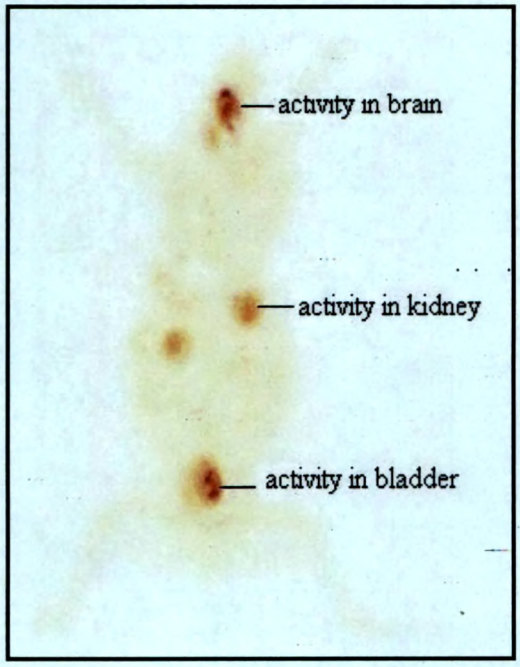
Chapter 6



Pharmacokinetic Study

1 INTRODUCTION:

Reports from the literature indicated the usefulness of radiolabeling techniques for pharmacokinetic and biodistribution studies. The radiolabeled formulations were better traced out in the biological system for their fate rather than conventional tissue extraction followed by instrumental analytical studies. Technetium-99m is a radionuclide of choice because of its unique properties like visualization of coupled complex in organs, sensitivity in detection even in extreme low levels in the organs.

6.2 MATERIALS:-

Stannous chloride dihydrate ($SnCl_2.2H_2O$) was purchased from Sigma Chemical Co.(St. Louis, MO), sodium pertechnetate, separated from molybdenom-99 (99m) by solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region) Board of Radiation and Isotope Technology (BRIT, Delhi, India)

Preparation and characterization of formulations:-

The formulations (solution S, microemulsion ME and mucoadhesive microemulsion MME) of clobazam (CZ) and clopidogrel bisulphate (CS) were prepared as described in section 4.4 and characterized as described in section 4.5. The formulations listed in the Table 5.5 & 5.6 and nasal gel of insulin like growth factor-1 were taken for the biodistribution study.

6.3 RADIOLABELING OF FORMULATIONS AND OPTIMIZATION:-

The formulations were radiolabeled using technetium-99m (^{99m}Tc) by direct-labeling method (Eckelman et al 1995; Babbar et al 2000). Radiolabeled technetium in sodium pertechnetate was reduced in the acidic medium in the presence of stannous chloride.

For carrying out the radiolabelling of the formulations, the required volume of formulation was treated with stannous chloride in 0.1Nhydrochloric acid and the pH was adjusted with sodium bicarbonate solution. Sterile sodium per ^{99m}Tc-pertechnetate (35 to 40 mCi/ml) was added with continuous mixing such that the resultant solution has the required radioactivity for the animal studies. The mixture was incubated at 30°C ± 5°C for 10 minutes. The final required volume was made up with 0.9 %w/v sterile sodium chloride solution.

Generally technetium is reduced in the presence of formulations, which enable the formulations tagged with technetium. In certain case, the previously reduced technetium is used for tagging of the formulations as like in the case of clopidogrel bisulphate formulations.

The radiochemical purity of the formulations was determined using ascending instant thin layer chromatography (TLC). Silica gel-coated fiberglass sheets (Gelman Sciences Inc, Ann Arbor, MI) were used as stationary phase while dual solvent systems consisting of acetone and pyridine: acetic acid: water (3:5:1.5 v/v) were used as mobile phases (Saha1993, Saha 2005). Since the free technetium is having R_f value of nearly 1 in acetone mobile phase, the ratio of radioactivity in the top $1/3^{rd}$ to lower $2/3^{rd}$ of the ITLC plates were used as the index of the percentage labeled. The contaminants redued/ hydrolysed (^{99m}Tc) collectively were called as colloids which were identified by their lower R_f values in pyridine: acetic acid: water (3:5:1.5 v/v) mobile phase.

The effect of incubation time, pH, and stannous chloride concentration on labeling were studied to achieve optimum labeling of the formulations and were tabulated. The in-vitro stability of radiolabeled formulations was evaluated in 0.9%w/v sodium chloride (normal saline) and mice serum by ascending ITLC (Garron et al 1991).

To evaluate stability and bonding strength of the labeled complex, the radio labeld formulations were challenged against various concentrations (1,2,3 and 4mM) of Diethylene Triamine Penta acetic acid (DTPA). Since 99mTc- DTPA complex have higher R_f values in pyridine: acetic acid: water (3:5:1.5 v/v) mobile phase, where the radiolabeled formulations retained at point of application. The effect of different molar concentrations and percent transchelation on radiolabeled formulations was tabulated. The optimized radiolabelled formulations were assessed for *in vitro* stability in normal saline and in mice serum (Theobald 1990). Consequently, the optimized stable radiolabeled formulations were used for *in vivo* studies.

6.4 BIODISTRIBUTION STUDIES:

The Social Justice and Empowerment Committee, Ministry of Government of India, approved all animal experiments were conducted for the purpose of control and supervision on animals and experiments. Balb/c mice (aged 4 to 5 months), weighing between 25 to 40 g were selected for the study. Three mice for each formulation per time point were used in the study. Radiolabeled complex of ^{99m}Tc-formulations was administered (10 µL) in each nostril. The mice were anaesthetized using chloroform inhalation from cholorform soaked cotton. Formulations were instilled into nostrils with the help of micropipette (10 to100 µL) attached with low-density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site. Similarly, 100 µL of radiolabeled complex of ^{99m}Tc-solution was injected through tail vein of the mice. The animals were killed humanely at different time intervals and the blood collected using cardiac puncture. Subsequently, brain and other tissues (liver, spleen, kidney, stomach and tail) were dissected, washed twice using normal saline, made free from adhering tissue/fluid, and weighed. Radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter. Radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose using equation:

$$\% \text{ Radioactivity /gm of tissue} = \frac{\text{counts in sample x 100}}{\text{wt of sample x total counts injected}}$$

The pharmacokinetic parameters, like C_{max}, T_{max}, AUC, brain /blood ratio in all time points were calculated. The area under the % radio activity per gram of tissue vs. time curve from zero to 24 hour (AUC) was calculated by standard trapezoidal rule. Terminal elimination rate constant (β) for drug following intranasal and intravenous administration was obtained by linear regression analysis of the terminal log-linear portion of % radio activity per gram of tissue vs. time curve. The corresponding half life of drug was calculated using the relationship 0.693 / β. The 0^{th} time concentration followed by IV route was calculated by interpolation of terminal elimination curve to the Y axis. The nasal bioavailability of the drugs from the formulations was calculated using equation (Zhao Y 2007).

$$\% \text{ Nasal bioavailability} = \frac{AUC_{IN}}{AUC_{IV}} \times 100$$

To evaluate the brain targeting efficiency, 2 indices [Drug targeting efficiency (DTE) (%) and direct nose-to-brain transport (DTP) (%)] were adopted as mentioned below. (Jung BH et al 2000, Zhang 2004). Brain targeting efficiency was calculated using two equations mentioned below. Drug targeting efficiency (DTE %) represents time average partitioning ratio.

$$DTE = \frac{AUC_{brain}}{AUC_{blood}}$$

Where, AUC indicates area under the curve.

Brain drug-direct-transport percentage [DTP%] was calculated using equations:

$$DTP\% = \frac{B_{IN} - B_X}{B_{IN}} \times 100$$

Where,

$\mathbf{B_X = (B_{IV}/P_{IV}) \times P_{IN}}$

$\mathbf{B_X}$ = Brain AUC fraction contributed by systemic circulation through the blood–brain-barrier (BBB) following intranasal administration.

$\mathbf{B_{IV}} = AUC_{0\rightarrow 24}$ (brain) following intravenous administration.

$\mathbf{P_{IV}} = AUC_{0\rightarrow 24}$ (blood) following intravenous administration.

$\mathbf{B_{IN}} = AUC_{0\rightarrow 24}$ (brain) following intranasal administration.

$\mathbf{P_{IN}} = AUC_{0\rightarrow 24}$ (blood) following intranasal administration.

AUC = Area under the curve.

6.5 GAMMA SCINTIGRAPHY IMAGING:-

The NewZealand rabbits (2.0 – 2.5 kg) were selected for the study. The radiolabeled complex of solutions was injected through the ear vein of the rabbits. Similarly, radiolabeled formulations ^{99m}Tc- S, ^{99m}Tc- ME, ^{99m}Tc- MME, ^{99m}Tc- IGF1 Gel were administered (50 µL) in each nostril (Eckelman 1995). The animals were anaesthetized using diazepam subcutaneous injection prior to administration of formulations. The animals were placed on board and images were captured using single positron 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 intravenous and intranasal administration of formulations were shown in Fig.6.1, Fig. 6.2. and Fig. 6.3

6.6. 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 $P<0.05$.

6.7 RESULTS

6.7.1 CLOBAZAM FORMULATIONS:

Table 6.1 Effect of quantity of $SnCl_2$ on radiolabeling of clobazam formulations

S.No.	Amount of Stannous Chloride (µgm)	% Radiolabeled		
		CZ Solution	CZ ME	CZ MME
1.	200	81.62 ± 1.08	86.29 ± 1.53	87.64 ± 2.4
2.	250	92.86 ± 2.41	81.87 ± 0.98	95.77 ± 1.35
3.	300	90.44 ± 1.32	78.34 ± 1.81	91.65 ± 2.6

Table 6.2 *Invitro* stability of labeled clobazam formulations

S. No	Time	In saline			In serum		
		CZS	CZ ME	CZMME	CZS	CZ ME	CZMME
1.	½ hr	92.86 ± 2.41	86.29 ± 1.53	95.77 ± 1.35	91.18 ± 1.35	92.56 ± 0.8	97.36 ± 2.4
2.	1 hr	91.42 ± 3.53	89.95 ± 2.17	95.71 ± 1.20	92.36 ± 2.31	94.35 ± 1.1	95.21 ± 1.3
3.	$2_{1/2}$ hr	90.75 ± 2.62	92.17 ± 1.18	94.78 ± 1.57	91.11 ± 1.41	95.71 ± 1.9	92.8 ± 1.04
4.	$4_{1/2}$ hr	90.01 ± 1.34	91.67 ± 2.11	94.16 ± 2.03	90.31 ± 0.98	91.25 ± 2.1	93.62 ± 0.9
5.	24 hr	89.14 ± 1.26	90.87 ± 1.98	91.84 ± 1.68	89.87 ± 2.54	90.45 ± 1.4	91.81 ± 1.1

Table 6.3 Effect of DTPA on radiolabeling of clobazam formulations

S.No.	DTPA concentration (mM)	% Transchelation		
		CZ Solution	CZ ME	CZ MME
1.	1.0	1.25 ± 0.29	1.18 ± 0.26	1.36 ± 0.81
2.	2.0	2.86 ± 0.35	1.23 ± 0.55	2.19 ± 0.57
3.	3.0	3.44 ± 0.34	2.26 ± 0.81	3.15 ± 0.81
4.	4.0	3.29 ± 0.42	3.49 ± 0.63	3.0 ± 0.14

Table 6.4 Radiolabelling summary of clobazam formulations

S.No.		CZ Solution	CZ ME	CZ MME
1.	Method	Direct labeling method	Labeling with reduced technetium	Direct labeling method
2.	Amount of $SnCl_2$(4mg/ml)	250µgm	200µgm	250µgm
3.	pH/ colour of pH paper	pH 6-7 / yellowish green	pH 6.5 -7	pH 8.5 /slightly green
4.	Incubation duration	½ hr	Needs more time, nearly an hour	½ hr
5.	Labelling efficiency (%)	92.86 ± 2.41	89.95 ± 2.17	95.77 ± 1.35

Table 6.5 Tissue/ organ distribution of ^{99m}Tc-CZS in Balb/c mice at predetermined time intervals of post intravenous administration

S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	1.5 ± 0.45	1.53 ± 0.28	1.23 ± 0.03	0.79 ± 0.18	0.33 ± 0.04	0.01 ± 0.002
2.	Brain	0.14 ± 0.06	0.09 ± 0.01	0.09 ± 0.05	0.08 ± 0.01	0.04 ± 0.01	0.002 ± 0.002
3.	Kidney	2.90 ± 0.54	4.12 ± 0.04	4.25 ± 0.08	4.37 ± 0.11	3.81 ± 0.22	0.98 ± 0.01
4.	Liver	65.32 ± 0.64	64.13 ± 0.32	58.35 ± 1.0	55.94 ± 0.41	37.44 ± 0.62	2.94 ± 0.32
5.	Spleen	12.07 ± 0.29	46.61 ± 0.65	35.26 ± 0.59	21.80 ± 0.68	10.24 ± 0.15	1.05 ± 0.4
6.	Stomach	0.55 ± 0.07	0.53 ± 0.08	0.55 ± 0.02	0.56 ± 0.09	0.38 ± 0.11	0.25 ± 0.02

Table 6.6 Tissue/ organ distribution of ^{99m}Tc-CZS in Balb/c mice at predetermined time intervals of post intranasal administration

S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	0.19 ± 0.03	0.21 ± 0.01	0.17 ± 0.04	0.073± 0.07	0.053 ± 0.02	0.002 ± 0.001
2.	Brain	0.05 ± 0.002	0.06 ± 0.003	0.058 ± 0.06	0.046 ± 0.01	0.028 ± 0.01	0.001 ± 0.001
3.	Kidney	0.25 ± 0.09	0.27 ± 0.02	0.25 ± 0.03	0.24 ± 0.01	0.12 ± 0.02	0.002 ± 0.001
4.	Liver	0.45 ± 0.04	0.32 ± 0.03	0.27 ± 0.01	0.23 ± 0.02	0.10 ± 0.01	0.003 ± 0.002
5.	Spleen	0.05 ± 0.03	0.085 ± 0.01	0.12 ± 0.02	0.15 ± 0.017	0.09 ± 0.01	0.002 ± 0.001
6.	Stomach	2.49 ± 0.03	3.46 ± 0.42	3.57 ± 0.22	3.31 ± 0.31	1.24 ± 0.05	0.21 ± 0.02

Table 6.7 Tissue/ organ distribution of ^{99m}Tc-CZME in Balb/c mice at predetermined time intervals of post intranasal administration

S.No	Organ/ tissue	Time (hours)					
		0.5	**1**	**2**	**4**	**8**	**24**
1.	Blood	0.26 ± 0.08	0.32 ± 0.09	0.278 ± 0.01	0.19 ± 0.12	**0.119 ± 0.02**	0.009 ± 0.001
2.	Brain	0.24 ± 0.17	0.14 ± 0.04	0.12 ± 0.02	0.102 ± 0.02	0.065 ± 0.015	0.013 ± 0.004
3.	Kidney	1.57 ± 0.37	0.91 ± 0.13	0.58 ± 0.032	0.17 ± 0.11	0.07 ± 0.011	0.002 ± 0.001
4.	Liver	0.64 ± 0.06	0.73 ± 0.04	0.65 ± 0.017	0.55 ± 0.07	0.35 ± 0.1	0.02 ± 0.01
5.	Spleen	0.31 ± 0.039	0.33 ± 0.018	0.42 ± 0.02	0.51 ± 0.08	0.41± 0.21	0.07 ± 0.01
6.	Stomach	0.58 ± 0.11	0.78 ± 0.02	0.71 ± 0.03	0.66 ± 0.03	0.45 ± 0.017	0.07 ± 0.02

Table 6.8 Tissue/ organ distribution of ^{99m}Tc-CZMME in Balb/c mice at predetermined time intervals of post intranasal administration

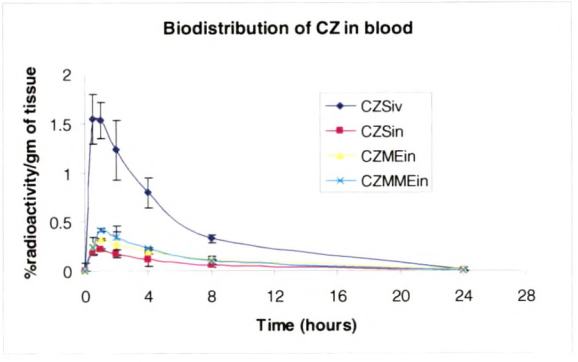
S.No	Organ/ tissue	Time (hours)					
		0.5	**1**	**2**	**4**	**8**	**24**
1.	Blood	0.25 ± 0.02	0.42 ± 0.02	0.34 ± 0.12	0.23 ± 0.01	0.11 ± 0.04	0.005 ± 0.001
2.	Brain	0.28 ± 0.01	0.19 ± 0.11	0.17 ± 0.04	0.13 ± 0.06	0.073 ± 0.03	0.008 ± 0.002
3.	Kidney	2.60 ± 0.4	1.23 ± 0.019	0.81 ± 0.03	0.54 ± 0.07	0.27 ± 0.04	0.06 ± 0.04
4.	Liver	1.03 ± 0.2	0.96 ± 0.051	0.75 ± 0.01	0.82 ± 0.04	0.68 ± 0.02	0.12 ± 0.02
5.	Spleen	0.28 ± 0.013	0.32 ± 0.036	0.44 ± 0.012	0.54 ± 0.04	0.28 ± 0.08	0.02 ± 0.001
6.	Stomach	0.48 ± 0.72	0.89 ± 0.074	0.68 ± 0.12	0.45 ± 0.05	0.35 ± 0.11	0.06 ± 0.01

Table 6.9 Distribution of ^{99m}Tc-CZS$_{IV}$, ^{99m}Tc-CZS$_{IN}$, ^{99m}Tc-CZME $_{IN}$, ^{99m}Tc-CZMME$_{IN}$ in BALB/c mice* at predetermined time intervals

Formulation	Organ/Tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
CZS$_{IV}$	Blood	1.543 ± 0.45	1.5315±0.28	1.2306±0.3	0.7947±0.18	0.3312±0.04	0.01±0.002
	Brain	0.1439±0.06	0.0920±0.01	0.0818±0.02	0.0846±0.01	0.0403±0.01	0.002±0.002
CZS$_{IN}$	Blood	0.1982±0.03	0.2138±0.01	0.1751±0.04	0.1173±0.07	0.0727±0.02	0.002±0.001
	Brain	0.0532±0.02	0.066±0.003	0.0584±0.06	0.0458±0.01	0.0282±0.01	0.001±0.001
CZME $_{IN}$	Blood	0.2539±0.08	0.3192±0.09	0.2763±0.01	0.1946±0.12	0.1192±0.02	0.009±0.001
	Brain	0.2361±0.17	0.1437±0.04	0.1219±0.02	0.1015±0.02	0.065±0.015	0.013±0.004
CZMME$_{IN}$	Blood	0.2439±0.02	0.4161±0.02	0.3417±0.12	0.2303±0.01	0.1047±0.04	0.005±0.001
	Brain	0.2750±0.01	0.1944±0.11	0.1689±0.04	0.1274±0.06	0.0725±0.03	0.008±0.002
CZS$_{IV}$	Brain/ Blood	0.0933	0.0601	0.0664	0.1064	0.1217	0.21
CZS$_{IN}$	Brain/ Blood	0.2688	0.3087	0.3337	0.3905	0.3878	0.5
CZME $_{IN}$	Brain/ Blood	0.9298	0.4505	0.4414	0.5215	0.5453	1.4518
CZMME$_{IN}$	Brain/ Blood	1.1277	0.4674	0.4944	0.5532	0.6927	1.7002

*Mice were administered with the radiolabeled complex of ^{99m}Tc- CZS, ^{99m}Tc- CZME, ^{99m}Tc- CZMME (20 µCi / 10 µL) containing 6-10µg clobazam (equivalent to 0.4mg/ kg BW)

Graph 6.1 Blood, Brain concentration versus time plot following administration of ^{99m} Tc-CZ formulations



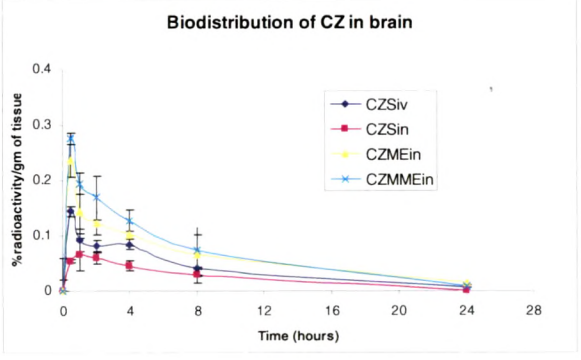


Table 6.10 Pharmacokinetics of ^{99m}Tc-CZS$_{IV}$, ^{99m}Tc-CZS$_{IN}$, ^{99m}Tc-CZME $_{IN}$, ^{99m}Tc-CZMME$_{IN}$ in BALB/c mice

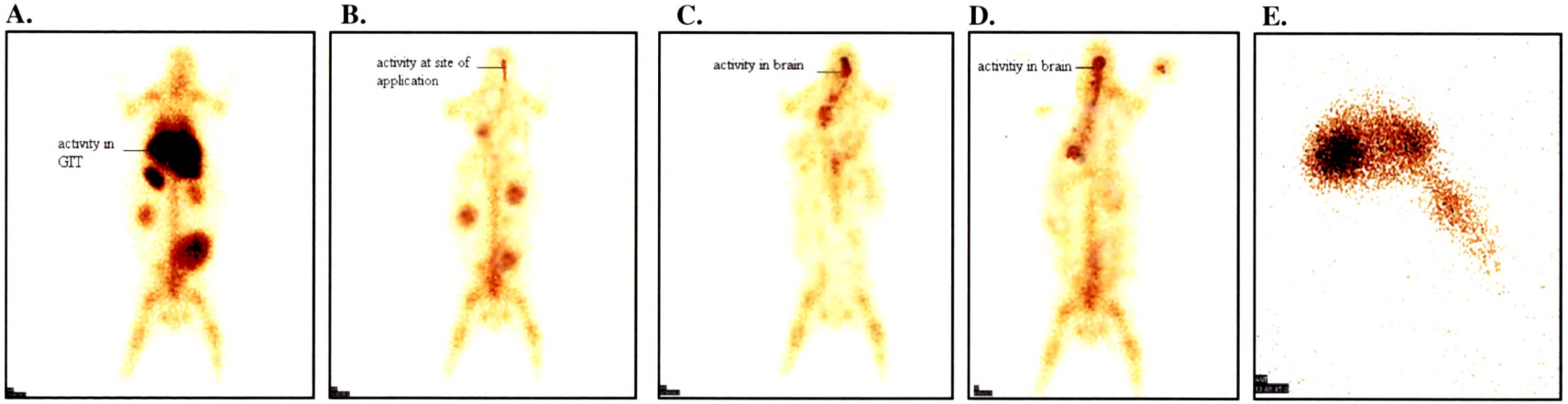
Formul -ation	Organ /Tissue	C_{max} (% radio activity/g)†	T_{max} (hours)	$AUC_{0\rightarrow 24hrs}$ (hours×% radioactivity/ g)	β(terminal) (hours^{-1})	$T_{1/2}$ (hours)	Nasal bioavaila bility (%)
CZS_{IV}	Blood	1.9058*	--	8.5624	0.2185	3.1708	--
	Brain	0.144±0.22	0.5	0.5252	0.1847	3.7520	
CZS_{IN}	Blood	0.214± 0.04	1.0	0.9041	0.2056	3.3696	10.55
	Brain	0.066± 0.08	1.0	0.2711	0.1962	3.5318	51.62
CZME $_{IN}$	Blood	0.319± 0.07	1.0	1.2844	0.1532	4.5249	15.00
	Brain	0.236± 0.19§	0.5	0.8347	0.1062	6.5273	158.93
$CZMME_{IN}$	Blood	0.416±0.02	1.0	1.4711	0.1971	3..5153	17.18
	Brain	0.275± 0.05‡	0.5	1.0311	0.1453	4.7688	196.32

C_{max} at 0th time was calculated by extrapolation of terminal linear portion till zero time. †Each value is the mean ± SD (n = 3)
§Significantly higher from corresponding values for CZS_{IV} and CZS_{IN} ‡Significantly higher from corresponding value for $CZME_{IN}$

Table 6.11 Brain targeting efficiency and Direct nose to brain transport percentage following intranasal administration of ^{99m}Tc-CZS, ^{99m}Tc-CZME and ^{99m}Tc-ZMME

Formulation	Route of administration	Brain targeting efficiency (DTE (%))	Direct nose to brain transport percentage (DTP (%))
CZS	Intranasal	29.9856	79.5442
CZME	Intranasal	64.9875	90.5674
CZMME	Intranasal	70.0904	91.2542

Figure 6.1 Gamma scintigraphy images of rabbits following A.^{99m}Tc- CZS $_{IV}$; B. ^{99m}Tc- CZS $_{IN}$; C. ^{99m}Tc- CZME $_{IN}$; D. ^{99m}Tc-CZMME$_{IN}$ (100 µCi / 100 µL) & E. ^{99m}Tc- CZMME$_{IN}$ (Lateral view)



6.7.2 CLOPIDOGREL BISULPHATE FORMULATIONS

Table 6.12 Effect of quantity of $SnCl_2$ on radiolabeling of clopidogrel bisulphate formulations

S.No.	Amount of Stannous Chloride (µgm)	% Radiolabeled		
		CS Solution	CS ME	CS MME
1.	100	75.25 ± 1.21	90.18 ± 1.17	87.36 ± 1.14
2.	200	88.86 ± 2.35	95.23 ± 0.95	93.79 ± 1.87
3.	300	85.44 ± 1.34	87.26 ± 2.07	90.25 ± 1.81

Table 6.13 *In vitro* stability of labeled complex (%) of clopidogrel bisulphate formulations

S. No	Time	In saline			In serum		
		CSS	CS ME	CSMME	CSS	CS ME	CSMME
1.	½ hr	86.86 ± 2.5	95.23 ± 0.9	93.79 ± 1.8	91.18 ± 1.3	92.36 ± 0.8	92.5 ± 0.4
2.	1½ hr	97.76 ± 1.1	96.75 ± 2.1	95.76 ± 1.5	96.25 ± 1.5	94.21 ± 1.9	91.81 ± 0.8
3.	3 hr	95.78 ± 1.5	95.79 ± 0.8	95.37 ± 1.4	96.27 ± 1.4	95.21. ± 1.2	92.35 ± 1.2
4.	5 hr	94.06 ± 0.9	94.27 ± 2.7	94.78 ± 1.8	92.64 ± 2.0	91.37 ± 1.3	93.11 ± 1.8
5.	24 hr	92.55 ± 3.1	90.16 ± 1.8	93.08 ± 2.1	88.29 ± 1.1	90.17 ± 0.4	90.85 ± 0.6

Table 6.14 Effect of DTPA on radiolabeling of clopidogrel bisulphate formulations

S.No.	DTPA concentration (mM)	% Radiolabeled		
		CS Solution	CS ME	CS MME
1.	1.0	0.95 ± 0.14	1.03 ± 0.21	1.06 ± 0.32
2.	2.0	1.98 ± 0.65	1.58 ± 0.23	2.01 ± 0.17
3.	3.0	2.56 ± 0.31	2.18 ± 0.72	2.15 ± 0.09
4.	4.0	2.3 ± 0.22	2.49 ± 0.62	2.5 ± 0.19

Table 6.15 Radiolabelling summary of clopidogrel bisulphate formulations

S.No.		CS Solution	CS ME	CS MME
1.	Method	Reduced method	Reduced method	Reduced method
2.	Amount of $SnCl_2$ (4mg/ml)	200µgm	200µgm	200µgm
3.	pH/ colour of pH paper	pH 6 - 7	pH 6.5	pH 6.5
4.	Incubation duration	Needs more time, nearly an hour	½ hr	½ hr
5.	Labelling efficiency (%)	97.76 ± 1.05	95.23 ± 0.95	93.79 ± 1.87

Table 6.16 Tissue/ organ distribution of ^{99m}Tc-CSS in Balb/c mice at predetermined time intervals of post intra -venous administration

S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	0.61 ± 0.02	0.59 ± 0.04	0.50 ± 0.02	0.37 ± 0.02	0.20 ± 0.04	0.0171 ± 0.002
2.	Brain	0.076 ± 0.006	0.19 ± 0.012	0.15 ±0.002	0.093 ± 0.003	0.036 ± 0.01	0.004 ± 0.002
3.	Kidney	10.60 ± 0.6	9.58 ± 1.0	7.48 ±0.4	4.52 ±0.85	2.31 ±0.0.55	0.52 ± 0.15
4.	Liver	33.37 ± 2.1	28.36 ± 2.9	15.33 ± 3.1	10.28 ± 1.5	7.48 ±1.25	1.03 ± 0.45
5.	Spleen	15.47 ± 2.3	10.21 ±3.4	8.17 ±1.3	4.11 ± 0.5	3.93 ± 0.35	0.75 ± 0.11
6.	Stomach	0.98 ± 0.2	0.65 ± 0.015	0.53 ± 0.02	0.25 ± 0.01	0.12 ±0.05	0.05 ± 0.01

Table 6.17 Tissue/ organ distribution of ^{99m}Tc-CSS in Balb/c mice at predetermined time intervals of post intranasal administration

S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	0.096 ± 0.002	0.29± 0.011	0.244 ± 0.004	0.172 ± 0.01	0.085 ± 0.02	0.005 ± 0.001
2.	Brain	0.039 ± 0.005	0.083± 0.002	0.0712± 0.001	0.0533± 0.001	0.029 ± 0.01	0.002 ± 0.001
3.	Kidney	0.127 ±0.011	0.151± 0.12	0.172 ± 0.013	0.190 ± 0.04	0.151 ± 0.03	0.011 ± 0.002
4.	Liver	0.149 ± 0.03	0.112 ± 0.05	0.10 ± 0.02	0.082 ± 0.022	0.059 ± 0.02	0.006 ± 0.001
5.	Spleen	0.046 ± 0.002	0.087 ±0.006	0.081 ± 0.001	0.073 ± 0.061	0.053 ± 0.01	0.003± 0.001
6.	Stomach	0.838 ± 0.06	0.63 ± 0.012	0.671 ± 0.02	0.539 ± 0.014	0.567 ± 0.1	0.042 ± 0.01

Table 6.18 Tissue/ organ distribution of ^{99m}Tc-CSME in Balb/c mice at predetermined time intervals of post intranasal administration

S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	0.35± 0.05	0.296 ± 0.021	0.262 ± 0.03	0.206 ± 0.02	0.127 ± 0.02	0.018 ± 0.001
2.	Brain	0.11± 0.01	0.204 ± 0.01	0.164 ± 0.02	0.106 ± 0.001	0.044 ± 0.015	0.0043 ± 0.004
3.	Kidney	0.21 ± 0.03	0.25 ± 0.02	0.32 ± 0.02	0.28 ± 0.017	0.161 ± 0.03	0.021 ± 0.01
4.	Liver	0.76 ± 0.08	0.63 ± 0.1	0.42 ± 0.07	0.33 ± 0.05	0.28 ± 0.017	0.11 ± 0.02
5.	Spleen	0.47 ± 0.02	0.94 ± 0.03	0.71 ± 0.034	0.51 ± 0.1	0.33 ± 0.02	0.12 ± 0.01
6.	Stomach	0.35 ± 0.2	0.16 ± 0.003	0.098 ± 0.02	0.076 ± 0.014	0.038 ± 0.01	0.002 ± 0.001

Table 6.19 Tissue/ organ distribution of ^{99m}Tc-CSMME in Balb/c mice at predetermined time intervals of post intranasal administration

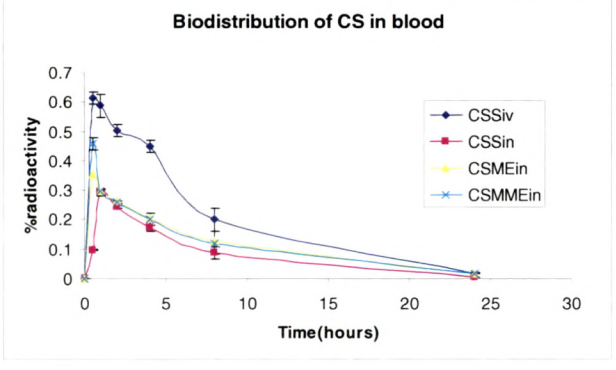
S.No	Organ/ tissue	Time (hours)					
		0.5	1	2	4	8	24
1.	Blood	0.45 ± 0.02	0.296 ± 0.01	0.261 ± 0.005	0.202 ± 0.02	0.121 ± 0.04	0.016 ±0.001
2.	Brain	0.22 ± 0.01	0.194 ± 0.006	0.153 ± 0.01	0.096 ± 0.01	0.075 ± 0.03	0.006 ±0.002
3.	Kidney	0.17 ± 0.014	0.197 ± 0.02	0.21 ± 0.021	0.18 ± 0.022	0.11 ± 0.01	0.06 ± 0.01
4.	Liver	0.53 ± 0.002	0.99 ± 0.024	0.71 ±0.031	0.68 ± 0.028	0.46 ± 0.02	0.24 ± 0.02
5.	Spleen	0.21 ± 0.01	0.16 ± 0.005	0.142 ± 0.04	0.138 ± 0.02	0.112± 0.31	0.55 ± 0.2
6.	Stomach	0.68 ± 0.2	0.57 ± 0.11	0.372 ±0.23	0.247 ± 0.41	0.134 ± 0.031	0.08 ± 0.011

Table 6.20 Distribution of ^{99m}Tc-CSS$_{IV}$, ^{99m}Tc-CSS$_{IN}$, ^{99m}Tc-CSME $_{IN}$, ^{99m}Tc-CSMME$_{IN}$ in BALB/c mice* at predetermined time intervals

Formulation	Organ/Tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
CSS$_{IV}$	Blood	0.6136 ± 0.02	0.5873± 0.04	0.5036 ±0.02	0.4702 ± 0.02	0.2001±0.04	0.0171±0.002
	Brain	0.0760±0.006	0.1904± 0.012	0.1498± 0.002	0.0926± 0.003	0.0355±0.01	0.0036±0.002
CSS$_{IN}$	Blood	0.0960± 0.002	0.2904± 0.011	0.2437± 0.004	0.1716 ± 0.01	0.0852±0.02	0.0052±0.001
	Brain	0.0392± 0.005	0.0834±0.002	0.0718± 0.001	0.0533± 0.001	0.0294±0.01	0.0015±0.001
CSME $_{IN}$	Blood	0.3537± 0.001	0.2959± 0.021	0.2622 ± 0.03	0.2059 ± 0.02	0.1269±0.02	0.0184±0.001
	Brain	0.1099± 0.01	0.2041± 0.01	0.1693± 0.02	0.1059± 0.001	0.044±0.015	0.0043±0.002
CSMME$_{IN}$	Blood	0.4572± 0.02	0.2959± 0.01	0.2606± 0.005	0.2019± 0.02	0.1214±0.04	0.0158±0.001
	Brain	0.2397± 0.01	0.2144± 0.006	0.1889 ± 0.01	0.1379 ± 0.01	0.0745±0.03	0.0063±0.002
CSS$_{IV}$	Brain/ Blood	0.1239	0.3247	0.2975	0.1970	0.1772	0.210
CSS$_{IN}$	Brain/ Blood	0.4086	0.2873	0.2948	0.3107	0.3446	0.2885
CSME $_{IN}$	Brain/ Blood	0.3107	0.6896	0.6255	0.5147	0.3484	0.2337
CSMME$_{IN}$	Brain/ Blood	0.5242	0.7246	0.7250	0.6829	0.6133	0.4006

*Mice were administered with the radiolabeled complex of ^{99m}Tc- CSS, ^{99m}Tc- CSME, ^{99m}Tc- CSMME (20 µCi / 10 µL) containing 8-13µg clopidogrel bisulphate (equivalent to 0.52mg/ kg BW)

Graph 6.2 Blood, Brain concentration versus time plot following administration of ^{99m} Tc-CS formulations



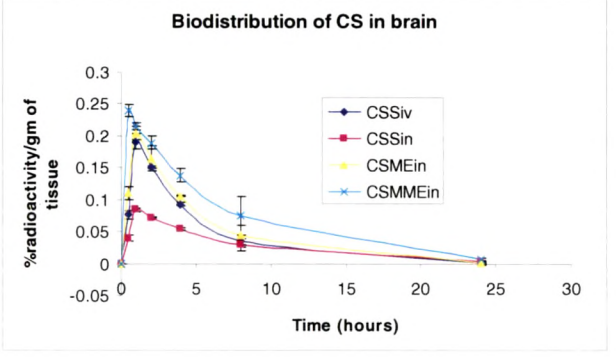


Table 6.21 Pharmacokinetics of ^{99m}Tc-CSS$_{IV}$, ^{99m}Tc-CSS$_{IN}$, ^{99m}Tc-CSME $_{IN}$, ^{99m}Tc-CSMME$_{IN}$ in BALB/c mice

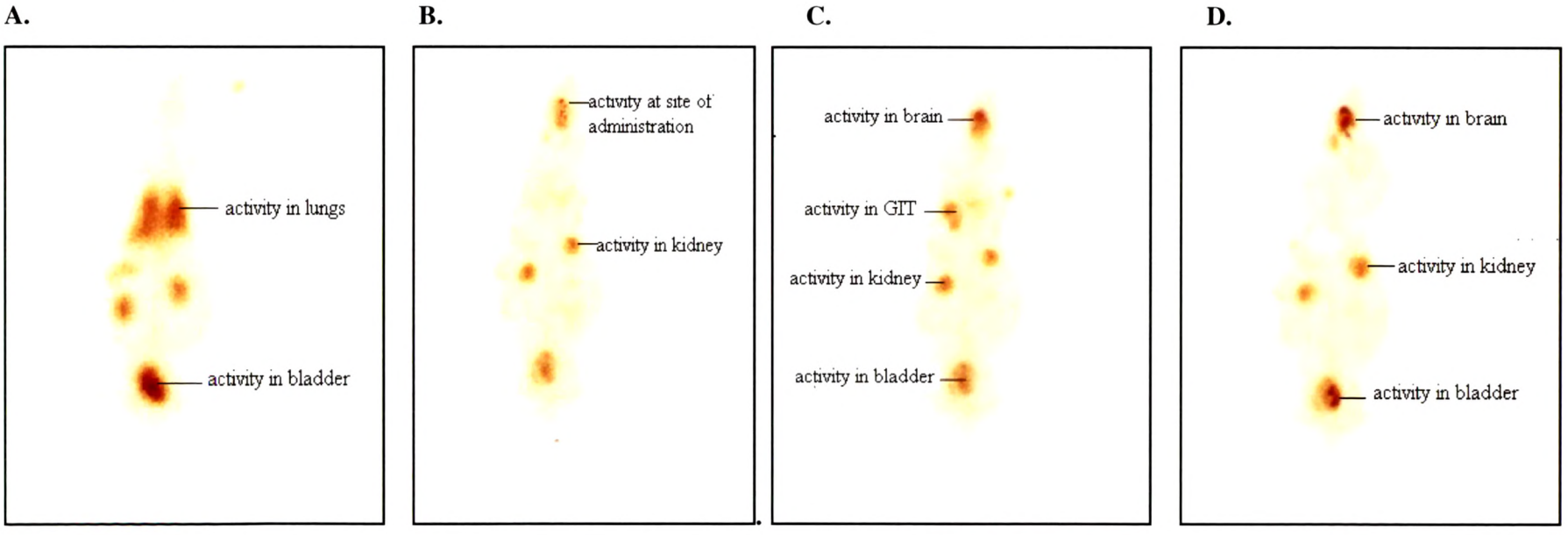
Formul -ation	Organ /Tissue	C_{max} (% radio activity/g) †	T_{max} (hours)	AUC $_{0\rightarrow24}$ $_{hrs}$(hours × %radioactivity/ g)	β(terminal) (hours^{-1})	$T_{1/2}$ (hours)	Nasal bioavaila bility (%)
CSS$_{IV}$	Blood	0.8502*	--	3.5443	0.1624	4.2682	--
	Brain	0.191± 0.012	0.5	0.5908	0.1568	4.4187	--
CSS$_{IN}$	Blood	0.29± 0.011	1.0	0.8882	0.1753	3.9542	25.0599
	Brain	0.083± 0.002	1.0	0.2821	0.1783	3.8878	47.7488
CSME $_{IN}$	Blood	0.354± 0.001	1.0	1.4456	0.1209	5.7317	40.7866
	Brain	0.204± 0.01§	0.5	0.6958	0.1561	4.4382	117.7725
CSMME$_{IN}$	Blood	0.457± 0.02	1.0	1.6488	0.1274	5.4414	46.5198
	Brain	0.2397± 0.01‡	0.5	1.0062	0.1541	4.4979	170.3114

* C_{max} at 0th time was calculated by extrapolation of terminal linear portion till zero time. †Each value is the mean ± SD (n = 3)
§Significantly higher from corresponding values for CSS$_{IV}$ and CSS$_{IN}$ ‡Significantly higher from corresponding value for CSME$_{IN}$

Table 6.22 Brain targeting efficiency and Direct nose to brain transport percentage following intranasal administration of ^{99m}Tc-CSS, ^{99m}Tc-CSME and ^{99m}Tc-CSMME

Formulation	Route of administration	Brain targeting efficiency (DTE(%))	Direct nose to brain transport percentage (DTP(%))
CSS	Intranasal	31.7609	47.5245
CSME	Intranasal	48.1323	65.3725
CSMME	Intranasal	61.0262	72.6893

Figure 6.2 Gamma scintigraphy images of rabbits following A.^{99m}Tc- CSS $_{IV}$; B. ^{99m}Tc- CSS $_{IN}$; C. ^{99m}Tc- CSME $_{IN}$; D. ^{99m}Tc- CSMME$_{IN}$ (100 µCi / 100 µL)



6.7.3 INSULIN LIKE GROWTH FACTOR-1 FORMULATIONS

Table 6.23 Effect of quantity of $SnCl_2$ on radiolabeling of insulin like growth factor1

S.No.	Amount of Stannous Chloride (µgm)	IGF 1 Solution
1.	100	95.49 ± 2.18
2.	200	82.65 ± 2.1
3.	300	90.69 ± 1.52

Table 6.24 Effect of pH on radiolabeling of insulin like growth factor1

S.No.	pH	IGF 1 Solution
1.	3	89.73 ± 2.17
2.	5	78.10 ± 2.34
3.	6.2-6.5	92.07 ± 1.56
4.	7.5	19.49 ± 2.35

Table 6.25 *In vitro* stability of labeled complex (%) of Insulin like growth factor 1

S.	Time (hour)		In saline		In serum	
No	Solution	Gel	Solution	Gel	Solution	Gel
1.	1 hr	$_{1/2}$ hr	92.48 ± 1.3	90.21 ± 1.2	94.21 ± 1.98	88.29 ± 1.87
2.	$1_{1/2}$ hr	1 hr	99.52 ± 2.3	93.22 ± 1.0	96.27 ± 1.37	93.86 ± 2.35
3.	$2_{1/2}$ hr	2 hr	99.0 ± 1.5	91.24 ± 1.1	95.27 ± 2.7	92.35 ± 1.37
4.	$3_{1/2}$ hr	3 hr	98.17 ± 0.89	91.82 ± 0.98	95.84 ± 1.68	91.01 ± 0.98
5.	$4_{1/2}$ hr	4 hr	95.36 ± 0.87	90.41 ± 2.0	92.79 ± 0.78	89.9 ± 1.25
6.	24	$23_{1/2}$ hr	91.11 ± 1.24	87.25 ± 1.4	86.75 ± 2.62	88.26 ± 0.93

Table 6.26 Effect of DTPA on radiolabeling of IGF-1formulations

S.No.	DTPA concentration (mM)	% Radiolabeled	
		IGF-1 Solution	IGF-1 gel
1.	1.0	0.5 ± 0.2	0.93 ± 0.1
2.	2.0	2.11 ± 0.5	2.81 ± 0.3
3.	3.0	2.43 ± 0.4	3.11 ± 0.4
4.	4.0	2.48 ± 0.2	2.37 ± 0.5

Table 6.27 Radiolabelling summary of Insulin like growth factor 1 formulations

S.No.		IGF 1 Solution	IGF 1 Gel
1.	Method	Direct method	--
2.	Amount of $SnCl_2$ (10mg/ml)	100µgm	--
3.	pH/ colour of pH paper	pH 6.2 – 6.5	--
4.	Incubation duration	½ hr	½ hr
5.	Labelling efficiency (%)	92.48 ± 1.3	90.21 ± 1.2

Table 6.28 Tissue/ organ distribution of ^{99m}Tc-IGF 1 Solution in Balb/c mice at predetermined time intervals of post intravenous administration

S.No	Organ / tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
1.	Blood	0.359 ±0.05	0.282 ±0.02	0.247±0.04	0.1903±0.01	0.1429 ± 0.03	0.0439 ±0.008
2.	Brain	0.043 ± 0.01	0.048 ±0.002	0.045±0.015	0.034±0.001	0.022 ± 0.001	0.0035 ±0.001
3.	Kidney	1.65 ± 0.3	1.45±0.02	1.137± 0.4	0.92±0.02	0.521±0.2	0.24± 0.01
4.	Liver	25.48 ±1.8	19.56±1.1	17.04± 1.2	10.52±0.85	8.612±0.6	1.06 ± 0.2
5.	Spleen	9.085± 0.2	7.95±0.25	6.67±0.51	2.48±0.45	1.12±0.11	0.35± 0.01
6.	Stomach	0.44±0.024	0.40±0.02	0.41±0.014	0.32±0.009	0.25±0.01	0.011± 0.007

Table 6.29 Tissue/ organ distribution of ^{99m}Tc-IGF 1 Solution in Balb/c mice at predetermined time intervals of post intranasal administration

S.No	Organ / tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
1.	Blood	0.119 ±0.011	0.162 ±0.02	0.143±0.01	0.109±0.005	0.091± 0.01	0.0178 ±0.002
2.	Brain	0.032 ±0.002	0.068 ±0.01	0.059±0.01	0.047±0.013	0.0456 ± 0.01	0.0041 ±0.003
3.	Kidney	0.138 ±0.05	0.184 ±0.04	0.091 ±0.01	0.075 ±0.005	0.035 ±0.01	0.007 ±0.001
4.	Liver	0.153 ±0.1	0.259 ± 0.2	0.182 ±0.02	0.170 ±0.017	0.15 ±0.02	0.011 ±0.002
5.	Spleen	0.045 ± 0.01	0.037 ±0.011	0.024±0.005	0.017 ±0.003	0.011 ±0.021	0.003 ±0.001
6.	Stomach	0.062 ± 0.02	0.043 ±0.01	0.052 ±0.02	0.045 ±0.013	0.028 ±0.002	0.01 ±0.004

Table 6.30 Tissue/ organ distribution of ^{99m}Tc-IGF 1 Gel in Balb/c mice at predetermined time intervals of post intranasal administration

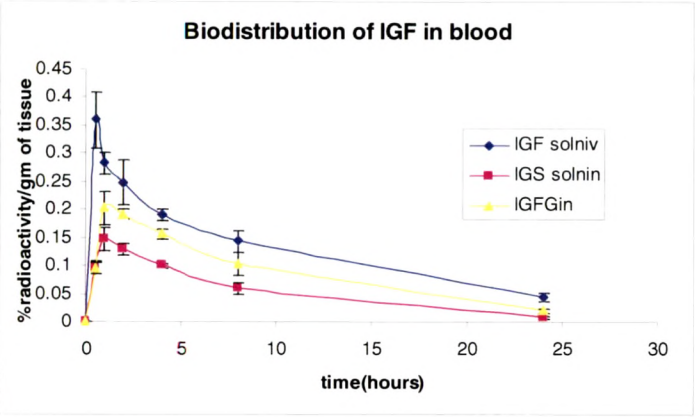
S.No	Organ / tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
1.	Blood	0.095 ± 0.01	0.1849 ±0.03	0.1713±0.01	0.146± 0.01	0.0733 ± 0.02	0.0149 ±0.004
2.	Brain	0.074 ±0.002	0.078 ±0.004	0.0721±0.02	0.0646±0.01	0.0472 ± 0.01	0.0084 ±0.002
3.	Kidney	0.108± 0.03	0.140 ±0.02	0.084±0.005	0.069±0.002	0.037 ±0.01	0.014±0.003
4.	Liver	0.194± 0.2	0.185±0.017	0.163±0.05	0.124±0.02	0.088 ±0.02	0.017±0.01
5.	Spleen	0.046±0.011	0.064± 0.02	0.061±0.013	0.058±0.011	0.028 ±0.02	0.009±0.001
6.	Stomach	0.059±0.005	0.082±0.01	0.071±0.002	0.045±0.01	0.026 ±0.003	0.013±0.003

Table 6.31 Distribution of ^{99m}Tc-IGF1S$_{IV}$, ^{99m}Tc-IGF1S$_{IN}$, ^{99m}Tc-IGF1G$_{IN}$ in BALB/c mice* at predetermined time intervals

Formulation	Organ/Tissue	0.5hr	1.0hr	2.0hr	4.0hr	8.0hr	24hr
IGF1S$_{IV}$	Blood	0.359 ±0.05	0.282 ±0.02	0.247±0.04	0.1903±0.01	0.1429 ± 0.03	0.0439 ±0.008
	Brain	0.043 ± 0.01	0.048 ±0.002	0.045±0.015	0.034±0.001	0.022 ± 0.001	0.0035 ±0.001
IGF1S$_{IN}$	Blood	0.119 ±0.011	0.162 ±0.02	0.143±0.01	0.109±0.005	0.091± 0.01	0.0178 ±0.002
	Brain	0.032 ±0.002	0.068 ±0.01	0.059±0.01	0.047±0.013	0.0456 ± 0.01	0.0041 ±0.003
IGF1G $_{IN}$	Blood	0.095 ± 0.01	0.1849 ±0.03	0.1713±0.01	0.146± 0.01	0.0733 ± 0.02	0.0149 ±0.004
	Brain	0.074 ±0.002	0.078 ±0.004	0.0721±0.02	0.0646±0.01	0.0472 ± 0.01	0.0084 ±0.002
IGF1S$_{IV}$	Brain/ Blood	0.1185	0.1688	0.1798	0.1778	0.1503	0.0795
IGF1S$_{IN}$	Brain/ Blood	0.2689	0.4197	0.4126	0.4311	0.5010	0.2304
IGF1G $_{IN}$	Brain/ Blood	0.7789	0.4218	0.4209	0.4443	0.6431	0.5637

*Mice were administered with the radiolabeled complex of ^{99m}Tc- IGF1S, ^{99m}Tc- IGF1S, ^{99m}Tc-IGF1G (20 µCi / 10 µL) containing 400ng insulin like growth factor1(equivalent to 20.02µg/ kg BW)

Graph 6.3 Blood, Brain concentration versus time plot following administration of ^{99m} Tc-IGF1 formulations



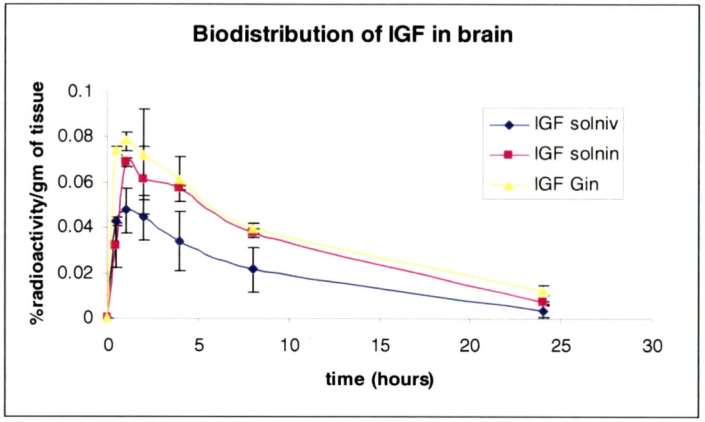


Table 6.32 Pharmacokinetics of ^{99m}Tc-IGF1S$_{IV}$, ^{99m}Tc-IGF1S$_{IN}$, ^{99m}Tc-IGF1G$_{IN}$ in BALB/c mice

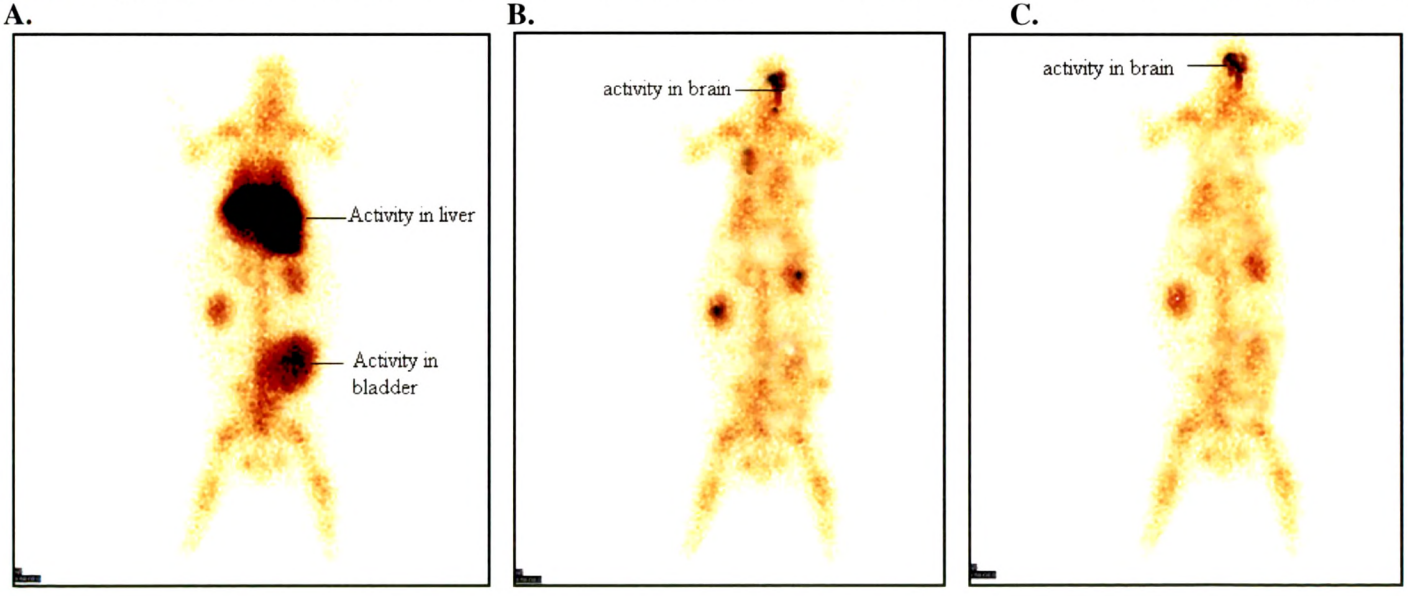
Formulation	Organ /Tissue	C_{max} (% radio activity/g) †	T_{max} (hours)	AUC $_{0\rightarrow 24\ hrs}$ (hours × % radioactivity / g)	β(terminal) (hours^{-1})	$T_{1/2}$ (hours)	Nasal bioavailability (%)
IGF1S$_{IV}$	Blood	3.6174*	--	5.0383	0.0690	10.039	--
	Brain	0.048±0.01	1.0	0.2058	0.0983	7.0522	--
IGF1S$_{IN}$	Blood	0.162 ±0.02	1.0	0.6455	0.0942	7.3573	12.8119
	Brain	0.068±0.002	1.0	0.2354	0.0879	7.8773	114.3828
IGF1G $_{IN}$	Blood	0.1849±0.03	1.0	0.6625	0.1096	6.3277	13.1493
	Brain	0.078±0.004§	1.0	0.3514	0.0925	7.4958	170.7483

* C_{max} at 0th time was calculated by extrapolation of terminal linear portion till zero time. †Each value is the mean ± SD (n = 3) §Significantly higher from corresponding values for IGFS$_{IV}$ and IGFS$_{IN}$

Table 6.33 Brain targeting efficiency and Direct nose to brain transport percentage following intranasal administration of ^{99m}Tc-IGF1S and ^{99m}Tc-IGF1G

Formulation	Route of administration	Brain targeting efficiency (DTE(%))	Direct nose to brain transport percentage (DTP(%))
IGF1S	Intranasal	36.4679	88.7983
IGF1G	Intranasal	53.0415	92.2985

Figure 6.3 Gamma scintigraphy images of rabbits following A.^{99m}Tc- IGF-1S$_{IV}$; B. ^{99m}Tc- IGF-1S$_{IN}$; C. ^{99m}Tc- IGF-1G$_{IN}$; (100 µCi / 100 µL)



6.8 DISCUSSION

6.8.1 CLOBAZAM:

Clobazam formulations (CZS, CZME, CZMME) were radiolabeled by direct labeling method. The radiolabeling was optimized by taking three factors in consideration, amount of stannous chloride, incubation time/ invitro stability of the radiolabeled complex and pH. The pH was adjusted ranging from 6 -8.5 and the amount of stannous chloride for optimum labeling (Theobald 1990; Saha 1993 & Saha 2005) was studied and the results were shown in Table 6.1. The stability studies of ^{99m}Tc-CZS/CZME/CZMME were carried out *in vitro* using normal saline and mice serum by ascending ITLC (Garron et al 1991). The stability of the complexes for 24hours was assessed and the results were shown in Table 6.2. The bonding strength of ^{99m}Tc-CZS/CZME/CZMME was assessed by DTPA (Diethylene triamine penta acetic acid) challenging test (Babbar et al 2000; Saha 2005). The effect of different molar concentrations of DTPA on ^{99m}Tc-CZS/CZME/CZMME and percent transchelation were studied and given in Table 6.3. The clobazam formulations were labeled successfully and their radio chemical purity/ labeling efficiency were found to be more than 90%. The radiolabeled ^{99m}Tc-CZS/CZME/CZMME complexes were found to be stable in normal saline and mice serum only with 10% degradation over 24 hours. The percent transchelation of ^{99m}Tc-CZS/CZME/CZMME were found to be less than 4% with 4mM DTPA.

Biodistribution of ^{99m}Tc-CZS following i.v. and ^{99m}Tc-CZS/CZME/CZMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 24hours. The results obtained are recorded in Table 6.5-6.8. The brain/blood ratio of drug at all time points for different formulations were calculated and recorded in Table 6.9. The biodistribution of clobazam in blood and brain following i.v of ^{99m}Tc-CZS and i.n. of ^{99m}Tc-CZS/CZME/CZMME were shown in Graph 6.1. The pharmacokinetic parameters C_{max}, T_{max}, AUC, terminal elimination rate constant, half life and nasal bioavailability were calculated using standard pharmacokinetic principles and given in Table 6.10. Lower T_{max} values were observed for i.n. CZME and CZMME. This may be attributed by preferential nose to brain transport following nasal administration. The brain/blood ratios of drug at all time points were found to be higher following i.n. administration

of the formulations than i.v solution. This further confirms direct nose to brain transport (Illum 2000; Lianli et al 2002). The higher T_{max} and lower C_{max} of clobazam in brain following i.n. CZS can be better explained by the rapid nasal ciliary clearance of the solution from the site of administration and high lipophilicity of the drug that renders more bioavailable in brain by i.v route of administration. The higher concentration of clobazam in brain following i.n administration of CZME and CZMME demonstrates the suitability/ capability of microemulsion as an effective delivery system across the nasal membrane (Lawrence and Rees 2000) and a larger extent of selective transport of clobazam from nose to brain. This is in agreement with many scientists who believe in this unique connection between the nose and brain and drug transport to brain circumventing the BBB after i.n. administration (Illum 2000; Lianli et al 2002; Vyas et al 2006a). The enhancement of AUC in brain followed by i.n. CZME and CZMME are in congruence with the observations reported by Lianli et al 2002 and Zhang et al 2004 that microemulsion enhances the transport of drug across nasal mucosa. CZMME was shown to have significantly higher C_{max} and AUC of CZMME demonstrated the importance of the mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of drug (Ugwoke et al 2001; Luessen et al 1995). The nasal bioavailability of clobazam in brain following i.n. of ^{99m}Tc-CZS /CZME / CZMME was 51.62 %, 158.93 % and 196.32 % respectively. The brain targeting efficiency of CZ formulations were calculated by the indices %DTE (Drug targeting efficiency) and %DTP (Direct nose-to-brain transport) and recorded in Table 6.11. Among all the three nasally administered formulations, CZMME showed highest %DTE and %DTP values followed by CZME and CZS. These findings demonstrated the mucoadhesive microemulsion has higher brain targeting efficiency by the virtue of bioadhesion and lipophilicity of the delivery system (Vyas et al 2005 and 2006).

Gamma scintigraphy imaging of rabbits administered with i.v of ^{99m}Tc-CZS and i.n. of ^{99m}Tc-CZS/CZME/CZMME were performed in order to confirm drug localization in brain. The gamma scintigraphy images of rabbits 30 min post i.v. and i.n. administrations were shown in Figure 6.1. The presence of radioactivity in the esophagus and GIT may be due to possible ingestion of formulation by the animal while i.n. administration. Accumulation of radioactivity in the rabbit brain following

different formulations and route of administration was observed and found that CZMME showed significantly high radioactivity in the brain. The scintigraphy images were consistent with the findings of the biodistribution studies.

6.8.2 CLOPIDOGREL BISULPHATE:

Clopidogrel bisulphate formulations (CSS, CSME, CSMME) were radiolabeled by direct labeling method with reduced technetium. The radiolabeling was optimized by taking three factors in consideration, amount of stannous chloride, incubation time/ invitro stability of the radiolabeled complex and pH. The pH was adjusted ranging from 6 - 7 and the amount of stannous chloride for optimum labeling (Theobald 1990; Saha 1993 & Saha 2005) was studied and the results were shown in Table 6.12. The stability studies of ^{99m}Tc-CSS/CSME/CSMME were carried out invitro using normal saline and mice serum by ascending ITLC (Garron et al 1991). The stability of the complexes for 24 hours was assessed and the results were shown in Table 6.13. The bonding strength of ^{99m}Tc-CSS/CSME/CSMME was assessed by DTPA (Diethylene triamine penta acetic acid) challenging test (Babbar et al 2000; Saha 2005). The effect of different molar concentrations of DTPA on ^{99m}Tc-CSS/CSME/CSMME and percent transchelation were studied and given in Table 6.14. The clopidogrel bisulphate formulations were labeled successfully and their radio chemical purity/ labeling efficiency were found to be more than 92%. The radiolabeled ^{99m}Tc-CSS/CSME/CSMME complexes were found to be stable in normal saline and mice serum only with 12% degradation over 24 hours. The percent transchelation of ^{99m}Tc-CSS/CSME/CSMME were found to be less than 3% with 4mM DTPA.

Biodistribution of ^{99m}Tc-CSS following i.v. and ^{99m}Tc-CSS/CSME/CSMME following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 24hours. The results obtained are recorded in Table 6.16 - 6.19. The brain/blood ratio of drug at all time points for different formulations were calculated and recorded in Table 6.20. The biodistribution of clopidogrel in blood and brain following i.v of ^{99m}Tc-CSS and i.n. of ^{99m}Tc-CSS/CSME /CSMME were shown in Graph 6.2. The pharmacokinetic parameters C_{max}, T_{max}, AUC, terminal elimination rate constant, half life and nasal bioavailability

were calculated using standard pharmacokinetic principles and given in Table 6.21. Lower T_{max} values were observed for i.n. CSME and CSMME. This may be attributed to preferential nose to brain transport following nasal administration. The brain/blood ratios of drug at all time points were found to be higher following i.n. administration of the formulations than i.v solution. This further confirms direct nose to brain transport (Illum 2000; Lianli et al 2002). The higher T_{max} and lower C_{max} of clopidogrel bisulphate in brain following i.n. CSS can be better explained by the rapid nasal ciliary clearance of the solution from the site of administration and dissociation of drug at nasal pH. The higher concentration of clopidogrel in brain following i.n administration of CSME and CSMME demonstrates the suitability/ capability of microemulsion as an effective delivery system across the nasal membrane (Lawrence and Rees 2000) and a larger extent of selective transport of drug from nose to brain. This is in agreement with many scientists who believe in this unique connection between the nose and brain and drug transport to brain circumventing the BBB after i.n. administration (Illum 2000; Lianli et al 2002; Vyas et al 2006a). The enhancement of AUC in brain followed by i.n. CSME and CSMME are in congruence with the observations reported by Lianli et al 2002 and Zhang et al 2004 that microemulsion enhances the transport of drug across nasal mucosa. CSMME was shown to have significantly higher C_{max} and AUC of CSMME demonstrated the importance of the mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of drug (Luessen et al 1995; Ugwoke et al 2001). The nasal bioavailability of clopidogrel in brain following i.n. of ^{99m}Tc-CSS /CSME / CSMME was 47.75%, 117.77% and 170.31% respectively. The brain targeting efficiency of CS formulations were calculated by the indices %DTE (Drug targeting efficiency) and %DTP (Direct nose-to-brain transport) and recorded in Table 6.22. Among all the three nasally administered formulations, CSMME showed highest %DTE and %DTP values followed by CSME and CSS. These findings demonstrated the mucoadhesive microemulsion has higher brain targeting efficiency by the virtue of bioadhesion and lipophilicity of the delivery system system (Vyas et al 2005 and 2006).

Gamma scintigraphy imaging of rabbits administered with i.v of ^{99m}Tc-CSS and i.n.of ^{99m}Tc-CSS/CSME/CSMME were performed in order to confirm drug localization in brain. The gamma scintigraphy images of rabbits 30 min post i.v. and i.n. administrations were shown in Figure 6.2. The presence of radioactivity in the esophagus and GIT may be due to possible ingestion of formulation by the animal while i.n. administration. Accumulation of radioactivity in the rabbit brain following different formulations and route of administration was observed and found that CSMME showed significantly high radioactivity in the brain. The scintigraphy images were consistent with the findings of the biodistribution studies.

6.8.3 INSULIN LIKE GROWTH FACTOR -1:

Insulin like growth factor -1 was radiolabeled by direct labeling method. The radiolabeling was optimized by taking three factors in consideration, amount of stannous chloride, incubation time/ invitro stability of the radiolabeled complex and pH. The pH was adjusted ranging from 3 -7.5 and the amount of stannous chloride for optimum labeling (Theobald 1990; Saha 1993 & Saha 2005) and pH was studied and the results were shown in Table 6.23 and 6.24. Radiolabeled IGF-1 was incubated with the gel and the stability of IGF1 in solution and gel was studied. The stability studies of ^{99m}Tc-IGF1S/ IGF1G were carried out *in vitro* using normal saline and mice serum by ascending ITLC (Garron et al 1991). The stability of the complexes for 24hours was assessed and the results were shown in Table 6.25. The bonding strength of ^{99m}Tc-IGF1S/ IGF1G was assessed by DTPA (Diethylene triamine penta acetic acid) challenging test (Babbar et al 2000; Saha 2005). The effect of different molar concentrations of DTPA on ^{99m}Tc-IGF1S/ IGF1G and percent transchelation were studied and given in Table 6.26. The formulations were labeled successfully and their radio chemical purity/ labeling efficiency were found tobe more than 90%. The radiolabeled ^{99m}Tc- IGF1S/ IGF1G complexes were found tobe stable in normal saline and mice serum only with 15% degradation over 24 hours. The percent transchelation of ^{99m}Tc- IGF1S/ IGF1G were found to be less than 3% with 4mM DTPA.

Biodistribution of ^{99m}Tc-IGF1S following i.v. and ^{99m}Tc- IGF1S/ IGF1G following i.n. administration in Balb/c mice were performed and the radioactivity was estimated at predetermined time intervals up to 24hours. The results obtained are

recorded in Table 6.28-6.30. The brain/blood ratio of drug at all time points for different formulations were calculated and recorded in Table 6.31. The biodistribution of clobazam in blood and brain following i.v of ^{99m}Tc- IGF1S and i.n. of ^{99m}Tc-IGF1S/ IGF1G were shown in Graph 6.3. The pharmacokinetic parameters C_{max}, T_{max}, AUC, terminal elimination rate constant, half life and nasal bioavailability were calculated using standard pharmacokinetic principles and given in Table 6.32.

The brain/blood ratios of drug at all time points were found tobe higher following i.n. administration of the formulations than i.v solution. This confirms direct nose to brain transport of IGF-1. (Xin-Feng Liu et al 2001a, 2001b, Throne et al 2004). The low bioavailability of IGF-I in brain following i.v. was due to the limitation of IGF-1 to pass through BBB (Loddick et al 1998). The lower C_{max} of IGF-1 solution can be better explained by the rapid nasal ciliary clearance of the solution from the site of administration. The higher concentration of IGF-1 following i.n administration of IGF1S / IGF1G demonstrates the effective delivery across the nasal membrane (Xin-Feng Liu et al 2001a, 2004 and Abdolhossein Rouholamini2004) and a larger extent of selective transport of drug from nose to brain. This is in agreement with many scientists who believe in this unique connection between the nose and brain and drug transport to brain circumventing the BBB after i.n. administration (Illum 2000; Lianli et al 2002; Vyas et al 2006a). The enhancement of AUC in brain followed by i.n. of IGF1S / IGF1G is in congruence with the observations reported by Xin-Feng Liu et al 2004 and Vig et al 2006. The substantial increment in C_{max} and brain concentrations following IGF-1G demonstrated the importance of the mucoadhesive agent in the delivery system. The muco adhesive polymer carbopol and HPMC were shown to prolong the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of drug (Luessen et al 1995; Ugwoke et al 2001). The nasal bioavailability of IGF-1 in brain following i.n. of ^{99m}Tc- IGF1S / IGF1G were 114.38% and 170.75 % respectively. The brain targeting efficiency of IGF-1 formulations were calculated by the indices %DTE (Drug targeting efficiency) and %DTP (Direct nose-to-brain transport) and recorded in Table 6.33. Among the solution and nasal gel, IGF-1 Gel showed higher %DTE and %DTP values than IGF-1 solution. These findings

demonstrated the mucoadhesive nasal gels are suitable delivery system for high molecular weight candidate drugs like peptides.

Gamma scintigraphy imaging of rabbits administered with i.v of ^{99m}Tc-IGF-1S and i.n.of ^{99m}Tc- IGF-1S / IGF-1G were performed in order to confirm drug localization in brain. The gamma scintigraphy images of rabbits 30 min post i.v. and i.n. administrations were shown in Figure 6.3. The presence of radioactivity in the esophagus and GIT may be due to possible ingestion of formulation by the animal while i.n. administration. Accumulation of radioactivity in the rabbit brain following different formulations and route of administration was observed and found that IGF-1G showed significantly high radioactivity in the brain. The scintigraphy images were consistent with the findings of the biodistribution studies.

6.9. REFERENCES

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