Glibenclamide Transdermal Patches: Physicochemical, Pharmacodynamic, and Pharmacokinetic Evaluations

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ABSTRACT: In the present study, matrix type transdermal patches containing glibenclamide were prepared using different ratios of ethyl cellulose (EC)/polyvinylpyrrolidone (PVP) and Eudragit RL-100 (ERL)/Eudragit RS-100 (ERS) by solvent evaporation technique. The possible drug and polymer interaction was studied by infrared spectroscopy, differential scanning calorimetry, and HPTLC analysis. All the prepared formulations were subjected to physicochemical studies (thickness, weight variation, drug content, moisture content and uptake, and flatness), in vitro release and in vitro permeation studies through mouse skin. The results suggested that there was no interaction between drug and polymers. Variations in drug release/permeation profiles among the formulations studied were observed. The microphotographs obtained by scanning electron microscopy showed the formation of pores on the surface of the patches after in vitro skin permeation studies. Based on physicochemical and in vitro skin permeation studies, the formulations with EC:PVP (3:2) and ERL:ERS (4:1) were selected for in vivo experiments. The hypoglycemic activity of the patches in comparison with oral glibenclamide administration was studied for acute (24 h) and long-term (6 weeks) effect in both normal and streptozotocin-induced diabetic mice. Various biochemical parameters (serum levels of high-density lipoprotein-cholesterol, triglycerides, total cholesterol, alanine transaminase, aspartate transaminase, urea, and creatinine and liver protein and glycogen content) and histopathological (liver, pancreas and stomach) studies were carried out in diabetic mice after treating for 6 weeks. The patches were subjected to skin irritation test (by both visual observation and histopathological evaluation), oral glucose tolerance test and pharmacokinetic evaluation in mice. The results revealed that the patches successfully prevented the severe hypoglycemia in the initial hours, which is the major side effect associated with oral route. The patches maintained similar effect during long-term treatment also. The transdermal systems produced better improvement with all the tested biochemical parameters compared to oral administration. They produced improved repair of the tissues after diabetes induced tissue injury and exhibited negligible skin irritation. The pharmacokinetic evaluation showed that the patches could maintain almost steady-state concentration of drug within the pharmacologically effective range for prolonged period of time. The better in vivo performance of the transdermal patches of glibenclamide in comparison with oral administration could be due to day-to-day glycemic control on long-term application. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:1577–1594, 2004

Keywords: transdermal; glibenclamide; ethyl cellulose; Eudragit; hypoglycemic; skin irritation; diabetes mellitus

INTRODUCTION

Diabetes mellitus is one of the important diseases involving the endocrine pancreas. Its major manifestations include disordered metabolism and
inappropriate hyperglycemia. A therapeutic classification includes two major types of diabetes mellitus. Type I diabetes (Insulin-Dependent Diabetes Mellitus; IDDM) is a severe form associated with ketosis in the untreated state. Type II (Noninsulin-Dependent Diabetes Mellitus; NIDDM) represents a heterogeneous group comprising a milder form of diabetes that occurs predominately in adults. Circulatory endogenous insulin is sufficient to prevent ketoacidosis, but is often subnormal or relatively inadequate because of tissue insensitivity.\(^1\)\(^2\) The vast majority of diabetic patients have NIDDM. In the United States, about 90% of all diabetic patients have NIDDM. The incidence rates of NIDDM increase with age, with a mean rate of about 440 per 100,000 per year by the sixth decade in males in the United States.\(^2\) Glibenclamide, an important drug of sulfonylurea class, is currently available for treating hyperglycemia in NIDDM. It increases the release of endogenous insulin as well as its peripheral effectiveness. It lowers the blood glucose level in both nondiabetic and diabetic individuals. The principal action of glibenclamide is on beta cells, stimulating insulin secretion and thus reducing plasma glucose.\(^1\)\(^2\) Many sulfonylureas like glibenclamide and glipizide have been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heartbeat, anorexia, and increased appetite after oral therapy. Glibenclamide is best avoided in the elderly and in patients with even mild renal impairment because of the risk of hypoglycemia.\(^3\)\(^4\) Because these drugs are usually intended to be taken for a long period, patient compliance is also very important.\(^5\)

Transdermal drug delivery offers many advantages such as reduced side effects, improved patient compliance, elimination of first-pass effect, sustained drug delivery, and interruption or termination of treatment when necessary.\(^6\) We previously reported the feasibility of application of transdermal delivery for glibenclamide.\(^7\)\(^8\) Glibenclamide (molecular weight: 494) showed favorable partition coefficients (\(\log \text{octanol/buffer: 0.32} \pm 0.07; \log \text{isopropylmyristate/buffer: 0.50} \pm 0.05\)) and negligible skin degradation. The enhancing effect of various penetration enhancers like propylene glycol, transcutol, Tween-20, polyethylene glycol, N-methyl-2-pyrrolidinone, geraniol, citral, eugenol, d-limonene, and varying concentrations of ethanol on the permeation of glibenclamide was investigated. The studies showed that target permeation rate of glibenclamide (193.8 \(\mu g/h\)) could be achieved by increasing the area of application in an appreciable range.\(^7\)\(^8\) In the present study, we have formulated the matrix-type transdermal systems of glibenclamide using the polymeric combinations of ethyl cellulose (hydrophobic) and polyvinylpyrrolidone (hydrophilic) and Eudragit RL-100 (hydrophilic) and Eudragit RS-100 (hydrophobic), and evaluated it with respect to various \textit{in vitro} parameters (physical characteristics like weight variation, thickness, drug content, moisture content/uptake, drug content, scanning electron microscopy, flatness, \textit{in vitro} release/permeation kinetics, etc.) and pharmacological, biochemical, and histopathological effects in an \textit{in vivo} normal and diabetic mouse model.

**MATERIALS AND METHODS**

Ethyl cellulose (EC; with an ethoxyl content of 47.5–53.5% by weight and a viscosity of 14 cps in a 5% w/w, 80:20 toluene:ethanol solution at 25°C) was purchased from SD Fine Chemicals Ltd., India. Polyvinylpyrrolidone-K30 (PVP) was procured from Loba Chemie, India. Sodium deoxycholate, anthrone, thiourea, streptozotocin, and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, MO. Glibenclamide was a gift from Bal Pharma, Modi-Mundi Pharma and Wallace Pharmaceuticals, India. All the other chemicals used were of analytical/reagent grade.

**Development of Transdermal Systems**

Matrix-type transdermal patches containing glibenclamide were prepared using different ratios of EC/PVP and ERL/ERS (Table 1). The polymers were weighed in requisite ratios keeping the total polymer weight 300 mg, and dissolved in chloroform. \(\text{di-}n\)-Butylphthalate was used as a plasticizer. Glibenclamide was added and mixed slowly with a mechanical stirrer. The polymeric solution of drug (5 mL) was poured on the mercury surface (25 cm\(^2\)), and dried at room temperature. After 24 h, the films were cut into a 12 cm\(^2\) area and backing membrane (biaxially oriented polypropylene film, obtained as a gift sample from Pidilite (P) Ltd., India) was then glued. A glossy paper having a smooth surface was used as release liner.
Drug–Polymer Interaction Studies

To search the possible interaction between glibenclamide and polymeric materials of the patches, infrared (IR) spectroscopy, differential scanning calorimetry (DSC), and high-performance thin-layer chromatographic (HPTLC) analysis were carried out on pure substances and their physical mixtures. The IR spectra were recorded using IR-Spectrophotometer (FTIR-8300, Shimadzu, Japan) by KBr pellet method. DSC analysis was performed using calorimeter (Perkin-Elmer, Norwalk, CT). All the samples were weighed and heated at scanning rate of 10°C/min between 50 and 300°C. The HPTLC analysis was performed using CAMAG-HPTLC system consisted of a CAMAG Linomat IV-automatic spotting device, a twin-trough chamber, a CAMAG TLC scanner-3, CAMAG Cats-4 software, and a 100-μL HPTLC syringe. The precoated TLC silica gel G60 F254 plates were procured from E. Merck, India. The mobile phase consisted of ethyl acetate and strong ammonia solution (10:0.1). On the plates, 5 μL of each solution in methanol containing drug alone or drug and different ratios of polymers was spotted using a Linomat IV-automatic spotting device. The plates were dried in the stream of warm air for 5 min and scanned using CAMAG Cats-4 software. The Rf values were calculated from the obtained chromatograms.

Evaluation of Transdermal Patches

Thickness

The thickness of the patches was assessed at six different points of the patch using Thickness Gauze (Mitutoyo, Japan). For each formulation, three randomly selected patches were used.

Weight Variation

Six films from each batch, as whole (25 cm²) and cut patches (12 cm²), were weighed individually and the average weight was calculated.

Drug Content

Patches (n = 3) of specified area were cut and weighed accurately. The pieces were taken into a 100-mL volumetric flask and dissolved in methanol. The solution was filtered through 0.45-μm membrane (Nulge Nunc, UK) prior to drug analysis.7,8

Moisture Content

The patches were weighed individually and kept in a desiccator containing calcium chloride at 40°C for 24 h. Then, the final weight was noted when there was no further change in the weight of individual patch. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.9

Moisture Uptake

The films were weighed accurately and placed in a desiccator containing 100 mL of saturated solution of aluminum chloride (79.50% RH). After 3 days, the films were taken out and weighed. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.10

Flatness

Longitudinal strips were cut out from the prepared patch, the length of each strip was measured, and then variation in the length due to the

<table>
<thead>
<tr>
<th>Ratios of Polymers</th>
<th>DBP (% w/w of Polymers)</th>
<th>Thickness (mm)</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC:PVP (4.5:0.5)</td>
<td>30</td>
<td>0.110 ± 0.009</td>
<td>371.5 ± 2.66 (178.9 ± 3.12)</td>
</tr>
<tr>
<td>EC:PVP (4:1)</td>
<td>30</td>
<td>0.105 ± 0.009</td>
<td>374.0 ± 3.64 (180.5 ± 2.49)</td>
</tr>
<tr>
<td>EC:PVP (3:2)</td>
<td>30</td>
<td>0.120 ± 0.007</td>
<td>372.0 ± 2.44 (179.2 ± 1.81)</td>
</tr>
<tr>
<td>ERL:ERS (4:1)</td>
<td>30</td>
<td>0.095 ± 0.005</td>
<td>371.9 ± 2.95 (176.8 ± 2.09)</td>
</tr>
<tr>
<td>ERL:ERS (3:2)</td>
<td>30</td>
<td>0.101 ± 0.003</td>
<td>372.3 ± 2.49 (179.1 ± 3.47)</td>
</tr>
<tr>
<td>ERL:ERS (2:3)</td>
<td>30</td>
<td>0.098 ± 0.003</td>
<td>373.1 ± 3.52 (180.2 ± 2.15)</td>
</tr>
</tbody>
</table>

The area of the patches was 25 cm². Amount of drug incorporated was 18 mg. Total weight of polymers was 300 mg. Volume of chloroform used was 5 mL. All values are expressed as mean ± SD, n = 3. The values given in parenthesis represent the weight of 12 cm² patches. DBP = di-n-Butylphthalate.
nonuniformity in flatness was measured. Flatness was calculated by measuring constriction of strips, and a 0% constriction was considered to be 100% flatness.¹¹

**In Vitro Release (Dissolution) Studies**

The dissolution of the patches was performed using USP basket-type dissolution apparatus. The patches were placed in respective baskets with their drug matrix exposed to dissolution medium (phosphate buffer, pH 7.4). All dissolution studies were performed at 50 rpm, with each dissolution jar having 900 mL of buffer. The samples withdrawn at different time intervals were passed through 0.45-μm membrane and analyzed for drug content.⁷,⁸

**In Vitro Skin Permeation Studies**

The in vitro skin permeation experiments were conducted using vertical type diffusion cells having receptor compartment capacity of 20 mL. Membrane for the permeability studies was the dorsal section of full thickness skin from Swiss albino mice, 6–8 weeks old, whose hair had been removed on the previous day with an electric clipper. The receiver compartment was filled with 20 mL of phosphate buffer, pH 7.4 (PB). The transdermal patches with backing membrane were firmly pressed onto the center of the mouse skin. Once adhesion to the skin surface had been confirmed, the skin was quickly mounted on the pregreased flange of the diffusion cell receptor compartment such that the patch was situated precisely over the flange aperture. The donor compartment was then placed in position and the two halves of the cell clamped together. The whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred using a magnetic bead. The samples were withdrawn at different time intervals and analyzed for drug content.⁷,⁸ Receptor phases were replenished with an equal volume of PB at each time interval. The cumulative amounts of drug permeated per square centimeter of patches were plotted against time.

**Scanning Electron Microscopy (SEM)**

The morphology of the transdermal patches before and after in vitro skin permeation experiments was analyzed by scanning electron microscopy (JEOL-JSM-840A, Japan). The patches were splutter coated with gold before scanning.

**In Vivo Studies**

The animals used for in vivo experiments were adult Swiss albino mice (6–8 weeks old) of either sex, weighing 25–30 g, from the Department of Radiobiology, Kasturba Medical College, Manipal. The animals were housed in polypropylene cages, four per cage, with free access to 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, Kasturba Medical College, Manipal.

**Hypoglycemic Activity in Normal Mice**

The hair on the backside of the mice was removed with an electric hair clipper on the previous day of the experiment. Following an overnight fast, mice were divided into four groups (n = 6). Group I served as control, was fed with 0.2 mL of 0.5% w/v sodium carboxymethyl cellulose (CMC). Group II was administered with glibenclamide (5 mg/kg; p.o.). The oral dose of glibenclamide was selected by conducting a series of experiments with graded doses ranging between 1 to 10 mg/kg. Group III animals were applied with 2.5 cm² transdermal matrix system prepared with EC and PVP (3:2), containing 1.8 mg of glibenclamide. Animals of group IV were applied with 2.5 cm² transdermal matrix system prepared with ERL and ERS (4:1), containing, 1.8 mg of glibenclamide. At time intervals between 2–24 h after treatment (acute study), blood was collected from orbital sinuses; blood glucose levels were determined using Accutrend Alpha Glucometer (Roche Diagnostics, Germany). In the long-term study, the above treatments were administered/applied once daily for 6 weeks. Blood glucose levels were determined once in every 2 weeks in over night fasted mice, 2 h after drug treatment as previously described.

**Induction of Diabetes Mellitus and Hypoglycemic Activity in Diabetic Mice**

Diabetes was induced in overnight fasted mice by injecting streptozotocin (150 mg/kg; i.p.) dissolved in citrate buffer (3 mM; pH 4.5).¹² Seven days later, mice with blood glucose levels between 300–400 mg/dL were selected. The acute and
long-term hypoglycemic activity of the transdermal patches was evaluated in overnight-fasted diabetic mice as described in the earlier section.

**Effect on Glucose Tolerance**

After an overnight fast, mice were divided into four groups \( n = 6 \). Control group was administered with 0.2 mL of CMC. The other three groups were administered with glibenclamide (5 mg/kg; p.o.) or applied with transdermal patches as described in earlier experiments. Two hours later, glucose was administered orally (2 g/kg) to all the four groups. Blood samples were collected just prior to and at 0.5, 1.0, and 2.0 h after the glucose feeding, and glucose level was determined. The percentage change in blood glucose was estimated in comparison with the control group.

**Biochemical and Histopathological Evaluation**

At the end of the treatment period in the long-term experiment, blood was collected from orbital sinuses of diabetic mice and serum was separated off. Lipid profile (high-density lipoprotein-cholesterol, triglycerides, and total cholesterol), alanine transaminase (ALT), aspartate transaminase (AST), urea, and creatinine levels were estimated in serum using an Auto-analyzer (Hitachi 911, Japan). Finally, the animals were sacrificed by cervical dislocation and the liver and pancreases were removed. A part of the liver was processed for glycogen estimation and total protein. Pieces of the liver, pancreas, and stomach were fixed in Bouin’s fixative and processed routinely for histological screening. The slides were stained with hematoxyline and eosin (H&E) and observed under a low-power microscope for any pathological changes.

**Skin Irritation Test (Visual and Histopathological Evaluation of Skin)**

The mice were divided into seven groups \( n = 6 \). On the previous day of the experiment, the hair on the backside area of mice was removed. The animals of group I were served as normal, without any treatment. One group of animals (Group II, control) was applied with adhesive tape (USP). Transdermal patches (blank, without drug and drug loaded) were applied onto nude skin of animals of the III, IV, V, and VI groups. A 0.8% v/v aqueous solution of formalin was applied as a standard irritant (Group VII). The animals were applied with new patch/formalin solution each day upto 7 days, and finally the application sites were graded according to a visual scoring scale, always by the same investigator. The erythema scale was as follows: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation. The edema scale was: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, severe. After visual evaluation of skin irritation, the animals were sacrificed by cervical dislocation and treated skin was dissected out. The skin samples were processed for histological examination as described in an earlier experiment and the slides of skin samples were observed under a low-power microscope for any pathological changes.

**Pharmacokinetic Evaluation**

Overnight-fasted mice, whose hair was previously removed, were divided into three groups \( n = 6 \). Group I was administered with glibenclamide orally (5 mg/kg). Group II animals were applied with 2.5 cm\(^2\) transdermal matrix system prepared with EC and PVP (3:2). Animals of group III were applied with a 2.5-cm\(^2\) transdermal patch prepared with ERL and ERS (4:1). The patches contained 1.8 mg of glibenclamide. Blood samples were withdrawn at different time intervals from orbital sinuses using heparinized capillaries. Plasma was separated by centrifugation using Biofuge-13 (Heraeus Instruments, Germany) and stored in vials at −70°C until further analysis.

Glibenclamide was estimated in plasma by an earlier reported reverse phase HPLC method with little modification. A Shimadzu Class VP series HPLC system with two LC-10AT pumps, a SPD-10A variable wavelength programmable UV/Vis detector, a SCL-10A system controller, and a RP C-18 column (Luna, Phenomenex, USA; 250 times; 4.6 mm; particle size 5 μm) were used. The system was equipped with Class VP series version 6.12 software. The pharmacokinetic parameters were calculated using noncompartmental pharmacokinetics data analysis software, PK Solutions 2.0™.

**Statistical Analysis**

The results were analyzed by a Student’s $t$-test using Graph Pad Instat Software (Version: 1.13). A difference below the probability level of 0.05 was considered statistically significant.
RESULTS AND DISCUSSION

In this study, various matrix-type transdermal patches containing glibenclamide and variable combinations of EC/PVP and ERL/ERS were prepared. It was desired to design a polymer matrix that allows one to provide an optimum drug release via the most appropriate choice of polymeric blends of EC/PVP and ERL/ERS among the formulations studied, using the different diffusion pathways of the individual polymeric composition to produce the desired overall prolonged/sustained drug release. The excellent film-forming ability of these polymeric combinations, EC/PVP and ERL/ERS, has already been well proven and, these polymeric combinations produced good films compared to the films prepared by a single polymer.10,19,20

Drug–Polymer Interaction Studies

The possible drug–polymer interaction was studied by IR spectroscopy, DSC, HPTLC analysis of pure substances, and their physical mixtures. The IR spectral analysis of glibenclamide alone showed that the principal peaks were observed at wave numbers of 1527.50, 1157.2, 1618.2, 1714.6, and 819.7, confirming the purity of the drug as per established standards.18 In the IR spectra of the physical mixture of glibenclamide, ERL, and ERS, the major peaks of glibenclamide were 1527.50, 1159.1, 1618.2, 1716.5, and 819.7 and 1527.50, 1157.2, 1618.2, 1714.6, and 819.7 wave numbers were observed for the mixture of glibenclamide, EC, and PVP. However, some additional peaks were observed with physical mixtures, which could be due to the presence of polymers. The DSC analysis (Fig. 1) of pure glibenclamide showed a sharp endotherm peak at 175.16 corresponding to its melting point. The DSC analysis of physical mixtures of drug and polymers revealed negligible change in the melting point of glibenclamide in the presence of any polymer mixture studied (174.06 and 169.94 °C for the mixtures of glibenclamide, EC, and PVP and glibenclamide, ERL, and ERS, respectively). In HPTLC analysis, the $R_f$ value of pure glibenclamide was found to be 0.92. In the presence of polymers, the $R_f$ value of the drug was unchanged and ranged from 0.90 to 0.92. No distinct difference in the IR peaks and melting point (DSC analysis) of drug in the physical mixtures and $R_f$ values of drug (HPTLC analysis) in the polymeric solution used in our study indicates that the polymers do not alter the performance characteristics of the drug from the patches studied. All these results suggest that there is no interaction between the drug and polymers used in the present study. It is already

![Figure 1. DSC thermograms: a = mixture of ERL and ERS; b = mixture of EC and PVP; c = mixture of glibenclamide, EC and PVP; d = glibenclamide; e = mixture of glibenclamide, ERL, and ERS.](image-url)
well known that the common polymers such as PVP, EC, ERL, and ERS are popular in controlled/sustained release matrix type patches because of their compatibility with a number of drugs.21

**Thickness and Weight Variation**

The results of thickness and weight variation test of the patches are shown in Table 1. The thickness and weights of the patches were found to be uniform among different batches. There was a proportionate relationship between the weight of the 25 cm² patch to that of the 12 cm² patch (used for other experiments), suggesting the uniform distribution of the drug and polymer over the mercury surface. Good uniformity in drug content among the batches was observed with all formulations and ranged from 98.02 ± 0.55 to 99.68 ± 0.29%.

**Moisture Content and Moisture Uptake Studies**

The results of the moisture content and moisture uptake (%) studies are shown in Figure 2. The moisture content in the formulations was found to be low and ranged from 2.25 ± 0.20 to 3.45 ± 0.12% for EC/PVP patches and 1.55 ± 0.20 to 3.25 ± 0.15% for ERL/ERS patches. The results revealed that the moisture content/uptake was found to increase with increasing concentration of hydrophilic polymers, PVP, and ERL. The small moisture content in the formulations helps them to remain stable and from being a completely dried and brittle film. Again, a low moisture uptake protects the material from microbial contamination and bulkiness of the patches.9–11

**Flatness**

The results of flatness study showed that none of the formulations had the difference in the strip lengths before and after their cuts, thus indicating 100% flatness. It shows that no amount of constriction in the patches and thus they could maintain a smooth surface when applied onto the skin.

**In Vitro Release—Dissolution Studies**

The results of *in vitro* drug release studies from transdermal patches are depicted in Figure 3. The formulations with EC:PVP (3:2) and ERL:ERS (4:1) exhibited the greatest (71.25 ± 8.55 and 63.55 ± 10.95%, respectively) percentage of drug release values, which were significantly (*p* < 0.05) different compared to the lowest values observed with the formulations containing EC:PVP (4.5:0.5) and ERL:ERS (2:3) (47.65 ± 9.55 and 40.25 ± 8.58%, respectively). The dissolution studies of the patches are very crucial, because one needs to maintain the drug concentration on the surface of stratum corneum consistently and substantially greater than the drug concentration in the body, to achieve a constant rate of drug permeation.22

In the present study, it was observed that as the concentration of hydrophilic polymers (PVP/ERL) increased in the formulations, the dissolution rate increased substantially. The addition of hydrophilic component to an insoluble film former tends to enhance the release rates, as reported by Bodmeier and Paeratakul.24 It has also been reported that PVP decreases the crystallinity of the drug in the patch, which accounts for the increased release of drug with an increase in the PVP concentration in the patches.20 The drug release was high from the formulations in the initial hours. This may be because of the presence of hydrophilic polymers, and their hydrophilic layers might need a very little “time lag” to establish a concentration profile in the patches resulting in a “burst release” in the dissolution studies.11

**In Vitro Skin Permeation Studies**

The results of *in vitro* skin permeation of glibenclamide from patches are shown in Figure 4.
The formulations (1 cm²) with EC:PVP (3:2) and ERL:ERS (4:1) exhibited the greatest (262.92 ± 15.25 and 254.58 ± 15.52 mg, respectively) cumulative amounts of drug permeation, which were significantly \( (p < 0.05) \) different compared to the lowest values observed with the formulations containing EC:PVP (4.5:0.5) and ERL:ERS (2:3) (216.25 ± 16.25 and 205.41 ± 13.25 mg, respectively) at the end of 24 h.

Variable permeation profiles of glibenclamide from different experimental patches composed of various blends of EC/PVP and ERL/ERS were observed. This can be explained in the following way. The process of drug release in most controlled release devices is governed by diffusion, and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. The alterations of the crosslinking and the modification of structural arrangements of polymers by using different blends of polymers were already reported. So, different drug release profiles from different formulations could be attributable to the varied crosslinking networks of polymeric chains of the different blends of polymeric transdermal experimental formulations as

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**Figure 3.** Cumulative percentage of glibenclamide released in *in vitro* dissolution studies from transdermal patches prepared by different proportions of EC/PVP (a) and ERL/ERS (b). Each point represents mean ± SD, \( n = 3 \). *Significant compared to EC:PVP (4.5:0.5). #Significant compared to ERL:ERS (2:3).

**Figure 4.** Cumulative amount of glibenclamide permeated (mg/cm²) across mouse skin from transdermal patches prepared by different proportions of EC/PVP (a) and ERL/ERS (b). Each point represents mean ± SD, \( n = 3 \). *Significant compared to EC:PVP (4.5:0.5). #Significant compared to ERL:ERS (2:3).
tortuosity and diffusion pathway varied, and they thereby have been reported to vary the release of drug and duration of diffusion.¹¹

In in vitro skin permeation experiments also, as the concentration of hydrophilic polymer was increased, the amount of drug permeated was increased. This may be a result of the initial rapid dissolution of the hydrophilic polymers when the patch is in contact with the hydrated skin, which results in accumulation of high amounts of drug on the skin surface and thus leads to the saturation of the skin with drug molecules at all times.²⁰ The rapid dissolution of the aqueous soluble fraction of the film also leads to the formation of pores, and hence, higher release rates.²⁰ Further, in the present study, the SEM evaluation also revealed the pore formation in the patch after skin permeation experiments.

The cumulative amounts of drug permeated per square centimeter of patches through the mouse skin when plotted against time, the permeation profiles of drug seem to follow mixed order/apparent zero-order kinetics (Fig. 4). Initially, up to 6–8 h the drug permeation followed zero-order kinetics because the drug matrix ensured constant concentration. Afterward, however, concentration-dependent kinetics changed the system toward a first-order pattern. Release of the drug from a transdermal drug delivery system mainly involves factors of diffusion.²⁵ Diffusion is related to the transport of drugs from a dosage matrix into the in vitro study fluid, depending on the concentration. As the gradient varies, the drug is released, and the distance for diffusion becomes increasingly greater. This could be an explanation as to why the drug diffuses comparatively at a slower rate as the distance for diffusion increases. If the release of drug from the transdermal film, when plotted against the square root of time yields a straight line, it indicates that the release pattern is obeying Higuchi’s kinetics.²⁶ In our experiments, the in vitro permeation profiles of all formulations did not fit into zero-order (R² = 0.9752 to 0.9853) and first-order behavior (R² = 0.8581 to 0.8927) completely, and they could be best expressed by Higuchi’s equation (R² = 0.9969 to 0.9982) for the permeation of drug from a homogeneous—polymer matrix-type delivery system that depends mostly on diffusion characteristics.⁹ The data was further treated as per the following equation:²⁷,²⁸

\[ \frac{M_t}{M_\infty} = K \cdot t^n \]

where, \( \frac{M_t}{M_\infty} \) is the fractional release of drug, \( M_t \) is the amount released at time \( t \), \( M_\infty \) is the total amount of drug contained in the transdermal patch, \( t \) is the release time, \( K \) is a kinetic constant, and \( n \) is the diffusional release exponent indicative of the operating release mechanism. The \( n \) values (0.6134 < \( n \) < 0.6561) obtained by this equation indicated that amount of released drug by Fickian diffusion predominated with all formulation. This observation thus supports the results obtained with Higuchi’s equation that the patches released the drug by diffusion-dominated mechanism. These results are in agreement with those reported by Guyot and Fawaz²⁸ and Verma and Murthy.²⁹

**Scanning Electron Microscopy**

Figure 5 shows the microstructure of EC/PVP (3:2) and ERL:ERS (4:1) films before and after the drug permeation experiments. The films prior to in vitro skin permeation studies showed uniform-smooth surface (a and c). After the permeation studies (b and d), the surface became rough and pores were formed on the surface of the patches due to the diffusion of the drug. The formation of pores in EC:PVP and Eudragit patches have already been demonstrated after in vitro permeation experiments.²⁰,³⁰ However, the films did not lose the integrity after the release, further indicating that the drug was released from the films predominantly by diffusion. Figure 5b and d also shows that after the release of drug molecules, the distorted portion of the membrane had a tendency of maintaining elasticity in an affected small area with little effect on the other part of the membrane. Thus, this shows that very little or almost no constriction, that is, 100% flatness of patches, persists even after the patches were deprived of the drug molecules. Therefore, it may be suggested that the formulations with EC/PVP (3:2) and ERL/ERS (4:1) are suitable for transdermal formulations in terms of their physical stability.

The aim of the present study was to overcome the initial hypoglycemia by transdermal administration, which is generally observed with oral administration of glibenclamide. The drug should permeate in a slow rate in the initial hours besides maintaining the suitable concentration of drug to elicit blood glucose-lowering activity. Although the amount of drug permeated in the initial hours was less, formulations with EC:PVP (4.5:0.5), EC:PVP (4:1), ERL:ERS (3:2), and ERL:ERS (2:3) could not achieve high cumulative amount of drug permeation at the end of 24 h; whereas the formulations with EC:PVP (3:2) and ERL:ERS (4:1) provided a
high cumulative amount of the drug permeation at the end of 24 h with comparatively negligible high permeation of the drug in the initial hours. Hence, formulations with EC:PVP (3:2) and ERL:ERS (4:1) were selected for in vivo experimentation. Further formulation development by increasing the ratio of hydrophilic polymers (PVP and ERL) was not attempted, as those systems showed burst release of the drug in the initial time period. The amount of drug in the $12 \text{cm}^2$ patch was 8.6 mg. We arrived to this combination by conducting a series of in vitro release and skin permeation experiments of the patches, loaded with different amounts of the drug.

Acute Hypoglycemic Activity

The results of acute hypoglycemic activity of transdermal patches in comparison with glibenclamide (5 mg/kg; p.o.) in both normal and diabetic mice are shown in Table 2. The hypoglycemic effect was significant in oral and transdermal patch-treated animal groups up to 10 h, compared with the control group ($p < 0.05$). Glibenclamide (oral) produced a decrease of $39.71 \pm 6.81$ (normal mice, $p < 0.05$ compared to control) and $38.12 \pm 2.12\%$ (diabetic mice, $p < 0.05$ compared to diabetic control) in blood glucose levels at 2 h. In the case of transdermal patches, the hypoglycemic response was gradual. A maximum hypoglycemic response was observed after 6 h, and thereafter remained stable up to 24 h. In the orally glibenclamide treated group, the blood glucose levels declined after 6 h and was only $10.58 \pm 5.22$ (normal mice) and $13.25 \pm 4.55\%$ (diabetic mice) after 24 h. On the other hand, both the patches produced a significant reduction in blood glucose levels up to 24 h compared to control ($p < 0.05$). The untreated group did not show any noticeable hypoglycemia. These results clearly show that the matrix could sustain the drug release for a period of 24 h when compared with oral administration where the effect declined after 6 h in agreement with the short half-life of glibenclamide. The glucose level reduction at 2 h was very high with oral administration, reflecting the severe hypoglycemia in the initial hours after administration. The blood glucose reduction observed with patches at 2 h was significantly less, but optimum, compared to oral glibenclamide, which
The reduction in blood glucose level (mg/dL) (percentage reduction in blood glucose levels) is shown in Table 2. Both the transdermal patches produced a significant ($p < 0.05$) hypoglycemic effect up to 6 weeks, indicating that transdermal systems provide optimum blood glucose reduction upon long-term application. The results also reveal that the severe hypoglycemia (significantly high compared to transdermal patches, $p < 0.05$) obtained in acute studies with oral administration of glibenclamide was continuously observed up to 6 weeks, which could be overcome by transdermal patches upon chronic application.

**Effect on Glucose Tolerance**

The results of oral glucose tolerance test (GTT) of transdermal patches are presented in Figure 6. The control group showed high-elevated blood glucose levels ($p < 0.05$) after glucose administration (+81.04 ± 2.81, +62.05 ± 3.41, and +15.01 ± 4.01% at 0.5, 1.0, and 2.0 h, respectively). The results showed that the GTT curve was completely inhibited in the treated groups. The hypoglycemia produced after transdermal delivery was significantly ($p < 0.05$) higher than the control group. On the contrary, the orally glibenclamide administered group showed severe hypoglycemia ranging from −30.95 ± 3.52 to −42.20 ± 2.22% at all intervals of the study period. The transdermal route effectively maintained the normoglycemic levels in contrast to the oral group, which produced remarkable hypoglycemia, an indication that a similar incident might be prevented in diabetic patients.

**Biochemical and Histopathological Evaluation**

Various complications including biochemical changes develop along with severe hyperglycemia as a consequence of the metabolic derangement in diabetes. Hence, it is necessary to investigate the efficacy of transdermal systems in reversing these changes in diabetes compared to oral administration of glibenclamide.

The results of biochemical studies are shown in Table 4. The glycogen levels in the liver of diabetic groups are shown in Table 3. Both the transdermal patches produced a significant ($p < 0.05$) hypoglycemic effect up to 6 weeks, indicating that transdermal systems provide optimum blood glucose reduction upon long-term application. The results also reveal that the severe hypoglycemia (significantly high compared to transdermal patches, $p < 0.05$) obtained in acute studies with oral administration of glibenclamide was continuously observed up to 6 weeks, which could be overcome by transdermal patches upon chronic application.
Reduction in Blood Glucose Levels after Oral and Transdermal Administration of Glibenclamide in Normal and Diabetic Mice (Long-Term Study)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Absolute Blood Glucose Level (mg/dL)</th>
<th>Reduction in Blood Glucose Level (mg/dL)</th>
<th>Percentage Reduction in Blood Glucose Levels</th>
<th>2 Week</th>
<th>4 Week</th>
<th>6 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td>Control (0.2 mL CMC)</td>
<td>128.8 ± 6.1</td>
<td>136.0 ± 4.08 (0.88 ± 1.01)</td>
<td></td>
<td>16.5</td>
<td>7.21</td>
<td>5.26</td>
</tr>
<tr>
<td></td>
<td>TP (EC:PVP, 3:2)</td>
<td>126.5 ± 6.24</td>
<td>131.5 ± 3.63 (2.21 ± 3.31)</td>
<td>4.08 (0.98 ± 1.01)</td>
<td>16.5</td>
<td>7.21</td>
<td>5.26</td>
</tr>
<tr>
<td>Diabetic mice</td>
<td>DC (0.2 mL CMC)</td>
<td>324.5 ± 12.1</td>
<td>321.2 ± 6.56 (0.91 ± 1.01)</td>
<td></td>
<td>16.5</td>
<td>7.21</td>
<td>5.26</td>
</tr>
<tr>
<td></td>
<td>TP (EC:PVP, 3:2)</td>
<td>321.5 ± 12.5</td>
<td>325.5 ± 4.25 (2.21 ± 3.25)</td>
<td>4.25 (2.14 ± 0.98)</td>
<td>16.5</td>
<td>7.21</td>
<td>5.26</td>
</tr>
<tr>
<td></td>
<td>GLB (5 mg/kg)</td>
<td>375.3 ± 12.2</td>
<td>90.7 ± 3.55 (40.00 ± 3.55)</td>
<td></td>
<td>16.5</td>
<td>7.21</td>
<td>5.26</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE; n = 6. *Significant compared to control (p < 0.05); †Significant compared to GLB (p < 0.05).

mice were significantly lowered compared to normal mice (p < 0.05). In the diabetic condition, the level of glycogen phosphorylase, an important enzyme of glycolytic pathway, is increased, and hence, liver glycogen content is decreased. The oral as well as transdermal treatment of glibenclamide significantly (p < 0.05) increased liver glycogen at the end of 6 weeks. Insulin, produced by the action of glibenclamide, stimulated glycogen synthesis, inhibited glycogenolysis and neoglucogenesis and inactivated liver phosphorylase. It also increases the activity of the enzymes that promote glycogen synthesis, especially glycogen synthase. The overall result is increase in the hepatic glycogen levels after glibenclamide treatment.

The liver protein levels of diabetic mice were significantly decreased compared to normal mice (p < 0.05) (Table 4). Insulin deficiency in diabetes stops protein synthesis and causes excessive catabolism of protein, which is utilized for gluconeogenesis. The glibenclamide treatment (both oral and transdermal) increased the protein levels significantly compared to diabetic untreated mice (p < 0.05). Hence, increased insulin release and peripheral uptake of glucose after glibenclamide treatment might be responsible for an elevated total protein content of the liver. Insulin stimulates amino acid uptake and protein synthesis and inhibits protein degradation. It inhibits the catabolism of proteins, thus decreasing the rate of amino acid release from the cells.
Table 4. Liver Protein and Glycogen Levels and Serum Lipid Profile (TC, TG, and HDL-C), Alanine Transaminase, Aspertate Transaminase, Urea, and Creatinine Levels in Diabetic Mice after Oral and Transdermal Administration of Glibenclamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Glycogen</th>
<th>Liver Protein</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (0 mL CMC)</td>
<td>3.71 ± 0.43</td>
<td>36.9 ± 4.96</td>
<td>101.1 ± 11.1</td>
<td>311.1 ± 5.41</td>
<td>126.5 ± 5.57</td>
<td>32.5 ± 2.29</td>
<td>30.2 ± 1.87</td>
<td>82.5 ± 4.25</td>
<td>5.52 ± 2.15</td>
</tr>
<tr>
<td>DC (0.2 mL CMC)</td>
<td>1.49 ± 0.15b</td>
<td>11.5 ± 1.88b</td>
<td>101.1 ± 4.11b</td>
<td>165.2 ± 8.11b</td>
<td>105.2 ± 7.59b</td>
<td>54.5 ± 5.51b</td>
<td>105.5 ± 7.54b</td>
<td>56.1 ± 6.42b</td>
<td>46.5 ± 0.72b</td>
</tr>
<tr>
<td>TP (EC:PVP, 3:2)</td>
<td>3.32 ± 0.25a</td>
<td>3.55 ± 0.29a</td>
<td>127.2 ± 4.25a</td>
<td>132.5 ± 7.54a</td>
<td>52.2 ± 6.56a</td>
<td>61.3 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
</tr>
<tr>
<td>GLB (5 mg/kg)</td>
<td>2.95 ± 0.32</td>
<td>24.5 ± 3.35</td>
<td>127.2 ± 7.54b</td>
<td>132.5 ± 7.54b</td>
<td>52.2 ± 6.56a</td>
<td>61.3 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
<td>61.2 ± 5.59a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE, n = 6; *NC = normal control; DC = diabetic control; CMC = carboxymethyl cellulose; TP = transdermal patch; GLB = glibenclamide; TC = total cholesterol; TG = triglycerides; HDL-C = high density lipoprotein cholesterol, ALT = alanine transaminase, AST = aspartate transaminase.

The serum lipid profile (total cholesterol, triglycerides, and high-density lipoprotein—cholesterol) was significantly increased in diabetic control mice compared to normal mice (p < 0.05) (Table 4). In the diabetic condition, all the effects of insulin that cause storage of fat are reversed. The most important effect is that the enzyme hormone-sensitive lipase in the fat cells becomes strongly activated. Insulin produced by glibenclamide treatment inhibited the action of hormone-sensitive lipase, which causes hydrolysis of the triglycerides. It promotes the conversion of all this excess glucose into fatty acids. These fatty acids are subsequently packaged as triglycerides in very low-density lipoproteins and transported in this form by way of the blood to the adipose tissue and deposited as fat. The final result is a significant (p < 0.05) reduction of lipid profile after glibenclamide (transdermal/oral) treatment.

The hepatic enzyme levels (ALT and AST) were significantly (p < 0.05) increased in diabetic control mice, reflecting the hepatic damage (Table 4), which was further confirmed by the histopathological studies (Table 5). When the blood glucose level is poorly controlled in diabetes mellitus, blood vessels in multiple tissues throughout the body begin to function abnormally and undergo structural changes that result in inadequate blood supply to the tissues. Besides this, other metabolic abnormalities on protein might also have contributed to liver damage, and thereby elevated the hepatic enzyme levels. The elevated levels of AST and ALT in the liver diabetic mice were significantly (p < 0.05) decreased upon treatment. Hence, improvement of liver function and subsequent increase in the uptake of glucose and its utilization after glibenclamide treatment, which increases insulin release, may be the possible mechanisms for this observation.

The serum urea and creatinine levels were significantly (p < 0.05) elevated in untreated diabetic mice, indicating the nephrotoxicity (Table 4). This could be due to thickening of the capillary basement membrane and other vascular changes that occur during diabetes mellitus. The cumulative effect is progressive narrowing of the vessel lumina, causing inadequate perfusion of critical regions of organs. These pathologic changes contribute to premature nephropathy. The observed nephroprotective effect of glibenclamide (both oral and transdermal) could be due to the increased release of insulin, which controlled the hyperglycemia and increased the uptake and utilization of glucose by the tissues.
The histopathological studies of the liver, pancreas, and stomach from diabetic mice are presented in Table 5. The liver and pancreas from untreated diabetic mouse showed severe/moderate cellular atypia, inflammation, necrosis, degeneration, and congestion. It is well known that diabetes mellitus is generally associated with tissue damage/toxicity, which could be due to the prolonged exposure of tissues to elevated concentrations of glucose. The stomach samples from diabetic mice showed moderate ulceration. Generally, diabetes is associated with gastric ulceration due to the back diffusion of $H^+$ ions in diabetic subjects. The severe/moderate toxic manifestations including gastric ulceration were considerably reversed with oral, but especially with transdermal, administration of glibenclamide by controlling the hyperglycemia. Hence, the results indicate that the transdermal administration of glibenclamide produces improved repair of the tissues after diabetes-induced tissue injury in comparison with oral administration.

Skin Irritation Test

The skin irritation test of the formulations in comparison with adhesive patch (USP) and standard irritant, formalin (0.8%), was carried out by both visual observation and histopathological examination of film-applied skin (Table 6). The results showed that the prepared patches (both blank and drug loaded) and USP adhesive tape produced negligible erythema and edema. On the other hand, standard irritant, formalin produced severe erythema and edema. Similar results have been reported by Krishna and Pandit and Kulkarni et al. The histopathological examination of the skin indicated that adhesive tape and prepared patches produced mild irritation and edema. Formalin produced high grade of irritation, indicated by “severe” inflammation and edema, besides showing discontinuity in epidermis, thin epidermis, ulceration, and hyperplasia. These results indicate that drug does not produce any cutaneous reaction and transdermal patches are well tolerated by the subjects.

Pharmacokinetic Studies

The plasma concentrations of glibenclamide after transdermal and oral administration against time are shown in Figure 7. Peak plasma concentration, $C_{\text{max}}$, after oral administration was $9.13 \pm 0.45 \, \mu g/mL$ and $t_{\text{max}}$ was 2.0 h. In the case of the EC:PVP (3:2) patch, the $C_{\text{max}}$ and $t_{\text{max}}$ were $6.72 \pm 0.20 \, \mu g/mL$ and 12.0 h, respectively; whereas the ERL:ERS (4:1) patch showed $6.25 \pm 0.22 \, \mu g/mL$ and 12.0 h $C_{\text{max}}$ and $t_{\text{max}}$ values. The pharmacokinetic parameters were calculated from the plasma concentrations of the drug and recorded in Table 7. All the pharmacokinetic parameters obtained with glibenclamide transdermal patches

### Table 5. Histopathological Evaluation of Liver, Pancreas, and Stomach from Diabetic Mice Treated with Oral and Transdermal Administration of Glibenclamide

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue</th>
<th>CA</th>
<th>Deg</th>
<th>Nec</th>
<th>Con</th>
<th>Inf</th>
<th>Ulcers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Liver</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Liver</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GLB-Oral</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TP (EC:PVP)</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TP (ERL:ERS)</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CA = cellular atypia; Deg = degeneration; Nec = necrosis; Con = congestion; Inf = inflammation; GLB = glibenclamide; TP = transdermal patch.

Histopathological scale: + = slight; ++ = moderate; +++ = severe.
were significantly \((p < 0.05)\) different from those obtained with oral glibenclamide administration. Glibenclamide absorption after oral administration was rapid, as indicated by high \(C_{\text{max}}\) and low \(t_{\text{max}}\) values. With transdermal patches, the increase of drug concentration was slower and the concentration remained high longer. Measurable concentrations of the drug were obtained within an hour of application of the patch, and relatively steady plasma concentration of drug was observed for over 24 h with a maximum glibenclamide concentration at 12 h. The calculated parameters also indicate that the biological half-life of glibenclamide is prolonged from 6.42 ± 1.23 h (oral) to 12.82 ± 0.49 h (transdermal) in mice. Hence, the drug administered through the transdermal patch will remain in the body for a longer period and thus will exert a sustained action. The significantly less elimination rate constants and high mean residential time values of glibenclamide obtained with transdermal patches \((p < 0.05, \text{ compared to oral glibenclamide})\) further support the sustained action of drug from the patches.

Although, the \(C_{\text{max}}\) was significantly \((p < 0.05)\) less with transdermal patches, the AUC values were significantly high compared to the oral route, which could be due to the maintenance of the concentration of the drug within the pharmacologically effective range for a longer period of time. The significantly \((p < 0.05)\) high AUC values observed with transdermal patches also indicate increased bioavailability of the drug from patches compared to oral administration.

In the present study, transdermal systems of glibenclamide produced better improvement with all the tested parameters compared to oral administration. This could be due to a slow and continuous supply of glibenclamide at a desirable rate to systemic circulation by a transdermal patch, which improved day-to-day glycemic control in diabetic subjects. This statement can be further supported by an earlier report of a multicenter, randomized clinical trial designed to compare intensive therapy with conventional diabetic therapy.\(^{39}\) The intensive therapy regimen was designed to achieve blood glucose levels as close to the normal range as possible with three or more daily insulin injections or with an external insulin pump. Conventional therapy consisted of one or

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Erythema</th>
<th>Edema</th>
<th>Inf</th>
<th>Edema</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.00 ± 0.0</td>
<td>0.00 ± 0.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adhesive tape (USP)</td>
<td>1.83 ± 0.16(^a)</td>
<td>1.50 ± 0.34(^a)</td>
<td>++</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>TP-EC:PVP</td>
<td>1.00 ± 0.25(^a)</td>
<td>1.16 ± 0.16(^a)</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>TP-ERL:ERS</td>
<td>1.16 ± 0.31(^a)</td>
<td>1.00 ± 0.25(^a)</td>
<td>++</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>TP-EC:PVP (Blank)</td>
<td>1.16 ± 0.45(^a)</td>
<td>1.33 ± 0.45(^a)</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>TP-ERL:ERS (Blank)</td>
<td>1.50 ± 0.42(^a)</td>
<td>1.16 ± 0.16(^a)</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Formalin (0.8% v/v)</td>
<td>3.67 ± 0.21</td>
<td>3.50 ± 0.22</td>
<td>+++</td>
<td>+++</td>
<td>Discontinuity in epidermis; Thin epidermis; Ulceration; Hyperplasia</td>
</tr>
</tbody>
</table>

Visual observation values are expressed as mean ± SE, \(n = 6\).

\(^a\)Significant compared to formalin \((p < 0.05); TP = \text{transdermal patch}; \text{Inf} = \text{inflammation}.\)

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.

Histopathological scale: + = slight; ++ = moderate; +++ = severe.

Figure 7. Plasma concentration–time profile of glibenclamide after oral and transdermal patch treatment in mice. \(^*\)Significant compared to GLB-Oral \((p < 0.05)\). Each point represents mean ± SE; \(n = 6\).
two daily injections of insulin. The results were definitive. The intensive therapy reduced the mean risk of retinopathy by 76%, nephropathy by 34%, hypercholesterolemia by 34%, and macrovascular disease by 41% compared to conventional therapy. Thus, it is clear that improving day-to-day glycemic control in diabetic mice by transdermal patches can dramatically reduce and slow the development complications of diabetes. Further, the slow and sustained release of the drug from the transdermal systems might reduce manifestations like sulfonylurea receptor downregulation and the risk of chronic hyperinsulinemia, a major risk factor for atherosclerosis frequently associated with oral therapy of glibenclamide.\textsuperscript{2,40,41}

In our earlier study, we calculated the target permeation rate for transdermal delivery of glibenclamide in man (60 kg) as 193.8 μg/h based on available pharmacokinetic data.\textsuperscript{7} In the present study, the cumulative amount of drug permeated from the patch (1 cm$^2$) prepared with EC:PVP (3:2) at the end of 24 h is 262.92 μg. Hence, the patch with an area of about 18 cm$^2$ would be sufficient to provide an optimum effect. But, it is well known that human skin is less permeable compared to mouse skin.\textsuperscript{42} However, in the view of encouraging results obtained in mice, it can be predicted that the required minimum effective concentration could be achieved within an appreciable range of application area in humans despite the greater barrier properties of human skin when compared to mouse skin.

In conclusion, the present study shows that transdermal systems of glibenclamide exhibited better control of hyperglycemia besides more effectively reversing the complications associated with diabetes mellitus than oral glibenclamide administration in mice. However, the pharmacodynamic and pharmacokinetic evaluation of these systems in human volunteers is necessary to confirm these findings.

ACKNOWLEDGMENTS

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REFERENCES


### Table 7. Pharmacokinetic Parameters of Glibenclamide after Oral and Transdermal Administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oral-GLB</th>
<th>TP (EC:PVP, 3:2)</th>
<th>TP (ERL:ERS, 4:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>9.13 ± 0.45</td>
<td>6.72 ± 0.20$^a$</td>
<td>6.25 ± 0.22$^a$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2.0 ± 0.00</td>
<td>12.0 ± 0.00$^a$</td>
<td>12.0 ± 0.00$^a$</td>
</tr>
<tr>
<td>$K_e$ (h$^{-1}$)</td>
<td>0.117 ± 0.024</td>
<td>0.054 ± 0.002$^a$</td>
<td>0.056 ± 0.001$^a$</td>
</tr>
<tr>
<td>AUC(0 → 24) (μg·h/mL)</td>
<td>93.56 ± 7.93</td>
<td>126.27 ± 4.41$^a$</td>
<td>116.07 ± 3.83$^a$</td>
</tr>
<tr>
<td>AUC (0 → ∞) (μg·h/mL)</td>
<td>103.13 ± 12.46</td>
<td>191.61 ± 10.21$^a$</td>
<td>171.62 ± 7.09$^a$</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>6.42 ± 1.23</td>
<td>12.82 ± 0.49$^a$</td>
<td>12.16 ± 0.23$^a$</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>10.10 ± 1.34</td>
<td>22.10 ± 0.69$^a$</td>
<td>21.12 ± 0.26$^a$</td>
</tr>
</tbody>
</table>

$^a$Significant compared to oral GLB ($p < 0.05$). $C_{\text{max}}$ = maximum concentration; $T_{\text{max}}$ = time of maximum concentration; $K_e$ = elimination rate constant; AUC = area under plasma concentration–time curve; $t_{1/2}$ = elimination half-life; MRT = mean residential time.


