Chapter 6

In vitro buccal permeation study

6.1 INTRODUCTION

The drug delivery through oral mucosa offers a number of advantages over oral delivery, especially for those drugs that have poor solubility, poor bioavailability, and/or those drugs that suffer from extensive first pass metabolism in the liver. Conceivably, buccal delivery systems may provide for easy administration with little or no irritation, there by increasing patient compliance. In addition, gastric acid- or digestive enzyme-mediated degradation in the gastrointestinal tract is also avoided. Moreover, absorption following oral mucosal administration is not influenced by the potential variation in the gastric-emptying rate or the presence of food[1-7].

Weak acids and weak bases are subject to pH-dependent ionization. It is presumed that ionized species penetrate poorly through the oral mucosa compared with non-ionized species. An increase in the amount of non-ionized drug is likely to increase the permeability of the drug across an epithelial barrier, and this may be achieved by a change of pH of the drug delivery system. It has been reported that pH has effect on the buccal permeation of drug through oral mucosa[8-15]. Previous drug absorption studies have demonstrated that buccal absorption through oral mucosa for drugs such as morphine sulphate[8], nicotine[9,10], flecainide[11], sotalol[11], propanol[12] and others[13-15] changed with change in pH.

The examination of penetration route for transbuccal drug delivery is important because it is fundamental to select the proper penetration enhancer to improve the drug permeability. The routes of drug transport have been visualized directly by scanning electron microscopy, autoradiography and confocal laser scanning microscopy[15]. An indirect way to examine the routes of drug transport across buccal mucosa is to study permeation in vitro. Previous drug absorption studies have demonstrated that oral mucosal absorption of amines and acids at constant concentration is proportional to their partition coefficients[16,17]. Similar dependencies on partition coefficients were obtained for acyclovir[15], β-adrenoceptor blocking agents[18], tetramethylpyrazine[19], substituted acetanilide[20] and others[16,17].

Based on the cellular structure of the oral mucosa, there are two possible pathways for passive drug transportation – the paracellular route and the transcellular route. The physicochemical properties of the diffusant determine the dominant route. For lipophilic compounds, the transcellular pathway is the main route. For hydrophilic compounds, paracellular transport is the primary route[21-23].

The flux of drug through the membrane under sink condition for paracellular route can be written as[15,19,24]:

$$J_p = \frac{D_p \varepsilon}{h_p} C_d \qquad (1)$$

Where, D_p = diffusion coefficient of the permeate in the intercellular spaces, h_p = path length of the paracellular route, ε = area fraction of the paracellular route and C_d = donor drug concentration.

The flux of drug through the membrane under sink condition for transcellular route can be written as the following equation[15,19,24]:

$$J_c = \frac{(1-\varepsilon) D_c K_c}{h_c} C_d \qquad (2)$$

Where, K_c= partition coefficient between lipophilic cell membrane and the aqueous phase, D_c = diffusion coefficient of the drug in the transcellular spaces and h_c = pathlength of the transcellular route.

The present study is to examine the effects of drug concentration at pH 7.4, pH in donor chamber, and 1-octanol/buffer partition coefficient on transbuccal permeation and the possible in-vitro transport route of both drugs across porcine buccal mucosa. The permeability of salbutamol sulphate, ondansetron hydrochloride and lamotrigine at

several pH values through guinea pig mucosa as a model membrane were measured to compare the permeability of ionized and nonionized species

6.2 EXPERIMENTALS

6.2.1 Materials

Salbutamol sulphate was as gift sample from Relax Pharmaceuticals Pvt. Ltd. (Baroda, India). Ondansetron hydrochloride was received as a gift sample from Skymax Laboratories, Rajkot, India. Lamotrigine was received as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad, India. Potassium dihydrogen phosphate and phosphoric acid were purchased from S. D. Fine Chem. (Mumbai, India). HPLC grade methanol and acetonitrile were purchased from Loba Chemicals Ltd., Baroda, India. Other reagents used were of analytical grade and were used without additional purification.

6.2.2 Methods

6.2.2.1 *Solubility measurement*

Solubility of salbutamol sulphate, ondansetron hydrochloride and lamotrigine were determined at several pH 4.0, 6.0, 6.8, 7.4, 8.0, 9.0. Excess of drug was added to 10 ml of McIlvaine buffer solutions at each level. The samples were stirred in a conical flask for 24 hour at 37 °C. The pH of the samples was checked, adjusting the pH with 0.1 M citric acid as necessary. The suspensions were filtered using a 0.45 micron whatman filter paper. The concentration of salbutamol sulphate, ondansetron hydrochloride and lamotrigine in the filtrate were determined spectrophotometrically by measuring absorbance at 276 nm, 249 nm and 305 nm respectively[27].

6.2.2.2 *Determination of partition coefficient*

1-Octanol was used to represent the biomembrane. The partition coefficients between 1-octanol and McIlvaine buffer solutions at different pH (from 4.0 to 9.0) at 37 °C were

determined by shake-flask method[27]. 1-octanol and buffer solution were co-saturated with each other for 24 hr at 37 °C before use. 1-Octanol (5 ml) was shaken with 5 ml buffer solution containing salbutamol sulphate (10 mg/ml) or ondansetron hydrochloride (10 mg/ml) or lamotrigine (1 mg/ml) for 8 hrs at 37 °C. The mixture was then centrifuged at 2000 rpm for 10 minutes and the concentration of drug in each phase was determined sphectrophotometrically by measuring absorbance at 276 nm (for salbutamol sulphate), 249 nm (for ondansetron hydrochloride) or at 305 nm (for lamotrigine) in aqueous phase. The partition coefficient (K_p) of each drug was calculated from the following equation:

$$K_p = \frac{|C_1 - C_2|}{C_2} \qquad (3)$$

Where C_1 is the original concentration of drug in aqueous phase and C_2 is the final concentration of drug in aqueous phase.

6.2.2.3 *Collection and preparation of buccal tissue*

Guinea pig buccal tissue was chosen, because its non-keratinized morphology is quite similar to human buccal epithelium[1,11]. Buccal tissue was removed after sacrificing the animal and was stored in Krebs buffer pH 7.4 and was immediately transported to the experimental set-up. The buccal mucosa membranes were separated by removing the underlying connective tissues using surgical scissors, making, sure that the basal membrane was still present[28-30]. Slice thickness was ranged from 500-600 μm and mounted between donor and receiver chambers of the diffusion cells for permeation studies.

6.2.2.4 *In vitro permeation study through buccal mucosa*

The prepared buccal mucosa membranes with an approximate area of 4.00 cm^2 were mounted between the donor and receiver chambers of Franz type diffusion cells with an available diffusion area of 1.76 cm^2. The receiver chambers were filled with 10 ml of

McIlvaine buffer solution at pH 7.4 and the donor chambers were filled with 5 ml solution of salbutamol sulphate or ondansetron hydrochloride or lamotrigine of different concentration at McIlvaine buffer pH 7.4 or solution of salbutamol sulphate (0.4 mg/ml) or ondansetron hydrochloride (10 mg/ml) or lamotrigine (0.4 mg/ml) in McIlvaine buffer solutions of different pH (4.0, 6.0, 6.8, 7.4, 8.0, 9.0). Samples (0.3 ml) were withdrawn from receptor compartment at predetermined time interval (1, 3, 5, 10, 30, 60, 90, 120, 180 minutes) replaced with the same volume of fresh medium and subsequently assayed by HPLC method. The amount of drug present in donor compartment was determined and was plotted as a function of time. The permeability coefficients (P) were calculated from the linear part of the curves as follows[19]:

$$P = \frac{dQ/dt}{AC_d} \qquad (4)$$

Where, A = the surface area of diffusion, dQ/dt = amount of drug permeated per unit time at steady state and C_d = donor drug concentration.

The permeabilities of salbutamol sulphate, ondansetron hydrochloride or lamotrigine were evaluated at different pH (from 4.0 to 9.0). The steady state flux ($J_{ss} = P \times C_d$) of each drug at pH 7.4 was calculated at different drug concentration. Permeability coefficients of unionized (P_u) and ionized species (P_i) of salbutamol sulphate, ondansetron hydrochloride or lamotrigine at different pH were also calculated.

6.2.2.5 *Drug retention in mucosa*

The mucosa was separated from the diffusion cell after the permeation run of donor solution at various pH and was homogenized using a mortar and pestle and extracted three times with 10 ml methanol. The organic layer was centrifuged at 7000 rpm for 10 min to separate the cellular components. The extract was diluted suitably and was analyzed by HPLC method.

6.2.3 *HPLC method for salbutamol sulphate*

The HPLC system consisted of an LC 290 pump (Model LC 290, Perkin-Elmer, Inc., MA, USA) with UV detector (Model 290, Perkin Elmer, Inc., MA, USA) set at 276 nm, PE Nelson 1020 integrator and PE Nelson 1020 computer system. The samples were injected manually using a Rheodyne injector (Model 7125, Rheodyne, Cotati, California, USA) with a 20-µl loop. HPLC column (250 × 4.6 mm) was a 5 micron C_{18} column (Hypersil, BDS, Reverse phase) from Thermo Electron Corporation, USA. The Mobile phase consisted of water (70 %), methanol (20 %) and acetonitrile (10 %) and was filtered through a 0.45-micron whatman filter paper before use. The chromatography was performed isocratically at a flow rate of 1.2 ml/min at room temperature (approximately 29 °C) and retention time of 3.5 min was obtained. The amount of salbutamol sulphate present in sample was calculated from prepared standard calibration curve having correlation coefficient value more than 0.9996. The calibration graph was linear in the concentration range of 0.2-2.0 µg/ml. The precision and accuracy of the method was between 0.96-2.28 % and 98.71-100.83 %, respectively.

6.2.4 *HPLC method for ondansetron hydrochloride*

The HPLC system consisted of an LC 290 pump (Model LC 290, Perkin-Elmer, Inc., MA, USA) with UV detector (Model 290, Perkin Elmer, Inc., MA, USA) set at 249 nm, PE Nelson 1020 integrator and PE Nelson 1020 computer system. The samples were injected manually using a Rheodyne injector (Model 7125, Rheodyne, Cotati, California, USA) with a 20-µl loop. HPLC column (250 × 4.6 mm) was a 5 micron C_{18} column (Hypersil, BDS, Reverse phase) from Thermo Electron Corporation, USA. The mobile phase consisted of water: methanol: acetonitrile (70:20:10) and was filtered through a 0.45-micron whatman filter paper before use. The chromatography was performed isocratically at a flow rate of 1.0 ml/min at room temperature (approximately 29 °C) and retention time of 4.8 min was obtained. The amount of ondansetron hydrochloride present in sample was calculated from prepared standard calibration curve having correlation coefficient value more than 0.9998. The calibration graph was linear in the concentration

range of 0.5-10 μg/ml. The precision and accuracy of the method were between 1.52-2.71 % and 98.04-101.52 %, respectively.

6.2.5 *HPLC method for lamotrigine*

The HPLC system consisted of an LC 290 pump (Model LC 290, Perkin-Elmer, Inc., MA, USA) with UV detector (Model 290, Perkin Elmer, Inc., MA, USA) set at 305 nm, PE Nelson 1020 integrator and PE Nelson 1020 computer system. The samples were injected manually using a rheodyne injector (Model 7125, Rheodyne, Cotati, California, USA) with a 20-μl loop. HPLC column (250 × 4.6 mm) was a 5 micron C_{18} column (Hypersil, BDS, reverse phase) from Thermo Electron Corporation, USA. The mobile phase consisted of 0.01M phosphate buffer: methanol: acetonitrile (70:20:10) and was filtered through a 0.45-micron whatman filter paper before use. The chromatography was performed isocratically at a flow rate of 1.0 ml/min at room temperature (approximately 29 °C) and retention time of 4.8 minutes.was obtained. The amount of lamotrigine present in sample was calculated from prepared standard calibration curve having correlation coefficient value more than 0.9997. The calibration graph was linear in the concentration range of 0.1-1.5 μg/ml. The precision and accuracy of the method were between 0.94-2.68 % and 98.83-101.45 %, respectively.

6.3 RESULTS AND DISCUSSION

6.3.1 Effect of pH on Solubility

The solubility of salbutamol sulphate at different pH buffer solution decreased with increasing pH. The solubility of the drug at pH 9.0 was about five times lesser than that of at pH 4.0. The results are shown in table 6.1.

Table 6.1. Solubility of salbutamol sulphate at different pH (n=5).

pH	4.0	6.0	6.8	7.4	8.0	9.0
Solubility (mg/ml) ± S.D.	409.58 ± 0.23	328.74 ± 0.55	298.20 ± 0.69	210.17 ± 0.031	**129.34 ± 0.87**	82.63 ± 1.09

The solubility of ondansetron hydrochloride at different pH buffer solution decreased with increasing pH. The solubility of the drug at pH 9.0 was about 300 times lesser than that of at pH 4.0. The results are shown in table 6.2.

Table 6.2. Solubility of Ondansetron Hydrochloride at Different pH (n=5).

pH	4.0	6.0	6.8	7.4	8.0	9.0
Solubility (mg/ml) ± S.D.	28.34 ± 0.10	26.32± 0.34	14.78 ± 0.32	2.42 ± 0.86	**0.19 ± 0.76**	0.093 ± 0.42

The solubility of lamotrigine at different pH buffer solution decreased with increasing pH. The results are shown in table 6.3.

Table 6.3. Solubility of lamotrigine at Different pH (n=5).

pH	4.0	6.0	6.8	7.4	8.0	9.0
Solubility (mg/ml) ± S.D.	1.05 ± 0.35	0.85 ± 0.63	0.56 ± 0.21	0.48 ± 0.08	0.4 ± 0.95	**0.37 ± 0.69**

6.3.2 Permeation Parameters of Salbutamol Sulphate

The permeation data and permeation profiles of salbutamol sulphate in McIlvaine buffer solution (10 mg/ml) having different pH values are shown in table 6.4 and figure 6.1 respcetively.

Table 6.4. Permeation data of salbutamol sulphate at various pH (n=5).

Time (min)	Salbutamol sulphate permeated (µg/cm^2) ± S.D.					
	pH 4.0	**pH 6.0**	**pH 6.8**	**pH 7.4**	**pH 8.0**	**pH 9.0**
1	0.52 ± 1.16	10.45 ± 1.16	9.25 ± 1.72	14.11 ± 1.08	15.44 ± 1.82	17.68 ± 1.72
3	1.56 ± 12.52	17.77 ± 12.52	16.49 ± 2.82	15.68 ± 12.93	20.97 ± 3.39	22.93 ± 14.89
5	10.50 ± 4.73	23.52 ± 4.73	24.53 ± 11.73	17.56 ± 5.93	43.11 ± 6.92	64.69 ± 1.93
10	20.13 ± 10.76	35.55 ± 10.76	34.99 ± 9.54	38.74 ± 6.56	65.63 ± 7.71	89.10 ± 17.03
30	52.80 ± 6.31	77.38 ± 6.31	93.71 ± 8.34	117.17 ± 8.43	129.34 ± 8.56	171.00 ± 8.345
60	113.46 ± 10.66	121.30 ± 10.66	172.54 ± 13.65	226.92 ± 10.54	245.82 ± 10.65	315.79 ± 10.54
90	153.72 ± 26.77	174.63 ± 26.77	236.49 ± 17.65	329.92 ± 13.76	364.23 ± 11.67	444.20 ± 11.67
120	207.57 ± 19.32	251.49 ± 19.32	323.77 ± 27.54	428.75 ± 23.75	503.23 ± 27.76	598.83 ± 10.68
150	252.02 ± 24.22	319.47 ± 24.22	423.52 ± 13.75	542.94 ± 15.75	666.69 ± 19.54	777.69 ± 12.75
180	293.32 ± 30.65	399.47 ± 30.65	521.25 ± 23.65	670.89 ± 28.87	830.47 ± 25.65	947.08 ± 23.76

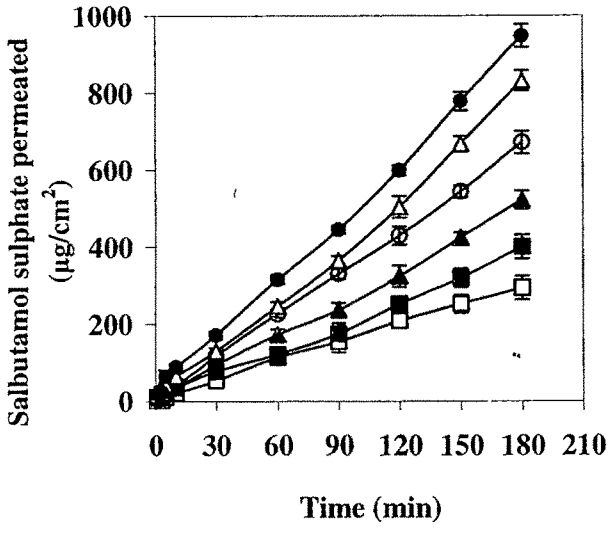

Figure 6.1. Permeation profiles of salbutamol sulphate through porcine buccal mucosa: (□) pH 4.0, (■) pH 6.0, (▲) pH 6.8, (○) pH 7.4, (Δ) pH 8.0, (•) pH 9.0. Each point represents the mean ± S. D. of five experiments.

Permeation of the drug was highest at pH 9.0 than that of the other pH studied. The permeated amount of drug increased linearly after 1 hr at each pH value. The permeated parameters such as steady state flux rate and permeability coefficient are given in Table 6.5.

Table 6.5. Permeation parameters of salbutamol sulphate (Donor Compartment Concentration-10 mg/ml) (n=5).

pH	$J^b \times 10^{-2}$ ($\mu g/cm^2/sec$) ± S.D.	$P^b \times 10^{-5}$ (cm/sec) ± S.D.
4.0	4.39 ± 0.36	0.25 ± 0.02
6.0	6.79 ± 0.48	0.38 ± 0.02
6.8	8.52 ± 0.62	0.48 ± 0.04
7.4	10.85 ± 0.81	0.61 ± 0.05
8.0	14.29 ± 0.94	0.81 ± 0.07
9.0	15.43 ± 0.87	0.87 ± 0.13

[b]The values of steady state flux (J, $\mu g/cm^2/sec$) and permeability coefficient (P, cm/sec) were calculated from the straight line obtained by plotting the permeated amount of drug ($\mu g/cm^2$) versus time (sec). Each value represents the mean ± S. D. (n=5).

The flux rate of the drug increased as the pH value of the donor solution increased, and the total flux at pH 9.0 was approximately 3.5 times higher than that of it at pH 4.0. The permeability coefficient increased with increasing the pH value. The steady state flux of salbutamol sulphate at pH 7.4 increased from 4.8 ± 0.18 × 10^{-5} to 31.36 ± 0.42 × 10^{-5} $mg/cm^2/sec$ as the drug concentration in donor compartment was increased from 5.0 to 25.0 mg/ml. The effect of donor concentration of the drug on steady state flux at pH 7.4 is shown in table 6 and figure 6.2.

Table 6.6. Steady state flux at different drug concentration (n=5).

Sr. No.	Concentration (mg/ml)	Steady state flux × 10^{-5} ($mg/cm^2/sec$) ± S.D.
1	5	4.805 ± 0.74
2	10	6.799 ± 0.99
3	15	18.328 ± 1.06
4	20	23.287 ± 0.84
5	25	31.365 ± 0.33

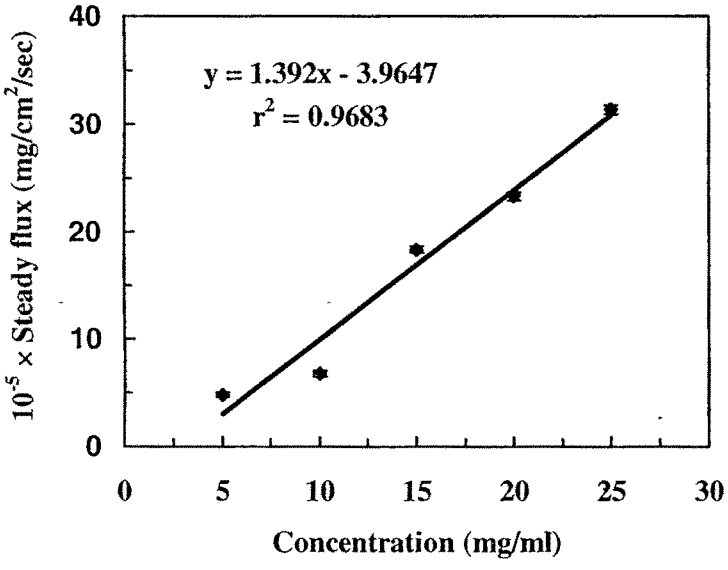

Figure 6.2. Effect of donor concentration of salbutamol sulphate on steady state flux at pH 7.4. Each point represents the mean ± S. D. of five experiments.

A linear relationship was observed between the flux and drug concentration (r^2=0.9683) which showed that the transport of drug through buccal mucosa over the range of concentrations investigated was a passive diffusion process. Both the apparent 1-octanol/buffer solution partition coefficient and the permeability coefficient increased

with increasing the pH of buffer solution from 4.0 to 9.0 in the donor compartment. The effect of pH on permeability coefficient and partition coefficient of the drug is shown in table 6.7 and figure 6.3.

Table 6.7. Effect of pH on permeability coefficient and partition coefficient of ondansetron hydrochloride (n=5).

pH	Partition coefficient ± S.D.	Fraction of ionized species	Fraction of unionized species	Permeability coefficient × 10^{-5} (cm/sec) ± S.D.
4.0	0.29 ± 0.004	0.99948	0.00052	0.2498 ± 0.0075
6.0	0.83 ± 0.017	0.98756	0.01244	0.3863 ± 0.0186
6.8	1.36 ± 0.058	0.96227	0.03773	0.4843 ± 0.0197
7.4	1.88 ± 0.0039	0.6661	0.3339	0.6166 ± 0.0183
8.0	2.6 ± 0.029	0.1663	0.8337	0.8120 ± 0.021
9.0	2.7 ± 0.073	0.019956	0.980044	0.8767 ± 0.0193

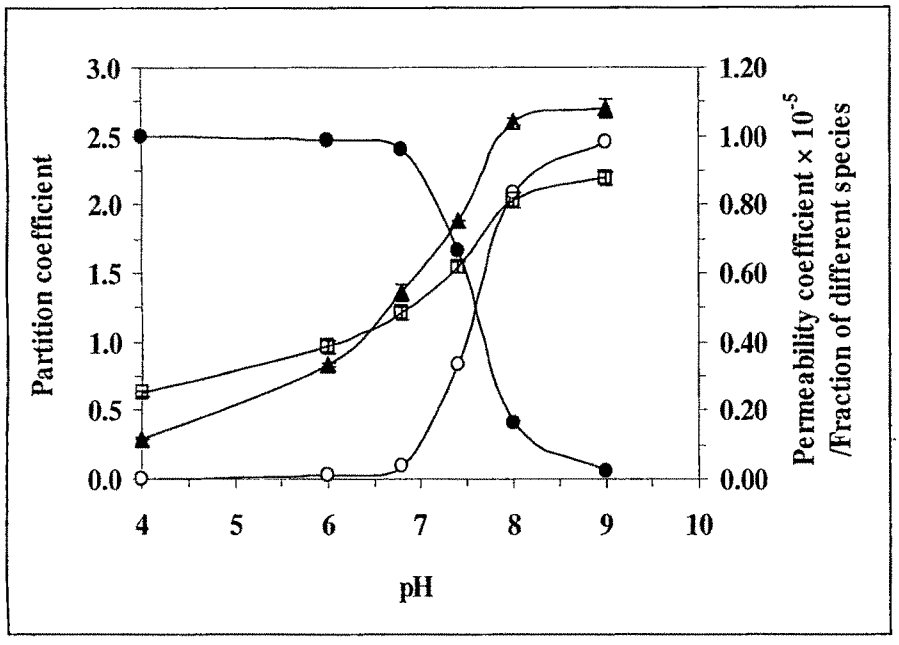

Figure 6.3. Effect of pH on permeability coefficient and partition coefficient of salbutamol sulphate: (□) Permeability coefficient × 10^{-5}, (▲) Partition coefficient, (●) Fraction of ionized species, (○) Fraction of unionized species. Each point represents the means ± S. D. of five experiments.

The partition coefficient and the total permeability coefficient increased with increasing the pH and with increasing the fraction of unionized form. The total permeability coefficient at pH 9.0 was 3.5 times higher than that of at pH 4.0. The statistically significant increased in permeation across porcine buccal mucosa may be due to the increased lipophilicity at pH 9.0 as compared with pH 4.0.

According to equations mentioned above and the assumption that drug will have the same partition tendency at 1-octanol and the biomembrane, the permeability of drug should have nothing to do with the partition coefficient if it goes through the paracellular route (Eq. 1), while the permeability shall vary with the partition coefficient if the drug was transported via the transcellular route (Eq. 2). Therefore, the transport route of drug permeation can be reflected since partition coefficient is pH dependent. In our study, the permabilities of drug was proportional to the partition coefficients and increased with

increasing pH (Fig. 3) slowly, which showed a paracellular route to be the main pathway for the buccal permeation of the drug. To further explain our experimental results, we assume that unionized form mainly goes through transcellular route and the ionized form transports via the paracellular route, the steady state flux can be expressed by the following equation[19]:

$$J_t = P_t C_t = J_u + J_i = P_u C_u + P_i C_i \qquad (5)$$

Where,

J_t = total flux of drug, J_u = transcellular flux, J_i = paracellular flux, C_u = concentration of unionized species, C_i = concentration of ionized species, C_t = total drug concentration, P_t = total drug permeability

So,

$$P_t = P_u \frac{C_u}{C_t} + P_i \frac{C_i}{C_t} \qquad (6)$$

Salbutamol sulphate is a basic drug with two pKa of 9.3 and 10.3. The percentage of different species at a given pH was calculated by using fitting the pKa value of 9.3 in the Henderson-Hesselbalch equation.

$$pH = pK_a + \log \frac{[\text{unionized}]}{[\text{ionized}]} \qquad (7)$$

P_u and P_i were calculated by fitting P_t, C_u/C_t, and C_i/C_t at different pH to Eq. (6). The calculated value for P_u was 8.89×10^{-6} cm/sec which is about four times higher than P_i, 2.49×10^{-6} cm/sec. The calculated permeability coefficient (P_{cal}) was plotted with the observed permeability coefficient at various pH which is shown in table 6.8 and figure 6.4.

Table 6.8. Observed permeability coefficient and calculated permeation coefficient at pH (n=5).

Sr. No.	pH	Observed permeability coefficient $\times 10^{-5}$ (cm/sec) ± S.D.	Calculated permeability coefficient $\times 10^{-5}$ (cm/sec)
1	4.0	0.2498 ± 0.0156	0.2497329
2	6.0	0.3863 ± 0.0128	0.3273628
3	6.8	0.4843 ± 0.0186	0.433551
4	7.4	0.6166 ± 0.0291	0.4631294
5	8.0	0.8120 ± 0.0204	0.7830514
6	9.0	0.8767 ± 0.0337	0.8767262

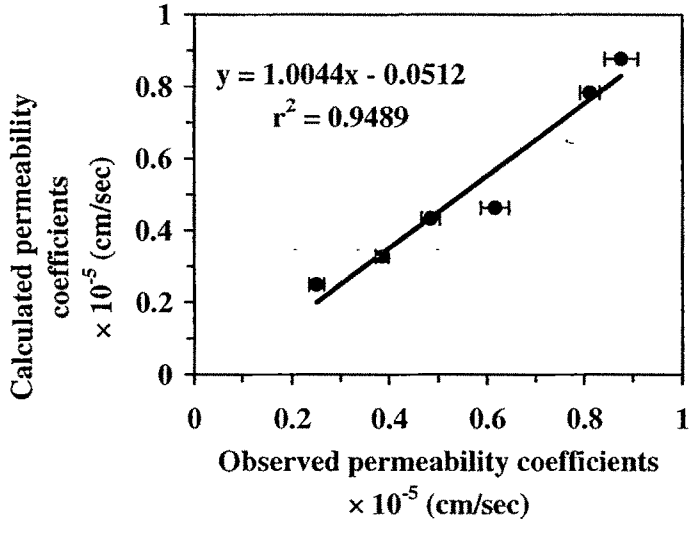

Figure 6.4. Correlation between observed permeability coefficient and calculated permeability coefficient. Each point represents the means ± S. D. of five experiments.

Good linearity ($r^2 = 0.9489$) was observed between the observed permeability coefficient and the calculated permeability coefficient at various pH which indicates the reliability of the mathematical model used for the calculation of P_l and P_u. The total permeability

coefficient and the partition coefficient of salbutamol sulphate increased with the fraction of unionized form slowly (Fig. 3). These results suggested that salbutamol sulphate transports via paracellular route based on our assumption. The transport pathway of drug depends on the lipophilicity of the drug. Hydrophilic compounds mainly cross the buccal mucosa via the paracellular route while the transcellular pathway is their main transport route for lipophilic compounds[11]. Salbutamol sulphate is a small hydrophilic molecule with a molecular weight of 576.70[31]. Therefore, the paracellular route is most likely the dominant pathway. A maximum partition coefficient (1-octanol/water) of 2.7 was found at pH 11.0, while the partition coefficient at pH 6.0 was 0.29. Here increased lipophilicity at pH 11.0 as compared with pH 6.0 resulted in a statistically significant increase in permeation across porcine buccal mucosa. Thus the increase in hydrophilicity made the paracellular pathway to play a significant role in the permeation of salbutamol sulphate.

6.3.3 Permeation Parameters of Ondansetron Hydrochloride

The permeation data and permeation profiles of ondansetron hydrochloride in McIlvaine buffer solution (10 mg/ml) having different pH values are shown in table 6.9 and figure 6.5 respectively.

Table 6.9. Permeation data of ondansetron hydrochloride at various pH (n=5).

Time (min)	Ondansetron hydrochloride permeated (µg/cm^2) ± S.D.					
	pH 4.0	**pH 6.0**	**pH 6.8**	**pH 7.4**	**pH 8.0**	**pH 9.0**
1	0.30 ± 3.68	0.13 ± 3.27	0.85 ± 3.82	1.31 ± 2.62	0.57 ± 3.12	0.68 ± 3.72
3	1.37 ± 2.73	0.49 ± 1.73	1.08 ± 2.82	14.88 ± 4.72	13.73 ± 1.71	14.31 ± 3.62
5	1.83 ± 5.7	1.31 ± 4.81	16.02 ± 3.73	16.60 ± 4.29	18.31 ± 4.62	19.46 ± 2.26
10	6.8 ± 4.85	1.48 ± 4.72	18.89 ± 8.92	21.18 ± 4.93	36.06 ± 6.21	34.92 ± 7.83
30	15.45 ± 4.92	17.74 ± 6.83	29.76 ± 7.93	38.92 ± 7.28	63.54 ± 7.27	70.98 ± 9.62
60	42.01 ± 8.36	44.65 ± 9.52	56.10 ± 11.74	72.70 ± 9.53	112.20 ± 9.26	127.66 ± 10.63
90	73.27 ± 12.74	87.01 ± 15.94	93.42 ± 16.83	108.19 ± 12.61	183.76 ± 8.38	196.35 ± 9.67
120	105.62 ± 14.67	121.93 ± 18.32	138.53 ± 15.84	163.15 ± 10.64	261.62 ± 12.39	282.23 ± 10.79
150	136.82 ± 15.63	151.90 ± 20.42	172.62 ± 14.93	219.25 ± 12.89	332.60 ± 15.70	368.10 ± 13.87
180	168.88 ± 18.95	186.05 ± 19.47	216.62 ± 12.54	274.21 ± 10.73	402.45 ± 14.84	442.52 ± 16.71

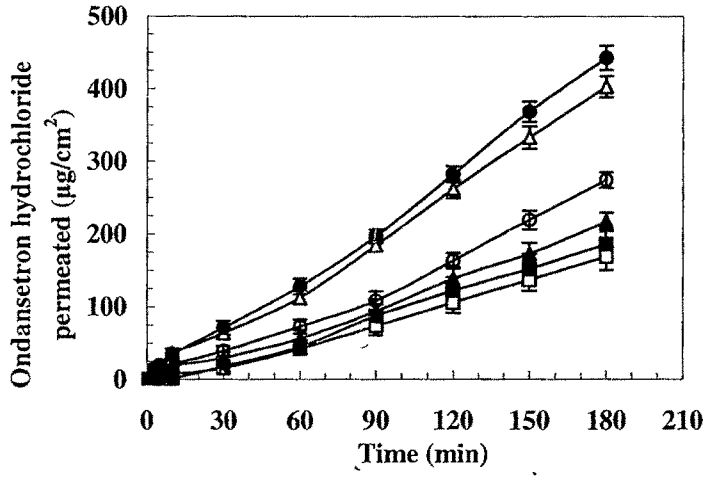

Figure 6.5. Permeation profiles of ondansetron hydrochloride through porcine buccal mucosa: (□) pH 4.0, (■) pH 6.0, (▲) pH 6.8, (○) pH 7.4, (Δ) pH 8.0, (●) pH 9.0. Each point represents the mean ± S. D. of five experiments.

Permeation of the drug was highest at pH 9.0 than that of the other pH studied. The permeated amount of drug increased linearly after 1 hr at each pH value. The permeated parameters such as steady state flux rate and permeability coefficient are given in Table 6.10.

Table 6.10. Permeation Parameters of Ondansetron hydrochloride (Donor compartment concentration-10 mg/ml) (n=5).

pH	$J^b \times 10^{-2}$ (µg/cm²/sec) ± S.D.	$P^b \times 10^{-5}$ (cm/sec) ± S.D.
4.0	3.10 ± 0.28	0.17 ± 0.01
6.0	3.45 ± 0.21	0.19 ± 0.02
6.8	3.92 ± 0.35	0.22 ± 0.04
7.4	4.92 ± 0.49	0.28 ± 0.06
8.0	7.09 ± 0.64	0.40 ± 0.07
9.0	7.69 ± 0.88	0.44 ± 0.06

[b]The values of steady state flux (J, μg/cm^2/sec) and permeability coefficient (P, cm/sec) were calculated from the straight line obtained by plotting the permeated amount of drug (μg/cm^2) versus time (sec). Each value represents the mean ± S. D. (n=5).

The flux rate of the drug increased as the pH value of the donor solution increased, and the total flux at pH 9.0 was approximately 2.5 times higher than that of it at pH 4.0. The permeability coefficient increased with increasing the pH value. The steady state flux of ondansetron hydrochloride at pH 7.4 increased from 0.91 ± 0.18 × 10^{-5} to 4.92 ± 0.42 × 10^{-5} mg/cm^2/sec as the drug concentration in donor compartment was increased from 0.5 to 2.24 mg/ml. The effect of donor concentration of the drug on steady state flux at pH 7.4 is shown in table 6.11 and figure 6.6.

Table 6.11. Steady state flux at different drug concentrations (n=5).

Sr. No.	Conc (mg/ml)	Steady flux × 10^{-5} (mg/cm^2/sec) ± S.D.
1	0.5	0.9161 ± 0.186
2	1.0	1.8730 ± 0.284
3	1.5	3.0366 ± 0.322
4	2.0	4.7816 ± 0.381
5	2.24	4.9216 ± 0.425

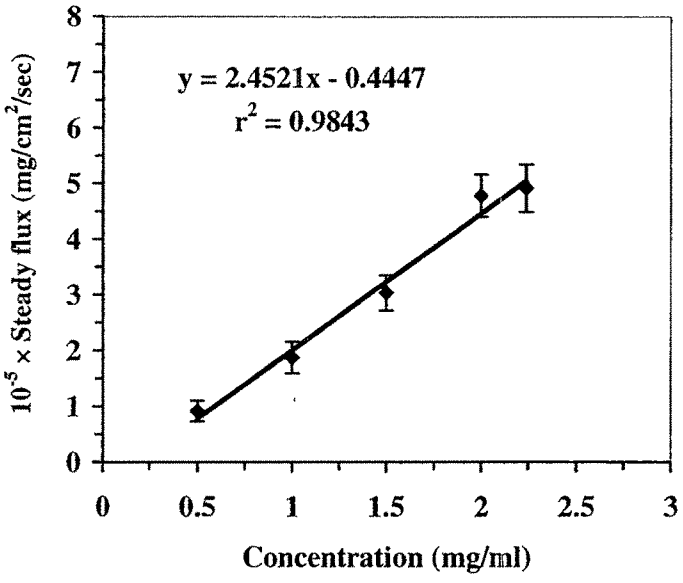

Figure 6.6. Effect of donor concentration of ondansetron hydrochloride on steady state flux at pH 7.4. Each point represents the means ± S. D. of five experiments.

A linear relationship was observed between the flux and drug concentration (r^2=0.9843) which showed that the transport of drug through buccal mucosa over the range of concentrations investigated was a passive diffusion process. Both the apparent 1-octanol/buffer solution partition coefficient and the permeability coefficient increased with increasing the pH of buffer solution from 4.0 to 9.0 in the donor compartment. The effect of pH on permeability coefficient and partition coefficient of the drug is shown in table 6.12 and figure 6.7.

Table 6.12. Effect of pH on permeability coefficient and partition coefficient of ondansetron hydrochloride (n=5).

pH	Permeability coefficient × 10^{-5} (cm/sec) ± S.D.	Partition coefficient ± S.D.	Fraction of ionized species	Fraction of unionized species
4.0	0.176 ± 0.092	0.091 ± 0.012	0.9984	0.0016
6.0	0.196 ± 0.083	0.184 ± 0.011	0.9617	0.0383
6.8	0.222 ± 0.055	0.301 ± 0.024	0.7993	0.2007
7.4	0.279 ± 0.046	0.757 ± 0.012	0.5000	0.5000
8.0	0.403 ± 0.054	1.384 ± 0.020	0.2007	0.7993
9.0	0.437 ± 0.069	1.47 ± 0.020	0.0417	0.9583

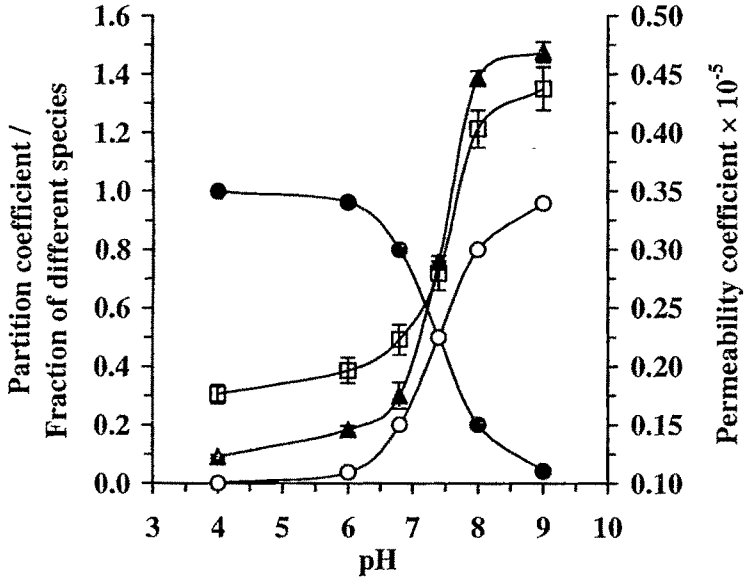

Figure 6.7. Effect of pH on permeability coefficient and partition coefficient of ondansetron hydrochloride: (□) Permeability coefficient × 10^{-5}, (▲) Partition coefficient, (●) Fraction of ionized species, (○) Fraction of unionized species. Each point represents the means ± S. D. of five experiments.

The partition coefficient and the total permeability coefficient increased with increasing the pH and with increasing the fraction of unionized form. A maximum partition coefficient (1-octanol/water) of 1.47 was found at pH 9.0, while the partition coefficient at pH 4.0 was 0.09. The total permeability coefficient at pH 9.0 was about more than two times higher than that of at pH 4.0. The statistically significant increased in permeation across porcine buccal mucosa may be due to the increased lipophilicity at pH 9.0 as compared with pH 4.0 In our study, the permeability of drug was proportional to the partition coefficient with the correlation coefficient (r^2) of 0.9911 and increased with increasing pH. The drug was permeated through transcellular route and paracellular route. The value of P_i and P_u were calculated by fitting the values of C_u/C_t and C_i/C_t in equation 6. The calculated values for P_u and P_i were 4.86×10^{-6} cm/sec and 7.18×10^{-7} cm/sec, respectively. The observed permeability coefficient and calculated permeability coefficient (P_{cal}) are shown in table 6.13. The calculated P_{cal} was plotted with the observed permeability coefficient at various pH which is shown in figure 6.8.

Table 6.13. Observed permeability coefficient and calculated permeation coefficient at pH (n=5).

pH	Permeability coefficient $\times 10^{-5}$ (cm/sec) ± S.D.	Calculated Permeability coefficient$\times 10^{-5}$ (cm/sec)
4.0	0.176 ± 0.092	0.724
6.0	0.196 ± 0.083	0.876
6.8	0.222 ± 0.055	1.549
7.4	0.279 ± 0.046	2.789
8.0	0.403 ± 0.054	4.028
9.0	0.437 ± 0.069	4.687

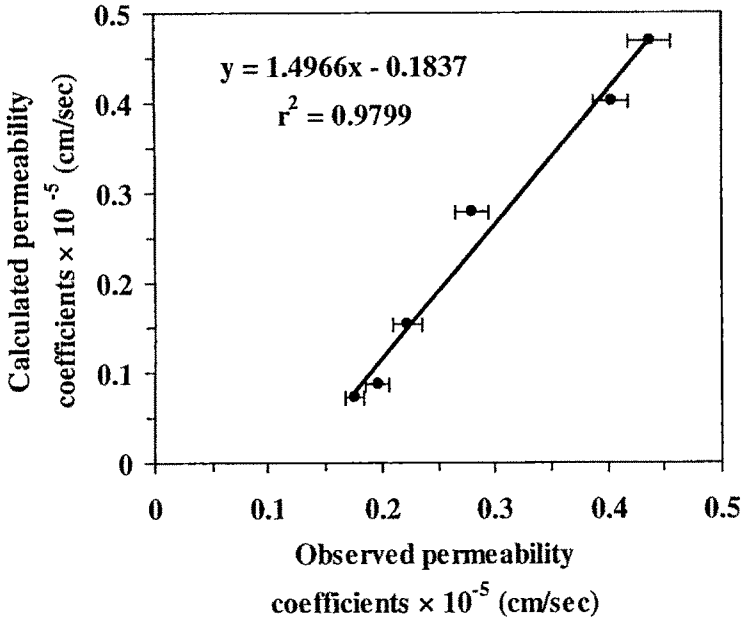

Figure 6.8. Correlation between observed permeability coefficient and calculated permeability coefficient. Each point represents the means ± S. D. of five experiments.

Good linearity ($r^2 = 0.9799$) was observed between the observed permeability coefficient and the calculated permeability coefficient at various pH which indicates the reliability of the mathematical model used for the calculation of P_i and P_u.

6.3.4 Permeation Parameters for Lamotrigine

The permeation data and permeation profiles of lamotrigine in McIlvaine buffer solution (0.4 mg/ml) having different pH values are shown in table 6.14 and figure 6.9.

Table 6.14. Permeation data of lamotrigine through porcine buccal mucosa (n=5).

Time (min)	Lamotrigine permeated (µg/cm^2) ± S.D.					
	pH 4.0	**pH 6.0**	**pH 6.8**	**pH 7.4**	**pH 8.0**	**pH 9.0**
1	0.56 ± 4.72	0.87 ± 4.16	4.10 ± 5.73	0.66 ± 4.72	0.82 ± 5.27	1.33 ± 3.61
3	1.80 ± 2.17	2.15 ± 1.72	13.87 ± 4.61	14.38 ± 5.28	13.35 ± 3.72	15.92 ± 4.72
5	3.19 ± 3.78	3.64 ± 3.26	16.43 ± 5.82	18.49 ± 6.72	18.49 ± 5.28	24.14 ± 2.72
10	18.01 ± 5.79	21.07 ± 6.78	20.54 ± 9.32	26.71 ± 6.38	26.71 ± 7.38	33.90 ± 9.28
30	47.34 ± 6.37	52.40 ± 7.38	59.59 ± 8.94	76.54 ± 8.66	76.54 ± 8.85	81.16 ± 10.66
60	88.52 ± 9.72	100.17 ± 10.72	137.67 ± 13.51	154.63 ± 10.04	196.75 ± 10.83	181.34 ± 12.03
90	134.84 ± 16.83	145.89 ± 18.66	250.18 ± 17.88	232.20 ± 13.82	299.50 ± 9.41	304.12 ± 14.96
120	169.83 ± 20.64	189.05 ± 25.80	374.50 ± 18.92	341.11 ± 13.76	401.22 ± 14.83	418.17 ± 12.82
150	220.27 ± 21.93	243.50 ± 24.23	474.37 ± 16.73	460.29 ± 16.37	535.30 ± 17.28	566.64 ± 15.06
180	277.40 ± 28.49	305.15 ± 29.57	567.66 ± 17.84	628.21 ± 18.93	688.39 ± 23.52	708.94 ± 18.39

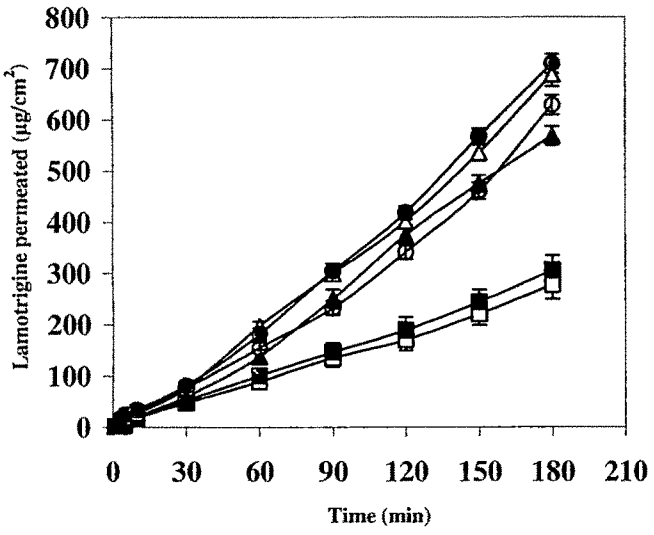

Figure 6.9. Permeation profiles of lamotrigine through porcine buccal mucosa. (□) **pH 4.0,** (■) **pH 6.0,** (▲) **pH 6.8,** (○) **pH 7.4,** (Δ) **pH 8.0,** (●) **pH 9.0. Each point represents the mean ± S. D. of five experiments.**

The permeated parameters such as steady state flux rate and permeability coefficient are given in table 6.15.

Table 6.15. Permeation parameters of lamotrigine (donor compartment concentration - 0.4 mg/ml) (n=5).

pH	$J^b \times 10^{-2}$ (µg/cm²/sec) ± S.D.	$P^b \times 10^{-5}$ (cm/sec) ± S.D.
4.0	4.61 ± 0.27	0.26 ± 0.02
5.0	5.01 ± 0.39	0.28 ± 0.04
6.8	10.51 ± 0.48	0.59 ± 0.07
7.4	11.57 ± 0.62	0.65 ± 0.12
8.0	12.01 ± 0.84	0.68 ± 0.14
9.0	12.89 ± 0.71	0.73 ± 0.08

[b]The values of steady state flux (J, μg/cm^2/sec) and permeability coefficient (P, cm/sec) were calculated from the straight line obtained by plotting the permeated amount of drug (μg/cm^2) versus time (sec). Each value represents the mean ± S. D. (n=5).

The permeated amount of drug increased linearly after 1 hr at each pH value. The steady state flux of lamotrigine at pH 7.4 increased with increasing the donor concentration. A linear relationship was observed between the flux and drug concentration (r^2=0.9639) which is shown in table 6.16 and figure 6.10.

Table 6.16 Effect of donor drug concentration on lamotrigine flux at pH 7.4 (n=5).

Sr. No.	Concentration (mg/ml)	Steady flux × 10^{-5} (mg/cm^2/sec) ± S.D.
1	0.1	5.42 ± 0.24
2	0.2	7.83 ± 0.37
3	0.3	8.56 ± 0.43
4	0.4	10.93 ± 0.48

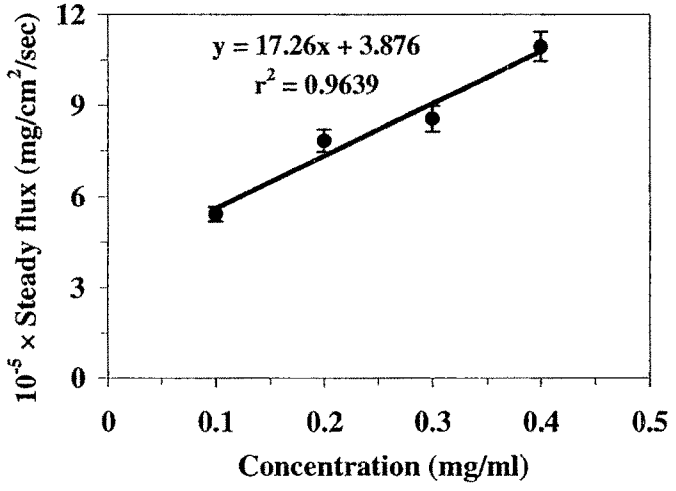

Figure 6.10. Effect of donor concentration of lamotrigine on steady state flux at ph 7.4. Each point represents the mean ± S. D. of five experiments.

The linear relationship between the steady state flux at pH 7.4 and donor drug concentration showed that the transport of drug through buccal mucosa at the concentration range from 0.1 to 0.4 mg/ml was a passive diffusion process. Both the apparent 1-octanol/buffer solution partition coefficient and the permeability coefficient increased with increasing the pH of buffer solution in donor chamber (table 6.17 and figure 6.11).

Table 6.17. Effect of pH on Partition Coefficient and Permeability (n=5).

pH	Permeability coefficient × 10^{-5} (cm/sec) ± S.D.	Partition coefficient ± S.D.
4.0	0.262 ± 0.032	0.308 ± 0.132
6.0	0.284 ± 0.027	1.165 ± 0.181
6.8	0.597 ± 0.029	4.966 ± 0.224
7.4	0.657 ± 0.038	6.354 ± 0.312
8.0	0.682 ± 0.026	7.136 ± 0.220
9.0	0.732 ± 0.023	7.73 ± 0.420

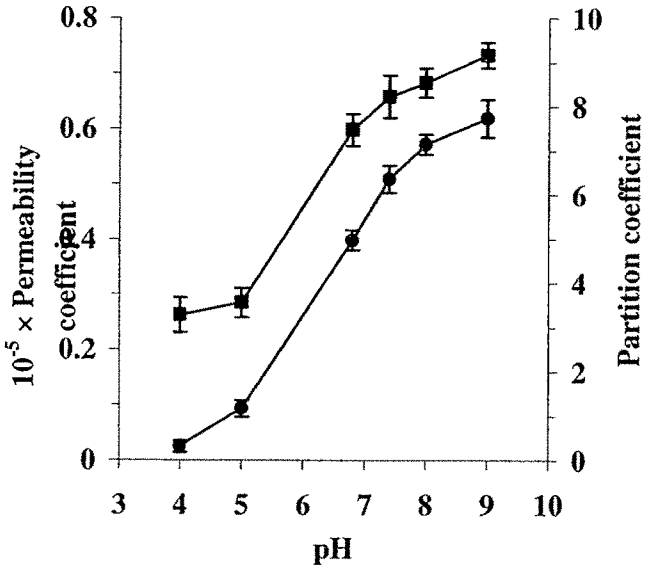

Figure 6.11. Effect of pH on permeability coefficient and partition coefficient of lamotrigine: (■) permeability coefficient, (●) partition coefficient. Each point represents the mean ± S. D. of five experiments.

Excellent linearity (r^2 = 0.9879) was observed between the permeability coefficient and the partition coefficient (figure 6.12).

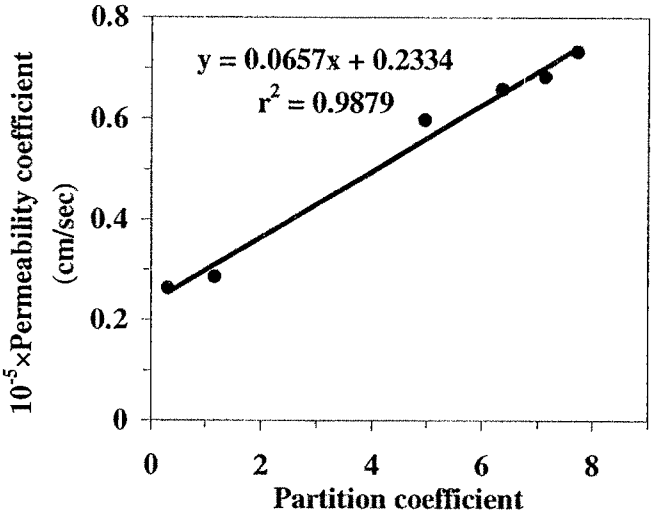

Figure 6.12. Correlation of 1-octanol/buffer partition coefficient and permeability coefficient Each point represents the means ± S. D. of five experiments.

Lamotrigine has one dissociation constant and its value is 1.995×10^{-06}. It is a basic drug with pKa value of 5.7 (30). P_u and P_i were calculated by fitting P_t, C_u/C_t, and C_i/C_t at different pH to equation 6. The calculated value for P_u was 0.7291×10^{-5} cm/sec which is about three times higher than P_i, 0.2500×10^{-5} cm/sec. The calculated permeability coefficient (P_{cal}) was plotted with the observed permeability coefficient at various pH values which is shown in table 6.18 and figure 6.13.

Table 6.18. Relationship between observed permeability and calculated permeability (n=5).

pH	Observed Permeability coefficient (cm/sec) ± S.D.	Calculated permeability coefficient
4.0	0.262 ± 0.032	0.262
6.0	0.284 ± 0.027	0.332
6.8	0.597 ± 0.029	0.503
7.4	0.657 ± 0.038	0.722
8.0	0.682 ± 0.026	0.729
9.0	0.732 ± 0.023	0.731

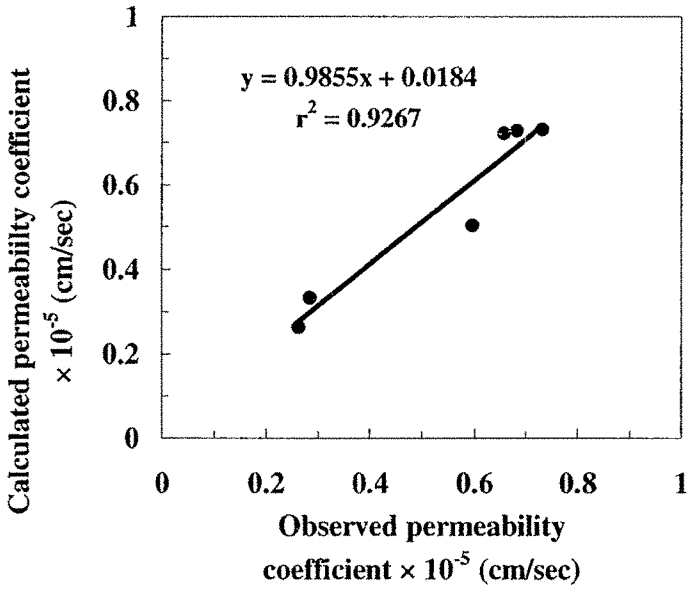

Figure 6.13. Correlation between observed permeability coefficient and calculated permeability coefficient. Each point represents the means ± S. D. of five experiments.

Figure 6.14 shows the relationship between the permeability coefficient and the fraction of different species of lamotrigine while figure 6.15 shows the relationship between the partition coefficient and the fraction of different species. Table 6.19 shows the relationship between permeability coefficient, partition coefficient and fraction of different species.

Table 6.19. Relationship between permeability coefficient, partition coefficient and fraction of different species (n=5).

pH	Permeability coefficient $\times 10^{-5}$(cm/sec) ± S.D.	Partition coefficient ± S.D.	Fraction of ionized species	Fraction of unionized species
4.0	0.262 ± 0.032	0.308 ± 0.132	0.9792	0.0208
5.0	0.284 ± 0.027	1.165 ± 0.181	0.8336	0.1664
6.8	0.597 ± 0.029	4.966 ± 0.224	0.4761	0.5239
7.4	0.657 ± 0.038	6.354 ± 0.312	0.0195	0.9805
8.0	0.682 ± 0.026	7.136 ± 0.220	0.0049	0.9951
9.0	0.732 ± 0.023	7.73 ± 0.420	0.0005	0.9995

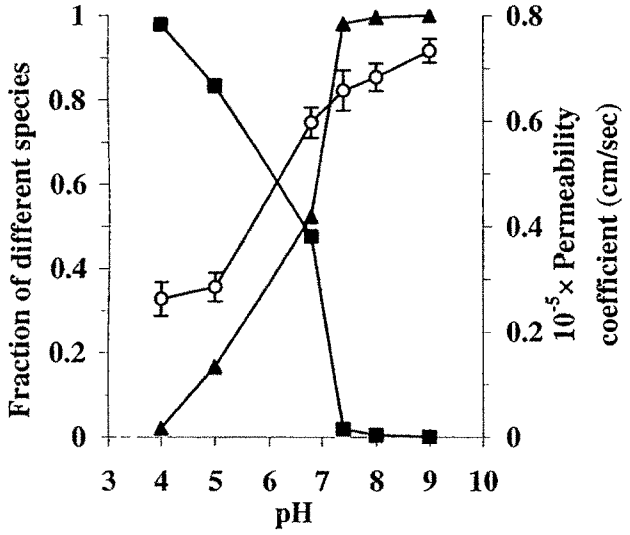

Figure 6.14. Relationship between permeability coefficient and the fraction of different species of lamotrigine: (■) fraction of ionized species, (○) permeability coefficient, (▲) fraction of unionized species. Each point represents the mean ± S. D. of five experiments.

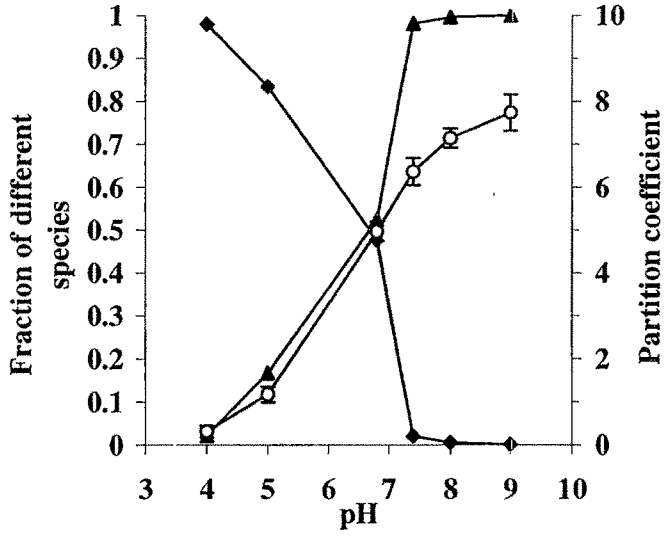

Figure 6.15. Relationship between partition coefficient and the fraction of different species of lamotrigine: (■) fraction of ionized species, (○) partition coefficient, (▲) fraction of unionized species. Each point represents the mean ± S. D. of five experiments.

The partition coefficient and the total permeability coefficient of lamotrigine increased with increasing the fraction of unionized form.

Drugs ability to diffuse across the membranes is frequently expressed in terms of their lipid-water partition coefficient, which is a measure of the relative affinity of a drug for the lipid and aqueous phases. But it is not true in vivo model for the transbuccal diffusion of drug and also does not take into account of the influence of pH of body fluids and pKa of drug. The transmembrane diffusion process is passive in nature and depends on a concentration differential as the driving force, each molecule requiring kinetic energy to effect a net movement down this gradient. Permeant molecules must therefore diffuse through the vehicle in which they are contained to the mucosal interface and have to partition from the vehicle into the upper layers of the tissue. From here molecules must diffuse within the mucosa, equilibrating laterally, and must emerge, eventually under steady-state conditions, from the distal surface of the tissue. Adsorptive interaction might be extensive in this layer, forming a reservoir of the permeant molecules. Further

partitioning into neighboring tissue strata or into the receptor fluid then takes place under the influence of the concentration gradient, and adsorption might occur once again. Initially the concentration gradient across the mucosa will not be linear as the permeant equilibrates within the tissue. However, after sufficient time has elapsed, steady state will be achieved and the effective permeant concentration at all points in the tissue will remain constant.

Penetration of drug into tissues is dependent on the ionization state of the drug molecule. At lower pH values of the buffer system used in this study, i.e., 4.0, approximately 98.05 % of the lamotrigine is present in their dissociated forms. Although this improves diffusion through the hydrophilic outer layers of the epithelium, it does not facilitate penetration of the lipoidal layer, which is thought to constitute the major permeability barrier of buccal mucosa[31,32]. At higher pH value, i.e., 9.0, approximately 5.01 % of the lamotrigine is present in their dissociated forms which improve diffusion through lipoidal layer. It is clear that the flux rates across buccal mucosa at pH 9.0 are statistically significantly higher than those of pH 4.0 (figure 6.9). This is also reflected in the permeability coefficients, showing the higher permeation of lamotrigine at higher pH than that of lower pH. The permeability coefficient increased as the pH value increased because the solubility of drug decreased with increasing the pH. Good linearity (r^2 = 0.9267) was observed between the observed permeability coefficient and the calculated permeability coefficient at various pH which indicates the reliability of the mathematical model used for the calculation of P_l and P_u.

According to the pH-partition hypothesis, only the nonionized form of the drug is able to cross lipodal membranes in significant amount. The effect of pH on drug absorption for ionizable compound has been extensively studied[15]. If a single species of drug is transported via the paracellular route (Eq.1), the permeability of the drug should be independent of partition coefficient. As shown in figure 6.11, this is not the case for lamotrigine. Conversely, if the drug is transported via the transcellular route (Eq. 2), the permeability of the drug should vary with the partition coefficient. Thus, the pH dependence of the permeation of ionizable drug actually reflects the drug penetration route, because the partition coefficient of ionizable drug is pH dependent. If a drug is

transported via the transcellular route, the drug absorption rate is also pH dependent. While in vitro permeation of lamotrigine varies with pH, the variation of the permeability coefficients of lamotrigine with pH far exceeds that of the partition coefficients. Therefore, a model of permeation of a single species would not be consistent with the data (figure 6.11).

The calculated value of permeability coefficient of unionized species was about three times higher than ionized species. In our study, the permeabilities of drug was proportional to the partition coefficients (figure 6.12) and increased with increasing the pH (Fig. 6.11), which showed a transcellular route to be the main pathway for the buccal permeation of the drug. The transport pathway of drug depends on the lipophilicity of the drug. Hydrophilic compounds mainly cross the buccal mucosa via the paracellular route while the transcellular pathway is main transport route for lipophilic compounds[11]. Lamotrigine is a lypophilic drug with a molecular weight of 265.1. Therefore, the transcellular route is most likely the dominant pathway. A maximum partition coefficient (1-octanol/water) of 7.74 was found at pH 9.0, while the partition coefficient at pH 4.0 was 0.31. The increased lipophilicity at pH 9.0 as compared with pH 4.0 resulted in a statistically significant increase in permeation across porcine buccal mucosa. The increased lipophilicity made the transcellular pathway to play a significant role in the permeation of lamotrigine.

Results of salbutamol sulphate remained in mucosa after different run at various pH is shown in figure 6.16. The salbutamol sulphate retention of mucosa at pH 9.0 was approximately two times higher than that of pH 4.0. The value of salbutamol sulphate content in mucosa increased with increasing pH from 4.0 to pH 9.0. The increase in drug content at higher pH may be due to higher partition coefficient at higher pH.

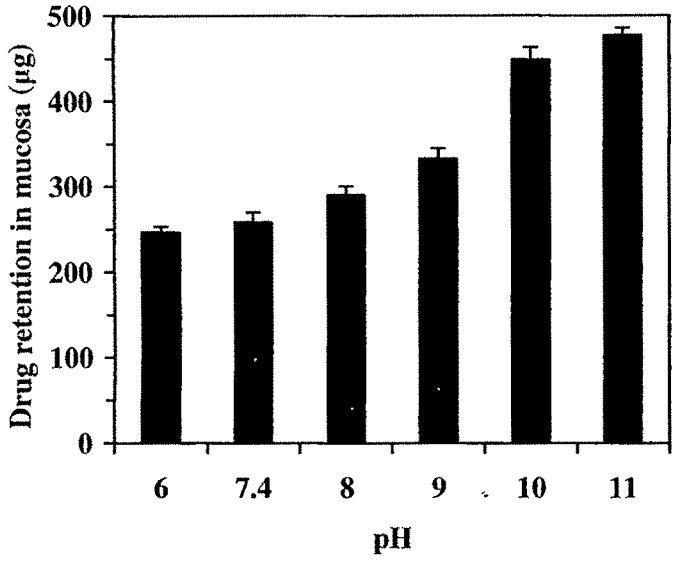

Figure 6.16. Content of salbutamol sulphate (µg) in mucosa after run at various pH. Each point represents the means ± S. D. of five experiments.

Results of ondansetron hydrochloride remained in mucosa after run at various pH is shown in figure 6.17. The drug retention of mucosa at pH 9.0 was approximately 2.2 times higher than that of pH 4.0. The value of drug content in-mucosa increased with increasing pH from 4.0 to pH 9.0. The increase in drug content at higher pH may be due a higher partition coefficient at higher pH.

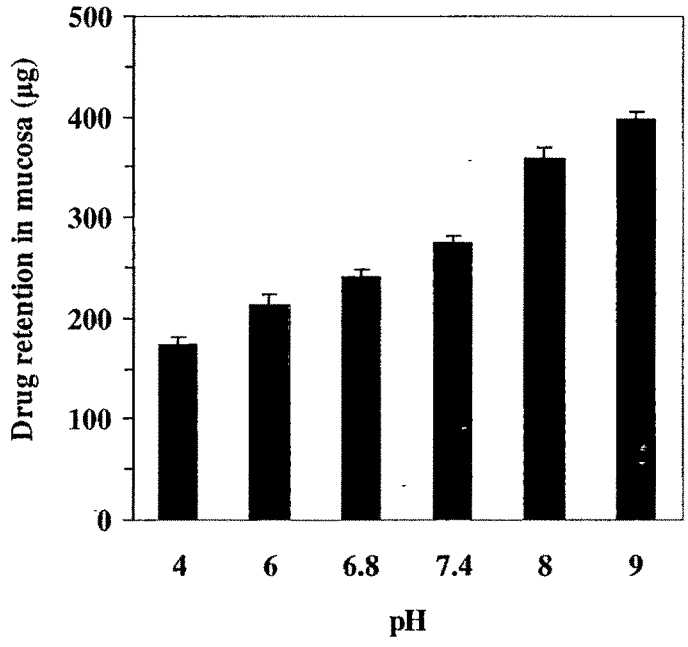

Figure 6.17. Content of ondansetron hydrochloride (µg) in mucosa after run at various pH. . Each point represents the means ± S. D. of five experiments.

Results of lamotrigine remained in mucosa after run at various pH is shown in figure 6.18. The value of drug content in mucosa increased with increasing the pH which indicated that baccal permeation of lamotrigine increased with increasing the pH. The fraction of unionized species of drug increases with increasing the pH. The increase of drug content in mucosa with increasing the pH might be due to permeation of more unionized species at higher pH value through the transcellular route across buccal mucosa.

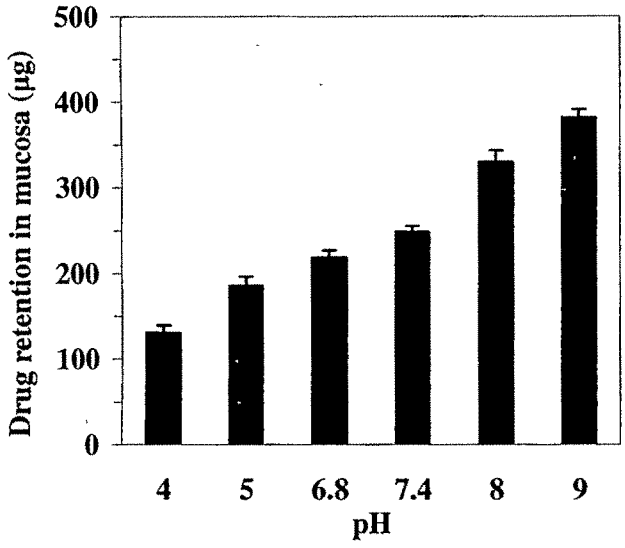

Figure 6.18. Content of lamotrigine (µg) in mucosa after run at various pH. . Each point represents the means ± S. D. of five experiments.

6.4 CONCLUSIONS

Salbutamol sulphate permeated through the buccal mucosa by passive diffusion over the range of concentrations examined. Permeability coefficient and steady state flux of salbutamol sulphate across porcine buccal mucosa increased with increasing pH values. The 1-octanol/buffer partition coefficient of the drug also increased with increasing pH. The permeability coefficient of unionized species was greater than that of ionized species which indicated that the nonionized species of salbutamol sulphate penetrated well through porcine buccal mucosa and the permeation was a function of pH. The results of this study indicate that the salbutamol sulphate penetrated well through porcine buccal mucosa this might be due to the paracellular route of permeation. Transbuccal delivery has been shown to be a potential route for the administration of salbutamol sulphate.

Ondansetron hydrochloride permeated through the buccal mucosa by passive diffusion over the range of concentrations examined. Permeability coefficient and steady state flux of ondansetron hydrochloride across porcine buccal mucosa increased with increasing pH values. The 1-octanol/buffer partition coefficient of the drug also increased with

increasing pH. The permeability coefficient of uionized species was greater than that of ionized species which indicated that the nonionized species of ondansetron hydrochloride penetrated well through porcine buccal mucosa and the permeation was a function of pH. Transbuccal delivery has been shown to be a potential route for the administration of ondansetron hydrochloride.

Permeability coefficient and steady state flux of lamotrigine across porcine buccal mucosa increased with increasing the pH values. The 1-octanol/buffer partition coefficient of the drug also increased with increasing the pH. The permeability coefficient of unionized species was greater than that of ionized species which indicated that the unionized species of lamotrigine penetrated well through porcine buccal mucosa and the permeation was a function of pH. It is concluded that main transport route of buccal permeation of lamotrigine is through transcellular route.

6.5 REFERENCES

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