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Permeation of Diclofenac through Buccal Mucosa

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Permeability coefficient, flux and other related values of diclofenac potassium through guinea pig buccal mucosa have been compared with those obtained through porcine buccal mucosa at pH 6.8 and 8.0. The permeation values of the species of diclofenac potassium have also been compared with diclofenac diethyl ammonium salt. Rodent buccal pouch being keratinised showed very poor permeation. Permeation through porcine buccal pouch at pH 6.8 and 8.0 was not significantly different. Permeability coefficient values were significantly higher for diclofenac potassium than those for diclofenac diethyl ammonium salt. Use of 5% polysorbate 80 significantly enhanced the permeation of diclofenac potassium.

Buccal mucosa in the oral cavity offers several advantages in the absorption of drugs[1]. Drug permeation through the mucosa depends on the physiological factors governing the oral mucosa and physical properties of the drug, the most important being the degree of ionization, partition coefficient and solubility. Diclofenac, a popular and widely used NSAID is used as a model drug for incorporation in this delivery system and evaluated. It is available in the forms diclofenac sodium and potassium and diclofenac diethyl ammonium salt.

The work presented here aims at evaluating the *in vitro* permeation of diclofenac through the buccal mucosa in animal model. The data would help in designing the buccal delivery of diclofenac. In case of certain drugs, buccal delivery gives low value of flux resulting in poor bioavailability. This has been reportedly overcome by the use of suitable permeation enhancers. They include bile salts[2-4], azone[5], surfactants[6,7] and chelating agents[8].

MATERIALS AND METHODS

Diclofenac potassium and diclofenac diethylammonium salt were obtained from Amoli Organics Ltd., Mumbai; bile salt (sodium tauroglycocholate) was purchased from Loba Chemie Pvt. Ltd., Mumbai. Isopropyl alcohol, methanol and n-hexane, all of HPLC grade; potassium dihydrogen phosphate, propylene glycol and sodium chloride AR grade were obtained from, Merck (India), Mumbai. Polysorbate 80 was procured from Sigma Chemical Company St. Louis, USA.; sodium hydroxide, sodium lauryl sulphate (SLS) and sodium phosphate dihydrate (IP grade) were also used in the study. A Hitachi HPLC unit (Model L-6000A) equipped with an UV/VIS detector (Model L-4200) and a Jasco Integrator (Model 807 IT) was used in the study.

Preparation of buffers:

Phosphate buffered saline (PBS)[9] and McIlvaine buffer (pH 6.8 and 8.0)[10] were prepared by standard methods. The pH of the buffers was measured using an Elico pH meter (Model L 1-120), previously standardized with buffer tablets of pH 4.0 and 7.0.

Isolation of the buccal pouch:

Guinea pig: The method suggested by Gandhi and Robinson[4] for isolation of buccal pouch of rabbits has been followed for the isolation of the buccal pouch of guinea pig. In all the experiments, the fresh buccal pouch was used.

Pig: Porcine buccal tissue was obtained from the

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abbatoir. The fresh tissue was kept at 37° in PBS (pH 7.4) upon removal. The buccal pouch was isolated using the method suggested by Gandhi and Robinson[4].

Permeation studies:

The procedure followed for the permeation studies is a modification of the method described by Coutel-Egros *et al.*[11] Diclofenac potassium (50 mg)/diclofenac diethyl ammonium salt (55.24 mg equivalent to 50 mg diclofenac potassium) was dissolved in 3 ml of a mixture of McIlIvaine buffer (pH 6.8 or 8.0) and propylene glycol (1:1). The solution was placed in the donor compartment of a jacketed all glass Franz cell (diameter 16 mm; height of donor 30 mm, receiver 65 mm) having guinea pig/porcine buccal pouch between the receiver and donor compartments maintained at 37±1°. The respective buffer (9.5 ml) was placed in the receptor compartment. The solution was stirred with a magnetic needle. Aliquots (1 ml) were withdrawn from the receptor compartment at 30 min interval for first 120 min and 60 min interval thereafter upto 300 min.

For the permeation enhancement studies, SLS at 1% and 5%, and bile salts and polysorbate 80 at 5% each were added to the donor solution and the experiment performed as above. All experiments were conducted six times over. The permeability coefficient (P) was calculated from the slope of graph of per cent drug transported vs time as, $P=Slope \times V_D / S$....(1), where, V_D = volume of donor solution = 3 ml, S = surface area of tissue = 0.503 cm^2 (for guinea pig); 1.23 cm^2 (for porcine) and Flux $J = P \times C_D$.....(2) where, CD = concentration of donor solution = 16.67 mg/ml and enhancement Ratio ER=Permeability coefficient of drug with enhancer/Permeability coefficient of drug alone....(3).

Analytical method development and validation:

The permeability of diclofenac required a sensitive method with validation of the analytical parameters (conforming to USP specifications). In the preliminary studies, the drug-free buffer eluate (DfE) obtained post permeation through the buccal pouch also showed absorbance at 276.8 nm, the wavelength at which diclofenac showed absorbance. By modifying the extraction system and by changing the mobile phase composition along with the chromatographic conditions, the interference from the contents of the buccal pouch in the analysis of diclofenac in buffer eluate was avoided. Spiking the DfE with the drug at different concentrations and injecting the same onto the HPLC column demonstrated the specificity in analysis of drug. The method finally arrived at was carefully validated. The chromatographic conditions after validation included a HPLC column [Lichrosphere RP 18 (10 µm)] with a flow rate of 1 ml/min. The mobile phase was methanol:phosphate buffer pH 7.0 (60:40). The retention time for diclofenac was 5.6 min. The samples were analyzed by the following method - To 0.5 ml DfE/ eluate sample, 0.5 ml of 0.83 M o-phosphoric acid was added. The mixture was vortexed for one minute. A mixture of n-hexane and IPA (9:1) (5 ml) was added and vortexed for 5 min. The mixture was centrifuged for 10 min at 1000 rpm. The organic layer was separated and evaporated to dryness under nitrogen. The residue was reconstituted with 0.2 ml of the mobile phase and injected onto the column. The absorbance was measured at 284 nm.

RESULTS

The degree of ionization of diclofenac potassium at pH 6.8 and 8.0 was 99.84 and 99.99 per cent, respectively and that for diclofenac diethyl ammonium salt was 12.13 and 68.63 per cent respectively at these pH values. The plot of diclofenac potassium permeating through guinea pig buccal mucosa vs time is shown in fig. 1 while the plot of diclofenac potassium and diclofenac diethyl ammonium salt permeating through porcine buccal mucosa vs time is shown in fig. 2.

The permeability coefficients and flux of diclofenac potassium through guinea pig buccal mucosa in presence and absence of absorption promoters are shown in Table 1. The lag time, diffusion coefficient, permeability coefficient and flux of diclofenac potassium through porcine buccal mucosa in the presence and absence of polysorbate 80 is

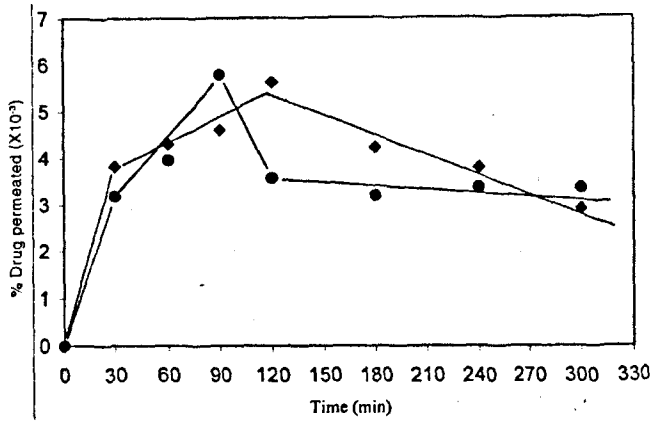

Fig. 1: Permeation of diclofenac potassium through guinea pig buccal mucosa.

(●) Indicates percent diclofenac potassium permeated at pH 6.8, whereas (◆) indicates per cent diclofenac potassium permeated at pH 8.0.

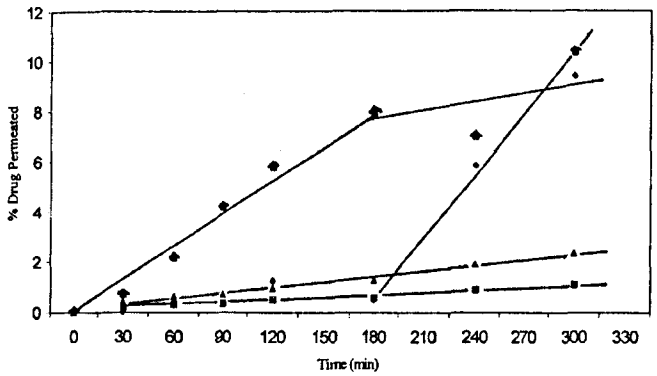

Fig. 2: Permeation of diclofenac potassium and diclofenac diethyl ammonium salt though porcine buccal mucosa.

(◆) indicates percent diclofenac potassium permeated at pH 6.8, (🏠) indicates percent diclofenac potassium permeated at pH 8.0, (▲) indicates percent diclofenac diethylammonium salt permeated at pH 6.8 and (■) indicates percent diclofenac diethylammonium salt permeated at pH 8.0.

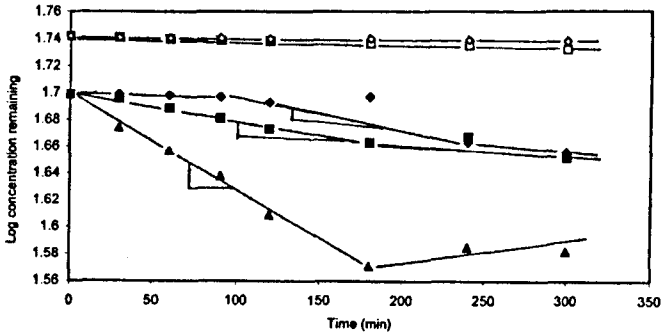

Fig. 3: Permeation of diclofenac potassium and diclofenac diethyl ammonium salt through porcine buccal mucosa.

(◆) Indicates diclofenac potassium remaining at pH 6.8, (🏠) indicates diclofenac potassium remaining at pH 8.0, (▲) indicates diclofenac potassium remaining at pH 6.8 in presence of 5% polysorbate 80 (◊) indicates diclofenac diethyl ammonium salt remaining at pH 6.8, and (□) indicates diclofenac diethyl ammonium salt remaining at pH 8.0.

shown in Table 2.

A parameter denoted as 'permeability constant', k (h^{-1}) was calculated from plot of log concentration vs time (fig. 3) using the relationship, $t_{1/2} = 0.693 / k$ ...(4), where k was determined from the slope of the graph. These values are shown in Table 3.

DISCUSSION

The amount of diclofenac potassium permeating through guinea pig buccal mucosa at pH 6.8 and 8.0 (fig. 1) was extremely small and independent of pH, the value of the flux, J, being 4.31×10^{-3} mg^2/cm^2min and 1.89×10^{-3} mg^2/cm^2min, respectively. This low amount permeating is attributed to the characteristic keratinised buccal pouch present in guinea pig and other rodents[12]. The solubility of diclofenac potassium at 30±1° at pH 6.8 and 8.0 were 1.49 and 2.98 mg/ml, respectively, while the degree of ionization was practically the same (99.84 and 99.99, respectively). These two pH values were considered for the study, since the former (pH 6.8) represented the average salivary pH[13] and the latter (pH 8.0) showed high solubility of diclofenac potassium. However, in case of permeation through porcine buccal mucosa (fig. 2), the degree of ionization governed by pH

TABLE 1: PERMEABILITY COEFFICIENTS AND FLUX FOR DICLOFENAC POTASSIUM THROUGH GUINEA PIG BUCCAL POUCH.

Absorption promoter	Permeability coefficient mg.cm/min	Flux $mg^2/cm^2.min$	Enhancement Ratio (ER*)
- (pH 6.8)	2.585×10^{-4}	4.309×10^{-3}	-
-(pH 8.0)	1.130×10^{-4}	1.890×10^{-3}	-
1% SLS	1.567×10^{-4}	2.612×10^{-3}	0.6
5% SLS	6.036×10^{-4}	1.006×10^{-2}	2.3
5% bile salt	3.494×10^{-4}	5.824×10^{-3}	1.4
5% polysorbate 80	2.498×10^{-3}	4.164×10^{-2}	9.7

* ER indicates ratio of Permeability coefficient with enhancer to Permeability coefficient without enhancer.

TABLE 2: LAG TIME, DIFFUSION COEFFICIENT, PERMEABILITY COEFFICIENT AND FLUX FOR DICLOFENAC POTASSIUM THROUGH PORCINE BUCCAL POUCH.

	Without Polysorbate 80 pH 6.8		Without Polysorbate 80 pH 8.0		With Polysorbate 80 pH 6.8	
	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Lag time (min)	27	175	13.33	-	0	-
Diffusion coefficient (mm^2/min)	$4.94x10^{-3}$	$7.62x10^{-4}$	0.01	-	-	-
Permeability coefficient (mg.cm/min)	$7.32x10^{-3}$	$9.05x10^{-2}$	0.12	-	0.35	$6.91x10^{-2}$
Flux ($mg^2/cm^2.min$)	0.12	1.51	2.00	-	5.81	1.15

Phase I indicates time upto 180 min and phase II indicates time upto 300 min.

TABLE 3: VALUES OF $T_{1/2}$ AND k CALCULATED FROM PLOT OF LOG CONCENTRATION VS TIME.

	Diclofenac potassium			Diclofenac diethyl ammonium salt	
	pH 6.8	pH 8.0	5% Polysorbate 80 (pH 6.8)	pH 6.8	pH 8.0
k (h^{-1})	0.0299	0.0276	0.0990	0.00370	0.00154
$t_{1/2}$(h)	23.15	25.11	6.99	187.30	450.00

$T_{1/2}$ indicates half life (h) and k indicates permeability constant (h^{-1}).

exhibited its influence on the amount of drug permeated 30 min from the commencement of the experiment. At steady state, the permeability coefficient can be calculated by using equation 5. $T\%=PS/V_D.t$...(5), where T is the percent of drug transported at time t; P is the permeability coefficient; S is the surface area of the tissue (the opening of the diffusion cell); and is the V_D is the volume of the donor chamber.

However, practically, the steady state is never reached because the drug concentrations in the donor and receiver are continuously changing. In such cases, Fick's second law of diffusion[14] has been employed.

The plot of percent diclofenac potassium transported vs time at pH 6.8 and 8.0 showed a biphasic release. At pH 6.8, in the first phase, the release is linear up to 180 min rising thereafter up to 300 min. At pH 8.0 after 180 min, the release became almost steady in the second phase. The values calculated from the graph are given in Table 3 for pH 6.8 and 8.0. The lag time is assumed to be the same as that obtained in Phase I.

The influence of absorption promoters on the permeability coefficient and flux through guinea pig buccal pouch can be better understood from the enhancement ratio (Table 1). While the addition of 1% SLS showed negative effect, results with 5% bile salt did not show significant difference between the presence and absence of enhancer. Addition of 5% SLS increased the permeability coefficient value to more than double the value obtained in absence of enhancer. The highest enhancement ratio was observed with 5% polysorbate 80, (9.663).

In case of porcine buccal pouch, the permeability coefficient value at pH 8.0 is 16.39 times the value obtained at pH 6.8. With 5% polysorbate 80, the permeability coefficient increased significantly (47.65 times) as compared to the permeability coefficient value at pH 6.8 without the use of enhancer. Kinetics of permeation of diclofenac potassium through guinea pig buccal pouch could not be calculated due to insignificant difference in the amount of diclofenac permeating during 30 to 60 min time interval over a period of five hours.

For diclofenac potassium using porcine buccal pouch, the permeability rate constant, k (h^{-1}) was found to be practically the same at pH 6.8 and 8.0 indicating that the rate

was not affected by pH. However, the addition of the enhancer increased the k value to almost four times at pH 6.8. The $t_{1/2}$ value was almost the same at pH 6.8 and 8.0 which was reduced by 70% when polysorbate 80 was used.

When compared with diclofenac diethyl ammonium salt, the k value for diclofenac potassium was 8.9 times higher than the k value obtained for diclofenac diethyl ammonium salt at pH 6.8 and 17.92 times higher than the k value obtained at pH 6.8. There was a significant difference in $t_{1/2}$ values between diethyl ammonium salt and the potassium salt indicating low permeation of diethyl ammonium salt as compared to potassium salt.

Both the species ionize to a different extent. At pH 6.8, diclofenac diethyl ammonium salt ionizes to an extent of 12.13% as compared to 99.84% for diclofenac potassium. At pH 8.0, diclofenac diethyl ammonium salt ionizes to 68.83% while diclofenac potassium ionizes to 99.99%. Diclofenac diethyl ammonium salt has a molecular weight of 369.29 while diclofenac potassium has a molecular weight of 318.1. Generally, the molecules are expected to penetrate the biological membranes in the unionized form when their molecular masses are the same. In the present situation, although there is difference in the degree of ionization between the two salts, the salt with lower molecular weight (diclofenac potassium) has shown higher degree of penetration as compared to the salt with the higher molecular weight (diclofenac diethyl ammonium). Thus, it is the molecular size which influences rate of penetration in this case.

Thus, it is evident that for *in vitro* evaluation of permeation of drugs, guinea pig buccal pouch cannot be employed due to its keratinised character. The porcine buccal pouch model is quite satisfactory. The values of permeability coefficient of diclofenac potassium at pH 6.8 and 8.0 through porcine buccal pouch did not differ significantly. However, permeability coefficient of diclofenac potassium was significantly higher than that of diclofenac diethyl ammonium salt at both the pH values indicating suitability of diclofenac potassium as a candidate for buccal drug delivery.

Amongst the enhancers employed, polysorbate 80 at 5% concentration showed enhancement in the permeability coefficient values. These findings were supported by the kinetics of drug permeation.

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