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Original Research Article

A Study of Transdermal Delivery of Glibenclamide Using Iontophoresis

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Abstract

Purpose: To assess iontophoretic transdermal delivery of glibenclamide across pigskin for its transdermal development.
Methods: *In vitro* iontophoretic transdermal delivery of glibenclamide across the pigskin was investigated at three different drug concentrations in the donor cell of the diffusion apparatus, using cathodal iontophoresis (current density 0.5 mA cm^{-2}) along with the passive controls.
Results: For passive permeation, the steady state flux significantly increased with the donor drug concentration. At all concentration levels, iontophoresis considerably increased the permeation rate compared to passive controls. Flux enhancement was highest at the lowest drug load and lowest at the highest drug load. The highest flux value obtained was 0.0603 µmol cm^{-2} h^{-1} and the target flux for glibenclamide was 0.3933 µmol h^{-1}. Required permeation rate was achieved by iontophoresis using a much smaller application area.
Conclusion: Permeation rate of drugs across the pigskin can be considerably enhanced by the use of Iontophoresis.

***Keywords*:** Glibenclamide; Iontophoresis; Pigskin; Transdermal drug delivery.

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Introduction

Transdermal route is especially suitable for the delivery of drugs for prolonged therapy in diseases like hypertension and diabetes. The first transdermal patch was launched in 1981,[1] with strong clinical benefits, industrial interest and regulatory precedence, and market prediction. Transdermal delivery can reduce the fluctuation of plasma level and bypass the first pass elimination.[2] Though it is hypothesized that skin is permeable to the lipophilic moieties of low molecular weight, in reality the extent of transdermal permeation is a composite parameter influenced by a number of physiochemical and biological factors. In addition to molecular weight (MW), partition co-efficient and solubility, the pK_a value, which determines the extent of ionization.

Iontophoresis may be defined as facilitated movement of compounds, in particular charged compounds, into or across a membrane by the application of an externally applied electrical potential difference across the membrane. Iontophoresis has provided a non-invasive delivery system for local or systemic delivery of drugs. This technique has gained growing acceptance for the topical delivery of drugs[3,4] hence attempts have been undertaken to develop oral hypoglycemic agent into transdermal systems.

Glibenclamide is an oral hypoglycemic agent, used for the treatment of non-insulin dependent diabetes mellitus[2,5]. The drug has plasma half-life 4-6 hrs [5,6] and needs frequent administration. Moreover, its oral use is associated with severe and some time fatal hypoglycemic symptoms like nausea, vomiting, heartburn, anorexia and increase in appetite[7]. In 1997, Takahshi and co-workers[8] had investigated the sulfonylureas for transdermal administration and reported promising results. Later, Mutalik and co-workers[9] studied the permeability of this drug in mouse skin with the objective of its transdermal development. The present work investigates the iontophoretic permeability of the glibenclamide to assess its potential for patient controlled active transdermal system.

Experimental

Materials

Glibenclamide was a gift sample from West-Coast Pharmaceutical Works (Gota, Ahmedabad, India). Silver plates (purity 99.99%, 5 mm diameters) were obtained from a goldsmith shop at Bangalore, India. Ethanol, methanol, sodium hydroxide, ootassium dihydrogen orthophosphate (KH_2PO_4), octanol and hydrochloric acid were obtained from SD Fine-Chem (Mumbai, India). All the reagents/chemicals used were of analytical grade. Experiments were conducted with ultra pure water (resistivity, 18.2 MΩ cm) obtained from Milli-Q Academic System (Millipore Pvt Ltd Bangalore, India).

Equipment

Hitachi high performance liquid chroma-tograph (HPLC) with a reversed phase Kromasil 300-4 C-18 , 10 μm column 150 X 4 mm i.d. and equipped with Hitachi L-7110 pump and L-7400 UV detector equipped with Winchrome-99 software was used. Iontophoretic DC source (digital display, current 0-10 mA, voltage 0-25 V) was purchased from C-tech Psu-2510/lab (Mumbai, India) and iontophoretic diffusion cell was fabricated by Navin Scientific Glass Product (Bangalore, India) as per given specifications. Silver/silver chloride electrode was prepared as per the standard procedure[10]. Silver wire (99.99% pure, 1.0 mm thickness) was used as connecting wire.

Preparation of skin membrane

From a local abattoir, ear was obtained from freshly slaughtered pigs. The skin was removed carefully from the outer regions of the ear and separated from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the

dermis side was removed using a scalpel and isopropyl alcohol. Finally the skin was washed with tap water and stored at -20 ˚C in aluminum foil packing and was used within two days [11].

Procedure of passive permeation

The *in vitro* passive permeation studies were conducted using vertical type Franz diffusion cell having a receptor compartment capacity of 10 ml. The excised skin was mounted between the half-cells with the dermis in contact with receptor fluid (phosphate buffer pH 7.4) and was equilibrated for 1 h. The area available for diffusion was about 1.21 cm^2. The donor cell was covered with an aluminum foil to prevent the evaporation of vehicle. The fluid in the receptor compartment was maintained at 37±0.5 ˚C. Under these conditions, the temperature at the skin surface was approximately 32 ˚C. Glibenclamide suspension (2 ml) was placed in the donor compartment. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from the receptor compartment at regular intervals and assayed for drug content [6].

Procedure of iontophoretic diffusion

For iontophoresis diffusion cell was modified as suggested by Glikfield et al[12]. The apparatus essentially consisted of a glass molded large receiving chamber provided with two parallel ports on the topside and a sampling port on the side. Two upper chambers are made from open-ended cylindrical glass tubes, the outer diameters of which were equivalent to the inner diameter of the parallel ports. The lower 10 mm of these tubes were slightly constricted to allow a clearance of 1 to 1.5 mm on the side. This ensured easy fitting. After the skin was tied at this constricted end, the effective diameter increased and became exactly equal to inner diameter of the extended ports. Once slipped into parallel ports, they stay attached by glass joints forming two separate chambers with skin at the base. Both the skin touched the receptor solution at the same depth and each chamber housed one electrode. Once the battery was switched on, current flowed through the skin placed in anodal compartment into receiving solution below and reached the cathodal electrode through the skin tied to cathodal end. Donor solution was filled in one of the top chambers depending on the polarity of the drug and the other serve as return electrode chamber. For our study, silver/silver chloride electrode was inserted into the donor compartment whereas silver plate was inserted into anodal chamber as return electrode. Direct current (0.5 mA cm^{-2}) was used throughout experiment. The receptor fluid (5 ml) was withdrawn at regular intervals and replaced with fresh buffer to maintain sink condition. Samples were assayed by the HPLC.

Solubility determination

Solubility study was carried out at different composition of phosphate buffer and ethanol. Excess amounts of drug were taken into glass vials and dissolved in measured amount of different solvent systems having varying proportion of phosphate buffer and ethanol to get saturated solutions. The solutions were kept at rest for 24 h to assist the attainment of equilibrium with the undissolved drug particles. From these solutions, the supernatant was filtered to separate the undissolved drug particles and diluted suitably and the concentrations were measured [13].

Estimation of the drug

Glibenclamide was estimated by HPLC as reported by Dhawan et al [14]. For calibration curve, working standards were prepared in phosphate buffer (5-80 µg ml^{-1}) and injected (20 µl) into the column. Eluted of the analyte was achieved with a mobile phase consisting of phosphate buffer (PB) 50 mM KH_2PO_4 and acetonitrile (65:35) adjusted to pH 3.5 by orthophosphoric acid. Delivered at a flow

rate of 1 ml min^{-1}, the retention time of the analyte was 16.87 min and detection was at 300 nm. The plots of peak area versus respective concentration of glibenclamide were found to be linear with a correlation coefficient (r) of 0.9989.

Partition coefficient

The octanol/water partition coefficient of the drugs was determined by shaking equal volume of octanol and phosphate buffer pH 7.4 in a separating funnel for 10 min and allowing to stand for 24 h. Aueous phase was assayed before and after partitioning to get the partition coefficients [13]. Skin/vehicle partition coefficients were determined by dipping the skin in known concentrations of drug in phosphate buffer for 24 h and estimating the concentration after partitioning.

Data analysis

The cumulative amount permeated was plotted against time, and the slope of the linear portion of the plot was estimated as the steady state flux. Permeability coefficient and diffusion coefficient were calculated using following formulas[15]:

$$K_P = J_{SS} / C_d \quad \text{..... (1)}$$
$$D = K_P h / K \quad \text{..... (2)}$$

where K_p represents permeability coefficient, J_{ss} is the steady-state flux, C_d is the concentration of drug in donor compartment, D is the diffusion coefficient, K is the skin/vehicle partition coefficient and h the thickness of the skin. Flux enhancement was calculated by dividing iontophoretic steady state flux by the corresponding passive steady state flux.

Statistical analysis

Statistical analysis was carried out using 2-way ANOVA. The effect of concentration on steady state flux was separately evaluated by one-way ANOVA followed by Bonferroni's test. [16] At 95% condifence intervals, p values less than 0.05 were considered to be significant.

Results

The solubility of glibenclamide in different phosphate buffer (PB): ethanol binary vehicle was increased with the composition 70:30 (ethanol: PB) showing the maximum value (Table 1). Experimentally determined octanol/buffer partition coefficient was found to be 2.0502±0.0019. The skin/vehicle and octanol/vehicle partition coefficients decreased with increasing ethanol concentration.

Passive and iontophoretic permeation profiles of glibenclamide

The permeability and diffusion coefficients of glibenclamide in different systems for passive diffusion and iontophoresis are provided in Table 2 while the passive and iontophoretic permeation profiles of glibenclamide at different donor concentrations are shown in Figures 1 and 2. The passive profiles are linear at all concentration levels indicating the permeation kinetics was more or less zero order. In the passive process, both the rate

Table 1: Solubility and partition coefficients of glibenclamide in ethanol: PB binary systems

Composition of donor system (ethanol: PB)	Concentration of drug (μmol ml^{-1})	Partition coefficients	
		Skin	Octanol
System-A (20:80)	0.1349±0.0051	20.495±1.932	1.055±0.068
System-B (50:50)	3.3246±0.0070	4.255±0.564	0.342±0.033
System-C (70:30)	4.9008±0.0092	3.065±0.124	0.217±0.022

Table 2: Permeability and diffusion coefficients of glibenclamide in different systems for passive diffusion and iontophoresis

Donor System	Permeability coefficient (cm h^{-1})		Diffusion coefficient (cm^2 s^{-1}) X 10^{-8}	
	Passive	Iontophoresis	Passive	Iontophoresis
System-A	0.1408±0.0066	0.3040±0.0106	19.1±0.181	41.4±0.398
System-B	0.0110±0.0010	0.0205±0.0109	7.25±0.897	13.5±0.167
System-C	0.0093±0.0062	0.0162±0.0135	8.43±0.343	14.6±0.060

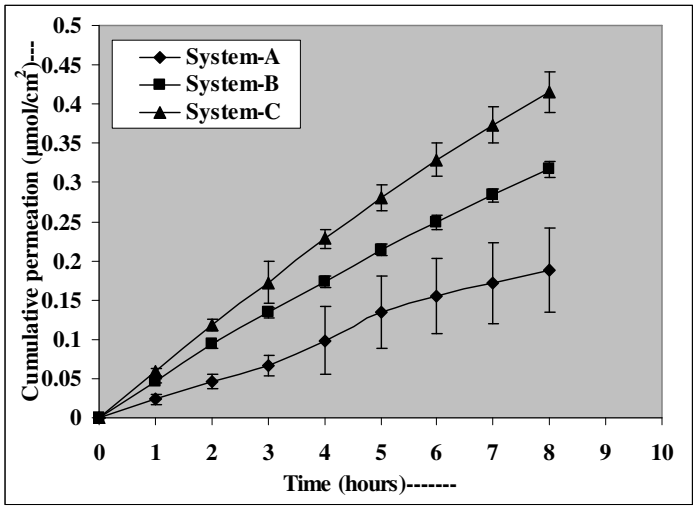

Figure 1: Permeation profile of glibenclamide (passive) at different donor concentrations (n=3)

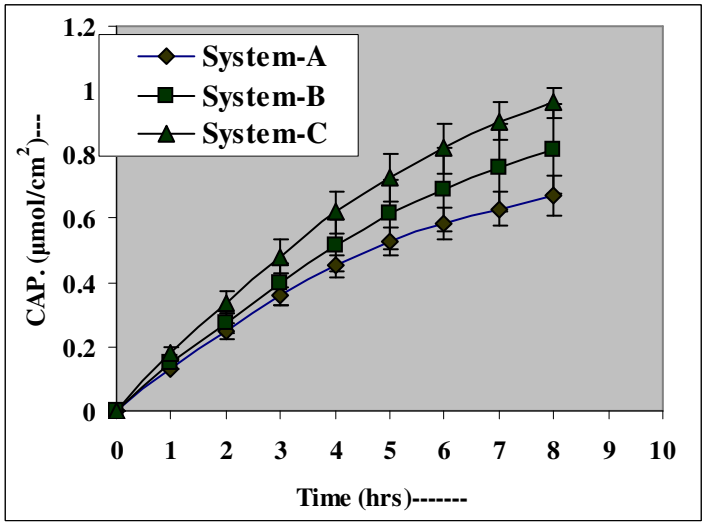

Figure 2: Permeation profile of glibenclamide (iontophoresis) at different donor concentrations (n=3)

and extent of permeation increased with increasing donor drug concentrations. In contrasts iontophoretic profiles were less linear indicating the involvement of multitude of factors. When the concentration of the drug was raised from low (system A) to medium (system B), the permeation rate increased but there no significant increase was found at the highest concentration (system C) over that of next lower concentration.

Figure 3 depicts the enhancement in iontophoretic flux compared to the passive flux of same donor concentration. Enhanced permeation was highest at the lowest drug load and lowest at the highest drug load.

The iontophoretic contribution was found to be slightly more at higher donor concentrations (Table 3).

Discussion

According to Doh et al[17], drug candidates for transdermal delivery should have MW around 200~500 Da. Glibenclamide, having a MW of around 495 fits into the category. As glibenclamide is poorly water soluble, ethanol: PB binary vehicle was used. The octanol-water partition determined is a measure of the relative lipophilicity of the gllibenclamide.

At pH 7, mammalian skins are negatively charged and glibenclamide being an acidic drug with the pKa value 5.1, is largely ionized[18], which reduces its natural affinity towards the skin[17]. In our preliminary study, intrinsic permeability was found to be low when the drug was delivered from aqueous saturated solution (data not shown).

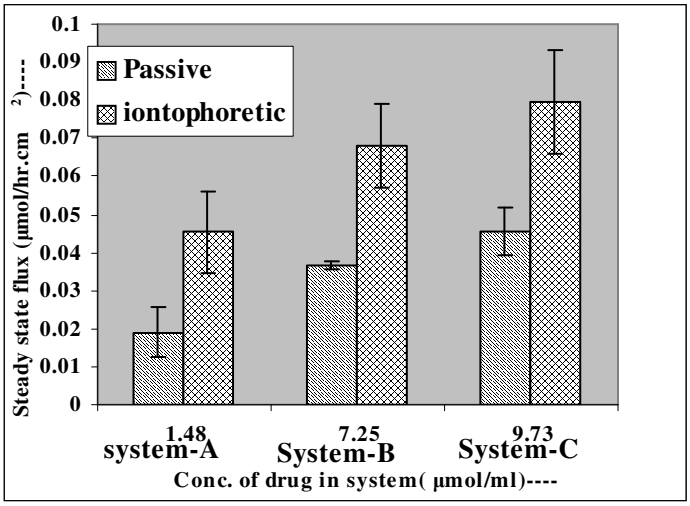

Figure 3: Comparison of steady state fluxes at different donor concentrations (n =3)

Table 3: Steady state fluxes glibenclamide in various systems

Donor System	Steady State flux (µmol $cm^{-2}h^{-1}$)		Enhancement Ratio (R)	Benefit by Iontophoresis (µmol $cm^{-2}h^{-1}$)
	Passive	Iontophoresis		
System-A	0.0190±0.0066	0.0453±0.0106	2.3842	0.0263
System-B	0.0367±0.0010	0.0682±0.0109	1.8583	0.0315
System-C	0.0455±0.0062	0.0795±0.0135	1.7472	0.0340

Because of low aqueous solubility, a high enough concentration gradient (which is the driving force of passive permeation) could not be achieved. Mutalik et al[9] had attempted to enhance the permeation rate using enhancers hence iontophoresis was attempted to enhance the permeation. To simulate the physiological condition, the diffusion cell was modified where both the electrodes are placed on the same side of skin. The receiving chamber with PB reflected the body.

Iontophoretic systems should have conductive medium, which support the movement of ions. The binary vehicle ethanol: PB was conductive of electricity that supported the movements of ions.

Of the factors that affect passive permeability, the concentration of the actives in the delivery system is the most crucial. To evaluate this effect, the experiment was designed at three different drug concentrations (Table 1). In all the systems, the drug was delivered as suspension. For drugs of low solubility this is particularly necessary as the amount required for prolonged maintenance often exceeds the limits of solubility. In suspension, the loss due to permeation is supplemented by the presence of solid drug in the reservoir and thermodynamic activity is maintained constant. Moreover, thermodynamic activity is a function of percent saturation in the vehicle and high thermodynamic activity results in higher partitioning into the stratum corneum[20]. Hence maximum flux of compounds can be achieved from suspension, which represents the highest saturation level. However, increasing the solubility by using ethanol reduced the drugs affinity to the skin. This is demonstrated in Table 3 where the skin/vehicle and octanol/vehicle partition coefficients decreased with increasing ethanol concentration.

The passive profiles of glibenclamide at the different donor concentrations are linear at all concentration levels indicating the permeation kinetics was more or less zero order. In the passive process, both the rate and extent of permeation increased with increasing donor drug concentration. This was expected as increase in the donor drug concentration enhances the concentration gradient, which is the driving force of mass transport [21]. In contrast iontophoretic profiles of glibenclamide at different donor concentrations were less linear indicating the involvement of multitude of factors. Raising the concentration from low (system A) to medium (system B) increased the permeation rate but no significant increase was found at the highest concentration (system C) over that of next lower concentration. This was an agreement with hypothesis that increasing the drug concentration increases iontophoretic delivery up to a certain point, but at still higher concentrations, the flux may become independent of concentration [10].

Athough ionic repulsion is the dominant force in iontophoresis, there is also a convective flow toward the direction of current. Permeability of skin also changes under this influence[22]. The total flux of a solute during iontophoresis is the sum of fluxes due to electro repulsion, convective flow, and passive diffusion[23]. Glibenclamide (pK_a 5.1) at pH 7.4 acquires a negative charge due to ionization of sulfonyl group and was delivered from cathodal chamber. Since the isoelectric point of the skin varies from 3 to 4 at physiological pH, the volume flow is directed towards the cathode. Hence at pH 7.4, only passive and electro-repulsive fluxes are likely to contribute the overall permeation. Electro-osmotic flow may even oppose the permeation from the cathodal compartment[24]. The iontophoretic profiles show, the initial permeation was high but the permeation rate declined in the later hours. This is contradictory as the voltage gradually dropped with time and hence the magnitude of electro-osmotic opposition was expected to be lower in the later part of the study. The opposite result suggests the involvement of a factor that negatively influenced the permeation as time progressed. It is

proposed that the decreasing flux values were due to the lesser electro repulsive contribution to the overall flux. During the passage of current, the cathodal electrode (Ag/AgCl) received a steady flow of electron, which resulted in the liberation of negatively charged chloride ions. As time progressed, the concentration of this newly released chloride ion was likely to increase in the cathodal compartment, which served as the drug reservoir. Since the drug was the negatively charged, the competition from the chloride ions increased with time. Chloride ion being much smaller than the drug ion is a powerful competitor, which reduced the transport efficiency of drug [25].

The values of permeation parameters are depicted in Table 4. Albeit steady state flux is the most therapeutically relevant parameter, permeability coefficients are usually used for comparison purpose[26]. It is evident the permeability coefficients decreased with the increase in donor concentration.

Finally, to analyze the net benefit of electrical energy, the active fluxes of drug at various donor drug concentrations were compared with the corresponding passive value (Table 3). Since the current density was maintained same in all experiments, iontophoretic contribution found to be slightly more at higher donor concentrations looks apparently contradictory. The varying proportion of phosphate buffer used in the different system may be the cause of this contradiction. The buffer carrying negative charge (PO_4^-) was a source of competitor co-ions for the drug. As the proportion of the buffer used were lesser in system B and C the drug ions vastly outnumbered the competitor co-ions which might have resulted in higher electro repulsive fluxes at high donor concentrations.

Glibenclamide is a potent medicine effective in low dosage range (5-15 mg). The non-compartmental analysis of the pharmacokinetic data indicates that to meet the demand of maintenance therapy for a 60 kg individual, 0.3933 µmol must be supplied to the systemic circulation every hour. The highest flux found in our study was 0.0603 µmol cm^{-2} h^{-1}. As patches in the market usually have wider area (10 cm^{-2} and above), even with the discount for species variation, iontophoretic systems are likely to achieve the therapeutic target.

Conclusion

The present work showed that the iontophoretic approach was feasible to enhance and control the rate of transdermal drug delivery of glibenclamide. In this study the effects of concentration on iontophoretic drug delivery have provided evidence to suggest that increasing the drug concentration increases iontophoretic delivery up to a certain point, but at still higher concentrations, the flux may become independent of concentration. Additional investigations, such as the effect of current density, type of electrodes, combined effect of natural enhancers and iontophoresis on the iontophoretic delivery will be useful for further studies.

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