of blood was taken directly into a 20 µL micropipette and put into tubes containing tetrachloric acid, and further analyzed for glucose estimation by the glucose oxidase-peroxidase method (*24*). The method involved the use of 4-aminophenazone as a color coupler with sulphonated 2,4-dichlorophenol for determination of hydrogen peroxide produced from glucose with glucose oxidase. The sensitivity of the method was such that 20 µg of glucose in a final volume of 4 mL gave an optical density of 0.61 at 515 nm with 10-mm cells, which corresponds to a molecular absorption of 22,000.

2.5. *Analysis of glibenclamide in TTS*

The transdermal film was dissolved in a small volume of isopropanol to produce 10 mL of solution A and filtered through Whatman filter paper No. 42. Then 1 mL of solution A was diluted to 10 mL with isopropanol to give solution B. One mL of the solution B was further diluted with isopropanol to produce solution C and it was analyzed at 238 nm using isopropanol as blank. Solution C had a dilution factor of 100. The concentration (µg/mL) of drug was read from the standard curve of the drug in isopropanol. The content of drug (in mg) was calculated as follows:

$$\text{Drug content (mg)} = \frac{\text{Concentration } (\mu\text{g/mL}) \times \text{Dilution factor}}{1000}$$

2.6. *Interaction studies*

Interaction between the excipients and drug were analyzed by ultraviolet (UV) scanning, drug assay, Fourier transform infrared (FT-IR) spectral analysis, and thin layer chromatography (TLC). The transdermal formulation was dissolved in isopropanol, the solution was filtered and scanned for UV absorption between 200-400 nm. The transdermal formulation was assayed using the assay method reported in analytical methodology and the drug content was calculated to estimate the percentage recovery of the loaded drug (*25*). FT-IR (Perkin Elmer, Rodgau, Germany) spectra of the dried medicated films were taken in the range of 400-4,000 cm^{-1}. TLC analysis was conducted using a silica gel GF_{250} plate with chloroform/cyclohexane/ethanol/glacial acetic acid (45:45:5:5, v/v) as a mobile phase and a UV chamber for visualization (*26*).

2.7. *Skin irritation studies*

The possibility of skin irritation arising from the application of the transdermal patches was assessed using a modified score test in rabbits by Draize *et al.* and Aqil *et al.* (*27*,*28*). The intact and abraded skin of rabbits were used for this purpose. The patches were placed on four areas, 10 cm apart (two intact and two abraded) on the back of the rabbit. The patches were placed on the rabbit with the help of adhesive to prevent patch removal due to animal movement. The rabbit was placed in an animal holder ensuring minimal movement during the 24 h patch exposure. Upon removal of the patches, the resulting reactions were also recorded after 72 h and the final skin irritation score represents an average of the 24 and 72 h reading. The score for erythma and edema formation (none = 0, very slight = 1, well defined = 2, moderate = 3, severe = 4) were estimated by visual inspection. The exercise was repeated three times for each formulation.

2.8. *Accelerated stability analysis*

Formulation C which produced satisfactory *ex vivo* and *in vivo* results was used for accelerated stability studies (*29*). Sufficient replicates of formulation C were prepared, packed in aluminium foil and stored in petridis at different temperatures of 40 ± 0.5°C, 50 ± 0.5°C, and 60 ± 0.5°C for 60 days. Three samples were withdrawn at intervals of 20, 40, and 60 days and analyzed for drug content by the assay method reported previously (*29*). The logarithms of percent drug remaining were plotted against time in days. The graph was plotted between percent drug remaining and time in days. This gave straight lines suggesting that the drug degradation followed first order kinetics. The degradation rate constant was calculated using the following formula.

$$slope = \frac{-K}{2.303}$$

where K is the degradation rate constant. K_{25} value was determined by Arrhenius plot and the shelf life was calculated by substituting the value of K_{25} in the following equation.

$$t_{0.9} = \frac{0.1054}{K_{25}}$$

2.9. *Statistical analysis*

Statistical analysis of the experimental data was performed by applying student's *t*-tests using GraphPad InStat 3.0 software (GraphPad Software, La Jolla, CA, USA). A p value of < 0.05 was considered significant.

3. Results

3.1. *Physicochemical parameters of polymer films*

The prepared polymeric films had an internal diameter of 2.93 cm and surface area of 6.74 cm^2. Physicochemical properties of the films were as follows: thickness was 250 ± 5, 256 ± 5, and 243 ± 5 μm for formulation A, B, and C respectively; weight was 247 ± 3, 242 ± 3, 221 ± 3 mg for formulations A-C, respectively; folding endurance was 268 ± 6, 163 ± 2, 208 ± 6 for formulations A-C, respectively; and percent elongation at break was 79 ± 2, 69 ± 1, 84 ± 2% for formulations A-C, respectively (data not shown).

3.2. *Ex vivo skin permeation studies*

The *ex vivo* skin permeation profile of glibenclamide TTS is shown in Figure 1. The cumulative percentages of drug permeated through rat skin from the three formulations in 48 h were 95.3, 98.8, and 99% for formulations A-C, respectively. After an initial lag period, permeation was observed to be gradually approaching a constant for the rest of the time, thus illustrating the controlled release behavior of these systems. In the graph between cumulative percent drug permeated *vs*. square root of time almost linear curves were obtained for all three optimized formulations A-C. This suggests that the formulation follows the Higuchian matrix mechanism of drug release (*11*). The result of kinetic analysis of *ex vivo* data showed a significantly lower coefficient of variation in the case of zero order *vs*. first order permeation kinetics except in formulation B in which the difference was not significant. Therefore, it is concluded that the permeation of the drug from the TTS followed zero order kinetics.

3.3. *In vivo studies*

Normoglycemic rabbits were used instead of hyperglycemic as hyperglycemia is induced by injecting streptozotocin which causes death in a large number of cases (*30*), however, in normoglycemic subjects, a dose dependent fall in blood glucose level was seen on transdermal administration of glibenclamide (*9*). As shown in Figure 2, a sufficiently high reduction in blood glucose level was obtained within 2 h for all formulations. The reduced level remained almost constant with a slight rise in blood glucose level on administration of food (Figure 2). These observations corroborated the *ex vivo* release pattern of the drug.

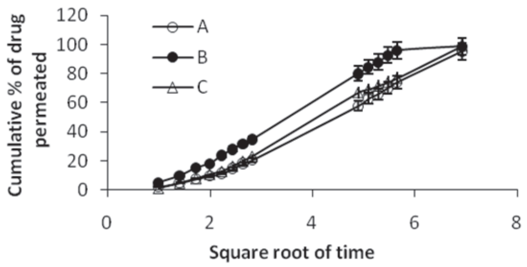

Figure 1. Cumulative percent of glibenclamide permeated in *ex vivo* study through rat skin from matrix type TTS. A, Eudragit RL 100 (600 mg) + PVP K-30 (400 mg); **B**, Eudragit RL 100 (400 mg) + PVP K-30 (600 mg); **C**, Eudragit RL 100 (900 mg) + polyvinyl acetate (100 mg). Data are the mean ± S.D. (n = 3).

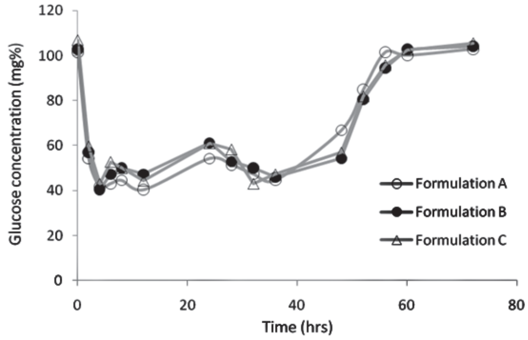

Figure 2. Effect of formulation A, B and C on blood glucose level of albino rabbits.

Normal glucose levels were restored in approximately 8 h after the removal of the transdermal system. There was insignificant variation ($p > 0.05$) in the blood glucose level when the placebo formulations were applied on the skin of albino rabbits, *vis-à-vis* the baseline values of normoglycemic rabbits (data not shown). Only slight variation on administration of food was observed.

3.4. *Interaction studies*

In the interaction studies, the UV spectra revealed that the transdermal formulation and pure drug (API) solution exhibited the same absorption maxima at 246 nm (data not shown), but there was a slight deviation in the absorption pattern of formulations due to the combined effect of API and excipients. The assay of drug from TTS (percentage recovery) was found to be in the range of 99.1 to 99.2% (data not shown). Formulation C was selected for FT-IR spectral analysis and it showed an identical spectral pattern compared to that of pure drug, although it showed a less intense peak to that of API (Figure 3). A few peaks were merged in the spectrum of the formulation and this might be due to physical but not chemical interaction between the drug and polymer. In TLC studies, almost the same R_f values and single spots were observed for the API and the drug in formulation (data not shown). From the results of all these interaction studies, it can be inferred that the glibenclamide remained intact in the TTS and no chemical interaction seemed to occur between API and the excipients therein.

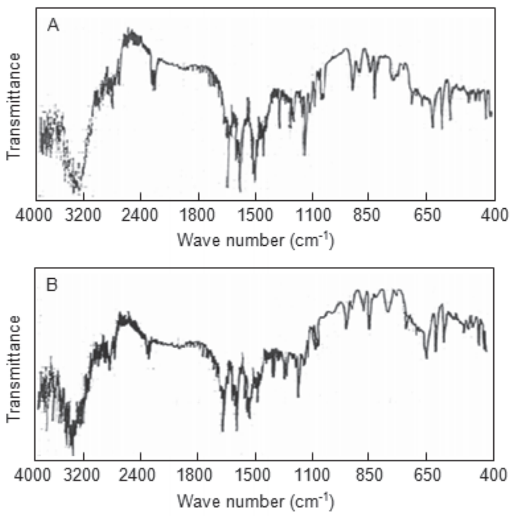

Figure 3. FT-IR spectra of pure drug (A) and formulation C (B).

3.5. *Skin irritation studies*

On performing the visual score test (*27,28*), the combined average for intact and abraded rabbit skin irritation score for the test formulations was found to be less than one (Table 2). The compound producing a score of 2 or less at 24 h was considered negative for irritation. Since the test formulation fulfilled the above condition, it was concluded that the TTS was devoid of skin irritation potential and could be well tolerated during the course of treatment.

3.6. *Accelerated stability analysis*

The formulation was subjected to accelerated stability analysis. On the basis of first order degradation kinetics the shelf life ($t_{0.9}$) was calculated to be 5.5 years (data not shown), which predicts very high stability of the drug in the TTS. The result conformed to the extremely high stability of the API with only 1% degradation in 3 years (data not shown). Various physical characteristics, thickness, weight, folding endurance, and percent elongation at break varied to negligible proportions and drug content declined insignificantly in 12 weeks (data not shown).

4. Discussion

Glibenclamide is a potent hypoglycemic drug indicated in the management of NIDDM. However, the drug shows an erratic absorption pattern due to interpatient and formulation factors resulting in variable oral biological half life. Also the erratic absorption of the

Table 2. Skin irritation data of formulation A, B and C

Formulations	Intact skin (i)			Abraded skin (ii)			Combined average (i + ii)
	24 h	72 h	Average	24 h	72 h	Average	
A	1	0	0.33	2	1	0.67	0.50
	0	0		0	0		
	1	0		1	0		
B	1	1	0.33	1	1	0.83	0.58
	0	0		1	0		
	0	0		1	1		
C	1	0	0.50	2	0	0.83	0.66
	1	1		1	1		
	0	0		1	0		

Criteria of scores for visual observation were as follows. Erythma scale: none, 0; very slight, 1; well defined, 2; moderate, 3; severe, 4. Edema scale: none, 0; very slight, 1; well defined, 2; moderate, 3; severe, 4.

drug leads to its major side effect, *i.e.*, hypoglycemia which is an extension of the pharmacological response (*31*). Also there is no controlled release formulation of the drug available in the market. These factors prompted us to develop Glibenclamide TTS to provide for the controlled and sustained release of the drug for a period of 48 h. The drug sample (API) was characterized on the basis of physicochemical and spectral analysis; the result confirmed it to be the pure sample of glibenclamide (*32*).

Since the amount of drug to be incorporated in the system was higher, monolithic matrix type TTS were fabricated by a film casting technique on an aluminum surface (*33*). Methyl alcohol/dichloromethane (3:5, v/v) was found to be an ideal solvent system for film formation (*34*). An elevated temperature condition was selected for solvent evaporation as the time required was reduced and complete removal of solvent was achieved. The polymer selected were non adhesive, therefore, a micro porous tape which forms an adhesive rim which could be stuck onto the skin was used. The Eudragit polymer based placebo films which yielded satisfactory results on physical characterization (thickness, weight, folding endurance, and % elongation at break) were selected for incorporation of the drug and the penetration enhancer.

Due to a moderate skin permeation rate of glibenclamide by passive diffusion (*32*), different chemical methods of permeation enhancement were used with various penetration enhancers such as dimethyl sulfoxide, propylene glycol, *N*,*N*-dimethylformamide, citral, and oleic acid. In the preliminary study, Tween 20 and turpentine oil were tried (data not shown). It was found that citral was the most effective enhancer for glibenclamide yielding 5.0-fold enhancements in permeability coefficients over controls. In conclusion, 5% citral was selected as enhancer for incorporation in medicated patches.

An apparatus similar to Keshary-Chein's diffusion cell (*35*) was fabricated for the evaluation of *ex vivo* skin permeation of the drug from the formulations. Formulations containing Eudragit RL polymer exhibited satisfactory results. These formulations were modified in quest of improved performance. The modified patches were coded as A, B and C containing Eudragit RL100/PVP K-30 (6:4), Eudragit RL100/PVP K-30 (4:6), and Eudragit RL100/PVP K-30 (8:2), respectively. The formulation containing the maximum ratio of RL100 and minimum ratio of PVP K-30 shows the maximum permeation (99%) of the drug, it suggests that the release pattern of drug from formulation "C" is more favorable. Whereas formulation A containing polymer ratio 6:4 showed poor permeability (95.3%) in comparison to formulation C (Figure 1). After an initial lag period permeation was observed to be gradually approaching a constant for the rest of the time, which exhibited the controlled release behavior of these systems.

The plots of cumulative percentage drug permeated (Q) *vs*. square root of time showed an almost linear curve for the three optimized formulations which endorsed the Higuchi matrix diffusion mechanism of drug release for these formulations.

The *in vivo* studies on selected transdermal formulation (A, B and C) containing 0.46 mg/kg of drug were carried out by determining the reduction in blood glucose level of albino rabbits. Sufficiently high reduction in blood glucose level ($p < 0.05$) was obtained within 2 h for all formulations. The reduced levels were maintained almost constant with a slight rise in blood glucose level on administration of food. Normal glucose levels were restored in approximately 8 h, after the removal of the transdermal system. There was no reduction in blood glucose level when the placebo formulations were applied on the skin of albino rabbits ($p > 0.05$). Only slight variation in glucose level was observed on administration of food.

The result of the interaction studies indicates that the drug remained intact in the formulation and there was no chemical interaction between the drug and excipient therein. The fabricated system produced no or very mild skin irritation, which could be well tolerated during the course of therapy. The accelerated stability studies at elevated temperature and humidity (*29*) revealed that the developed TTS held promise for high stability ($t_{0.9}$ = 5.5 years) and satisfactory performance if stored at optimum temperature and humidity conditions.

5. Conclusion

On the basis of above *ex vivo* and *in vivo* evaluations, it could be concluded that glibenclamide, a potent hypoglycemic drug, could be administered successfully from the matrix type monolithic TTS for controlled and sustained management of NIDDM for a period of 48 h. The systems were free of any hazardous skin irritation. Further work needs to be done to establish the therapeutic utility of these systems through long term pharmacokinetic and pharmacodynamic studies on healthy human subjects and patients.

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