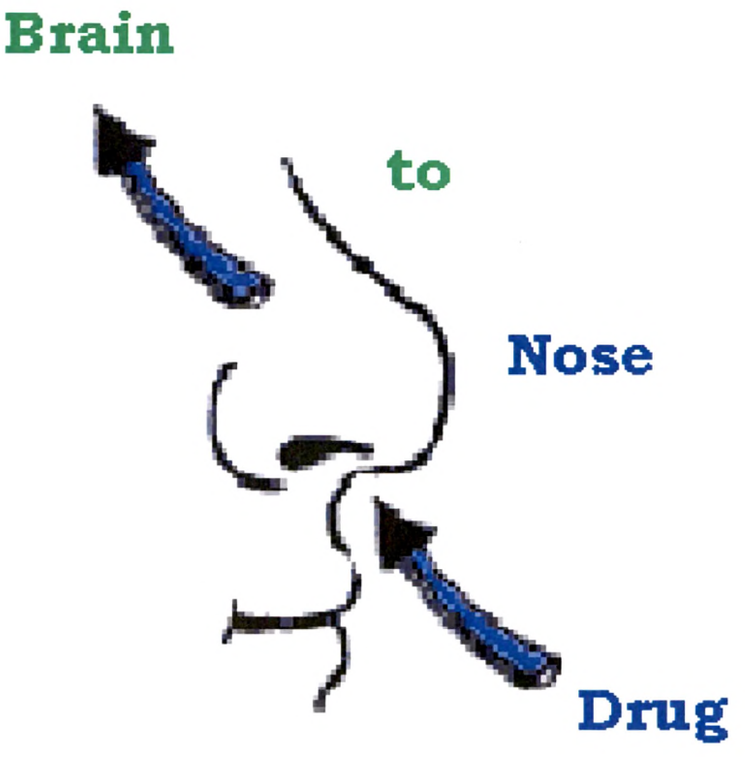


CHAPTER 7

BIODISTRIBUTION AND PHARMACOKINETICS

7.1 Tacrine

7.1.1 Biodistribution Studies:

All experiments conducted on animals were approved by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. Balb/c mice (aged 4 to 5 months), weighing between 30 to 40 g were selected for the study on the basis of randomization technique.

Three mice (male, weighing between 30 to 40 g) for each formulation per time point were used in the study. The radiolabeled complex of ^{99m}Tc-TS (100 μCi/50 μL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg/ kg body weight (B.W.)) was injected intravenously (i.v.) through tail vein of mice. Similarly, radiolabeled complex of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME (100 μCi/10 μL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg/kg B.W.) was administered (5 μL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 μL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site. The mice were held from the back in slanted position during nasal administration of the formulations. Blood was collected using cardiac puncture at predetermined time intervals (15, 30, 60, 120, 240, and 480min). After collecting the blood, the mice were sacrificed with mercy by exposure to diethyl ether. Subsequently, different tissues/organs including brain were dissected, washed twice using normal saline solution, and made free from adhering tissue/fluid and weighed. The radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter (Capintec Inc., New Jersey, USA) (Theobald 1990; Saha 1993). The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (Babbar et al. 2000). The results of radioactivity in different organs are recorded in Table 7.1, 7.2, 7.3 and 7.4. In order to comprehensively understand the drug distribution behavior in blood and brain, the drug concentration as percent of radioactivity per g of tissue vs. time were plotted and shown in Figure 7.1. The brain/blood ratio were also calculated at all time points and recorded in Table 7.5. The pharmacokinetic parameters

were derived from Table 7.5 using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) and recorded in Table 7.6.

Table 7.1 Tissue/organ distribution of ^{99m}Tc-TS in Balb/c mice at predetermined time intervals post intravenous administration.*

TISSUE/ ORGAN	15 min	30 min	60 min	120 min	240 min	480 min
Blood	4.17±0.47	3.58±0.36	2.45±0.32	1.75±0.25	0.84±0.14	0.37±0.04
Liver	1.15±0.10	1.49±0.14	2.68±0.18	1.74±0.20	1.41±0.14	0.40±0.13
Kidney	1.85±0.18	2.16±0.22	2.62±0.16	1.55±0.17	1.38±0.15	0.23±0.08
Brain	0.28±0.07	0.33±0.10	0.36±0.04	0.41±0.09	0.29±0.05	0.11±0.02

* The mice were administered intravenously with 100 µCi ^{99m}Tc-TS and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.2 Tissue/organ distribution of ^{99m}Tc-TS in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	15 min	30 min	60 min	120 min	240 min	480 min
Blood	0.71±0.07	0.82±0.08	1.13±0.12	1.23±0.18	0.80±0.12	0.43±0.06
Liver	0.34±0.08	0.40±0.08	0.54±0.10	0.40±0.07	0.34±0.06	0.09±0.03
Kidney	0.40±0.08	0.47±0.10	0.55±0.09	0.52±0.06	0.44±0.09	0.16±0.04
Brain	0.46±0.12	0.48±0.09	0.53±0.10	0.50±0.06	0.38±0.06	0.14±0.03

* The mice were administered intranasally with 100 µCi ^{99m}Tc-TS and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.3 Tissue/organ distribution of ^{99m}Tc-TME in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	15 min	30 min	60 min	120 min	240 min	480 min
Blood	0.56±0.09	0.76±0.11	1.45±0.14	1.72±0.12	1.43±0.11	0.36±0.06
Liver	0.60±0.11	0.72±0.12	0.93±0.08	0.82±0.07	0.77±0.08	0.15±0.06
Kidney	0.71±0.10	0.85±0.11	0.93±0.13	0.90±0.13	0.85±0.11	0.20±0.08
Brain	0.54±0.10	0.62±0.09	0.88±0.07	0.83±0.07	0.67±0.09	0.23±0.06

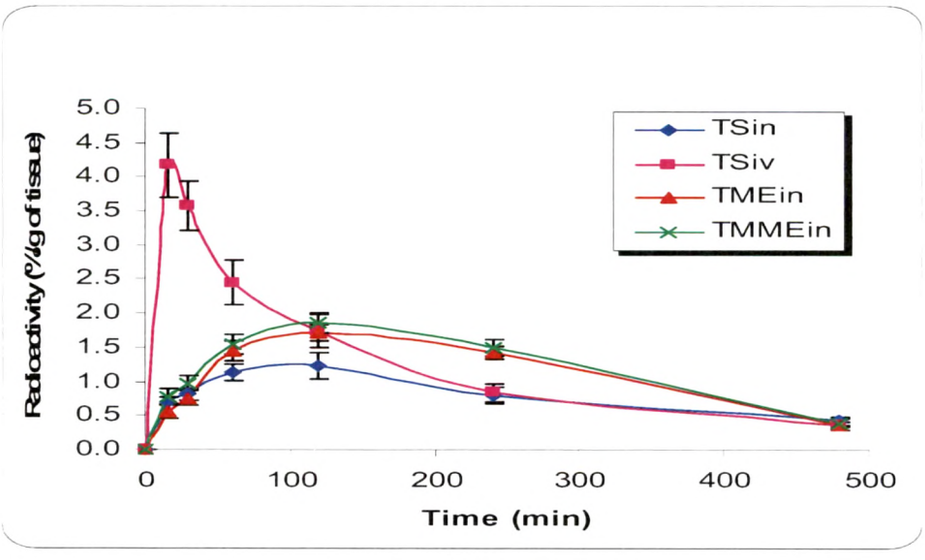
* The mice were administered intranasally with 100 μCi ^{99m}Tc-TME and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.4 Tissue/organ distribution of ^{99m}Tc-TMME in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	15 min	30 min	60 min	120 min	240 min	480 min
Blood	0.78±0.11	0.98±0.10	1.54±0.14	1.86±0.13	1.50±0.12	0.40±0.06
Liver	0.66±0.07	0.80±0.08	0.98±0.11	0.85±0.06	0.71±0.08	0.17±0.05
Kidney	0.94±0.13	1.07±0.10	1.33±0.08	1.13±0.11	0.89±0.08	0.25±0.07
Brain	0.72±0.10	0.87±0.12	1.22±0.08	1.07±0.08	0.90±0.10	0.25±0.07

* The mice were administered intranasally with 100 μCi ^{99m}Tc-TMME and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

(A)



(B)

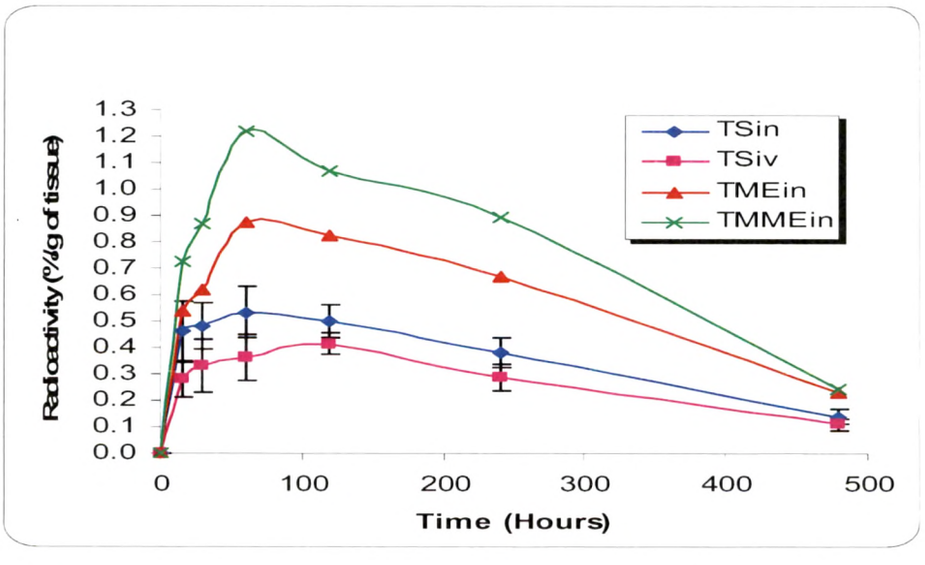


Figure 7.1 Tacrine concentration in mice (n = 3) (A) blood and (B) brain at different time intervals following ^{99m}Tc-TS$_{i.v.,}$ ^{99m}Tc-TS$_{i.n.,}$ ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ administrations. Error bars represents SD

Table 7.5 Distribution of ^{99m}Tc-TS$_{i.v.}$, ^{99m}Tc-TS$_{i.n.}$, ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ in Balb/c mice[$] at predetermined time intervals

Formulation$_{\text{route of administration}}$	Organ/Tissue	Sampling time points (min)					
		15	30	60	120	240	480
TS$_{i.v.}$	Blood	4.17 ± 0.47	3.58 ± 0.36	2.45 ± 0.32	1.75 ± 0.25	0.84 ± 0.14	0.37 ± 0.04
	Brain	0.28 ± 0.07	0.33 ± 0.10	0.36 ± 0.04	0.41 ± 0.09	0.29 ± 0.05	0.11 ± 0.02
TS$_{i.n.}$	Blood	0.71 ± 0.07	0.82 ± 0.08	1.13 ± 0.12	1.23 ± 0.18	0.80 ± 0.12	0.43 ± 0.06
	Brain	0.46 ± 0.12	0.48 ± 0.09	0.53 ± 0.10	0.50 ± 0.06	0.38 ± 0.06	0.14 ± 0.03
TME$_{i.n.}$	Blood	0.56 ± 0.09	0.76 ± 0.11	1.45 ± 0.14	1.72 ± 0.12	1.43 ± 0.11	0.36 ± 0.06
	Brain	0.54 ± 0.10	0.62 ± 0.09	0.88 ± 0.07	0.83 ± 0.07	0.67 ± 0.09	0.23 ± 0.06
TMME$_{i.n.}$	Blood	0.78 ± 0.11	0.98 ± 0.10	1.54 ± 0.14	1.86 ± 0.13	1.50 ± 0.12	0.40 ± 0.06
	Brain	0.72 ± 0.10	0.87 ± 0.12	1.22 ± 0.08	1.07 ± 0.08	0.90 ± 0.10	0.25 ± 0.07
TS$_{i.v.}$	Brain/Blood	0.07 ± 0.01	0.09 ± 0.03	0.15 ± 0.04	0.24 ± 0.07	0.35 ± 0. 09	0.30 ± 0.06
TS$_{i.n.}$	Brain/Blood	0.64 ± 0.10	0.59 ± 0.14	0.48 ± 0.14	0.42 ±0.12	0.48 ± 0.07	0.33 ± 0.09
TME$_{i.n.}$	Brain/Blood	0.99 ± 0.27	0.82 ± 0.03	0.61 ± 0.03	0.48 ±0.03	0.47 ± 0.05	0.68 ± 0.32
TMME$_{i.n.}$	Brain/Blood	0.93 ± 0.18	0.89 ± 0.04	0.79 ± 0.06	0.58 ±0.01	0.60 ± 0.02	0.62 ± 0.12

[$] The mice were administered with 100 μCi ^{99m}Tc-tacrine formulations and the radioactivity was measured in %/g of tissue of the administered dose. Radio activity was measured at 0 min and all the measurements were performed using 0 min sample of corresponding tissue/organ as blank sample. Each value is the mean ± SD (n = 3).

Table 7.6 Pharmacokinetics of ^{99m}Tc-TS$_{i.v.}$, ^{99m}Tc-TS$_{i.n.}$, ^{99m}Tc-TME$_{i.n.}$ and ^{99m}Tc-TMME$_{i.n.}$ in Balb/c mice$^{\$}$

Pharmacokinetic Parameter	^{99m}Tc-TS$_{i.v.}$		^{99m}Tc-TS$_{i.n.}$		^{99m}Tc-TME$_{i.n.}$		^{99m}Tc-TMME$_{i.n.}$	
	Blood	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**
C_{max} (% radioactivity/g)$^{\#}$	4.17 ± 0.47	0.41 ± 0.09	1.23 ± 0.18	0.53 ± 0.10	1.72 ± 0.12	0.88 ± 0.07	1.86 ± 0.13	1.22 ± 0.08
T_{max} (min)	15	120	120	60	120	60	120	60
$AUC_{0\rightarrow480}$ (min*% radioactivity/g)	606.45	130.13	386..25	171.75	546.15	284.55	588.45	373.58
$AUC_{0\rightarrow\infty}$ (min*% radioactivity/g)	683.29	160.37	533.44	211.63	631.29	349.77	684.34	446.67
K_{el} (L/min)	0.0045	0.0037	0.0029	0.0036	0.0045	0.0037	0.0044	0.0038
$T_{1/2}$ (min)	155.03	186.85	241.36	191.20	152.52	187.74	155.91	181.62
Bioavailability (%)	-	-	63.69	131.99	90.06	218.67	97.03	287.09

$^{\$}$ The mice were administered with 100 μCi ^{99m}Tc-tacrine formulations and the radioactivity was measured in percent per gram of tissue of the administered dose.

$^{\#}$ Each value is the mean ± SD (n = 3)

To evaluate the brain targeting efficiency, two indices (DTE (%) and DTP (%)) were adopted as mentioned below (Chow et al. 1999; Zhang et al. 2004; Vyas et al. 2005a, 2006a, 2006b).

Drug targeting efficiency (DTE (%)): DTE (%) represents time average partitioning ratio.

$$DTE\ (\%) = \frac{\left(AUC_{brain}/AUC_{blood}\right)_{i.n.}}{\left(AUC_{brain}/AUC_{blood}\right)_{i.v.}} \times 100 \quad \ldots\ldots (1)$$

The brain drug direct transport percentage (DTP (%)): In order to define nose to brain direct transport clearly, DTP (%); which has been derived from equations (2) and (3) was calculated.

$$DTP\ (\%) = \frac{B_{i.n.} - B_X}{B_{i.n.}} \times 100 \quad \ldots\ldots (2)$$

$$B_x = \left(B_{i.v.}/P_{i.v.}\right) \times P_{i.n.} \quad \ldots\ldots (3)$$

Where,

AUC = Area under the curve for blood/brain concentration vs. time.

B_x = Brain AUC fraction contributed by systemic circulation through the BBB following i.n. administration.

$B_{i.v.} = AUC_{0\rightarrow480}$ (brain) following i.v. administration.

$P_{i.v.} = AUC_{0\rightarrow480}$ (blood) following i.v administration.

$B_{i.n.} = AUC_{0\rightarrow480}$ (brain) following i.n. administration.

$P_{i.n.} = AUC_{0\rightarrow480}$ (blood) following i.n. administration.

Reports in the literature reveal that the drug uptake into the brain from the nasal mucosa mainly occurs via three different pathways (Illum 2003; Illum 2004; Thorne et al. 2004; Vyas et al. 2005b; Dhanda et al. 2005). One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The others are the olfactory and the trigeminal neural pathway by which part of the drug is transported directly from the nasal cavity to CSF and brain tissue (Illum 2004; Thorne et al. 2004). We can deduce that the amount of drug that reaches in the brain tissue after nasal administration is attributed to these three pathways. Since, the amount of drug is proportional to AUC, we can assume that the brain AUC fraction

contributed by systemic circulation through BBB (represented by B_x), divided by blood AUC from nasal route is equal to that of i.v. route (see Equation (3)). Therefore, DTP (%) represents the percentage of drug directly transported to the brain via the olfactory pathway and the trigeminal neural pathway. DTP (%) and DTE (%) were calculated using tissue/organ distribution data following i.n. and i.v. administrations and are recorded in Table 7.7.

7.1.2 Gamma Scintigraphy Imaging:

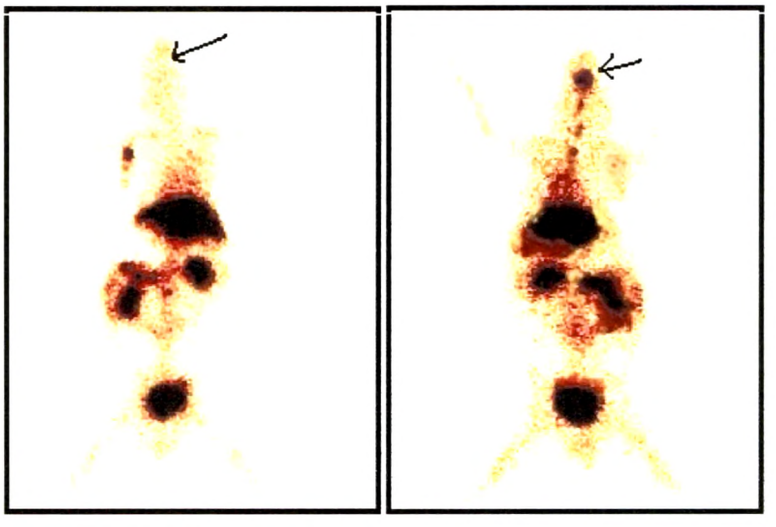
The New Zealand rabbits (2.00–2.50 kg) were selected for the study. The radiolabeled complex of ^{99m}Tc-TS (100 µCi/100 µL) containing 0.94–1.18 mg tacrine (equivalent to 0.47 mg/kg B.W.) was i.v. injected through the ear vein of the rabbit. Similarly, the radiolabeled complex of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME (100 µCi/100 µL) containing 0.94–1.18 mg tacrine (equivalent to 0.47 mg/kg B.W.) was administered i.n. (50 µL in each nostril) (Eckelman 1995). The rabbits were held from the back in slanted position during nasal administration of formulations. The rabbits were anaesthetized using 1 mL ketamine hydrochloride intramuscular injection (50 mg/mL) and placed on the imaging platform. Imaging was performed using Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens AG; Erlanger, Germany) gamma camera (Capala et al. 1997; Babbar et al. 2000). The scintigraphy images following i.v. and i.n. administrations of ^{99m}Tc-tacrine formulations are shown in Figure 7.2.

7.1.3 Statistical Analysis:

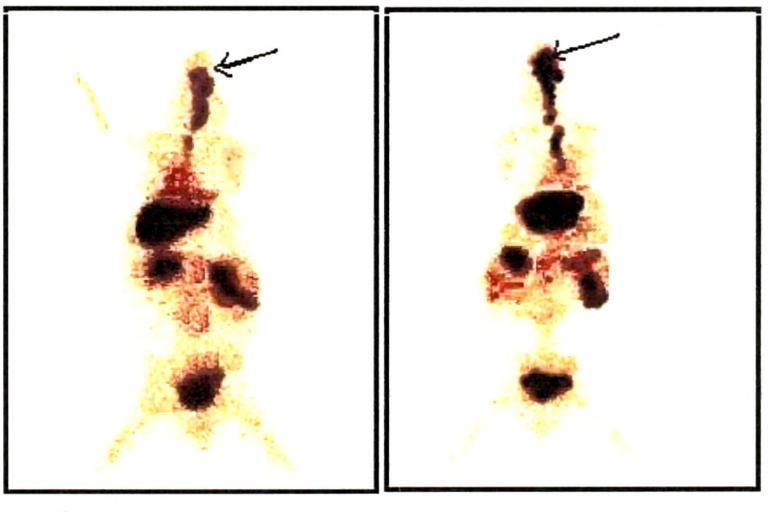
All data are reported as mean ± SD and the difference between the groups were tested using Student's t test at the level of $P< 0.05$. More than two groups were compared using ANOVA and differences greater at $P< 0.05$ were considered significant.

Table 7.7 Drug targeting efficiency (DTE (%)) and direct nose to brain transport (DTP (%)) following intranasal administration of ^{99m}Tc-TS/^{99m}Tc-TME/^{99m}Tc-TMME Formulations.

Formulation$_{\text{route of administration}}$	**DTE (%)**	**DTP (%)**
$TS_{i.n.}$	207.23	51.75
$TME_{i.n.}$	242.82	58.82
$TMME_{i.n.}$	295.87	66.20



(A) (B)



(C) (D)

Figure 7.2 Gamma scintigraphy images of rabbit showing the presence of radioactivity into the brain (arrows). (A) ^{99m}Tc-TS$_{i.v.}$ (100 μCi); (B) ^{99m}Tc-TS$_{i.n.}$ (100 μCi); (C) ^{99m}Tc-TME$_{i.n.}$ (100 μCi); and (D) ^{99m}Tc-TMME$_{i.n.}$ (100 μCi)

7.2 Donepezil

7.2.1 Bio-distribution Studies:

All experiments conducted on animals were approved by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. Balb/c mice (aged 4 to 5 months), weighing between 30 to 40 g were selected for the study on the basis of randomization technique.

Three mice (male, weighing between 30 to 40 g) for each formulation per time point were used in the study. The radiolabeled complex of ^{99m}Tc-DS (100 μCi/50 μL) containing 0.0195–0.0260 mg donepezil (equivalent to 0.65 mg/ kg B.W.) was injected intravenously (i.v.) through tail vein of mice. Similarly, radiolabeled complex of ^{99m}Tc-DS/^{99m}Tc-DME/^{99m}Tc-DMME (100 μCi/10 μL) containing 0.0195–0.0260 mg donepezil (equivalent to 0.65 mg/ kg B.W.) was administered (5 μL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 μL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site. The mice were held from the back in slanted position during nasal administration of the formulations. Blood was collected using cardiac puncture at predetermined time intervals (0.25, 0.50, 1.00, 2.00, 4.00, and 8.00 h). After collecting the blood, the mice were sacrificed with mercy by exposure to diethyl ether. Subsequently, different tissues/organs including brain were dissected, washed twice using normal saline solution, and made free from adhering tissue/fluid and weighed. The radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter (Capintec Inc., New Jersey, USA) (Theobald 1990; Saha 1993). The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (Babbar et al. 2000). The results of radioactivity in different organs are recorded in Table 7.8, 7.9, 7.10 and 7.11. In order to comprehensively understand the drug distribution behavior in blood and brain, the drug concentration as percent of radioactivity per g of tissue vs. time were plotted and shown in Figure 7.3. The brain/blood ratio were also calculated at all time points and recorded in Table 7.12. The

pharmacokinetic parameters were derived from Table 7.12 using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) and recorded in Table 7.13 (Rey et al. 1999; Wermling et al. 2001)

In order to evaluate the brain targeting efficiency, two indexes were adopted as described under tacrine biodistribution studies (section 7.1.1). DTP represents the percentage of drug directly transported to the brain via olfactory/trigeminal neural pathway. DTP (%) and DTE (%) derived from tissue/organ distribution data following intranasal and intravenous administration is shown in Table 7.14.

7.2.2 Gamma Scintigraphy Imaging:

The New Zealand rabbits (2.00–2.50 kg) were selected for the study. The radiolabeled complex of ^{99m}Tc-DS (100 µCi/100 µL) containing 0.467–0.583 mg donepezil (equivalent to 0.233 mg/kg B.W.) was i.v. injected through the ear vein of the rabbit. Similarly, the radiolabeled complex of ^{99m}Tc-DS/^{99m}Tc-DME/^{99m}Tc-DMME (100 µCi/100 µL) containing 0.467–0.583 mg donepezil (equivalent to 0.233 mg/kg B.W.) was administered i.n. (50 µL in each nostril) (Eckelman 1995). The rabbits were held from the back in slanted position during nasal administration of formulations. The rabbits were anaesthetized using 1 mL ketamine hydrochloride intramuscular injection (50 mg/mL) and placed on the imaging platform. Imaging was performed using Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens AG; Erlanger, Germany) gamma camera (Capala et al. 1997; Babbar et al. 2000). The scintigraphy images following i.v. and i.n. administrations of ^{99m}Tc-donepezil formulations are shown in Figure 7.4.

7.2.3 Statistical Analysis:

All data are reported as mean ± SD and the difference between the groups were tested using Student's 't' test at the level of $P< 0.05$. More than two groups were compared using ANOVA and differences greater at $P< 0.05$ were considered significant.

Table 7.8 Tissue/organ distribution of ^{99m}Tc-DS in Balb/c mice at predetermined time intervals post intravenous administration.*

TISSUE/ ORGAN	0.25 h	0.50 h	1.00 h	2.00 h	4.00 h	8.00 h
Blood	3.07±0.33	2.39±0.30	1.94±0.20	1.71±0.19	1.67±0.17	1.61±0.16
Liver	0.99±0.14	1.19±0.18	1.77±0.21	1.31±0.13	1.07±0.11	0.45±0.07
Kidney	1.49±0.15	1.74±0.17	1.94±0.19	1.25±0.11	1.09±0.12	0.19±0.07
Brain	0.26±0.05	0.32±0.06	0.35±0.06	0.39±0.07	0.25±0.05	0.10±0.03

* The mice were administered intravenously with 100 µCi ^{99m}Tc-DS and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.9 Tissue/organ distribution of ^{99m}Tc-DS in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	0.25 h	0.50 h	1.00 h	2.00 h	4.00 h	8.00 h
Blood	0.61±0.10	0.76±0.09	1.03±0.11	1.16±0.10	0.14±0.09	1.10±0.08
Liver	0.28±0.07	0.35±0.09	0.46±0.11	0.37±0.05	0.31±0.06	0.07±0.03
Kidney	0.35±0.07	0.42±0.10	0.50±0.09	0.45±0.07	0.37±0.09	0.13±0.04
Brain	0.37±0.10	0.40±0.09	0.49±0.11	0.43±0.10	0.34±0.08	0.12±0.04

* The mice were administered intranasally with 100 µCi ^{99m}Tc-DS and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.10 Tissue/organ distribution of ^{99m}Tc-DME in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	0.25 h	0.50 h	1.00 h	2.00 h	4.00 h	8.00 h
Blood	0.64±0.08	0.79±0.09	1.20±0.12	1.44±0.11	1.41±0.13	1.36±0.09
Liver	0.45±0.10	0.54±0.12	0.68±0.13	0.57±0.10	0.48±0.08	0.13±0.05
Kidney	0.61±0.09	0.70±0.11	0.79±0.04	0.74±0.04	0.66±0.05	0.16±0.06
Brain	0.45±0.12	0.53±0.13	0.74±0.15	0.65±0.16	0.57±0.13	0.19±0.06

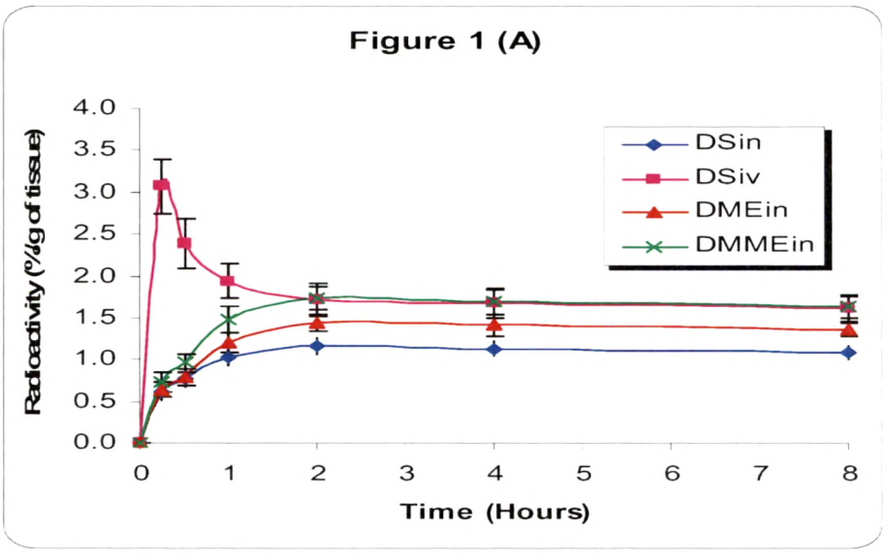
* The mice were administered intranasally with 100 µCi ^{99m}Tc-DME and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

Table 7.11 Tissue/organ distribution of ^{99m}Tc-DMME in Balb/c mice at predetermined time intervals post intranasal administration.*

TISSUE/ ORGAN	0.25 h	0.50 h	1.00 h	2.00 h	4.00 h	8.00 h
Blood	0.73±0.12	0.96±0.11	1.48±0.15	1.73±0.14	1.69±0.15	1.63±0.12
Liver	0.59±0.09	0.75±0.12	0.92±0.10	0.80±0.09	0.61±0.07	0.18±0.06
Kidney	0.81±0.13	0.94±0.12	1.08±0.13	0.98±0.11	0.77±0.11	0.21±0.06
Brain	0.59±0.08	0.70±0.12	1.04±0.14	0.92±0.09	0.75±0.12	0.26±0.06

* The mice were administered intranasally with 100 µCi ^{99m}Tc-DMME and the radioactivity was measured as percent per g of tissue of the administered dose. Each value is the mean ± SD (n=3).

(A)



(B)

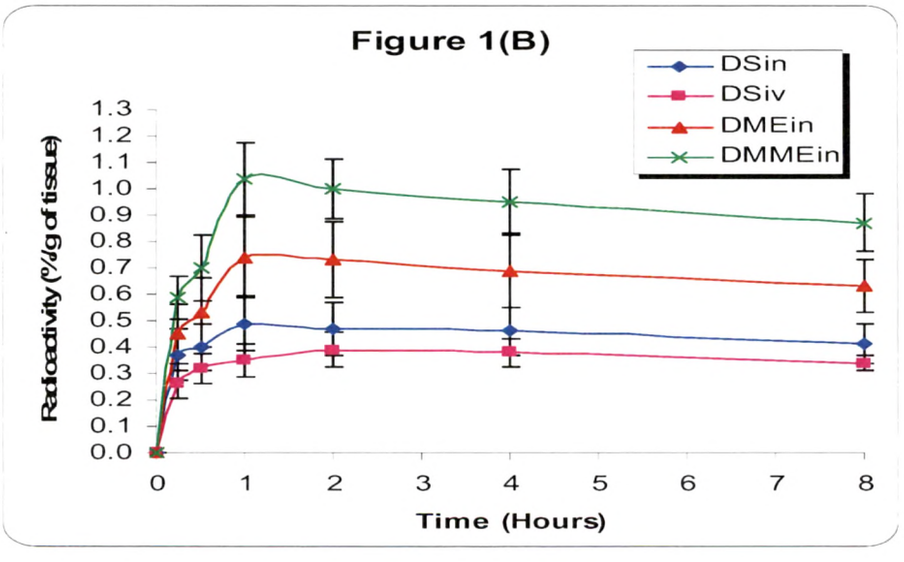


Figure 7.3 Donepezil concentration in mice (n = 3) (A) blood and (B) brain at different time intervals following ^{99m}Tc-DS$_{i.v.,}$ ^{99m}Tc-DS$_{i.n.,}$ ^{99m}Tc-DME$_{i.n.}$and ^{99m}Tc-DMME$_{i.n.}$ administrations. Error bars represents SD

Table 7.12 Distribution of ^{99m}Tc-DS$_{i.v.}$, ^{99m}Tc-DS$_{i.n.}$, ^{99m}Tc-DME$_{i.n.}$ and ^{99m}Tc-DMME$_{i.n.}$ in Balb/c mice[$] at predetermined time intervals

Formulation$_{route\ of\ administration}$	Organ/Tissue	Sampling time points (h)					
		0.25	0.50	1.00	2.00	4.00	8.00
DS$_{i.v.}$	Blood	3.07±0.33	2.39±0.30	1.94±0.20	1.71±0.19	1.67±0.17	1.61±0.16
	Brain	0.26±0.05	0.32±0.06	0.35±0.06	0.39±0.07	0.38±0.05	0.34±0.03
DS$_{i.n.}$	Blood	0.61±0.10	0.76±0.09	1.03±0.11	1.16±0.10	0.13±0.09	1.09±0.08
	Brain	0.37±0.10	0.40±0.09	0.49±0.11	0.47±0.10	0.46±0.09	0.41±0.08
DME$_{i.n.}$	Blood	0.64±0.08	0.79±0.09	1.20±0.12	1.44±0.11	1.41±0.13	1.36±0.09
	Brain	0.45±0.12	0.53±0.13	0.74±0.15	0.73±0.15	0.69±0.14	0.63±0.10
DMME$_{i.n.}$	Blood	0.73±0.12	0.96±0.11	1.48±0.15	1.73±0.14	1.69±0.15	1.63±0.12
	Brain	0.59±0.08	0.70±0.12	1.04±0.14	1.00±0.12	0.95±0.12	0.87±0.11
DS$_{i.v.}$	Brain/Blood	0.09 ± 0.03	0.14 ± 0.04	0.18 ± 0.05	0.23 ± 0.07	0.23 ± 0. 06	0.21 ± 0.04
DS$_{i.n.}$	Brain/Blood	0.60 ± 0.08	0.52 ± 0.06	0.47 ± 0.06	0.40 ±0.06	0.40 ± 0.05	0.37 ± 0.04
DME$_{i.n.}$	Brain/Blood	0.72 ± 0.24	0.69 ± 0.22	0.62 ± 0.15	0.51 ±0.14	0.50 ± 0.15	0.47 ± 0.10
DMME$_{i.n.}$	Brain/Blood	0.83 ± 0.24	0.74 ± 0.20	0.71 ± 0.16	0.58 ±0.10	0.57 ± 0.10	0.54 ± 0.10

[$] The mice were administered with 100 μCi ^{99m}Tc-donepezil formulations and the radioactivity was measured in %/g of tissue of the administered dose. Radio activity was measured at 0 h and all the measurements were performed using 0 h sample of corresponding tissue/organ as blank sample. Each value is the mean ± SD (n = 3).

Table 7.13 Pharmacokinetics of ^{99m}Tc-DS$_{i.v.}$, ^{99m}Tc-DS$_{i.n.}$, ^{99m}Tc-DME$_{i.n.}$ and ^{99m}Tc-DMME$_{i.n.}$ in Balb/c mice[$]

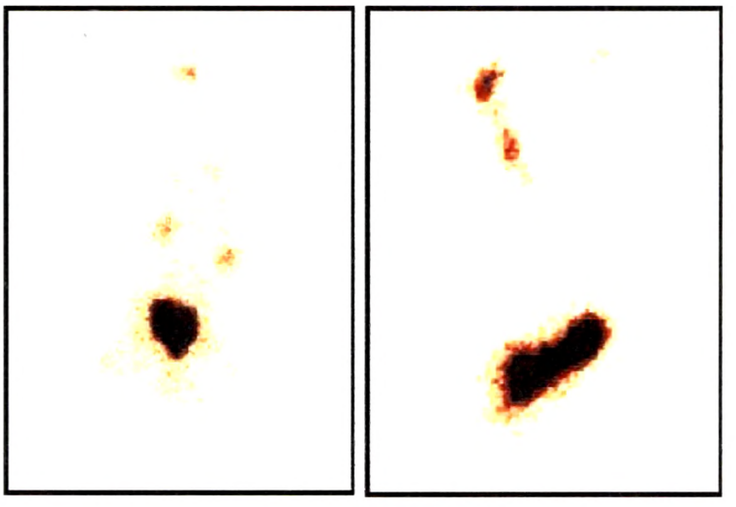
Pharmacokinetic Parameter	^{99m}Tc-DS$_{i.v.}$		^{99m}Tc-DS$_{i.n.}$		^{99m}Tc-DME$_{i.n.}$		^{99m}Tc-DMME$_{i.n.}$	
	Blood	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**	**Blood**	**Brain**
C_{max} (% radioactivity/g)[#]	3.07±0.33	0.39±0.07	1.16±0.10	0.49±0.11	1.44±0.11	0.74±0.15	1.73±0.14	1.04±0.14
T_{max} (h)	0.25	2.00	2.00	1.00	2.00	1.00	2.00	1.00
$AUC_{0\rightarrow480}$ (h*% radioactivity/g)	13.91	2.85	8.52	3.52	10.47	5.29	12.58	7.28
$AUC_{0\rightarrow\infty}$ (h*% radioactivity/g)	176.17	17.34	115.48	20.41	154.25	32.01	178.83	45.01
K_{el} (L/h)	0.0099	0.0236	0.0102	0.0244	0.0095	0.0236	0.0098	0.0230
$T_{1/2}$ (h)	69.91	29.41	68.10	28.45	73.31	29.41	70.75	30.09
Bioavailability (%)	-	-	61.23	123.23	75.22	185.50	90.40	255.21

[$] The mice were administered with 100 μCi ^{99m}Tc-donepezil formulations and the radioactivity was measured in percent per gram of tissue of the administered dose.

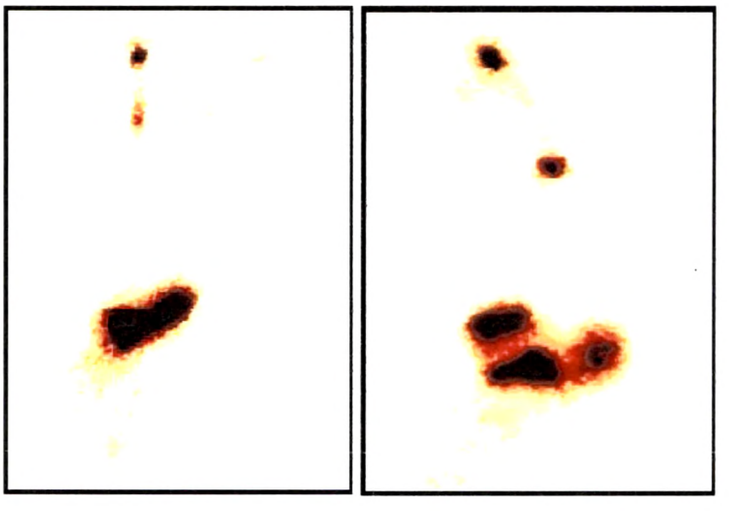
[#] Each value is the mean ± SD (n = 3)

Table 7.14 Drug targeting efficiency (DTE (%)) and direct nose to brain transport (DTP (%)) following intranasal administration of ^{99m}Tc-DS/^{99m}Tc-DME/^{99m}Tc-DMME Formulations.

Formulation$_{\text{route of administration}}$	**DTE (%)**	**DTP (%)**
$DS_{i.n.}$	201.24	50.31
$DME_{i.n.}$	246.60	59.45
$DMME_{i.n.}$	282.33	64.58



(A) **(B)**



(C) **(D)**

Figure 7.4 Gamma scintigraphy images of rabbit showing the presence of radioactivity into the brain (arrows). (A) ^{99m}Tc-DS$_{i.v.}$ (100 µCi); (B) ^{99m}Tc-DS$_{i.n.}$ (100 µCi); (C) ^{99m}Tc-DME$_{i.n.}$ (100 µCi); and (D) ^{99m}Tc-DMME$_{i.n.}$ (100 µCi)

7.3 Results and Discussion

7.3.1 Tacrine:

Biodistribution studies of ^{99m}Tc-TS following i.v. and ^{99m}Tc-TS/TME/TMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 480min. The results obtained are recorded in Table 7.1, 7.2, 7.3 and 7.4. The brain/blood ratios of the drug at all time points for different formulations were calculated and recorded in Table 7.5. The pharmacokinetic parameters of the drug were calculated from the data in Table 7.5 using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) and recorded in Table 7.6. After nasal administration, tacrine was delivered to the brain quickly compared to i.v. administration (T_{max} – 60min versus 120min). Similarly, lower T_{max} values for brain (60 min) compared to blood (120 min) were observed for all the three nasally administered formulations. This may be attributed to preferential nose to brain transport following nasal administration. The brain/blood ratios of the drug at all time points were found to be higher following i.n. administration of formulations (Table 7.5). This further confirms direct nose to brain transport (Liu et al. 2001; Lianli et al. 2002). The concentrations of drug in the brain following i.n. administration were found to be significantly higher at all sampling time points compared to i.v. administration up to 480 min. The substantially higher uptake in the brain with intranasal administration suggests a larger extent of selective transport of tacrine from nose-to brain. This is in agreement with many scientists who believe in this unique connection between the nose and the brain, and drug transport to brain circumventing the BBB after i.n. administration (Behl et al. 1998; Illum 2000). The $T_{1/2}$ and K_{el} of drug in blood was found to be significantly different for i.v. and i.n. administration of different tacrine formulations, but insignificant differences in these values were observed in brain irrespective of the routes of administration and the type of the formulations (Table 7.6). These differences in the results may be due to more selective distribution of the drug to the brain after i.n. administration. Significantly higher C_{max} (brain) and AUC (brain) were observed when $TS_{i.n.}$, $TME_{i.n.}$ and $TMME_{i.n.}$ were compared to $TS_{i.v.}$. The bioavailability of tacrine in brain after i.n. compared to i.v. administrations was 131.99%, 218.67% and 287.09% for TS, TME and TMME respectively (Table 7.6). This is suggestive of direct nose to brain transport of the drug

following i.n. administration (Sakane et al. 1999). When $TME_{i.n.}$ was compared to $TS_{i.n.}$, significantly higher AUC and C_{max} were observed. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa (Lawrence and Rees 2000). These findings are in congruence with the observations reported by Lianli et al. (2002) and Zhang et al. (2004), that microemulsion enhances transport of drug across nasal mucosa resulting in direct nose to brain transport of the drugs. Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance. However, when mucoadhesive agent was incorporated in the formulation (TMME), significantly higher C_{max} and AUC were observed compared to $TS_{i.n.}$ and $TME_{i.n.}$. The results demonstrated the importance of mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of the drug (Ugwoke et al. 2001; Praspari and Parkpoom 2003).

To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were also calculated, from the pharmacokinetics data, for nasally administered formulations and are recorded in Table 7.7. Amongst all the three nasally administered formulations, TMME showed highest DTE (%) and DTP (%) values followed by TME and then TS. These results demonstrated the significance of the mucoadhesive microemulsion formulation in prolonging the residence time in nasal cavity which resulted in higher uptake of the drug in the brain. The higher DTE (%) and DTP (%) demonstrated that $TMME_{i.n.}$ has greater brain targeting efficiency compared to $TME_{i.n.}$ and $TS_{i.n.}$, may be because of preferential nose to brain transport (Dragphia et al. 1995; Fehm et al. 2000; Dorman et al. 2002).

In order to ascertain the brain uptake following i.v. and i.n. administrations of ^{99m}Tc-tacrine formulations, we used gamma scintigraphy imaging. The gamma scintigraphy images of rabbit 15 min post i.v. and i.n. administrations are shown in Figure 7.2. The presence of some radioactivity in the esophagus following i.n. administration led to absorption of part quantity of formulation from gastrointestinal tract. Accumulation of significantly higher radioactivity in the rabbit brain following i.n. administration of tacrine compared with i.v. administration was observed. Amongst i.n. formulations, TMME shows higher radioactivity compared to TS and TME. Scintigraphy images are consistent with the observations of biodistribution studies.

7.3.2 Donepezil:

Biodistribution studies of ^{99m}Tc-DS following i.v. and ^{99m}Tc-DS/DME/DMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 8 h. The results obtained are recorded in Table 7.8, 7.9, 7.10and 7.11. The brain/blood ratios of the drug at all time points for different formulations were calculated and recorded in Table 7.12. The pharmacokinetic parameters of the drug were calculated from the data in Table 7.12 using WinNonlin® software (version 5.0.1, Pharsight Corporation, North Carolina, USA) and recorded in Table 7.13. After nasal administration, donepezil was delivered to the brain quickly compared to i.v. administration (T_{max} – 1 h versus 2 h). Similarly, lower T_{max} values for brain (1 h) compared to blood (2 h) were observed for all the three nasally administered formulations. This may be attributed to preferential nose to brain transport following nasal administration. The brain/blood ratios of the drug at all time points were found to be higher following i.n. administration of formulations (Table 7.12). This further confirms direct nose to brain transport (Liu et al. 2001; Lianli et al. 2002). The concentrations of drug in the brain following i.n. administration were found to be significantly higher at all sampling time points compared to i.v. administration up to 8 h. The substantially higher uptake in the brain with intranasal administration suggests a larger extent of selective transport of donepezil from nose-to brain. This is in agreement with many scientists who believe in this unique connection between the nose and the brain, and drug transport to brain circumventing the BBB after i.n. administration (Behl et al. 1998; Illum 2000). The $T_{1/2}$ of 68.10 – 73.31 h (blood), 28.45 – 30.09 h (brain), and K_{el} of 0.0095 – 0.0102 (blood), 0.0230 – 0.0244 (brain) were observed. The $T_{1/2}$ and K_{el} of drug in blood were found to be significantly different from the corresponding values in brain for i.v. and i.n. administration of different donepezil formulations, but insignificant differences in these values were observed in individual tissues irrespective of the routes of administration and the type of the formulations (Table 7.13). Significantly higher C_{max} (brain) and AUC (brain) were observed when $DS_{i.n.}$, $DME_{i.n.}$ and $DMME_{i.n.}$ were compared to $DS_{i.v.}$. The bioavailability of donepezil in brain after i.n. compared to i.v. administrations was 123.23%, 185.50% and 255.21% for DS, DME and DMME respectively (Table 7.6). This is suggestive of direct nose to brain transport of the drug following i.n. administration (Sakane et al. 1999). When $DME_{i.n.}$ was compared to $DS_{i.n.}$, significantly higher AUC and

C_{max} were observed. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa (Lawrence and Rees 2000). These findings are in congruence with the observations reported by Lianli et al. (2002) and Zhang et al. (2004), that microemulsion enhances transport of drug across nasal mucosa resulting in direct nose to brain transport of the drugs. Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance. However, when mucoadhesive agent was incorporated in the formulation (DMME), significantly higher C_{max} and AUC were observed compared to $DS_{i.n.}$ and $DME_{i.n.}$. The results demonstrated the importance of mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of the drug (Ugwoke et al. 2001; Praspari and Parkpoom 2003).

To evaluate the brain targeting efficiency, DTE (%) and DTP (%) were also calculated, from the pharmacokinetics data, for nasally administered formulations and are recorded in Table 7.14. Amongst all the three nasally administered formulations, DMME showed highest DTE (%) and DTP (%) values followed by DME and then DS. These results demonstrated the significance of the mucoadhesive microemulsion formulation in prolonging the residence time in nasal cavity which resulted in higher uptake of the drug in the brain. The higher DTE (%) and DTP (%) demonstrated that $DMME_{i.n.}$ has greater brain targeting efficiency compared to $DME_{i.n.}$ and $DS_{i.n.}$, may be because of preferential nose to brain transport (Dragphia et al. 1995; Fehm et al. 2000; Dorman et al. 2002).

In order to ascertain the brain uptake following i.v. and i.n. administrations of ^{99m}Tc-donepezil formulations, we used gamma scintigraphy camera. The gamma scintigraphy images of rabbit 15 min post i.v. and i.n. administrations are shown in Figure 7.4. The presence of some radioactivity in the esophagus following i.n. administration led to absorption of part quantity of formulation from gastrointestinal tract. Accumulation of significantly higher radioactivity in the rabbit brain following i.n. administration of donepezil compared with i.v. administration was observed. Amongst i.n. formulations, DMME shows higher radioactivity compared to DS and DME. Scintigraphy images are consistent with the observations of biodistribution studies.

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