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Effect of pH and complexation on transdermal permeation of gliquidone

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Gliquidone, a second generation sulfonylurea has been investigated for transdermal delivery. The poor aqueous solubility of the drug prompted the use of hydroxypropyl-β-cyclodextrin (HP-β-CD), a cyclic oligosaccharide, which is known to facilitate transdermal permeation of many drugs by enhancing the solubility and thus improving the diffusible species of the drug molecules at the skin-vehicle interface. In order to optimize the transdermal delivery of gliquidone, the effect of pH along with complexation on the solubility and permeation has been investigated. The solubility profiles of the drug, on increasing the concentration of HP-β-CD were of Higuchi's A_L type at the three pH values evaluated. However, the solubilization slope of the drug at pH 7.0 was 22 times that at pH 3.0 as a result of greater intrinsic solubility of the ionized form of the drug at pH 7.0. Transdermal flux of gliquidone at pH 7.0 was significantly greater than the flux at pH 3.0 in the presence of 15% w/v HP-β-CD, attributable to the better solubility of the drug at pH 7.0 in the presence of HP-β-CD. The effect of increasing concentrations of HP-β-CD investigated at variable drug loading in the donor phase at pH 7.4 endorsed the earlier observations from studies on other drugs, that the drug has to be present at saturation in HP-β-CD aqueous vehicle to achieve an optimized flux. While at saturation, the steady state flux of gliquidone from the aqueous HP-β-CD (25% w/v) vehicle was enhanced 31 times compared to pure drug suspension at pH 7.4, unsaturation in the donor phase resulted in the decreased flux of gliquidone. It was concluded from the present study that enhanced transdermal flux of gliquidone can be achieved by adjusting the pH and the concentration of HP-β-CD to achieve a better solubility of the drug.

1. Introduction

The transdermal route has been an attractive alternative to the oral application of drugs with its advantages of improving patient compliance, bypassing hepatic first pass metabolism and easy retractability in case of severe side effects. However, molecular size (<500 D), lipophilicity and dose restricts the number of therapeutic entities that can be considered to be administered transdermally [1]. To circumvent these difficulties, several physical and chemical enhancement techniques were developed.

One of the successful approaches of chemically enhancing the transdermal delivery of drugs is incorporating an agent, which increases the saturation solubility of the drug in the reservoir [1]. Cyclodextrins, cyclic oligo saccharides known to improve the solubility of lipophilic drugs through molecular encapsulation and hence improve transdermal flux have received considerable attention in the recent years [2]. Transdermal delivery is also influenced by the pH of the delivery phase and pK_a of the drug for ionizable drugs [3]. Both molecular encapsulation with cyclodextrins and transdermal delivery are known to be significantly higher with the unionized form of the drug compared to the ionized species. However, this belief is being questioned in separate investigations, which concluded that both molecular encapsulation [4] and transdermal permeation [3, 5] are a summation of both ionized as well as non-ionized species. Studies combining the use of complexation and optimization of pH to improve solubility and transdermal bioavailability have been limited.

In this context the present study addresses the combined effect of pH and complexation on transdermal permeation of a lipophilic anti-diabetic drug, gliquidone. The study also aims at investigating the effect of concentration of hydroxypropyl-β-cyclodextrin (HP-β-CD) at different drug loadings on transdermal permeation of gliquidone.

Gliquidone, a short acting second generation sulphonyl urea, with its molecular weight of 527.6, lipophilicity, ($\log P = 2.8$ at pH 7.4) and virtual insolubility in water is hardly an obvious candidate for passive diffusion via transdermal route. Hence, optimization of pH and HP-β-CD concentration was attempted to facilitate the passive diffusion of gliquidone through skin. Apart from serving as a model molecule for evaluation of the above strategy, transdermal delivery can improve the patient compliance of the drug by avoiding multiple dosing. Inter individual variation, a characteristic of the drug due to intense hepatic metabolization [6] can be avoided by transdermal application. The possibility of controlled delivery in predetermined quantities reduces the possibility of chronic hyperinsulinemia and oversensitization of insulin receptors, the major risk factors with sulphonyl urea therapy [7, 8]. Rapid termination of therapy in the event of a hypoglycemic reaction would be an additional advantage.

2. Investigations, results and discussion

2.1. Phase solubility studies

The solubility profiles of gliquidone under three pH conditions (pH 3.0, 5.0 and 7.0) as a function of increasing concentration of HP-β-CD are shown in the Fig. The phase solubility curves were all of the Higuchi's A_L type [9], though the slopes differ significantly. The slopes calculated by linear regression analysis are found to be 0.0006, 0.0007 and 0.0131 at pH 3.0, 5.0 and 7.0, respectively. The solubilization slope of gliquidone at pH 7.0 where the drug is predominantly in the ionized form is approximately 22 times that of the slope at pH 3.0. The slopes at pH 3.0 (τu) and pH 7.0 (τi) were substituted into the equations proposed by Li et al. [4] to calculate the stability constants for the complexation of unionized (Ku) and ionized (Ki) drug with cyclodextrins. Briefly, the solubilization slopes for the unionized (τu) and ionized drugs (τi) are described by the following equations.

$$\tau u = \frac{Ku\,[Du]}{1 + Ku\,[Du]} \qquad (1)$$

where [Du] is the intrinsic solubility of the unionized drug and Ku is the complexation constant. Similarly the con-

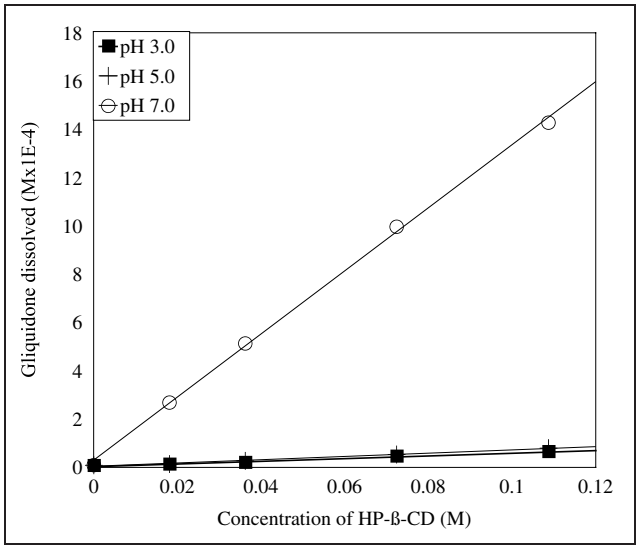

Fig.: Effect of pH and HP-β-CD on total solubility of gliquidone, n = 3 (rsd < 5%)

stant for ionized drug, Ki, was calculated from the solubilization slope τi for the complexation of the ionized drug and is described as

$$\tau i = \frac{Ki\,[Du] \times 10^{(pH-pKa)}}{1 + Ki\,[Du] \times 10^{(pH-pKa)}}$$

The stability constants for the unionized (Ku) and ionized (Ki) drug were found to be 1200 M^{-1} and 516 M^{-1}, respectively. Despite the complexation constant being only 0.44 times that of unionized species, the intrinsic solubility of the ionized drug at pH 7.0 is approximately 60 times higher than the solubility of the unionized drug (Du) at pH 3.0. It may be noted here that the larger solubilization slope of ionized gliquidone is a product of its complexation constant and its higher intrinsic solubility at pH 7.0 where 98% of the drug is ionized ($pK_a = 5.3$), which led to a greater solubilization slope (22 times) of the ionized drug. This trend is in agreement with earlier studies [4], which suggest that the solubility of an ionized complex (DiL) can exceed the solubility of free unionized drug and the solubility of unionized drug complex (DuL) i.e. when Di/Du > Ku/Ki , [DiL] > [DuL].

2.2. Skin permeation studies

The *in vitro* skin permeation studies were conducted using rat abdominal skin as the membrane. The steady state flux of gliquidone in the presence of 15% w/v HP-β-CD buffered at the three pH values (pH 3.0, 5.0 and 7.0) correlates with the solubility data (Table 1). The flux of the pure drug suspension at different pH conditions could not compared meaningfully. While the flux was undetectable at pH 3.0 and pH 5.0, it was negligibly low at pH 7.0 (0.103 μg/cm^2/h) as a result of poor intrinsic solubility of the drug. The flux was measurable at all the three pH values on addition of HP-β-CD. However, the flux was significantly higher at pH 7.0 than at pH 3.0 or 5.0 for a similar concentration of HP-β-CD.

As noted in solubility studies, at a higher pH, the ability of cyclodextrin to solubilize a greater quantity of drug creates a higher concentration gradient at unit thermodynamic activity, the basis for improving the Fickian diffusion. This together with the rapid equilibration of the drug between the complex and the continuous buffer phase improves the flux significantly. Also, the considerably lipophilic (log P = 2.8 at pH 7.4) anionic form of the drug could partition easily into the stratum corneum. The permeability co-efficient showed insignificant change at three pH values suggesting that solubility is responsible for the increase in flux. An ion pairing mechanism has been suggested to explain the permeation of ionized drugs [3].

The influence of HP-β-CD concentration on the transdermal flux of gliquidone at different drug loadings was investigated at pH 7.4 in phosphate buffer saline since determinable intrinsic flux of the drug could be obtained in the vehicle. The solubility study conducted in phosphate buffer saline of pH 7.4 as a function of HP-β-CD concentration yielded an A_L-type curve with a K 1 : 1 constant of 1625 M^{-1}.

The effect of increasing concentrations of HP-β-CD at different drug loading in the donor phase on transdermal flux of the drug is shown in Table 2. The flux increased linearly with increasing concentrations of HP-β-CD. The addition of cyclodextrin resulted in a greater quantity of solubilized drug thus allowing a greater quantity of the drug to be loaded into the donor phase in a diffusible form. Though the cyclodextrin complex is not known to penetrate the stratum corneum barrier, it is established [10] that the drug molecules encapsulated in the cyclodextrin cavity are in dynamic equilibrium with the bulk phase and when proximate to the lipophilic membrane like skin these molecules preferentially partition into it thus improving the flux significantly. In other words, cyclodextrin acted as a carrier by delivering the gliquidone molecules in a solubilized form to the surface of the skin from where it partitioned into the skin. However, at low drug concentration (0.25% w/v) addition of 25% w/v HP-β-CD caused a reduction in flux, which was a result of complete solubilization of the drug by HP-β-CD. At this drug concentration,

Table 1: Flux of gliquidone at different pH values in aqueous vehicles containing 15% w/v HP-β-CD (n = 3)

pH	3.0	5.0	7.0
Solubility (μg/ml)	33.87	42.15	754.46
Steady state flux (μg/cm^2/h)	0.218 ± 0.01	0.312 ± 0.052	4.78 ± 0.471
Permeability coefficient(× 10^{-3})	6.435 ± 0.28	7.408 ± 1.22	6.33 ± 0.63
Fraction of the drug ionized (fi)	0.5%	33.4%	98.0%

Table 2: Flux vs hydroxypropyl-β-cyclodextrin at pH 7.4 with variable concentration of gliquidone in the donor phase (n = 3)

Concentration of drug (%w/v)	Concentration of HP-β-CD (%w/v)	Flux ± SD (μg/cm^2/h)	Enhancement factor
0.25	0	0.34 ± 0.02	1.00
	5	1.62 ± 0.19	4.75
	10	3.64 ± 0.25	10.70
	15	5.13 ± 0.24	15.08
	20	7.76 ± 0.49	22.82
	25	1.14 ± 0.11	3.35
0.5	0	0.37 ± 0.03	1.00
	5	1.63 ± 0.29	4.41
	10	3.96 ± 0.43	10.70
	15	5.55 ± 0.94	15.00
	20	8.11 ± 1.22	21.70
	25	11.42 ± 0.98	30.9

excess cyclodextrin in the reservoir led to unsaturation, thus shifting the equilibrium of the reversible complexation phenomenon towards more drug entering into the complex and reducing the concentration of the free drug that is capable of permeating the skin. Increasing the initial drug loading to 0.5%w/v restores the saturation to maintain unit thermodynamic activity and hence an increase in flux was observed. Hence, it is essential to maintain the HP-β-CD concentration, so that the drug is saturated in the vehicle to achieve an optimized flux, an observation confirmed by similar studies [11].

From the study, it is evident that HP-β-CD forms a 1 : 1 molecular complex with gliquidone at the concentrations investigated and is capable of enhancing its transdermal flux. Choosing the optimum concentrations of the drug, cyclodextrin and controlling the pH comprises a vital part in optimizing the transdermal delivery of gliquidone.

3. Experimental

3.1. Materials

Gliquidone was a kind gift from M/s Boehringer Ingelheim, Germany. Hydroxypropyl-β-cyclodextrin (HP-β-CD), molar substitution 0.6 was purchased from Fluka Chemie GmbH, Switzerland. All the other chemicals were of HPLC grade and were purchased from E.Merck (India) Ltd., Mumbai, India.

3.2. Quantitative analysis

The quantitative determination of gliquidone [12] was performed on a HPLC system (Shimadzu LC-10Ai, Japan) using a photodiode array detector set at a wavelength of 229 nm using a Shimpack ODS 5 μm (4.6×25 mm) column. The mobile phase consisted of acetonitrile and 0.1 M acetic acid (85 : 15) and pumped at a flow rate of 1.5 ml/min. The retention time was 2.7 min and a calibration graph of peak areas vs concentration followed a linear regression equation: concentration = [1.6 E-02] area – [1.31 E + 0.2] with a correlation coefficient $r^2 = 0.999$ and inter – intraday variation of rsd $\leq$ 1.2%.

3.3. Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors [9]. An excess of gliquidone was added to the vials containing HP-β-CD concentration in the range of 0.036 M to 0.181 M in aqueous buffer samples, and then briefly sonicated and shaken at 32 °C [13] (to represent the surface temperature of skin) for 3 days. After attainment of equilibrium, the samples were filtered through membranes of pore size 0.45 μm. The gliquidone concentrations were determined using HPLC. Citrate phosphate buffers were used for studies at pH 3.0, 5.0 and 7.0 and phosphate buffered saline was the vehicle at pH 7.4.

3.4. Skin permeation studies

Vertical diffusion cells with a diffusional surface area of 4.9 cm^2 and receptor phase volume of 30 ml were used. Male wistar rats (150 ± 10 g) were sacrificed by cervical dislocation and their abdominal skins were excised and separated from subcutaneous fat by blunt dissection. The hair in the region was clipped prior to the experiment. The skins were immediately clamped between two halves of the diffusion cells using a rubber-o-ring to seal the epidermis side to the donor half with dermis side in contact with receptor phase maintained at 37 °C. Two kinds of receptor phases were used for practical reasons. For pH related experiments the receptor phase was normal saline with 2.5% HP-β-CD to maintain sink conditions. All the other experiments were conducted in phosphate buffered saline of pH 7.4 with 2.5% w/v HP-β-CD as a solubilizer. Gentamycin (0.01% w/v) was used as a preservative [14] and did not interfere with the transdermal permeation or analytical determination of the drug.

For evaluation of the effect of pH, the donor phase always contained a little excess drug in crystalline form in citrate – phosphate buffer to maintain unit thermodynamic activity and an infinite dose. The other permeation experiments were carried out with varying concentrations of cyclodextrin and drug in phosphate buffered saline (pH 7.4) as the vehicle. Samples were withdrawn at fixed time interval up to 24 h and analyzed for the drug concentration by HPLC. The steady state flux was obtained from the linear portion of the cumulative drug permeated Vs time profiles and results shown were an average of three determinations. Enhancement factor (EF) was calculated as in eq. (3).

$$EF = \frac{\text{Flux on addition of HP-β-CD}}{\text{Flux without HP-β-CD}} \quad (3)$$

The fraction of ionized drug (fi) was calculated from

$$fi = 100/[1 + 10^{(pKa-pH)}] \quad (4)$$

and the permeability coefficient (K_p) was calculated from the equation $K_p = J_{ss}/C_0$ where C_0 is the saturation solubility of the drug in the vehicle.

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