

Research Article

Effect of Bile Salts on Transbuccal Permeability of Carbamazepine through Porcine Buccal Mucosa

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ABSTRACT

The delivery of drug through buccal mucosa provides potential advantages like prevention of enzymatic degradation in gastro intestinal tract and bypassing hepatic first-pass metabolism. However the significant aspect in transbuccal drug delivery is the very restrictive passage of drug through the buccal mucosa. In our earlier work we confirmed the ability of Carbamazepine (CBZ) to permeate through the porcine buccal mucosa. Our earlier study proved that CBZ crosses the membrane by passive diffusion. But the measured Flux (J_s) and permeation coefficient (K_p) of CBZ was insufficient to achieve plasma therapeutic concentration. In this study an approach was made to improve permeability of CBZ using some bile salts as chemical permeation enhancers like sodium taurocholate (STC) and sodium taurodeoxycholate (STDC) which are capable to lessening the barrier property of the mucosal tissue. These patches were subjected to *ex vivo* permeation studies. The six buccal patches of CBZ selected from our earlier reported formulations were subjected to this permeability improvement study. These patches were either based on HPMC K15M or Carbopol 934p or Chitosan in combination of PVA or PVP K30 or Sodium alginate. The significant change in J_s and K_p of formulations in presence and absence of chemical permeation enhancers STC or STDC (in two different concentrations 0.1% and 0.5 % w/w) was evaluated by *ex vivo* permeation studies through porcine buccal mucosa under modified Franz diffusion cell. The calculated value of Flux (J_s) and Permeation coefficient (K_p) confirmed that there was a significant increase in permeated amount of CBZ after the inclusion of permeation enhancers. The compatibility of bile salts with buccal mucosa was examined by histological studies and kinetic studies.

KEYWORDS: Carbamazepine, buccal patch, permeation enhancer, bile salt

INTRODUCTION

Drug delivery through buccal mucosa directly access systemic circulation by internal jugular vein. It helps to evade hepatic first pass metabolism of drugs. Buccal route shows very less enzymatic degradation compared to oral route of administration. The smooth muscle of buccal mucosa has considerable larger surface area, immobility and robustness to hold retentive dosage forms like buccal patches. The rapid cell turn over process protects buccal mucosa from tissue damage and local irritation quicker than other mucosal route¹.

CBZ is an iminostilbene derivative used as antiepileptic and antineuralgic agent. The traditional oral route of administration of CBZ is comparatively slow and results uneven gastrointestinal absorption due to poor water solubility (113 $\mu\text{g/mL}$, at 25°C). This drug is classified as class-II drugs under Biopharmaceutics Classification System (BCS). Moreover this drug undergoes hepatic first-pass metabolism and enzymatic auto induction of metabolism in the oral route of administration. These factors are beside the selection of CBZ as a candidate for buccal patch formulations².

The plasma therapeutic drug concentration of CBZ required to produce pharmacological response is ranged from 4-12 mg/L for adult individuals³. The clinical recognition of buccal mucosal drug delivery is based on the achievement of required therapeutic drug concentration within specified duration. But buccal dosage forms exhibit low bioavailability due to poor permeability across buccal mucosa. An approach was initiated to improve the flux of CBZ across buccal mucosa using chemical permeation enhancers to minimize barrier capacity of mucosa.

The bile salts and surfactants alter barrier properties of buccal mucosa by various mechanisms that include extraction or denaturation of protein, entrapment of intercellular lipids by micelles, by enzyme inactivation, increasing fluidity of membrane and by disrupting cellular structure by swelling. Buccal mucosa permits drug penetration either by paracellular or transcellular route. The lipophilic drugs predominantly follow transcellular route to permeate buccal mucosa. Because of lipophilic nature, the buccal epithelial cell membrane can permit partitioning of lipophilic drugs like CBZ through transcellular route^{4,5}.

The rate of transport of hydrophilic drugs (flux) (J_p) through the buccal mucosa under sink condition for paracellular route can be determined by the following equation.

$$J_p = \frac{\epsilon D_p K_c}{h_p} C_d$$

Where, D_p - diffusion coefficient of the drug through paracellular route, h_p - thickness of the membrane, ϵ - area fraction of the membrane and C_d - initial donor drug concentration.

The flux (J_c) of lipophilic drugs across buccal mucosa under sink condition through transcellular route can be assessed by the following equation.

$$J_c = \frac{(1 - \epsilon) D_c K_c}{h_c} C_d$$

Where, K_c - Partition coefficient of drug, D_c - diffusion coefficient of the drug, h_c - thickness of membrane, and C_d - initial concentration of drug in donor compartment.

Chemical permeation enhancers like sodium taurocholate (STC) and sodium taurodeoxycholate (STDC) are natural bile salts and were used in previous study to enhance permeation of drugs and to increase flux across buccal mucosa⁶. Bile salts exerted less toxicity, than nonionic surfactants Tween 80 and Poloxamer F68⁷.

The main goal of this research work was to investigate the effect of bile salts as chemical permeation enhancers on the permeability of CBZ across buccal mucosa under *ex vivo* conditions. The compatibility of bile salts with buccal epithelium also to be examined by histological studies.

MATERIALS AND METHODS

Materials:

CBZ was received as a gift sample from Caplin point Pharma Ltd, Puducherry, India. The polymers hydroxypropyl methyl cellulose-K15M (HPMC), carbopol 934p (Cp), chitosan (CH), polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP K-30), and sodium alginate (SA) and bile salts sodium taurocholate (STC) and sodium taurodeoxycholate (STDC) were obtained from Sigma-Aldrich, Bangalore. Propylene glycol (PG) and Polyethylene glycol (PEG-400) were purchased from SD Fine Chem Ltd, Bangalore, India. Biaxially-oriented polypropylene (BOPP) film was supplied by Pidilite®, India. All other chemicals and solvents of analytical grade were used for this study.

Methods:

Determination of distribution Coefficient of CBZ:

The distribution coefficient of CBZ between n-octanol-water system was determined by shake flask method. The passive diffusion of drug molecules across lipophilic biological membranes is resembled by distribution of drug into n-octanol layer. Each 5 mL of n-octanol and phosphate buffer pH 7.4 were taken in a separating funnel. These two solvents were saturated with each other prior to the experiment for

12 h at room temperature. The weighed quantity of CBZ (5mg/mL) was mixed with solvents in separating flask and shaken for 8 h at temperature of 37°C. Then the aqueous phase was separated and centrifuged at 2000 rpm. The drug content of clear aqueous layer was estimated by UV-spectrophotometer at 285 nm. The distribution coefficient (log D_{7.4}) was determined by applying the following equation⁸.

$$\log D_{7.4} = \log \left[\left(\frac{C_1 - C_2}{C_2} \right) \times \left(\frac{V_{\text{buf}}}{V_{\text{oct}}} \right) \right]$$

Where, C₁ is initial concentration of aqueous layer, C₂ is final concentration of aqueous layer, V_{buf} is Volume of aqueous buffer and V_{oct} is volume of n-octanol⁹.

Formulation of CBZ buccal patches:

Buccal patches of carbamazepine were prepared by solvent casting method in a specially fabricated Teflon coated Petri dish. In our preliminary study total 108 formulations were designed and prepared by using different ratio of mucoadhesive polymers HPMC K15M or Carbopol 934p or Chitosan in combination of PVA or PVP K30 or Sodium alginate (Table 1). The 2% w/v solution of HPMC K15M, Carbopol 934p, Chitosan and 1% w/v solution of PVA, PVP K30 and Sodium alginate were prepared separately using deionized water and continuously stirred for 24 h. These solutions were mixed according to the formulation ratio and the total volume of polymer solution used was maintained constant as 30 mL. To this polymer mixture ethanolic solution of CBZ (5mL) and 2mL of plasticizer either PG or PEG- 400 was added and homogenized.

Total six formulations FA10, FB16, FA25, FB36, FA46 and FB49 were selected and renamed as A,B,C,D,E and F. Their composition was incorporated with chemical permeation enhancers STC or STDC (in two different concentrations 0.5% and 1.0% w/w). Total 24 formulations were designed (Table 2). This mixture was allowed to swell for 2 h and the air bubble free solution was casted on the surface of Petri dish (9 cm diameter) placed on a horizontal surface. After drying at room temperature for 2 h, the Petri plates were transferred carefully to hot air oven and dried at 50°C

for 48 h. The dried patches were carefully removed from the surface of Petri dish and cut into 3 cm diameter circular patches (equivalent to 20 mg CBZ). These patches were made water impermeable on one side by pasting BOPP film (Pidilite®) to allow unidirectional permeation of CBZ. These buccal patches were packed in aluminium foil and preserved in a desiccator¹⁰.

Evaluation of Buccal Patches:

Collection and Preparation of Porcine buccal mucosa:

Porcine buccal mucosa was selected for the study due to non-keratinized cell structure like human buccal membrane. Buccal mucosa was obtained from local slaughter house within 2h of slaughter and preserved immediately in simulated saliva (pH 6.2). The buccal mucosa with basal membrane was carefully separated from adjacent connective tissue and rinsed with deionized water and then with simulated saliva (pH 6.2). The thickness of the mucous membrane was measured in the range of 1.73 to 2.2 mm¹¹.

Study of Permeation through Porcine buccal mucosa:

The porcine buccal mucosa of 4 cm diameter was used for the study. A modified Franz diffusion cell with 3 cm inner diameter and can hold 100 ml receptor fluid was used for the study. The freshly collected buccal mucosa was mounted between donor and receptor compartment by placing smooth mucosal surface towards donor compartment. A 3 cm diameter CBZ loaded buccal patch was fixed on the surface of buccal mucosa by mucoadhesion. The donor compartment was moistened with 1 mL of simulated saliva (pH 6.2) and the receptor compartment was filled completely till to touch the membrane with a 100 mL of receptor fluid containing ethanol and isotonic phosphate buffer (pH 7.4) in the ratio of 20:80. The non-aqueous solvent ethanol was added to the buffer to prevent saturation of carbamazepine in aqueous medium. The receptor compartment was stirred at 50 r /min and maintained at 37 ± 0.2 °C. The 2mL sample of receptor medium was collected at pre-determined time intervals and replaced with fresh medium. The

Table 1: Composition of buccal patches without permeation enhancers

Composition (per 9.2 cm diameter Petri dish)	Formulation code					
	FA10*	FB16*	FA25*	FB36*	FA46*	FB49*
	A	B	C	D	E	F
Carbamazepine (mg)	188	188	188	188	188	188
HPMC K 15 M (ml) (2% w/v) (ml)	10	13.3	3.3	5	10	6
Polyvinyl alcohol (ml) (1% w/v) (ml)	-	-	-	5	-	-
Polyvinyl alcohol (ml) (2% w/v) (ml)	10	3.3	-	-	-	-
PVP K 30 (1% w/v) (ml)	10	13.3	-	-	-	-
Carbopol 934p (1% w/v) (ml)	-	-	-	-	10	12
Carbopol 934p (2% w/v) (ml)	-	-	13.3	20	-	-
Sodium alginate (1% w/v) (ml)	-	-	13.3	-	-	-
Chitosan (2% w/v) (ml)	-	-	-	-	10	12
Propylene Glycol (ml)	2	-	2	-	2	-
Poly Ethylene Glycol 400 (ml)	-	2	-	2	-	2
Aspartame (mg)	5	5	5	5	5	5

* Selected formulations codes were renamed A-F.

The concentration of 0.1 and 0.5 % w/W of permeation enhancers used in each patch of CBZ.

samples were analysed spectrophotometrically at 285 nm. The percentage of drug permeated was plotted against time. The slope of the linear portion of the curve was used to calculate permeability coefficient (P)¹².

Calculation of permeation data:

The rate of permeation of drug at steady state per unit area of buccal mucosa at unit time (Flux J_c) was measured using the following relationship.

$$\text{Flux } (J_c) = \frac{Q_r}{A \cdot t} \quad (\mu\text{g cm}^{-2}\text{h}^{-1})$$

Where Q_r is amount of CBZ permeated through mucosa (μg), A is the surface area of buccal mucosa exposed for permeation and t is the time of measurement (h). The amount of drug permeated per hour was measured from the slope of the linear

portion of the graph plotted between percentages of drug permeated versus time.

The permeation coefficient (K_p) was calculated from by the following equation:

$$\text{Permeation coefficient } (K_p) = \frac{J_s}{C_d} \quad (\text{cmh}^{-1})$$

Where J_c Flux of drug at steady state ($\mu\text{g cm}^{-2}\text{h}^{-1}$), C_d is concentration of drug in donor compartment ($\mu\text{g cm}^{-3}$).

Two different Permeation enhancers (STC and STDC) in two different concentrations (0.5% and 1.0%w/W) were used for the study.

The change in permeation data and efficacy of permeation enhancers was determined by finding enhancement ratio (ER) by following equation¹².

$$\text{Enhancement Ratio (ER)} = \frac{\text{Flux with Permeation Enhancer}}{\text{Flux without Permeation Enhancer}}$$

Histological study of porcine buccal mucosa:

The porcine buccal mucosa exposed to permeation enhancers during permeation study were removed from the Franz diffusion cell and a small portion was cut and preserved in 10% formalin solution. A control tissue was also preserved separately without treating chemical permeation enhancers. All dehydrated tissue specimens were embedded in paraffin wax. Then vertical microscopic sections were made by microtome. The mounted tissues were stained with hematoxylin and eosin and examined under optical microscope¹³.

RESULTS AND DISCUSSION

Distribution Coefficient of CBZ:

The distribution coefficient of CBZ in n-octanol and Phosphate buffer pH7.4 (log D7.4) was determined as 1.62 at room temperature. The distribution coefficient of n-octanol- water system is a measure of lipophilic nature of drugs. This measure is useful to understand the pharmacokinetic properties like absorption, distribution and binding of drugs. The value of log D between 0 to 3 indicates the drug candidate possess optimal lipophilicity and may undergo sufficient absorption and penetration into the biological membrane¹⁴.

Ex vivo Study of Permeation through Porcine buccal mucosa:

The ex vivo permeation study revealed CBZ released from buccal patches were permeated across the porcine buccal mucosa. The tested formulations without permeation enhancers were released maximum percentage of drug from 60 to 140 min of study. The formulation D showed comparably less permeation rate of $99 \pm 0.9\%$ of drug in 140 min (Fig 1). Whereas the formulation D11 with STC 0.1% showed $99.8 \pm 2.2\%$ in 90 min and D12 with STC 0.5% permeated 99.7% in 60min. The formulation containing STDC 0.1% (D21) and 0.5% (D22) were showed permeation of $99.9 \pm 0.97\%$ in 90 min and $99.9 \pm 2.6\%$ in 75 min respectively (Fig 5).

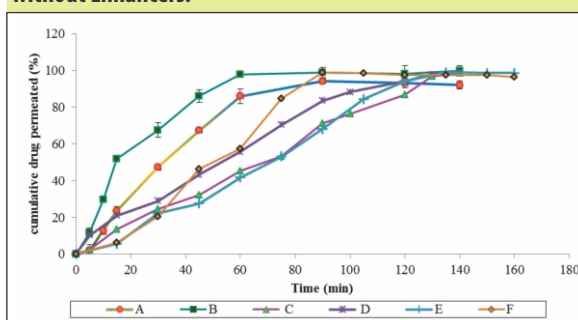
Table 2: Formulation of buccal patches with permeation enhancers

Preamection enhancers	Formulation code and percentage of permeation enhancer used		
	0 % w/W	0.1% w/W	0.5% w/W
STC	A	A11	A12
STDC		A21	A22
STC	B	B11	B12
STDC		B21	B22
STC	C	C11	C12
STDC		C21	C22
STC	D	D11	D12
STDC		D21	D22
STC	E	E11	E12
STDC		E21	E22
STC	F	F11	F12
STDC		F21	F22

STC - Sodium taurocholate

STDC- Sodium taurodeoxycholate

Fig. 1: Study of Permeation of CBZ from buccal patches without Enhancers.



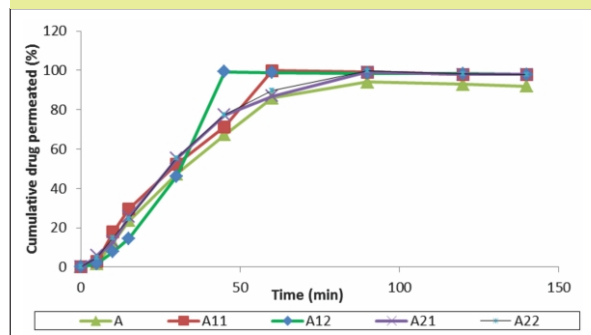
Effect of permeation enhancers on permeability of CBZ:

The permeation study through porcine buccal mucosa was conducted for all the formulations with and without permeation enhancers (Fig 2-7). The rate

Table 3: Permeation data and Effect of permeation enhancers and on CBZ buccal patches

Formulation code	Flux (J_s) ($\mu\text{g}/\text{cm}^2/\text{h}$)			Permeation coefficient (K_p) (cm/h)			Enhancement Ratio (ER)	
	Control	(A11-F11) Enhancer STC (0.1% w/W)	(A12-F12) Enhancer STC (0.5% w/W)	Control ($\times 10^{-3}$)	(A11-F11) Enhancer STC (0.1% w/W) ($\times 10^{-3}$)	(A12-F12) Enhancer STC (0.5% w/W) ($\times 10^{-3}$)	(A11-F11) Enhancer STC (0.1% w/W)	(A12-F12) Enhancer STC (0.5% w/W)
A	60.21	62.40	65.97	3.041	3.151	3.332	1.04	1.10
B	54.82	54.99	61.69	2.768	2.777	3.116	1.00	1.13
C	61.58	63.65	67.11	3.109	3.215	3.389	1.03	1.10
D	61.29	65.38	71.07	3.095	3.302	3.590	1.07	1.16
E	58.97	60.47	62.56	2.978	3.054	3.160	1.03	1.06
F	56.64	57.96	63.57	2.860	2.927	3.211	1.02	1.12
Formulation code	Flux (J_s) ($\mu\text{g}/\text{cm}^2/\text{h}$)			Permeation coefficient (K_p) (cm/h)			Enhancement Ratio (ER)	
	Control	(A21-F21) Enhancer STDC (0.1% w/w)	(A22-F22) Enhancer STDC (0.5% w/w)	Control ($\times 10^{-3}$)	(A21-F21) Enhancer STDC (0.1% w/w) ($\times 10^{-3}$)	(A22-F22) Enhancer STDC (0.5% w/w) ($\times 10^{-3}$)	(A21-F21) Enhancer STDC (0.1% w/W)	(A22-F22) Enhancer STDC (0.5% w/W)
A	60.21	62.39	62.66	3.041	3.151	3.165	1.03	1.04
B	54.82	54.76	56.82	2.768	2.766	2.870	1.00	1.04
C	61.58	63.49	65.87	3.109	3.207	3.327	1.03	1.07
D	61.29	65.11	65.94	3.095	3.289	3.330	1.06	1.08
E	58.97	60.40	61.85	2.978	3.051	3.124	1.02	1.05
F	56.64	57.17	62.17	2.860	2.887	3.140	1.01	1.01

* The Flux and Permeation coefficient were calculated from the average value of cumulative drug permeated ($n=3$)

Fig. 2: Permeation of CBZ from Formulation A with and without enhancers.

of permeation at unit surface and unit time (Flux) at steady state was determined from the linear portion of the graph plotted between percentage of drug permeated versus time. The calculated Flux ($\mu\text{g}/\text{cm}^2/\text{h}$) and permeation coefficient (cm/h) and enhancement ratio were given in (Table 3).

The flux (J_s) of CBZ was calculated without permeation enhancer as control (Formulations A to F) and the values were ranged between 54.82 to 61.29 $\mu\text{g}/\text{cm}^2/\text{h}$. The permeation coefficient of control groups were found to be from 2.768×10^{-3} to 3.109×10^{-3}

³ cm/h. The formulations showed notable increase in their rate of permeation when used with permeation enhancers STC and STDC. The formulations A11 to F11 were composed of enhancer STC (0.1% w/w) and showed increase in flux in the range of 54.99 to 65.38 $\mu\text{g}/\text{cm}^2/\text{h}$ and permeation coefficient was ranged from 2.777×10^{-3} to 3.302×10^{-3} cm/h. Whereas enhancer STDC (0.1% w/w) composed formulations (A21 to F21) showed increase in flux in the range of 61.69 to 71.07 $\mu\text{g}/\text{cm}^2/\text{h}$ and permeation coefficient was calculated in between 3.116×10^{-3} and 3.590×10^{-3} cm/h.

The flux of CBZ across porcine buccal mucosa was enhanced in presence of enhancers STC and STDC than the control formulations. The Flux (J_s) of formulation D was increased in the order Control < STC 0.1% < STC 0.5% (i.e. D < D11 < D12) was noted as $61.29 < 65.38 < 71.02 \mu\text{g}/\text{cm}^2/\text{h}$ and Control < STDC

Fig. 3 : Permeation of CBZ from Formulation B with and without enhancers

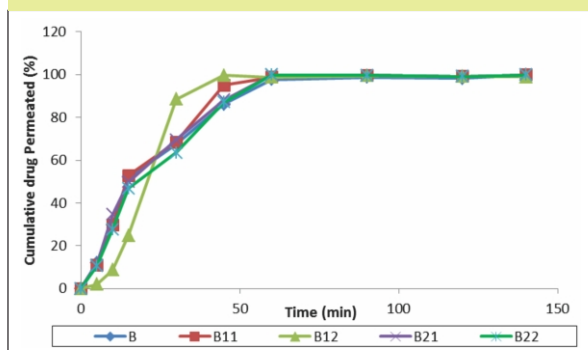


Fig. 4 : Permeation of CBZ from Formulation C with and without enhancers.

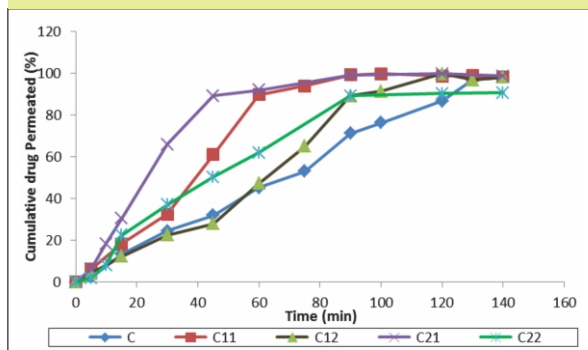


Fig. 5: Permeation of CBZ from Formulation D with and without enhancers.

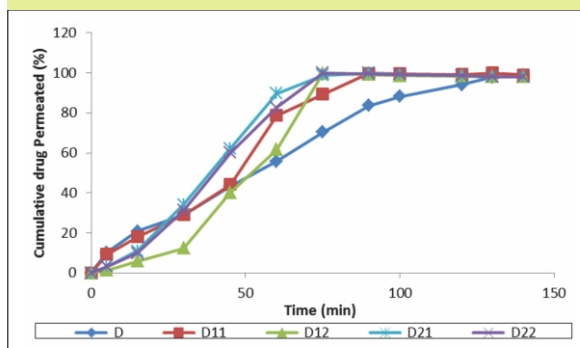
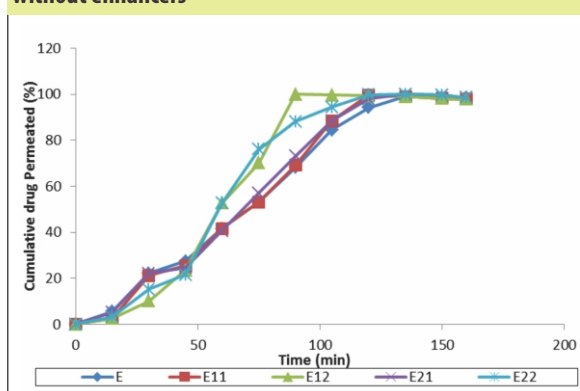


Fig. 6 : Permeation of CBZ from Formulation E with and without enhancers



0.1% < STDC 0.5% (i.e. D < D21 < D22) was calculated as $61.29 < 65.11 < 65.94 \mu\text{g}/\text{cm}^2/\text{h}$. This indicates there is an increase in permeation of CBZ with the increase in concentration of enhancer. The flux of STC used formulations showed remarkable increase than STDC.

The permeation coefficient of formulation D for control, STC 0.1% and STC 0.5% was found to be 3.095×10^{-3} , 3.302×10^{-3} and 3.590×10^{-3} respectively and for formulation D of control, STDC 0.1% and STDC 0.5% reported permeation coefficient of 3.095×10^{-3} , 3.289×10^{-3} and 3.330×10^{-3} respectively.

The enhancement ratio (ER) indicates formulation D12 with enhancer STC 0.5% undergo more permeation with the ratio of 1.16 and with STDC 0.5% (D22) it was calculated as 1.08 (Fig 8).

The results indicated that Flux and permeation coefficient of all the tested formulations were

Fig. 7: Permeation of CBZ from Formulation F with and without enhancers.

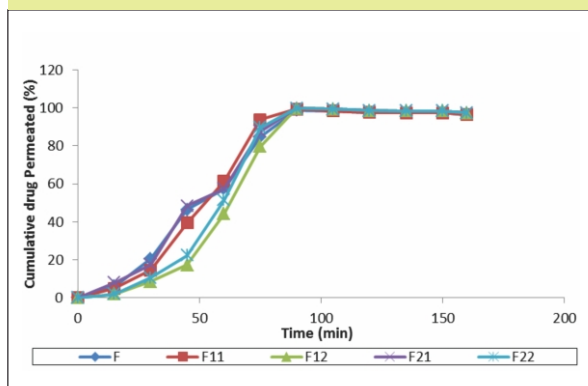
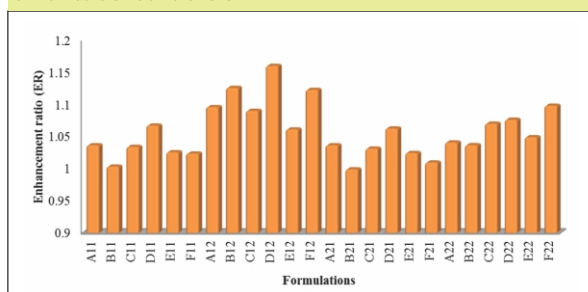


Fig. 8: Enhancement ratio of buccal patches of CBZ with enhancers STC and STDC



increased significantly in presence of sodium taurocholate (STC) (Table 3). The flux of CBZ increased as the concentration of permeation enhancer increases. The actual mechanism of enhancement of permeability by bile salts may be due to the increased fluidization of the buccal membrane. The subsequent increase in intercellular spaces resulted more penetration of the epithelium. These reversible structural changes caused by enhancers to the buccal mucosa were examined by histological studies¹⁵.

Histological study of porcine buccal mucosa:

The porcine buccal mucosa used for the *ex vivo* permeation study in Franz diffusion cell was preserved immediately after the study. The Histological observations are done to understand the changes in cellular structure caused by chemical permeation enhancers and solvents used for the study. The histological observation of microscopic cross section of tissue of control, STC treated and STDC

treated were given in Fig 9 a, b and c. The microscopical view clearly shows the outer layer of epithelial cells, basement membrane and adjacent lamina propria in all the specimens.

The microscopic view of tissue treated with permeation enhancer STC 0.5% and STDC 0.5% during

Fig. 9 (a): Microscopic image of (40X) normal buccal mucosa as control

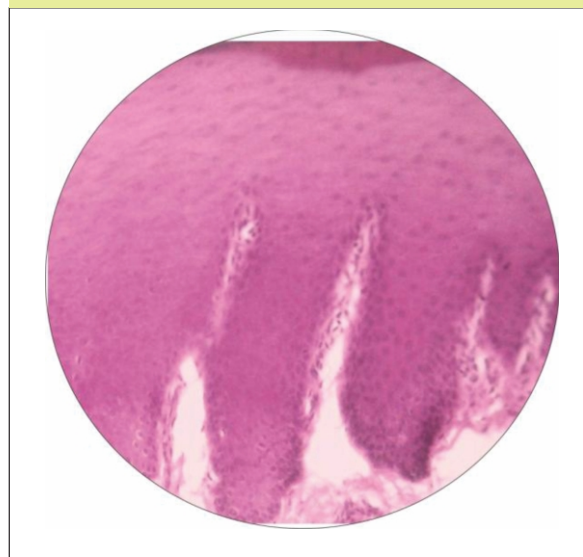


Fig. 9 (b): Microscopic image of (40X) buccal mucosa used for permeation with STC 1%

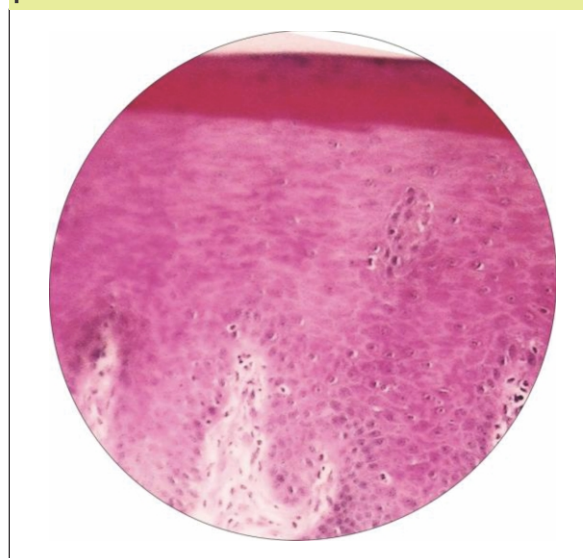
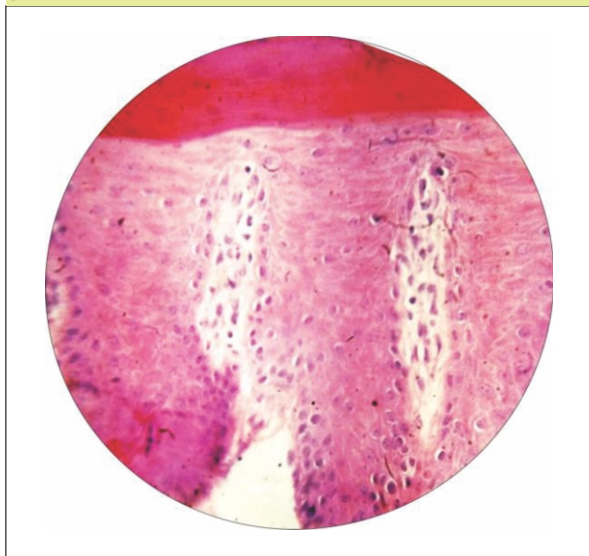


Fig. 9 (c): Microscopic image of (40X) buccal mucosa used for permeation with STDC 1%



150 min of permeation study [Fig 9(a) and Fig 9(b)] shows undamaged epithelial layer and

adjacent lamina propria. No abnormal changes were noticed in the cell morphology of mucosa. In the epithelium some vacuoles and increased intercellular spaces were observed. These histological changes may be due to the effect of bile salt which disrupts cellular structure by swelling. These swelled epithelial cells facilitate increased permeation of CBZ through transcellular route.

The appearance of swelling and vacuoles on the epithelial layer indicates penetration of drug and permeation enhancer across the membrane. But it was not noticed in control specimens. These structural changes caused by bile salts are reversible due to rapid cell turn over process of buccal mucosa and can be minimized by adjusting the concentration^{13,16}.

CONCLUSIONS

The buccal route of administration of CBZ was aimed to minimize first pass metabolism and to related adverse drug reactions. This route would be a better alternative to oral route of CBZ administration and convenient to unconscious epileptic patient. The potential drawbacks faced during formulation of CBZ

buccal patches was its low permeation across buccal mucosa. This study was aimed to improve permeability of drug by using chemical permeation enhancers of natural origin. The selected permeation enhancers of bile salts STC and STDC exhibited remarkable increase in rate of permeation and improved Flux (J_s) and Permeation coefficient (K_p) to a satisfactory level.

The permeation coefficient of CBZ across porcine buccal mucosa increased with increasing concentration of Permeation enhancers. STC performed better than STDC in the enhancement of permeation. The alteration of cellular structure of buccal mucosa by fluidization and swelling by bile salts facilitated more permeation of CBZ. These reversible histological changes were confirmed by microscopic examination of tissue specimens. This concluded that the lipophilic drug CBZ underwent permeation by intercellular route.

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