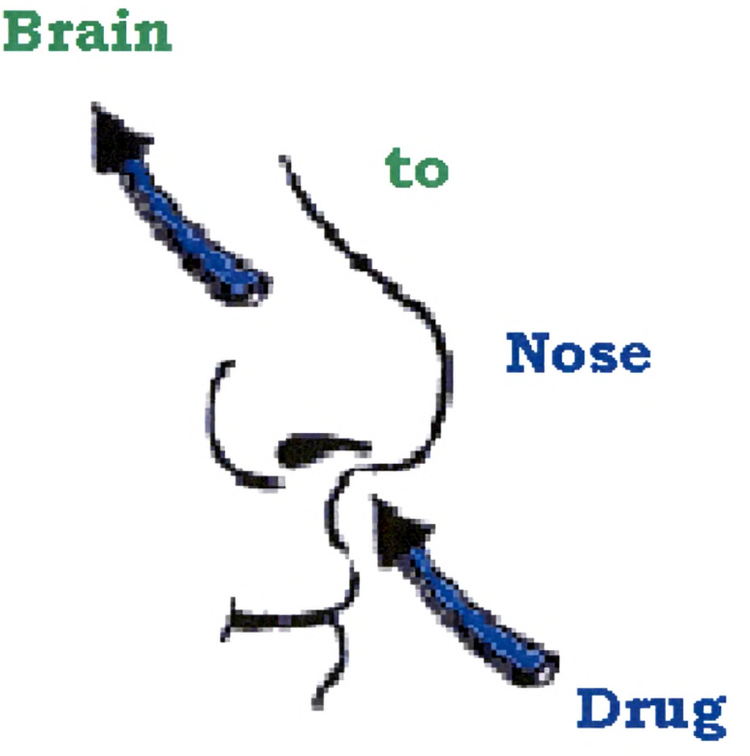


CHAPTER 5

DRUG DIFFUSION STUDIES

In vitro diffusion of formulations is a valuable tool to predict behavior of a particular formulation with respect to drug transport across the membrane. According to Gemmell and Morrison (1957), *in vitro* model may have limitations in terms of prediction of drug transport across the mucosal membrane nevertheless; under the testing conditions *in vitro* studies can be helpful to assess the relative drug transport behavior across the mucosa. Various physicochemical parameters pertaining to formulations such as flux, partition coefficient, diffusion coefficient can be derived using *in vitro* evaluation techniques. One of the disadvantages of *in vitro* evaluation techniques is that method does not mimic the behavior of living tissues/organs, for example, degradation of drug compound in presence of enzymes, capricious blood supply or metabolism etc. In practice, it virtually becomes difficult task to perform the biological studies using animals or on humans for the assessment of different formulations from the perspective of economy and time requirement. At the same time, *in vitro* models can serve as second line option which will be indicative kind of tool prior to proceeding for animal or human studies.

In this investigation, all the test formulations were assessed for *in vitro* diffusion across the sheep nasal mucosa in triplicate and the physicochemical parameters were calculated as mentioned below (Higuchi 1961).

(A) Percent Drug Diffused

The percent drug diffused across the sheep nasal mucosa at predetermined sampling time interval was determined using formula mentioned below.

$$\%\,Drug\ Diffused = \frac{c_r v_r}{c_d v_d} \times 100$$

Where, Cr = Concentration of the drug in receptor compartment

Vr = Volume of the receptor compartment

Cd = Initial concentration of the drug in donor compartment

Vd = Initial volume in the donor compartment

(B) Kinetics of Release

In order to investigate the mechanism of drug release from the formulation, the release rates were integrated into each of the following equation and the regression coefficient was investigated from each of the regressed graph.

Zero-order equation:

$$Q = K_0 t$$

Where, Q = Amount of drug released at time t

t = Time in hours

K_0 = Zero-order release rate constant

First-order equation:

$$Q = Q_0 e^{-K_1 t}$$

Where, Q = Amount of drug released at time t

t = Time in hours

K_1 = First-order release rate constant

Higuchi's equation:

$$Q = K_H \times \sqrt{t}$$

Where, Q = Amount of drug released at time t

t = Time in hours

K_H = Higuchi's diffusion rate constant

The order of drug release was determined by performing the regression over the mean values of percent drug diffusion vs. t (for Zero-order), log percent drug diffusion vs. t (for First-order) and percent drug diffusion vs. square root of t (for Higuchi).

(C) Mean Steady State Flux

The flux across the nasal mucosa was calculated using following equation

$$J = V \times \left(dc/dt\right)$$

Where J = Flux of the drug across nasal mucosa

(dc/dt) = Rate of change of concentration

V= Volume of diffusion medium in receptor compartment

Note: Mean steady flux is the mean of individual flux values at all the sampling points

(D) Diffusion coefficient

The diffusion coefficient of the drug at every sampling point was calculated using following equation.

$$R = 200 \times \sqrt{Dt / \pi h^2}$$

Where, R = Percent drug diffused

h = Thickness of the nasal mucosa

t = time in seconds

D = Diffusion coefficient (cm^2/sec)

The diffusion coefficient derived is the mean of the value (D) obtained at each sampling point.

5.1 Experimental Set Up

5.1.1 Preparation of Nasal Mucosa:

Sheep nasal mucosa (approx. 10 mm round in size) was incised from the nasal cavity of freshly sacrificed sheep. Nasal mucosa was washed with phosphate buffered saline (pH 6.4) thrice to remove adhered tissues. Selective samples of 0.20 mm thickness were taken for the studies to maintain homogeneity. The mucosae were stored in Kreb's solution at -2 °C to 2 °C till further processing. The nasal mucosa was sandwiched tightly between the receptor and donor compartment prior to initiation of studies and receptor compartment was filled with 13 mL of the corresponding diffusion medium and stirred for 30 min before charging solution/formulation in the donor compartment.

5.1.2 Design of Diffusion Cell:

The proposed *in vitro* studies were carried out using Franz diffusion cell (Figure 5.1). The diffusion cell consists of a hollow glass tube in the center having diameter of 8 mm. The cell has two compartments viz. (1) Donor compartment and (2) Receptor compartment. The donor compartment was used for holding the test formulation while the receptor compartment was used for holding the respective diffusion medium. The sheep nasal mucosa was sandwiched between donor and receptor compartments. The donor compartment was tightly fixed using lid and pharmaceutical grade grease after placing the formulation into it. The diffusion medium of the receptor compartment was continuously stirred (approx. 50 RPM) using magnetic stirrer. The temperature of receptor compartment was maintained at 37.0 °C ± 0.50 °C (Chien and Valia 1984).

5.1.3 Validation of Diffusion Cell:

The hydrodynamic characteristics of the Franz diffusion cell was established using benzoic acid disc method. (Chein and Valia 1984)

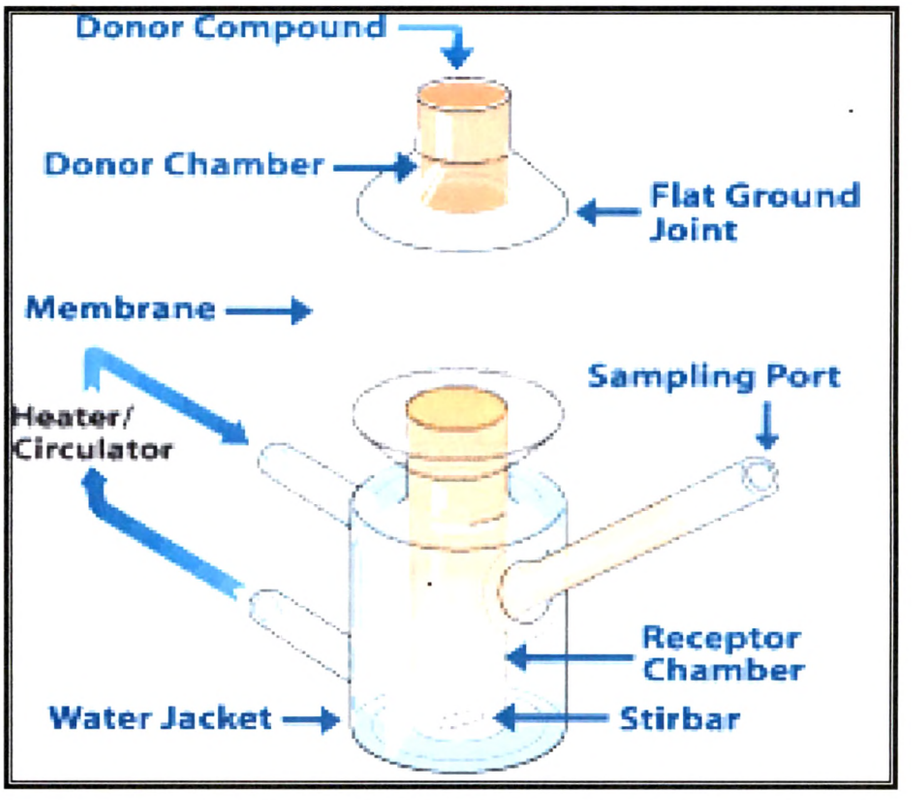


Figure 5.1 Franz Diffusion Cell

5.2 Tacrine *in vitro* Diffusion Studies

In vitro drug diffusion study was performed using Franz type diffusion cell with a diameter of 8 mm and mucosa thickness 0.20 mm (Willimann et al 1992). The promising compositions of tacrine which have been selected for *in vitro* diffusion study are shown in Table 5.1.

Sheep nasal mucosa was excised immediately after sacrificing and washed with phosphate buffer saline pH 6.4. Then, sheep nasal mucosa was sandwiched between the donor compartment (upper) and the recipient compartment (lower). Formulations, 0.30 mL each containing 10 mg drug were placed in the donor compartment onto the sheep nasal mucosa. Recipient compartment containing 13 mL of phosphate buffer saline pH 6.4 with 5 % (w/v) propylene glycol (PG) at 37.0 ± 0.5 °C was stirred at 50 RPM with Teflon® coated magnetic stirrer. Samples (0.2 mL) from the recipient compartment were withdrawn after predetermined time intervals and analyzed using UV-Visible double beam spectrophotometer (Shimadzu UV-1601, Japan) as mentioned in Chapter 3, Section 3.2.3. Each sample removed was replaced by an equal volume of phosphate buffer saline pH 6.4 with 5 % (w/v) PG. The experiments were run in triplicate and the mean cumulative % drug diffused is shown in Figure 5.2 and Table 5.2. The mean flux rate and diffusion coefficient have been calculated along with standard deviation (SD) from the cumulative drug diffused and are recorded in Table 5.3 and represented graphically in Figure 5.3. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, first order, and Higuchi's equations. The regression coefficients for the different formulations of tacrine are recorded in Table 5.4.

Table 5.1 Promising compositions of tacrine microemulsions for *in vitro* diffusion studies

System	Ratio of S:CoS	Formulation	O (%)	S (%)	CoS (%)	AQ (%)
TME 1	3:1	05	15.00	41.25	13.75	30.00
TME 2	3:1	05	15.00	41.25	13.75	30.00
TME 3	3:1	05	15.00	48.75	16.25	20.00
TME 4	3:1	05	15.00	48.75	16.25	20.00

Table 5.2 Cumulative % drug diffused for different tacrine microemulsions at different time intervals

Time (h)	Cumulative % drug diffused[#] System (Formulation)			
	TME 1 (05)	TME 2 (05)	TME 3 (05)	TME 4 (05)
0.25	**0.92 ± 0.24**	0.75 ± 0.15	0.59 ± 0.18	0.51 ± 0.20
0.50	**4.23 ± 0.67**	3.98 ± 0.54	3.16 ± 0.46	2.89 ± 0.58
1.00	**13.14 ± 1.02**	12.10 ± 0.87	9.67 ± 0.51	8.48 ± 1.05
2.00	**26.91 ± 1.14**	25.56 ± 1.24	18.13 ± 0.92	17.45 ± 0.97
3.00	**39.07 ± 1.31**	37.67 ± 1.13	28.76 ± 1.17	27.44 ± 1.22
4.00	**51.23 ± 1.19**	48.43 ± 1.74	37.74 ± 0.87	36.38 ± 1.48
5.00	**56.70 ± 1.22**	53.19 ± 1.52	44.13 ± 1.09	43.39 ± 1.18
6.00	**60.21 ± 1.89**	56.43 ± 1.61	48.96 ± 1.76	47.65 ± 1.38
7.00	**63.90 ± 1.41**	58.42 ± 1.12	51.24 ± 1.33	50.29 ± 1.63
8.00	**67.85 ± 1.56**	60.76 ± 1.45	53.10 ± 1.15	52.04 ± 1.57

[#] All values are represented as mean ± SD (n=3).

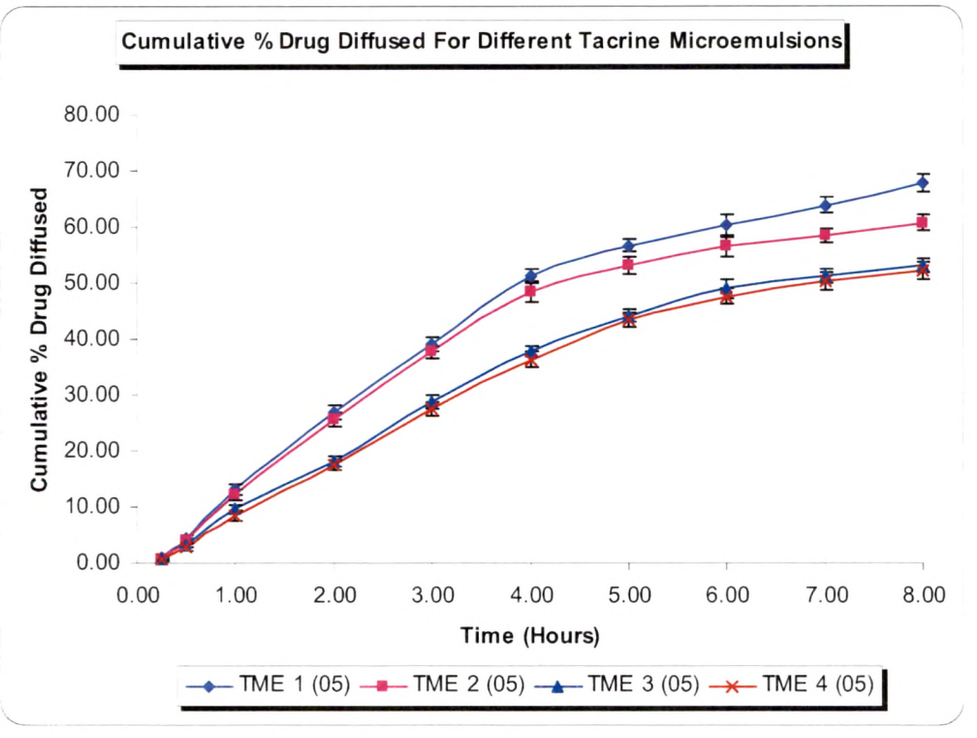


Figure 5.2 Cumulative % drug diffused for different tacrine microemulsions at different time intervals. Error bars represent SD (n=3).

Table 5.3 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for different tacrine microemulsions

	TME 1 (05)	TME 2 (05)	TME 3 (05)	TME 4 (05)
Mean flux (µg/min)	**2.06**	1.87	1.61	1.55
Diffusion coefficient (cm^2/sec)	**3.61E-09**	3.15E-09	2.12E-09	1.99E-09

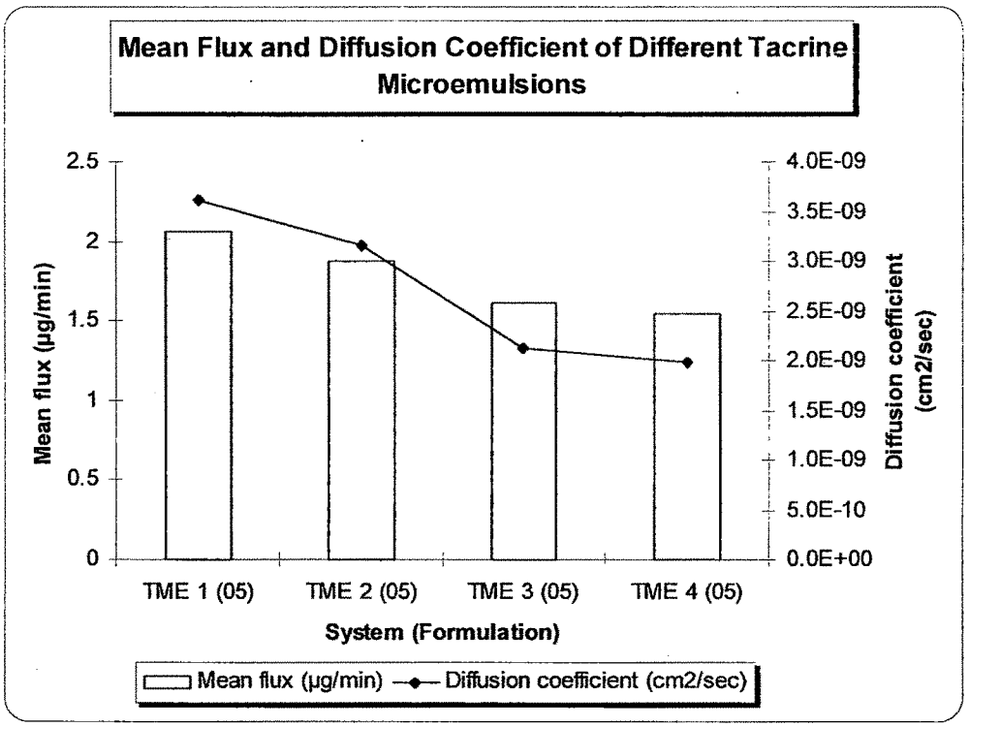


Figure 5.3 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) of different tacrine microemulsions.

Table 5.4 Regression coefficients of different tacrine microemulsions derived using regressed graphs

System (Formulation)	Zero-order equation	First-order equation	Higuchi's equation
	r^2	r^2	r^2
TME 1 (05)	0.9344	0.6606	**0.9882**
TME 2 (05)	0.9134	0.6408	**0.9796**
TME 3 (05)	0.9525	0.6738	**0.9891**
TME 4 (05)	0.9558	0.6783	**0.9885**

5.3 Donepezil *in vitro* Diffusion Studies

The i*n vitro* drug diffusion study was performed using Franz type diffusion cell with a diameter of 8 mm and mucosa thickness 0.20 mm (Willimann et al. 1992). The promising compositions of donepezil which have been selected for *in vitro* diffusion study are shown in Table 5.5.

Sheep nasal mucosa was excised immediately after sacrificing and washed with phosphate buffer saline pH 6.4. Then, sheep nasal mucosa was sandwiched between the donor compartment (upper) and the recipient compartment (lower). Formulations, 0.30 mL each containing 5 mg drug were placed in the donor compartment onto the sheep nasal mucosa. Recipient compartment containing 13 mL of phosphate buffer saline pH 6.4 with 5 % (w/v) PG at 37.0 ± 0.5 °C was stirred at 50 RPM with Teflon® coated magnetic stirrer. Samples (0.2 mL) from the receptor phase were withdrawn after predetermined time intervals and analyzed using UV-Visible double beam spectrophotometer (Shimadzu UV-1601, Japan) as mentioned in Chapter 3, Section 3.4.3. Each sample removed was replaced by an equal volume of phosphate buffer saline pH 6.4 with 5 % (w/v) PG. The experiments were run in triplicate and the mean cumulative % drug diffused is shown in Figure 5.4 and Table 5.6. The mean flux rate and diffusion coefficient have been calculated along with SD from the cumulative drug diffused and are recorded in Table 5.7 and represented graphically in Figure 5.5. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, first order, and Higuchi's equation. The regression coefficients for the different formulations of donepezil are recorded in Table 5.8.

Table 5.5 Promising compositions of donepezil microemulsions for *in vitro* diffusion studies

System	Ratio of S:CoS	Formulation	O (%)	S (%)	CoS (%)	AQ (%)
DME 1	1:1	05	25.00	22.50	22.50	30.00
DME 2	3:1	05	15.00	41.25	13.75	30.00
DME 3	3:1	05	15.00	48.75	16.25	20.00

Table 5.6 Cumulative % drug diffused for different donepezil microemulsions at different time intervals

Time (h)	Cumulative % drug diffused[#] System (Formulation)		
	DME 1 (05)	**DME 2 (05)**	DME 3 (05)
0.25	0.68 ± 0.17	**1.13 ± 0.27**	0.74 ± 0.10
0.50	2.67 ± 0.44	**5.03 ± 0.69**	3.48 ± 0.35
1.00	8.46 ± 0.85	**15.98 ± 0.74**	11.49 ± 0.88
2.00	19.35 ± 0.78	**29.31 ± 1.07**	24.17 ± 1.14
3.00	27.64 ± 1.41	**42.29 ± 1.36**	36.67 ± 1.36
4.00	41.28 ± 1.27	**54.34 ± 1.05**	47.59 ± 1.52
5.00	46.59 ± 1.58	**59.82 ± 1.67**	52.15 ± 1.08
6.00	49.92 ± 1.14	**64.46 ± 1.28**	56.28 ± 1.49
7.00	52.31 ± 1.69	**68.74 ± 1.44**	59.04 ± 1.23
8.00	54.29 ± 1.33	**73.56 ± 1.51**	61.20 ± 1.40

[#] All values are represented as mean ± SD (n=3).

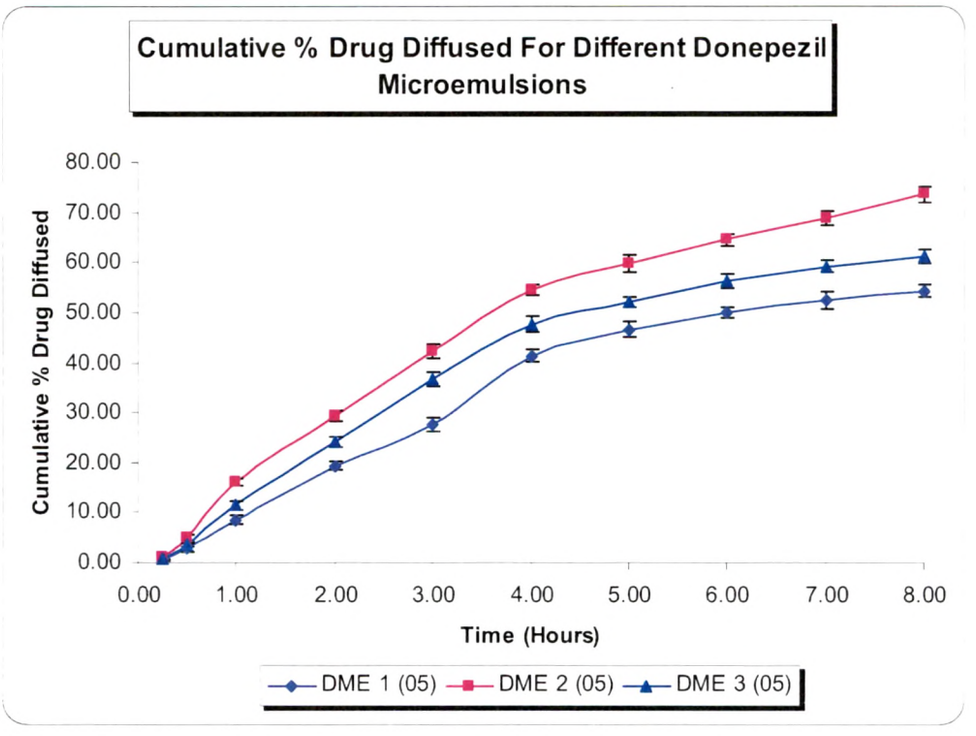


Figure 5.4 Cumulative % drug diffused for different donepezil microemulsions at different time intervals. Error bars represent SD (n=3).

Table 5.7 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for different donepezil microemulsions

	DME 1 (05)	**DME 2 (05)**	DME 3 (05)
Mean flux (µg/min)	1.57	**2.29**	1.85
Diffusion coefficient (cm^2/sec)	2.24E-09	**4.22E-09**	3.06E-09

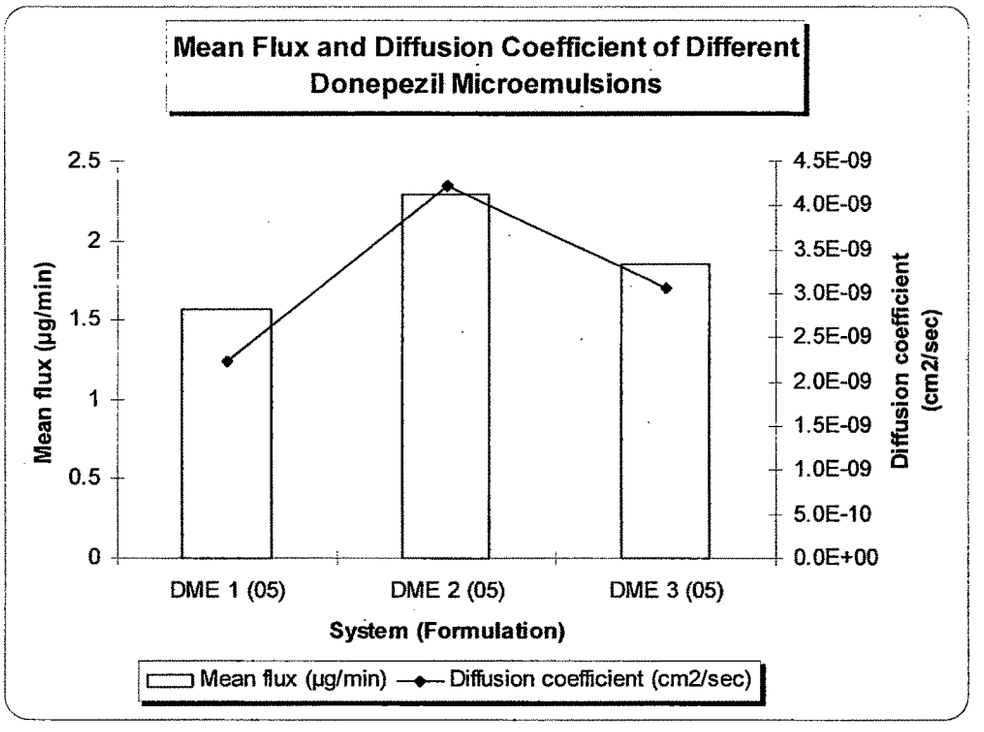
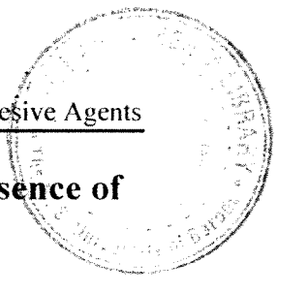


Figure 5.5 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) of different donepezil microemulsions.

Table 5.8 Regression coefficients of different donepezil microemulsions derived using regressed graphs

System (Formulation)	Zero-order equation	First-order equation	Higuchi's equation
	r^2	r^2	r^2
DME 1 (05)	0.9413	0.6953	**0.9812**
DME 2 (05)	0.9397	0.6577	**0.9920**
DME 3 (05)	0.9263	0.6562	**0.9839**



5.4 Diffusion Studies of Tacrine and Donepezil in Presence of Mucoadhesive Agents

Microemulsions of tacrine and donepezil were studied for mean flux rate and diffusion coefficient. The formulations which demonstrated higher cumulative % drug diffusion, flux rate, and diffusion coefficient (shown in bold face fonts in Table 5.2, 5.3 and Table 5.6, 5.7) have been taken and were formulated as mucoadhesive microemulsions (containing 0.25, 0.50 and 1.0 %w/w of chitosan or Carbopol 934 P) of respective drugs as stated in Chapter 4 under the preparation of mucoadhesive microemulsions of the corresponding drugs (Section 4.1.2 and 4.1.4). *In vitro* diffusion studies have been carried out on solutions and mucoadhesive microemulsions and compared with microemulsions (Akiyama et al. 1995; Apsden et al. 1995). The promising formulations have been further comparatively evaluated for *in vivo* biodistribution, brain targeting efficiency, brain scintigraphy imaging and pharmacodynamic performance in scopolamine induced amnesic mice.

The tacrine solution and mucoadhesive microemulsions were studied in triplicate for diffusion studies as per the method mentioned under tacrine *in vitro* diffusion studies and the mean cumulative % drug diffused are shown in Table 5.9. The mean flux rate and diffusion coefficient have been calculated along with SD from the cumulative % drug diffused (Gupta et al. 1990; Morimoto et al. 1985) and are recorded in Table 5.10. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, first order, and Higuchi's equation. The regression coefficients for the different formulations of tacrine are recorded in Table 5.11. Comparison of cumulative % drug diffused for tacrine solution, optimized tacrine microemulsion (shown in bold face fonts in Table 5.2) and optimized tacrine mucoadhesive microemulsion (shown in bold face fonts in Table 5.9) were represented graphically in Figure 5.6. Similarly, comparison of mean flux and diffusion coefficient for tacrine solution, optimized tacrine microemulsion (shown in bold face fonts in Table 5.3) and optimized tacrine mucoadhesive microemulsion (shown in bold face fonts in Table 5.10) were represented graphically in Figure 5.7.

The donepezil solution and mucoadhesive microemulsions were studied in triplicate for diffusion studies as per the method mentioned under donepezil *in vitro* diffusion studies and the mean cumulative % of drug diffused are shown in Table 5.12. The mean flux rate and diffusion coefficient have been calculated along with SD from the cumulative % drug diffused and are recorded in Table 5.13. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, first order, and Higuchi's equations. The regression coefficients for the different formulations of donepezil mucoadhesive microemulsion and solution are recorded in Table 5.14. Comparison of cumulative % drug diffused for donepezil solution, optimized donepezil microemulsion (shown in bold face fonts in Table 5.6) and optimized donepezil mucoadhesive microemulsion (shown in bold face fonts in Table 5.12) were represented graphically in Figure 5.8. Similarly comparison of mean flux and diffusion coefficient for donepezil solution, optimized donepezil microemulsion (shown in bold face fonts in Table 5.7) and optimized donepezil mucoadhesive microemulsion (shown in bold face fonts in Table 5.13) were represented graphically in Figure 5.9.

Table 5.9 Cumulative % drug diffused for different tacrine mucoadhesive microemulsions and solution at different time intervals

Time (h)	Cumulative % drug diffused[#] System (Formulation)						
	TS	TCH 0.25%	TCH 0.5%	TCH 1.0%	TCP 0.25%	**TCP 0.5%**	TCP 1.0%
0.25	0.78 ± 0.17	0.97 ± 0.34	1.12 ± 0.22	1.20 ± 0.14	1.01 ± 0.26	**1.16 ± 0.15**	1.13 ± 0.20
0.50	1.99 ± 0.43	4.31 ± 0.79	5.45 ± 0.60	5.22 ± 0.52	4.98 ± 0.84	**5.38 ± 0.57**	5.42 ± 0.45
1.00	6.03 ± 0.39	14.46 ± 1.12	17.65 ± 0.73	18.14 ± 0.95	16.12 ± 1.16	**18.88 ± 0.71**	17.12 ± 0.85
2.00	17.33 ± 0.76	24.88 ± 1.08	29.87 ± 1.15	28.79 ± 1.30	28.76 ± 1.04	**31.25 ± 1.42**	32.47 ± 1.13
3.00	27.85 ± 0.94	40.23 ± 1.67	44.35 ± 1.40	45.14 ± 1.16	44.62 ± 1.61	**48.29 ± 1.27**	47.85 ± 1.20
4.00	35.96 ± 1.41	54.66 ± 1.32	58.78 ± 1.23	59.82 ± 1.46	57.13 ± 1.44	**62.14 ± 1.10**	63.38 ± 0.95
5.00	39.32 ± 1.18	62.78 ± 1.45	67.52 ± 1.80	68.82 ± 1.51	68.76 ± 1.87	**73.24 ± 1.53**	74.71 ± 1.48
6.00	46.39 ± 1.32	70.21 ± 1.88	74.56 ± 1.62	73.45 ± 1.72	76.55 ± 2.04	**82.23 ± 2.15**	83.22 ± 1.55
7.00	49.86 ± 1.66	78.43 ± 2.10	83.32 ± 1.95	84.05 ± 2.24	83.21 ± 2.31	**89.54 ± 1.83**	90.08 ± 1.76
8.00	53.83 ± 1.30	82.76 ± 2.41	88.67 ± 2.16	90.13 ± 2.05	89.92 ± 2.56	**95.36 ± 2.36**	96.14 ± 2.44

[#] All values are represented as mean ± SD (n=3).

Table 5.10 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for different tacrine mucoadhesive microemulsions and solutions

	TS	TCH 0.25%	TCH 0.5%	TCH 1.0%	TCP 0.25%	TCP 0.5%	TCP 1.0%
Mean flux (µg/min)	**1.46**	2.45	2.71	2.74	2.70	**2.90**	2.88
Diffusion coefficient (cm^2/sec)	**1.89E-09**	4.53E-09	5.37E-09	5.45E-09	5.35E-09	**6.22E-09**	6.31E-09

Table 5.11 Regression coefficients of different tacrine mucoadhesive microemulsions and solution derived using regressed graphs

Formulation	Zero-order equation	First-order equation	Higuchi's equation
	r^2	r^2	r^2
TS	0.9680	0.7373	**0.9902**
TCH 0.25 %	0.9751	0.7007	**0.9918**
TCH 0.5 %	0.9720	0.6800	**0.9960**
TCH 1.0 %	0.9711	0.6870	**0.9940**
TCP 0.25 %	0.9753	0.6878	**0.9948**
TCP 0.5 %	0.9719	0.6823	**0.9952**
TCP 1.0 %	0.9707	0.6868	**0.9950**

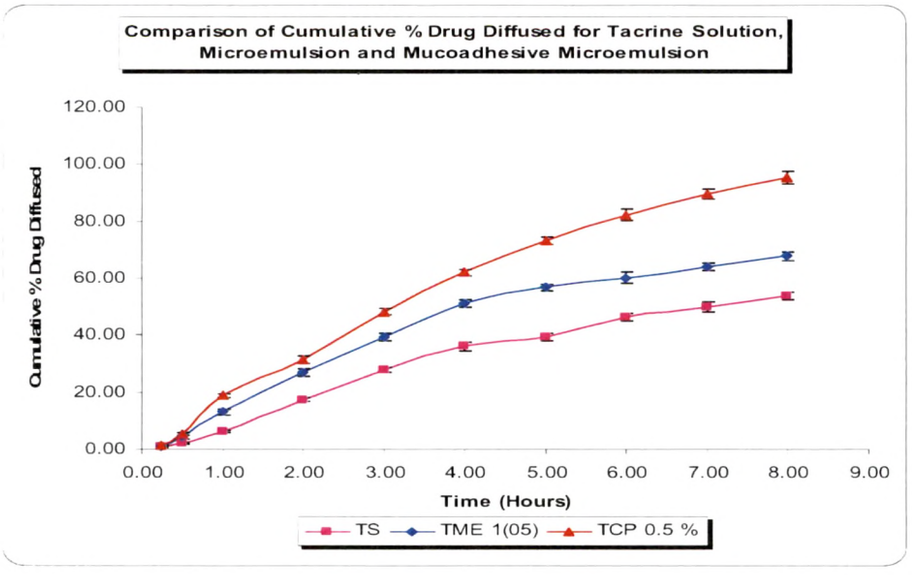


Figure 5.6 Comparison of cumulative % drug diffused for Tacrine Solution (TS), Tacrine Microemulsion (TME 1(05)) and Tacrine Mucoadhesive Microemulsion (TCP 0.5 %). Error bars represent SD (n=3).

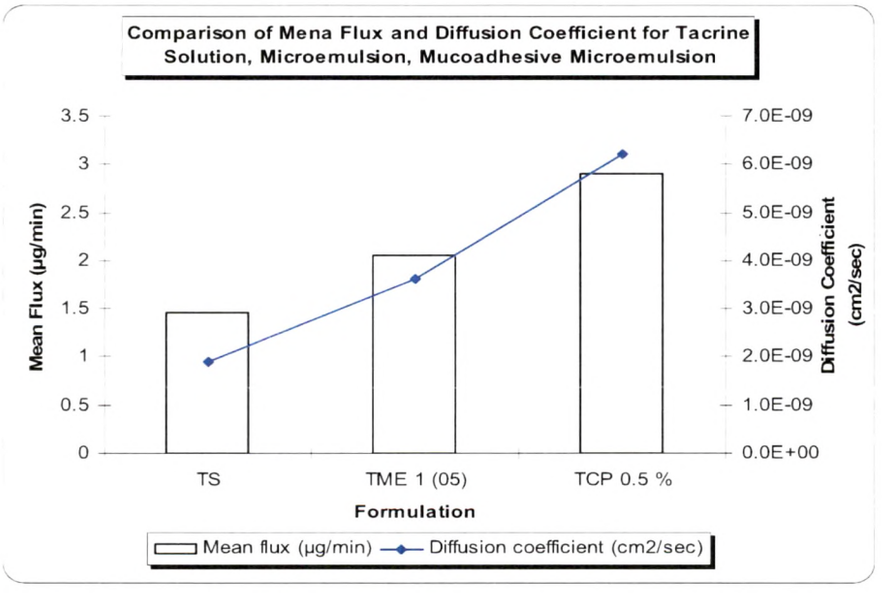


Figure 5.7 Comparison of Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for Tacrine Solution (TS), Tacrine Microemulsion (TME 1(05)) and Tacrine Mucoadhesive Microemulsion (TCP 0.5 %).

Table 5.12 Cumulative % drug diffused for different donepezil mucoadhesive microemulsions and solution at different time intervals

Time (h)	Cumulative % drug diffused[#] System (Formulation)						
	DS	DCH 0.25%	DCH 0.5%	DCH 1.0%	DCP 0.25%	**DCP 0.5%**	DCP 1.0%
0.25	0.52 ± 0.11	0.74 ± 0.34	0.96 ± 0.29	0.85 ± 0.23	0.91 ± 0.30	**1.08 ± 0.19**	1.02 ± 0.23
0.50	2.12 ± 0.27	3.43 ± 0.42	4.89 ± 0.46	5.01 ± 0.69	4.15 ± 0.64	**4.86 ± 0.41**	5.10 ± 0.72
1.00	5.87 ± 0.68	12.21 ± 0.59	16.75 ± 0.98	15.89 ± 0.77	14.23 ± 0.94	**17.68 ± 1.02**	16.89 ± 0.67
2.00	16.23 ± 1.03	23.02 ± 0.98	30.05 ± 1.24	30.36 ± 1.11	24.67 ± 1.17	**30.45 ± 1.28**	31.60 ± 1.30
3.00	28.30 ± 1.17	38.45 ± 1.26	43.17 ± 1.52	44.59 ± 1.04	40.33 ± 1.32	**46.53 ± 1.44**	47.14 ± 1.36
4.00	37.23 ± 1.38	51.89 ± 1.14	54.66 ± 1.30	55.29 ± 1.60	53.85 ± 1.65	**59.62 ± 1.23**	60.79± 1.54
5.00	41.56 ± 1.06	60.46 ± 1.83	64.19 ± 1.45	65.44 ± 1.28	62.09 ± 1.34	**70.44 ± 1.28**	71.84 ± 1.36
6.00	44.38 ± 1.25	68.38 ± 1.57	71.90 ± 2.02	73.05 ± 1.45	71.89 ± 1.57	**79.81 ± 1.46**	81.04 ± 1.87
7.00	47.04 ± 1.53	74.67 ± 2.03	77.54 ± 2.13	79.16 ± 1.86	78.10 ± 1.89	**86.35 ± 1.76**	88.46 ± 1.61
8.00	49.76 ± 1.36	79.13 ± 2.29	82.25 ± 1.97	84.28 ± 1.75	86.92 ± 2.14	**92.23 ± 1.93**	94.30 ± 2.20

[#] All values are represented as mean ± SD (n=3).

Table 5.13 Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for different donepezil mucoadhesive microemulsions and solution.

	DS	DCH 0.25%	DCH 0.5%	DCH 1.0%	DCP 0.25%	DCP 0.5%	DCP 1.0%
Mean flux (µg/min)	**1.39**	2.19	2.53	2.57	2.55	**2.78**	2.82
Diffusion coefficient (cm^2/sec)	**1.82E-09**	4.10E-09	4.83E-09	4.99E-09	4.59E-09	**5.77E-09**	5.98E-09

Table 5.14 Regression coefficients of different donepezil mucoadhesive microemulsions and solution derived using regressed graphs

Formulation	Zero-order equation	First-order equation	Higuchi's equation
	r^2	r^2	r^2
TS	0.9380	0.7010	**0.9799**
TCH 0.25 %	0.9743	0.6997	**0.9902**
TCH 0.5 %	0.9650	0.6641	**0.9972**
TCH 1.0 %	0.9664	0.6600	**0.9973**
TCP 0.25 %	0.9834	0.7047	**0.9913**
TCP 0.5 %	0.9727	0.6834	**0.9955**
TCP 1.0 %	0.9739	0.6820	**0.9959**

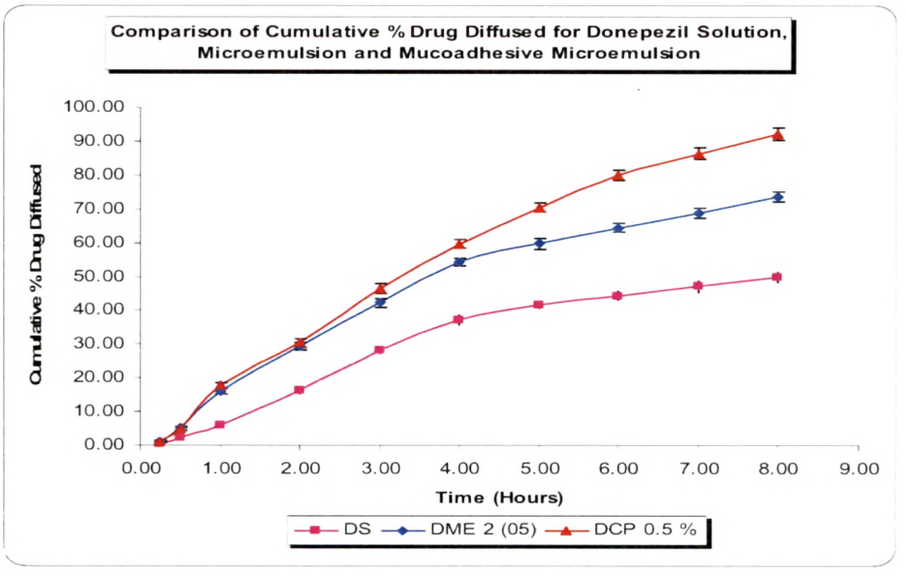


Figure 5.8 Comparison of cumulative % drug diffused for Donepezil Solution (DS), Donepezil Microemulsion (DME 2(05)) and Donepezil Mucoadhesive Microemulsion (DCP 0.5 %). Error bars represent SD (n=3).

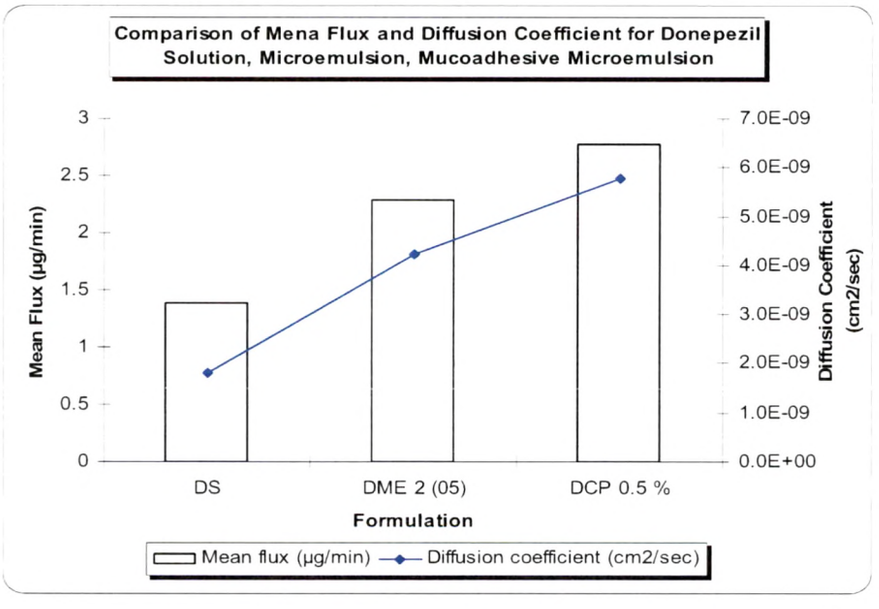


Figure 5.9 Comparison of Mean flux (µg/min) and diffusion coefficient (cm^2/sec) for Donepezil Solution (DS), Donepezil Microemulsion (DME 2(05)) and Donepezil Mucoadhesive Microemulsion (DCP 0.5 %).

5.5 Results and Discussion

In vitro diffusion studies were performed to evaluate relative diffusion behavior of different formulations of tacrine and donepezil. Cumulative drug diffused across sheep nasal mucosa of tacrine formulations (Table 5.1) up to 8 h have been recorded in Table 5.2 and graphically in Figure 5.2. As shown in the data, TME 1 (formulation 05) was found to have substantially higher diffusion across the sheep nasal mucosa. Furthermore, mean kinetic flux and diffusion coefficient were calculated from the cumulative % drug diffused and concentration gradient at specific time intervals. The data (Table 5.3 and Figure 5.3) indicated that TME 1 (formulation 05) has the highest mean kinetic flux (2.06 µg/min) and diffusion coefficient (3.61E-09 cm^2/sec) amongst the tested formulations. The mechanism of drug diffusion was also predicted by inputting the regressed data into the excel spread sheet and the result are recorded in Table 5.4. It was found that all the tested formulations of tacrine follow Higuchi's kinetics whereas, the regression coefficient values were found less for zero-order and first-order compared to Higuchi's kinetic fit.

Diffusion kinetics of donepezil formulations (Table 5.5) was studied and cumulative drug diffused up to 8 h across the sheep nasal mucosa are recorded in Table 5.6 and graphically in Figure 5.4. As seen from the data, DME 2 (formulation 05) has shown better drug diffusion across the sheep nasal mucosa. The diffusion kinetics data indicated that DME 2 (formulation 05) has the highest mean kinetic flux (2.29 µg/min) and diffusion coefficient (4.22E-09 cm^2/sec) amongst the tested formulations (Table 5.7 and Figure 5.5). The mechanism of drug diffusion was also predicted by inputting the regressed data into the excel spread sheet and the result are recorded in Table 5.8. It was found that all the tested formulations of donepezil follow Higuchi's kinetics.

Following evaluation of microemulsions, mucoadhesive agents such as Carbopol 934 P (0.25%, 0.5% and 1.0%) and chitosan (0.25%, 0.5% and 1.0%) were incorporated and diffusion kinetics of the drug in solution (in propylene glycol), and mucoadhesive microemulsions were evaluated and cumulative % drug diffused were recorded in Table 5.9 (Tacrine) and Table 5.12 (Donepezil). Mean flux and diffusion coefficients were recorded in Table 5.10 (Tacrine) and Table 5.13 (Donepezil). The mechanism of drug diffusion was also predicted by inputting the regressed data into the excel spread sheet and the result are recorded in Table 5.11 (Tacrine) and Table 5.14 (Donepezil). It was observed that all formulations follow Higuchi's kinetics. Further, it was observed that

mucoadhesive microemulsions containing Carbopol 934 P shown better drug diffusion across sheep nasal mucosa. Mean flux and diffusion coefficients were also higher for Carbopol 934 P containing mucoadhesive microemulsions. This may be attributed to the fact that Carbopol 934 P may deplete calcium ions from the nasal mucosa which in turn result into channel formation due to stretching of the tight junctions (Illum 1997; Illum 2000). Moreover, higher viscosity of the formulations may facilitate interaction formulation with nasal mucosa due to close proximity and hence, more concentration gradient between the donor and the recipient compartment (Colombo 1997). Amongst the different Carbopol 934 P containing mucoadhesive microemulsions, concentration beyond 0.5 % does not result into significant increase in % drug diffused, mean flux and diffusion coefficient. Hence, mucoadhesive microemulsion TCP 0.5% and DCP 0.5% was selected for further evaluation in *in vivo* studies. On comparing tacrine solution, optimized tacrine microemulsion (TME 1(05)) and tacrine mucoadhesive microemulsion (TCP 0.5%) it was observed that microemulsion and mucoadhesive microemulsion showed better drug diffusion compared to solution (Figure 5.6). Tacrine mucoadhesive microemulsion (TCP 0.5%) showed 2-fold mean flux and 3-fold diffusion coefficient compared to tacrine solution (Figure 5.7). This may be attributed to the fact that microemulsion enhances transport of drug across mucosa (Lawrence and Rees 2000). Similar results were obtained for donepezil mucoadhesive microemulsions (DCP 0.5%) as shown in Figure 5.8 and 5.9.

In conclusion, *in vitro* diffusion study across the sheep nasal mucosa may be a reasonable tool for comparative evaluation of different formulations. However, absolute rate and extent of diffusion and its intricacies on the *in vivo* studies prediction may not be appropriate. The non linearity of percent drug diffused vs. time graphs suggested that the diffusion pattern does not follow zero order kinetics (Vincent and Robinson 1995). However, the correlation coefficients indicated that Higuchi's model was found to be the best-fit curve for all the tested formulations. This may be attributed to the fact that the systems tested has reservoir compartment and a sheep nasal mucosa as a barrier or controlling membrane hence, the drug diffusion will more mimic and closer to reservoir system rather than zero-order or first-order (concentration gradient) diffusion (Swardick and Boylan 1994). Consequently, following *in vitro* evaluation, promising formulations such as microemulsions and 0.5% Carbopol 934 P containing mucoadhesive microemulsions were taken up for comparative *in vivo* evaluation including solutions.

5.6 References

AkiyamaY, Nagahara N, Kashihara T, Hirai S, Toguchi H. In vitro and in vivo evaluation of Mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and poly (acrylic acid) derivatives. *Pharm Res* **1995;** 12:397-405.

Arthurson P, Lindmark T, Davis SS, Illum L. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (caco-2). *Pharm Res* **1994;** 1:1358-1361.

Aspden TJ, Adler J, Davis SS, Skaugrud Q, Illum L. Chitosan as a nasal delivery system: evaluation of the effect of chitosan on mucociliary clearance rates in the frog palate model. *Int J Pharm* **1995;** 122:69-78.

Chien YW, Valia KH. *Drug Dev Ind Pharmacy* **1984;** 10:575.

Colombo P. Mucosal drug delivery, nasal, In: Swarbrick J, Boylan JC (eds.), Encyclopedia of Pharmaceutical Technology: Vol. 7, Dekker Incorporated, Marcel. **1997;** 592-604.

Gemmell DH, Morrison JC. The release of medicinal substances from topical applications and their passage through the skin. *J Pharm Pharmacol* **1957;** 9(10):641-656.

Gupta PK, Leung SHS, Robinson JR. Bioadhesives/Mucoadhesives in drug delivery to the gastrointestinal tract. In: Lenaerts V, Gurny R. (Eds.), Bioadhesive Drug Delivery Systems, CRC Press, Boca Raton, FL, **1990;** 65-92.

Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm Sci* **1961;** 50:874-875.

Illum L. 3rd Ann. Meet. Nasal Drug Delivery Focus Group, Am Assoc Pharm Sci. Boston, **1997.**

Illum L. Transport of drugs from the nasal cavity to the central nervous system. *Eur J Pharm Sci* **2000;** 11:1-18.

Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Del Rev* **2000;** 45:89-121.

Morimoto K, Morisaka K, Kamada A. Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. *J Pharm Pharmacol* **1985;** 37(2):134-136.

Swardick J, Boylan JC. Mucosal Adhesive Preparation. Encyclopedia of Pharmaceutical Technology, Vol. 10, Marcel and Dekker Inc., NY, **1994;** 144.

Vincent HL, Robinson RJ. In Controlled Drug Delivery, Marcel Dekker, Inc. New York and Basel, **1995;** 99.

Willimann H, Walde P, Luisi PL, Gazzaniga A, Stropopolo F. Lecithin organogel as matrix for transdermal transport of drugs. *J Pharm Sci* **1992;** 81(9):871-874.