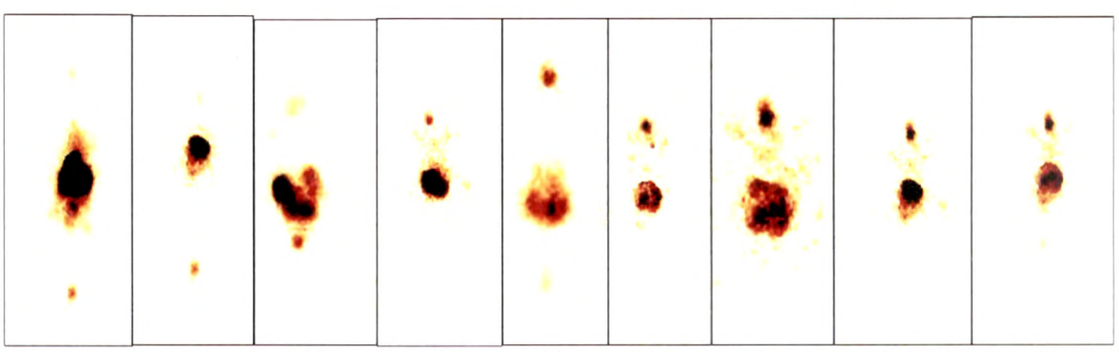
Chapter 9

Biodistribution Studies



9.1. Introduction

Radiolabeling of drugs and drug delivery systems has been widely applied to study these biological distribution patterns. Particularly, the radiolabeling with short lived radionuclides has been preferred due to their rapid decay and hence low toxicity. Drugs or colloidal drug carriers are linked to the radionuclides that are tailored for preferable concentration by a particular organ or physiologic process. In practice, the majority of radiopharmaceuticals are used for diagnosis (Mishra et al., 1999), but there a number of radionuclides available for the treatment of some disorders, especially cancer (Babbar et al., 2003). In the typical radiopharmaceutical formulation, the quantities of radionuclides and pharmaceutical agent used are normally quite less. The radiopharmaceutical differs from the conventional pharmaceutical in that it is not intended to elicit a pharmacological response due to the sub therapeutic doses administered. Hence, the radiopharmaceutical does not disturb the normal physiological process being measured, function as a true tracer, and they are generally free from hypersensitivity reactions. Since the dose administered is very low, the control of parameters such as tonicity and pyrogenicity is also not so important. The natural decay process may result in change in the final radionuclide composition and in the degradation of the stable materials. Variation in quality of radiopharmaceutical can greatly affect the biodistribution pattern and thereby the ultimate scan quality, causing problems in interpretation.

Quality control is an important aspect in the formulation and use of radiopharmaceuticals as it decides the efficacy for the purpose used. Before using the radionuclide for linking to the compound, the quality control testing is necessary to assure the efficacy of radionuclide. They include – radioactivity, radionuclide concentration, radionuclide purity and identity, radiochemical purity, chemical purity, sterility, apyrogenicity, absence of foreign particulate matter, particle Size (Babbar et al., 2003).

The emergence of scintigraphy or imaging techniques for studying the biodistribution patterns in the sixties and seventies has lead to the increase in the popularity of the application of nuclear medicine. These techniques allow non invasive biodistribution study by tracing using an external detection system viz. gamma camara (Single Photon Emission Computed Tomography - SPECT). SPECT imaging represents methods for acquiring and processing the scintigraphic data to reconstruct a three dimensional tomographic image displaying the distribution of radioactivity within

certain organ system using emitted gamma rays upon administration of a radio tracer (Budingeret al., 1980). Gamma imaging has lead to an increase in the demand for short lived radio tracers which can be safely administered in larger doses with minimal radiation dose. For biological experiments, the radionuclides are linked to the compounds of interest by various techniques. The effective binding of radiolabeled compound is determined by the quality control tests such as labelling efficiency, stability of radiolabeled complexes, challenge tests using substances having high affinity to the radiolabel compound and serum stability.

In practice, the radiopharmaceutical preparation is administered to the species of interest, using by the parenteral route. At specified time intervals, the organs or tissues of interest are removed and measured for radioactivity using a gamma counter. The images of organs/tissues can also be taken without sacrificing the host using the SPECT camera. Various radionuclides are used for the above mentioned purposes include ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{99}Mo, ^{131}I, ^{123}I, ^{133}Xe, ^{201}Tl, ^{99m}Tc, ^{67}Ga, ^{111}In .

Various reports are available where ^{99m}Tc has been widely used for the pharmacokinetic and biodistribution studies of many drugs and their delivery systems. Technetium is prepared by the following reaction from Uranium (^{235}U)

$$^{235}U \xrightarrow{\text{Irradiated with neutron flux}} {}^{99}Mo \longrightarrow {}^{99m}Tc$$

Common methods of separation of ^{99m} Technicium and ^{99} Molybdenum

1. Column Chromatography over acidic alumina
2. Solvent extraction of ^{99m}Tc with methyl ethyl ketone
3. Sublimination of Tc oxides from Mo compounds

The principle involved in the measurement of radioactivity is as follows: the gamma rays emitted by the isotopes enter a stainless steel casing and generate electrons, which are absorbed by the sodium iodide (NaI) crystal. The NaI crystal undergoes excitation and further de-excitation to produce a flash of light. This flash of light passes through an optically coupled photomultiplier tube. In the photomultiplier tube, the intensity of light is enhanced and passes through a pre-amplifier and linear amplifier and consequently to the pulse height analyzer. The signals are then tuned in a tuner and recorded in the recorder in case of gamma camera. The gamma camera is equipped with a scaler instead of recorder. In scaler, the signals are converted into digits in terms of counts.

Physical Properties of ^{99m}Technicium

^{99m}Tc decays by isomeric transition with the physical half life of 6.02 h. The principle photon useful for the detection and imaging studies is gamma-2 with the mean energy of 140.5keV. The specific gamma ray constant for ^{99m}Tc is 0.8R/mCi-hr at 1cm (5.58μCi/kg/hr/MBq at 1cm). The use of 2.5mm thickness of lead can effectively attenuate the radiation emitted by a factor of 1000.

Principles of radiolabeling of compounds with ^{99m}Tc

The majority of ^{99m}Tc compounds employ the stannous chloride reduction method, which makes use of the fact that stannous chloride is one of the most powerful reducing agent. ^{99m}Tc obtained from the Mo / Tc generator is in chemical form of TcO_4^-, or pertechnetate. While the anion has an overall negative charge of -1, the oxidation number of technetium is +7. The chelating agents commonly used to prepare ^{99m}Tc products are also anions with an overall negative charge due to the presence of N, O and P atoms, each of which has 1 or more extra pairs of electrons. These negative charges repel each other so pertechnetate will not form chelates. A reducing agent is therefore required to convert the ^{99m}Tc into an electropositive cationic form capable of binding to chelating agents. ^{99m}Tc sulfur colloid and ^{99m}Tc DMSA are the only two commercially available compounds that do not use the stannous reduction method. In the reaction, the stannous ion is the reducing agent, and therefore the substance oxidized, while pertechnetate is the oxidizing agent and therefore the substance reduced. Most soluble ^{99m}Tc compounds, excluding those containing a protein have octahedral structures and are said to be hexa coordinated since there are typical 6 binding sites available consisting of N, O, or P atoms.

9.2. Materials

Stannous chloride dehydrate (S_nCl_2. 2 H_2O) was purchase from Sigma Chemicals Co. (St. Louis, MO), Sodium pertechnetate, separated from Molybdenom-99 (99m) by solvent extraction method, was provided by Regional Centre for Radiopharmaceuticals Division (Northern Region) Board of Radiation and Isotope Technology (BRIT, Delhi, India).

9.3. Radiolabeling of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) formulations and Optimization

The radiolabeling of TZ solution, CBZ solution, LMC-TZ NPs, MMC-TZ NPs, LMTC-TZ NPs, MMTC-TZ NPs, LMTMC-TZ NPs, MMTMC-TZ NPs, LMC-CBZ NPs, MMC- CBZ NPs, LMTC- CBZ NPs, MMTC- CBZ NPs, LMTMC- CBZ NPs,

MMTMC- CBZ NPs was performed with ^{99m}Tc by direct labelling method (Richardson et. al, 1977, Babbar et al., 1991). Radiolabeled technetium in sodium pertechnetate was reduced in the acidic medium in the presence of stannous chloride. For carrying out the radiolabeling of the formulations, 1ml of drug solution and NPs dispersion were mixed with 50µl of stannous chloride solution (5mg/ml). The pH was adjusted to 6.5 with 0.5M sodium bicarbonate solution. Further, the preparation was incubated with 100 µl of freshly eluted ^{99m}Tc-pertechnetate solution (2 mCi) for 30minutes at room temperature. The quality control (percentage labeling efficiency and stability of the labelled complexes) was performed as described earlier. The labeling efficiency of ^{99m}Tc- TZ solution/CBZ solution/ LMC-TZ NPs/ MMC-TZ NPs/ LMTC-TZ NPs/ MMTC-TZ NPs/ LMTMC-TZ NPs/ MMTMC-TZ NPs/ LMC-CBZ NPs/ MMC- CBZ NPs/ LMTC- CBZ NPs/ MMTC- CBZ NPs/ LMTMC- CBZ NPs/ MMTMC- CBZ NPs was determined using ascending instant thin layer chromatography (ITLC) using silica gel (SG)-coated fibre glass sheets (Gelman Sciences Inc, Ann Arbor, MI). The ITLC was performed using acetone as the mobile phase. Approximately 2 to 3 µL of the radiolabeled complex was applied at a point 1 cm from one end of an ITLC-SG strip. The strip was eluted in acetone. The solvent front was allowed to reach 7-8 cm from the point of application. The strip was cut horizontally into 2 halves, and the radioactivity in each half was determined in a gamma ray counter (Gamma ray spectrometer, Captec-R, Capintec, USA). The free ^{99m}Tc-pertechnetate that moved with the solvent (R_f= 0.9) was determined.

The radiocolloids (reduced/hydrolyzed) technetium along with the labelled complex remained at the point of application. The amount of radiocolloids was determined using ITLC with pyridine: acetic acid: water (3:5:1.5 v/v) as mobile phase. The radiocolloids remained at the point of application, while both the free pertechnetate and the labelled complex moved away with the solvent front. The activity migrate using pyridine: acetic acid: water as a mixture was subtracted from that with the solvent front using acetone, the net amount of ^{99m}Tc- TZ solution/CBZ solution/ LMC-TZ NPs/ MMC-TZ NPs/ LMTC-TZ NPs/ MMTC-TZ NPs/ LMTMC-TZ NPs/ MMTMC-TZ NPs/ LMC-CBZ NPs/ MMC- CBZ NPs/ LMTC- CBZ NPs/ MMTC-CBZ NPs/ LMTMC- CBZ NPs/ MMTMC- CBZ NPs, was calculated.

The radiolabelling was optimized for incubation time and the concentration of $SnCl_2.2H_2O$. The pH of the solution and the formulations was maintained at around 6.5. 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 radiolabeled solution/NPs, one mL of the radiolabeled formulation was challenged against various concentrations (10, 30 and 50 mM) of diethylene triamine penta acetic acid (DTPA) (Babbar et al., 2000). The mixtures were incubated for 4 hours at 37°C and the labeling efficiency was measured using ITLC-SG; acetone and PAW system as mobile phase. Approximately 2 to 3 µL complex was applied at 1 cm distance on the ITLC-SG and mobile phase was allowed to run up to 8 cm from the point of application. The separated pertechnetate and DTPA complex was separated at migration value 0.90 ($R_f = 0.90$) while ^{99m}Tc-drug solution/NPs remained at the point of application ($R_f = 0$).

9.4. Biodistribution studies

All animal experiments conducted were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision on animals and experiments, Ministry of Government of India. Swiss albino mice weighing between 25-30gm were selected for biodistribution studies. Three mice were used at each time point for each formulation. ^{99m}Tc (technetium) labeled NPs and drug solutions were administered through intranasal route, intravenous and oral routes and drug brain targeting efficiency were compared among three route of administration. The animals were anaesthetized using ketamine injection prior to administration of radiolabeled formulations. The animals were held from back in slanted position and formulations (^{99m}Tc labelled-TZ solution (2 mCi/100 µL) equivalent to 0.0208 mg of TZ /mice and ^{99m}Tc-CBZ solution equivalent to 0.026 mg of CBZ/mice was injected through tail vein and orally in Swiss albino mice. Similarly, ^{99m}Tc- TZ loaded chitosan/ thiolated chitosan/trimethyl chitosan NPs (2 mCi/100 µL) equivalent to 0.0208 mg of TZ /mice and ^{99m}Tc-CBZ loaded chitosan/ thiolated chitosan/trimethyl chitosan NPs (2 mCi/100 µL) equivalent to 0.026 mg of CBZ/mice was administered intravenous, oral, and intranasal route in mice. Formulations were instilled into nostrils (10 µl in each nostril) with the help of micropipette (10µL to100µL) attached with low-density polyethylene tubing, having 0.1mm internal diameter at the delivery site. The animals were killed humanely at different time intervals and the blood collected using cardiac

puncture. Subsequently, brain and other tissues (liver, kidney, spleen, tail, stomach, intestine) were dissected, washed twice with normal saline to remove adhering tissue/fluid, and weighed (Vyas et al., 2006). Radioactivity present in each tissue/organ was measured using gamma scintillation counter. Radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose using equation:

$$\%\text{Radio activity/gm of tissue} = \frac{\text{Counts in sample X 100}}{\text{Wt. of sample X Total counts injected}}$$

The results of radioactivity in different organ and blood are recorded in Table 9.11 to 9.13. The pharmacokinetic parameters were derived from results of Table 9.11 to 9.13 and Figure 9.1 & 9.2 using Kinetica software is tabulated in Table 9.17. The degree of drug targeting to CSF after intranasal administration can be evaluated by the drug targeting index (DTI), which can be derive from the ratio of the value of AUC_{CSF}/AUC_{plasma} following intranasal administration to that following intravenous injection. The higher the DTI is the more degree of drug targeting to CSF can be expected after intranasal administration and DTI can be determine using equation:

$$DTI = \frac{(AUC_{\text{brain tissue}}/AUC_{\text{plasma}})_{i.n.}}{(AUC_{\text{brain tissue}}/AUC_{\text{plasma}})_{i.v,}}$$

Moreover, to understand the direct transfer of drug between nose and brain clearly after intranasal administration, drug targeting percentage (DTP) was introduced, the equations are as follows:

$$DTP\ (\%) = \frac{B_{i.n.} - B_x}{B_{i.n.}} X\ 100$$

$$\text{Where}\quad B_x = (B_{i.v.}/P_{i.v.}) * P_{i.n.}$$

Where, B_x = AUC_{CSF} fraction that contributed by systemic circulation through the blood–brain barrier (BBB) following intranasal delivery

$B_{i.v.}$ = AUC_{CSF} following intravenous administration

$P_{i.v.}$ = AUC_{plasma} after intravenous administration

$B_{i.n.}$ = AUC_{CSF} flowing intranasal delivery

$P_{i.n.}$ = AUC_{plasma} by intranasal administration.

The DTI and DTP (%) of TZ/CBZ formulations are recorded in Table 9.18.

Statistical differences between i.n. and i.v. administration were concluded using the unpaired Student's t-test and a value of $P < 0.05$ was considered statistically significant. Results were presented as mean values ± SD.

9.5. Gamma scintigraphy imaging

The animals (Swiss albino mice) were anaesthetized using ketamine injection prior to administration of radiolabeled formulations. The animals were held from back in slanted position and formulations (^{99m}Tc-TZ solution (2 mCi/100 µL) equivalent to 0.0208 mg of TZ /mice and ^{99m}Tc-CBZ HCl solution equivalent to 0.026 mg of CBZ/mice was injected through tail vein and orally in Swiss albino mice. Similarly, radiolabeled complex of ^{99m}Tc-TZ HCl loaded chitosan/ thiolated chitosan/trimethyl chitosan NPs (2 mCi/100 µL) equivalent to 0.0208 mg of TZ /mice and ^{99m}Tc-CBZ loaded chitosan/ thiolated chitosan/trimethyl chitosan NPs (2 mCi/100 µL) equivalent to 0.026 mg of CBZ/mice were administered via intravenous, oral and intranasal route in mice. Formulations were instilled into nostrils (10 µl in each nostril) with the help of micropipette (10µL to100µL) attached with low-density polyethylene tubing, having 0.1mm internal diameter at the delivery site. Then, the anaesthetized animals were placed on board and images were captured using gamma camera. Imaging was performed using Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens AG; Erlanger, Germany) gamma camera. (Vyas et al., 2006). Figure 9.2 shows the gamma scintigraphy imaging of TZ/CBZ solution and TZ/CBZ loaded chitosan/thiolated chitosan/trimethyl chitosan NPs following intravenous, oral and intranasal administrations.

9.6. Statistical analysis

All data reported as mean±SD and the difference between the groups were tested using Student's t test at the level of $P<0.05$.

9.7. Results

9.7.1. Radiolabeling of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) formulations and Optimization

Table 9.1: Influence of incubation time on the labeling efficiency of ^{99m}Tc-tizanidine HCl formulations

Incubation time (minutes)	% Radiolabeling						
	^{99m}Tc- TZ solution	^{99m}Tc-LMC-TZ NPs	^{99m}Tc-MMC-TZ NPs	^{99m}Tc-LMTC-TZ NPs	^{99m}Tc-MMTC-TZ NPs	^{99m}Tc-LMTMC-TZ NPs	^{99m}Tc-MMTMC-TZ NPs
0	90.21± 1.3	85.12 ± 3.1	92.34± 1.7	90.98 ± 3.9	93.43± 1.9	88.33 ± 3.9	92.30 ± 1.7
10	93.09 ± 2.2	90.54 ± 1.1	94.43 ± 2.9	95.54 ± 3.2	94.12 ± 2.9	92.51 ± 3.4	93.66 ± 1.8
15	94.21 ± 1.6	92.09 ± 2.3	95.89 ± 1.5	96.45 ± 2.1	97.65 ± 1.4	96.98 ± 2.9	96.21 ± 1.1
30	**97.00 ± 1.3**	**97.5 ± 1.2**	**97.9±1.3**	**99.2±2.8**	**98.5±3.1**	**99.1±2.1**	**98.89 ± 2.4**
60	96.78 ± 1.9	96.9 ± 1.9	96.5±1.7	98.6±1.7	97.8±2.8	98.7±1.8	97.25 ± 2.1

(Mean ± S.D., n = 3)

Table 9.2: Influence of incubation time on the labeling efficiency of ^{99m}Tc-cyclobenzaprine HCl formulations

Incubation time (minutes)	% Radiolabeling						
	^{99m}Tc- CBZ solution	^{99m}Tc-LMC-CBZ NPs	^{99m}Tc-MMC- CBZ NPs	^{99m}Tc-LMTC-CBZ NPs	^{99m}Tc-MMTC-CBZ NPs	^{99m}Tc-LMTMC-CBZ NPs	^{99m}Tc-MMTMC-CBZ NPs
0	91.56± 1.8	89.33 ± 2.2	91.34± 2.7	87.45 ± 3.1	86.33± 2.9	93.49 ± 1.4	91.78 ± 2.1
10	94.65 ± 2.9	94.14 ± 1.9	93.56 ± 2.9	93.89 ± 2.2	91.44 ± 2.4	96.52 ± 3.2	94.67 ± 2.9
15	97.11 ± 2.7	96.12 ± 1.8	97.32 ± 2.8	95.33 ± 1.6	94.98 ± 2.9	98.46 ± 2.1	97.21 ± 3.2
30	**98.12 ± 2.8**	**98.89 ± 1.9**	**99.32±3.8**	**98.59±2.1**	**97.99± 2.1**	**99.21±2.9**	**98.99 ± 3.4**
60	97.67 ± 1.3	97.8 ± 2.9	98.11±2.9	97.56±2.4	96.32±2.3	98.43±2.2	97.48 ± 2.7

(Mean ± S.D., n = 3)

Table 9.3: Influence of the amount of Stannous Chloride on the Labelling Efficiency of ^{99m}Tc-tizanidine HCl formulations

Amount of Stannous chloride (µg)	% Radiolabeling						
	^{99m}Tc- TZ solution	^{99m}Tc-LMC-TZ NPs	^{99m}Tc-MMC-TZ NPs	^{99m}Tc-LMTC-TZ NPs	^{99m}Tc-MMTC-TZ NPs	^{99m}Tc-LMTMC-TZ NPs	^{99m}Tc-MMTMC-TZ NPs
150	91.55± 1.6	92.17 ± 3.6	92.21± 1.5	91.11 ± 2.9	94.55± 1.3	89.25 ± 3.1	93.44 ± 1.5
250	**97.11 ± 1.5**	**97.33 ± 1.9**	**97.45±1.8**	**99.17±2.1**	**98.37±2.5**	**99.54±3.2**	**98.74 ± 3.4**
350	91.32 ± 1.9	92.78 ± 2.7	95.11 ± 1.4	95.45 ± 2.7	96.63 ± 1.8	97.88 ± 2.2	96.77 ± 1.9

(Mean ± S.D., n = 3)

Table 9.4: Influence of the amount of Stannous Chloride on the Labelling Efficiency of ^{99m}Tc-cyclobenzaprine HCl formulations

Amount of Stannous chloride (µg)	% Radiolabeling						
	^{99m}Tc- CBZ solution	^{99m}Tc-LMC-CBZ NPs	^{99m}Tc-MMC-CBZ NPs	^{99m}Tc-LMTC-CBZ NPs	^{99m}Tc-MMTC-CBZ NPs	^{99m}Tc-LMTMC-CBZ NPs	^{99m}Tc-MMTMC-CBZ NPs
150	90.16± 1.5	9-.42 ± 1.2	92.32± 1.6	89.66 ± 2.5	91.21± 1.4	94.33 ± 1.8	93.68 ± 3.1
250	**98.32 ± 2.3**	**98.67 ± 2.3**	**99.14±2.8**	**98.43±1.8**	**97.59± 2.6**	**99.01±2.3**	**98.76 ± 2.7**
350	97.71 ± 1.7	97.14 ± 2.4	98.90 ± 1.8	96.32 ± 2.5	96.48 ± 1.3	97.36 ± 3.2	96.98 ± 2.5

(Mean ± S.D., *n* = 3)

Table 9.5: *In-vitro* stability of ^{99m}Tc-tizanidine HCl formulations in 0.9% w/v sodium chloride

Time (hrs)	% Radiolabeling						
	^{99m}Tc- TZ solution	^{99m} Tc-LMC-TZ NPs	^{99m} Tc-MMC-TZ NPs	^{99m} Tc-LMTC-TZ NPs	^{99m} Tc-MMTC-TZ NPs	^{99m} Tc-LMTMC-TZ NPs	^{99m} Tc-MMTMC-TZ NPs
05	91.31± 1.5	86.23 ±2.2	91.54± 2.7	88.32 ± 1.9	91.44± 3.2	89.89 ± 2.4	90.76 ± 2.9
1.5	**97.13 ± 2.3**	**97.34 ± 1.6**	**97.67±1.9**	**99.33±2.8**	**98.78±4.2**	**99.34±2.9**	**98.78 ± 3.1**
3	94.43 ± 2.7	93.26 ± 1.7	95.49 ± 2.3	95.49 ± 1.1	96.22 ± 2.3	96.87 ± 4.3	97.21 ± 1.4
5	92.13 ± 2.3	91.45 ± 1.9	92.99±4.1	92.54±2.2	93.21±2,9	93.54±1.9	95.89 ± 2.7
24	90.32 ± 1.9	89.43 ± 3.2	90.33±2.9	87.87±2.4	91.89±3.2	90.79±2.1	91.25 ± 2.9

(Mean ± S.D., *n* = 3)

Table 9.6: *In-vitro* stability of ^{99m}Tc-tizanidine HCl formulations in mice serum at 37°C

Time (hrs)	% Radiolabeling						
	^{99m}Tc- TZ solution	^{99m} Tc-LMC-TZ NPs	^{99m} Tc-MMC-TZ NPs	^{99m} Tc-LMTC-TZ NPs	^{99m} Tc-MMTC-TZ NPs	^{99m} Tc-LMTMC-TZ NPs	^{99m} Tc-MMTMC-TZ NPs
05	89.56± 2.1	87.21± 2.4	91.43± 1.6	89.22± 2.1	88.12± 2.7	91.19 ± 2.3	92.58 ± 3.2
1.5	**98.32 ± 3.2**	**98.56 ± 2.7**	**99.72±3.2**	**98.69±3.3**	**97.56± 2.4**	**99.43±2.1**	**98.59 ± 3.1**
3	97.87 ±2.5	95.16 ± 1.9	96.33 ± 2.6	96.67 ± 2.5	93.18 ± 2.1	97.16 ± 2.5	94.11 ± 2.2
5	94.65 ± 2.9	92.39 ± 1.4	92.39±2.8	93.11±3.2	91.43± 2.8	94.51±2.3	92.96± 2.4
24	91.67 ± 1.8	90.65 ±1.5	89.32±1.7	90.23±2.7	89.12±2.4	90.93±2.9	87.88 ± 2.1

(Mean ± S.D., *n* = 3)

Table 9.7: *In-vitro* stability of ^{99m}Tc-cyclobenzaprine HCl formulations in 0.9%w/v sodium chloride

Time (hrs)	% Radiolabeling						
	^{99m}Tc- CBZ solution	^{99m}Tc-LMC-CBZ NPs	^{99m}Tc-MMC-CBZ NPs	^{99m}Tc-LMTC-CBZ NPs	^{99m}Tc-MMTC-CBZ NPs	^{99m}Tc-LMTMC-CBZ NPs	^{99m}Tc-MMTMC-CBZ NPs
05	89.32± 1.9	87.43 ± 2.4	91.67± 2.3	89.32 ± 2.8	91.54± 3.2	87.61 ± 1.6	91.65 ± 3.1
1.5	**97.12 ± 2.3**	**97.78 ± 2.9**	**97.76±3.1**	**99.43±2.1**	**98.79±2.6**	**99.56±2.8**	**98.66 ± 2.9**
3	93.71 ± 1.7	93.76 ± 2.7	94.19 ± 2.6	95.76 ± 2.9	95.25 ± 3.1	95.77 ±1.6	94.32 ± 2.3
5	90.55 ± 2.5	91.23 ± 1.8	91.66±3.4	93.29±1.6	92.44±2.5	91.53±2.3	91.56 ± 2.1
24	86.89± 1.4	88.32±2.6	90.15±1.9	90.48±1.4	90.12±2.9	88.91±2.6	87.21 ± 2.9

(Mean ± S.D., n = 3)

Table 9.8: *In-vitro* stability of ^{99m}Tc- cyclobenzaprine HCl formulations in mice serum at 37°C

Time (hrs)	% Radiolabeling						
	^{99m}Tc- CBZ solution	^{99m}Tc-LMC-CBZ NPs	^{99m}Tc-MMC-CBZ NPs	^{99m}Tc-LMTC-CBZ NPs	^{99m}Tc-MMTC-CBZ NPs	^{99m}Tc-LMTMC-CBZ NPs	^{99m}Tc-MMTMC-CBZ NPs
05	89.21± 3.2	90.41 ± 2.6	90.56 ± 2.2	89.32 ± 2.1	88.43± 1.8	91.21 ± 1.9	90.43 ±3.3
1.5	**98.34 ± 2.1**	**98.56 ± 1.3**	**99.45±3.1**	**98.67±2.5**	**97.43± 3.1**	**99.78±2.4**	**98.75 ± 3.1**
3	95.21 ± 2.8	94.88 ± 1.7	95.38 ± 2.2	96.89 ±3.6	94.23 ± 2.1	95.90 ± 1.1	96.34 ± 2.9
5	92.19 ± 3.1	91.82 ± 2.3	93.11±3.2	92.22±1.5	91.45± 2.8	93.67±2.3	92.12 ±2.5
24	90.83 ± 2.1	90.32 ± 2.6	89.23±2.4	91.45±2.8	89.67±2.7	88.56±2.7	90.23 ± 2.1

(Mean ± S.D., n = 3)

Table 9.9: Effect of Variable molar concentration of DTPA on radiolabeled ^{99m}Tc-tizanidine HCl formulations

DTPA concentration (mM)	% Transchelation						
	^{99m}Tc- TZ solution	^{99m} Tc-LMC-TZ NPs	^{99m} Tc-MMC-TZ NPs	^{99m} Tc-LMTC-TZ NPs	^{99m} Tc-MMTC-TZ NPs	^{99m} Tc-LMTMC-TZ NPs	^{99m} Tc-MMTMC-TZ NPs
10	1.21±0.10	1.83±0.18	1.43±0.16	1.32±0.14	1.98±0.19	1.87±0.11	1.62±0.20
30	2.78±0.24	2.29±0.31	2.87±0.28	2.76±0.25	2.43±0.24	2.54±0.29	2.87±0.27
50	3.22±0.43	3.24±0.47	3.29±0.37	3.13±0.39	3.33±0.38	3.39±0.41	3.32±0.36

(Mean ± S.D., n = 3)

Table 9.10: Effect of Variable molar concentration of DTPA on radiolabeled ^{99m}Tc-cyclobenzaprine HCl formulations

DTPA concentration (mM)	% Transchelation						
	^{99m}Tc- CBZ solution	^{99m}Tc-LMC-CBZ NPs	^{99m}Tc-MMC-CBZ NPs	^{99m}Tc-LMTC-CBZ NPs	^{99m}Tc-MMTC-CBZ NPs	^{99m}Tc-LMTMC-CBZ NPs	^{99m}Tc-MMTMC-CBZ NPs
10	1.13±0.14	1.78±0.19	1.45±0.14	1.33±0.17	1.65±0.11	1.44±0.22	1.23±0.18
30	2.56±0.23	2.87±0.21	2.54±0.28	2.87±0.22	2.73±0.27	2.69±0.32	2.19±0.29
50	3.14±0.45	3.23±0.39	3.31±0.34	3.45±0.38	3.21±0.33	3.11±0.41	3.35±0.35

(Mean ± S.D., n = 3)

9.7.2. Biodistribution studies

Table 9.11: Tissue/organ distributions of ^{99m}TC-TZ/CBZ solution in swiss albino mice at predetermine time intervals of intravenous, oral and intranasal administration

Time (hrs)	Blood	Liver	Spleen	Kidney	Stomach	Intestine	Brain
Intravenous administration of ^{99m}TC -Tizanidine HCl solution							
0.5	13.21±1.1	25.42±2.1	14.32±1.1	7.34±0.760	1.14±0.211	0.789±0.065	0.010±0.001
1	10.59±1.3	33.57±3.1	19.54±1.1	9.85±1.5	1.28±0.123	0.969±0.078	0.068±0.003
2	5.67±0.983	43.72±3.2	29.56±1.1	11.39±1.9	1.48±0.145	1.35±0.127	0.189±0.033
4	3.70±0.656	22.7±2.1	78.98±2.1	9.04±1.1	0.879±0.078	1.08±0.099	0.088±0.013
8	1.47±0.214	8.93±1.1	43.98±2.1	4.71±0.973	0.321±0.034	1.09±0.124	0.006±0.001
Oral administration of ^{99m}TC -Tizanidine HCl solution							
0.5	0.045±0.001	2.67±0.211	0.021±0.003	0.087±0.003	23.34±1.2	2.32±0.221	0.020±0.002
1	0.323±0.033	3.89±0.234	0.387±0.044	0.566±0.048	49.87±2.3	46.98±1.5	0.037±0.001
2	0.569±0.043	5.78±0.456	0.684±0.065	1.123±0.111	77.72±2.5	24.90±1.1	0.034±0.003
4	0.289±0.014	6.90±0.567	0.562±0.032	0.705±0.045	62.12±3.1	2.56±0.245	0.088±0.007
8	0.089±0.011	3.89±0.112	0.070±0.006	0.140±0.011	33.20±1.4	1.90±0.231	0.010±0.001
Intranasal administration of ^{99m}TC -Tizanidine HCl solution							
0.5	0.965±0.078	0.087±0.001	0.002±0.001	0.234±0.022	0.119±0.021	0.131±0.012	0.053±0.002
1	1.98±0.212	0.092±0.003	0.256±0.067	0.341±0.056	0.346±0.025	0.211±0.023	0.589±0.033
2	3.90±0.321	0.415±0.033	0.543±0.054	0.521±0.043	0.499±0.039	0.278±0.033	0.347±0.027
4	2.78±0.342	0.345±0.045	0.467±0.043	0.632±0.034	0.436±0.026	0.468±0.047	0.201±0.029
8	0.976±0.078	0.134±0.005	0.017±0.002	0.245±0.021	0.203±0.018	0.219±0.032	0.035±0.003
Intravenous administration of ^{99m}TC- Cyclobenzaprine HCl solution							
0.5	3.04±0.123	29.35±1.2	17.63±2.1	1.11±0.222	0.343±0.041	0.191±0.013	0.099±0.007
1	2.36±0.111	49.78±1.8	28.10±1.6	1.28±0.128	0.693±0.053	0.263±0.028	0.181±0.019
2	2.22±0.113	69.82±2.4	34.90±2.1	3.10±0.203	0.815±0.076	0.883±0.043	0.256±0.014
4	2.02±0.189	47.90±2.1	89.43±4.1	9.31±1.2	1.495±0.124	0.780±0.051	0.241±0.022
8	1.88±0.154	30.64±2.1	42.48±2.7	0.481±0.065	0.454±0.034	0.435±0.043	0.201±0.018
Oral administration of ^{99m}TC -Cyclobenzaprine HCl solution							
0.5	0.234±0.022	1.98±0.123	0.479±0.45	0.572±0.026	57.43±2.4	7.31±0.987	0.119±0.013
1	0.976±0.074	2.78±0.178	2.17±0.121	0.351±0.039	69.65±2.8	85.15±2.6	0.143±0.016
2	1.78±0.221	4.98±0.236	12.31±1.5	9.11±1.3	90.32±3.9	29.57±1.2	0.178±0.020
4	1.57±0.201	5.45±0.289	1.60±0.232	0.631±0.034	72.54±3.4	3.89±0.234	0.159±0.023
8	1.41±0.167	2.84±0.211	0.231±0.032	0.241±0.022	50.39±2.1	2.34±0.134	0.141±0.011
Intranasal administration of ^{99m}TC -Cyclobenzaprine HCl solution							
0.5	0.235±0.021	0.054±0.001	0.086±0.005	0.282±0.034	0.134±0.017	0.129±0.012	0.154±0.010
1	0.346±0.032	0.099±0.005	1.19±0.143	0.366±0.043	0.387±0.038	0.201±0.018	0.344±0.033
2	0.678±0.045	0.107±0.009	0.847±0.087	0.562±0.021	0.501±0.055	0.289±0.024	0.299±0.042
4	0.964±0.078	0.063±0.004	4.62±0.321	0.615±0.054	0.399±0.047	0.498±0.037	0.263±0.023
8	0.564±0.066	0.043±0.001	0.246±0.066	0.267±0.036	0.233±0.031	0.231±0.021	0.212±0.036

(Mean ± S.D., $n = 3$)

Table 9.12: Tissue/organ distributions of ^{99m}TC-LMC-TZ NPs/MMC-TZ NPs/LMTC-TZ NPs/MMTC-TZ NPs/LMTMC-TZ NPs/MMTMC-TZ NPs in swiss albino mice at predetermine time intervals of intranasal administration

Time (hrs)	Blood	Liver	Spleen	Kidney	Stomach	Intestine	Brain
Intranasal administration of ^{99m}TC-LMC-TZ NPs							
0.5	0.678±0.012	0.089±0.007	0.014±0.001	0.237±0.051	0.112±0.003	0.129±0.043	0.743±0.093
1	1.71±0.0.16	0.276±0.039	0.261±0.094	0.339±0.065	0.301±0.075	0.209±0.029	0.957±0.063
2	3.76±0.212	0.395±0.032	0.539±0.054	0.523±0.054	0.401±0.045	0.281±0.031	0.689±0.035
4	2.67±0.301	0.183±0.015	0.458±0.088	0.627±0.078	0.387±0.032	0.465±0.043	0.564±0.047
8	0.997±0.154	0.088±0.009	0.013±0.002	0.238±0.043	0.189±0.008	0.211±0.021	0.321±0.032
Intranasal administration of ^{99m}TC-MMC-TZ Ps							
0.5	0.845±0.088	0.078±0.007	0.021±0.004	0.221±0.031	0.118±0.009	0.117±0.13	0.458±0.054
1	1.36±0.123	0.289±0.043	0.245±0.034	0.332±0.043	0.295±0.054	0.201±0.022	0.876±0.054
2	2.45±0.152	0.389±0.032	0.551±0.076	0.511±0.065	0.399±0.035	0.254±0.054	0.634±0.036
4	2.23±0.167	0.171±0.015	0.444±0.043	0.621±0.043	0.367±0.027	0.438±0.032	0.532±0.013
8	0.646±0.076	0.077±0.009	0.021±0.005	0.231±0.015	0.178±0.012	0.203±0.012	0.312±0.021
Intranasal administration of ^{99m}TC-LMTMC-TZ NPs							
0.5	0.678±0.032	0.058±0.002	0.004±0.001	0.225±0.032	0.109±0.021	0.132±0.014	1.11±0.127
1	0.986±0.045	0.234±0.032	0.239±0.021	0.321±0.041	0.313±0.056	0.199±0.032	1.89±0.156
2	1.34±0.178	0.344±0.054	0.539±0.087	0.524±0.043	0.407±0.047	0.267±0.045	1.19±0.132
4	0.896±0.021	0.179±0.021	0.467±0.076	0.621±0.056	0.337±0.035	0.456±0.057	0.954±0.078
8	0.233±0.016	0.074±0.008	0.006±0.001	0.219±0.022	0.176±0.015	0.209±0.033	0.589±0.054
Intranasal administration of ^{99m}TC-MMTMC-TZ NPs							
0.5	0.598±0.065	0.068±0.004	0.010±0.001	0.209±0.011	0.116±0.018	0.125±0.019	1.05±0.102
1	0.954±0.022	0.267±0.053	0.249±0.022	0.331±0.032	0.297±0.043	0.209±0.023	1.67±0.108
2	1.22±0.121	0.380±0.065	0.521±0.043	0.515±0.061	0.401±0.065	0.243±0.065	1.13±0.118
4	0.843±0.044	0.165±0.010	0.451±0.054	0.619±0.055	0.332±0.023	0.412±0.046	0.998±0.098
8	0.567±0.053	0.089±0.002	0.015±0.001	0.217±0.021	0.167±0.012	0.204±0.043	0.567±0.061
Intranasal administration of ^{99m}TC-LMTC-TZ NPs							
0.5	0.678±0.078	0.069±0.005	0.011±0.002	0.217±0.033	0.098±0.008	0.116±0.022	1.21±0.111
1	1.10±0.131	0.240±0.056	0.238±0.021	0.313±0.025	0.289±0.021	0.216±0.029	1.72±0.156
2	1.32±0.109	0.358±0.032	0.532±0.067	0.508±0.037	0.398±0.054	0.265±0.045	1.23±0.134
4	0.921±0.043	0.168±0.008	0.432±0.054	0.599±0.067	0.312±0.032	0.438±0.034	1.09±0.121
8	0.256±0.026	0.067±0.003	0.009±0.001	0.198±0.013	0.176±0.017	0.179±0.022	0.654±0.078
Intranasal administration of ^{99m}TC-MMTC-TZ NPs							
0.5	0.664±0.044	0.062±0.003	0.008±0.001	0.201±0.019	0.107±0.018	0.114±0.011	1.29±0.232
1	0.997±0.031	0.209±0.031	0.233±0.021	0.320±0.054	0.265±0.065	0.206±0.035	1.67±0.221
2	1.28±0.133	0.332±0.044	0.511±0.054	0.505±0.033	0.387±0.043	0.231±0.022	1.18±0.098
4	0.911±0.089	0.148±0.021	0.429±0.076	0.611±0.032	0.321±0.041	0.408±0.021	1.10±0.087
8	0.239±0.019	0.040±0.006	0.012±0.001	0.179±0.021	0.178±0.019	0.167±0.033	0.632±0.062

(Mean ± S.D., n = 3)

Table 9.13: Tissue/organ distributions of ^{99m}TC-LMC-CBZ NPs/MMC-CBZ NPs/LMTC-CBZ NPs/MMTC-CBZ NPs/LMTMC-CBZ NPs/MMTMC-CBZ NPs in swiss albino mice at predetermine time intervals of intranasal administration

Time (hrs)	Blood	Liver	Spleen	Kidney	Stomach	Intestine	Brain
Intranasal administration of ^{99m}TC-LMC- CBZ NPs							
0.5	0.645±0.28	0.078±0.006	0.079±0.006	0.214±0.024	0.122±.011	0.127±0.012	0.698±0.078
1	0.886±0.096	0.258±0.013	1.19±0.212	0.347±0.043	0.365±0.034	0.198±0.016	0.936±0.065
2	0.987±0.074	0.377±0.025	0.836±0.065	0.566±0.056	0.479±0.045	0.278±0.29	0.835±0.047
4	0.931±0.065	0.179±0.021	4.56±0.456	0.621±0.078	0.365±0.038	0.476±0.032	0.789±0.077
8	0.814±0.045	0.068±0.006	0.239±0.043	0.216±0.021	0.211±0.012	0.219±0.013	0.674±0.032
Intranasal administration of ^{99m}TC-MMC- CBZ Ps							
0.5	0.634±0.034	0.065±0.003	0.068±0.007	0.211±0.014	0.118±0.010	0.120±0.016	0.665±0.054
1	0.843±0.078	0.245±0.034	1.12±0.234	0.351±0.023	0.346±0.030	0.187±0.017	0.925±0.098
2	0.967±0.084	0.367±0.045	0.821±0.056	0.537±0.037	0.456±0.039	0.265±0.028	0.814±0.089
4	0.869±0.067	0.159±0.011	4.32±0.654	0.612±0.045	0.328±0.041	0.461±0.025	0.720±0.065
8	0.808±0.065	0.057±0.002	0.230±0.022	0.204±0.021	0.209±0.013	0.206±0.012	0.648±0.049
Intranasal administration of ^{99m}TC-LMTMC- CBZ NPs							
0.5	0.656±0.047	0.081±0.009	0.062±0.034	0.221±0.018	0.115±0.018	0.113±0.012	0.865±0.067
1	0.888±0.078	0.265±0.033	1.10±0.111	0.340±0.024	0.345±0.029	0.201±0.017	1.98±0.201
2	0.956±0.097	0.367±0.041	0.831±0.056	0.571±0.036	0.463±0.049	0.245±0.032	1.55±0.167
4	0.776±0.081	0.189±0.012	4.48±0.690	0.607±0.049	0.334±0.043	0.451±0.067	1.41±0.145
8	0.745±0.068	0.071±0.005	0.225±0.027	0.217±0.011	0.206±0.021	0.204±0.019	1.25±0.115
Intranasal administration of ^{99m}TC-MMTMC- CBZ NPs							
0.5	0.632±0.051	0.770±0.067	0.052±0.002	0.208±0.019	0.113±0.007	0.109±0.019	0.845±0.091
1	0.865±0.073	0.248±0.023	1.13±0.245	0.356±0.028	0.331±0.035	0.171±0.016	1.87±0.113
2	0.949±0.082	0.339±0.034	0.829±0.057	0.519±0.034	0.452±0.048	0.243±0.033	1.59±0.187
4	0.775±0.089	0.162±0.013	4.39±0.278	0.602±0.067	0.321±0.036	0.443±0.028	1.45±0.154
8	0.732±0.034	0.066±0.007	0.221±0.022	0.197±0.021	0.213±0.024	0.198±0.020	1.29±0.131
Intranasal administration of ^{99m}TC-LMTC- CBZ NPs							
0.5	0.645±0.047	0.071±0.004	0.062±0.005	0.213±0.011	0.126±0.010	0.116±0.009	0.888±0.078
1	0.899±0.099	0.256±0.021	1.11±0.189	0.329±0.25	0.378±0.038	0.213±0.028	2.103±0.212
2	0.959±0.076	0.355±0.035	0.818±0.078	0.532±0.038	0.456±0.049	0.231±0.036	1.89±0.231
4	0.798±0.065	0.178±0.019	4.21±0.345	0.619±0.056	0.357±0.032	0.432±0.043	1.77±0.236
8	0.765±0.034	0.064±0.006	0.218±0.032	0.202±0.018	0.217±0.022	0.178±0.027	1.55±0.121
Intranasal administration of ^{99m}TC-MMTC- CBZ NPs							
0.5	0.678±0.044	0.064±0.003	0.057±0.05	0.205±0.022	0.123±0.011	1.25±0.122	0.891±0.064
1	0.887±0.073	0.234±0.014	1.12±0.212	0.322±0.034	0.342±0.029	1.54±0.201	1.76±0.267
2	0.941±0.081	0.349±0.026	0.811±0.059	0.508±0.046	0.465±0.056	1.29±0.212	1.71±0.234
4	0.785±0.069	0.156±0.012	4.17±0.345	0.616±0.056	0.376±0.034	1.11±0.211	1.65±0.217
8	0.768±0.054	0.045±0.004	0.209±0.021	0.167±0.016	0.245±0.018	0.687±0.055	1.41±0.157

(Mean ± S.D., n = 3)

Table 9.14: Blood/brain ratio of ^{99m}TC-TZ/CBZ solution in swiss albino mice at predetermine time intervals of intravenous, oral and intranasal administration

Time (hrs)	Blood	Brain	Blood/Brain ratio
Intravenous administration of ^{99m}TC -Tizanidine HCl solution			
0.5	13.21±1.1	0.010±0.001	0.000757002
1	10.59±1.3	0.068±0.003	0.006421152
2	5.67±0.983	0.189±0.033	0.033333333
4	3.70±0.656	0.088±0.013	0.023783784
8	1.47±0.214	0.006±0.001	0.004081633
Oral administration of ^{99m}TC -Tizanidine HCl solution			
0.5	0.045±0.001	0.020±0.002	0.444444444
1	0.323±0.033	0.037±0.001	0.114551084
2	0.569±0.043	0.034±0.003	0.059753954
4	0.289±0.014	0.088±0.007	0.30449827
8	0.089±0.011	0.010±0.001	0.112359551
Intranasal administration of ^{99m}TC -Tizanidine HCl solution			
0.5	0.965±0.078	0.053±0.002	0.05492228
1	1.98±0.212	0.589±0.033	0.297474747
2	3.90±0.321	0.347±0.027	0.088974359
4	2.78±0.342	0.201±0.029	0.072302158
8	0.976±0.078	0.035±0.003	0.035860656
Intravenous administration of ^{99m}TC - Cyclobenzaprine HCl solution			
0.5	3.04±0.123	0.099±0.007	0.032565789
1	2.36±0.111	0.181±0.019	0.076694915
2	2.22±0.113	0.256±0.014	0.115315315
4	2.02±0.189	0.241±0.022	0.119306931
8	1.88±0.154	0.201±0.018	0.106914894
Oral administration of ^{99m}TC - Cyclobenzaprine HCl solution			
0.5	0.234±0.022	0.119±0.013	0.508547009
1	0.976±0.074	0.143±0.016	0.146516393
2	1.78±0.221	0.178±0.020	0.1
4	1.57±0.201	0.159±0.023	0.101273885
8	1.41±0.167	0.141±0.011	0.1
Intranasal administration of ^{99m}TC -Cyclobenzaprine HCl solution			
0.5	0.235±0.021	0.154±0.010	0.655319149
1	0.346±0.032	0.344±0.033	0.994219653
2	0.678±0.045	0.299±0.042	0.44100295
4	0.964±0.078	0.263±0.023	0.272821577
8	0.564±0.066	0.212±0.036	0.375886525

(Mean ± S.D., n = 3)

Table 9.15: Blood/brain ratio of ^{99m}TC-LMC-TZ NPs/MMC-TZ NPs/LMTC-TZ NPs/MMTC-TZ NPs/LMTMC-TZ NPs/MMTMC-TZ NPs in swiss albino mice at predetermine time intervals of intranasal administration

Time (hrs)	Blood	Brain	Blood/Brain ratio
Intranasal administration of ^{99m}TC –LMC-TZ NPs			
0.5	0.678±0.012	0.743±0.093	1.095870206
1	1.71±0.0.16	0.957±0.063	0.559649123
2	3.76±0.212	0.689±0.035	0.183244681
4	2.67±0.301	0.564±0.047	0.211235955
8	0.997±0.154	0.321±0.032	0.321965898
Intranasal administration of ^{99m}TC –MMC-TZ NPs			
0.5	0.845±0.088	0.458±0.054	0.542011834
1	1.36±0.123	0.876±0.054	0.644117647
2	2.45±0.152	0.634±0.036	0.25877551
4	2.23±0.167	0.532±0.013	0.238565022
8	0.646±0.076	0.312±0.021	0.482972136
Intranasal administration of ^{99m}TC –LMTC-TZ NPs			
0.5	0.678±0.078	1.21±0.111	1.784660767
1	1.10±0.131	1.72±0.156	1.563636364
2	1.32±0.109	1.23±0.134	0.931818182
4	0.921±0.043	1.09±0.121	1.1834962
8	0.256±0.026	0.654±0.078	2.5546875
Intranasal administration of ^{99m}TC – MMTC-TZ NPs			
0.5	0.664±0.044	1.29±0.232	1.942771084
1	0.997±0.031	1.67±0.221	1.675025075
2	1.28±0.133	1.18±0.098	0.921875
4	0.911±0.089	1.10±0.087	1.207464325
8	0.239±0.019	0.632±0.062	2.644351464
Intranasal administration of ^{99m}TC – LMTMC-TZ NPs			
0.5	0.678±0.032	1.11±0.127	1.637168142
1	0.986±0.045	1.89±0.156	1.9168357
2	1.34±0.178	1.19±0.132	0.888059701
4	0.896±0.021	0.954±0.078	1.064732143
8	0.233±0.016	0.589±0.054	2.527896996
Intranasal administration of ^{99m}TC –MMTMC-TZ NPs			
0.5	0.598±0.065	1.05±0.102	1.755852843
1	0.954±0.022	1.67±0.108	1.750524109
2	1.22±0.121	1.13±0.118	0.926229508
4	0.843±0.044	0.998±0.098	1.183867141
8	0.567±0.053	0.567±0.061	1

(Mean ± S.D., n = 3)

Table 9.16: Blood/brain ratio of ^{99m}TC-LMC-CBZ NPs/MMC- CBZ NPs/LMTC-CBZ NPs/MMTC- CBZ NPs/LMTMC- CBZ NPs/MMTMC- CBZ NPs in swiss albino mice at predetermine time intervals of intranasal administration.

Time (hrs)	Blood	Brain	Blood/Brain ratio
Intranasal administration of ^{99m}TC –LMC- CBZ NPs			
0.5	0.645±0.28	0.698±0.078	1.08217054
1	0.886±0.096	0.936±0.065	1.05643341
2	0.987±0.074	0.835±0.047	0.84599797
4	0.931±0.065	0.789±0.077	0.84747583
8	0.814±0.045	0.674±0.032	0.82800983
Intranasal administration of ^{99m}TC –MMC- CBZ NPs			
0.5	0.634±0.034	0.665±0.054	1.0488959
1	0.843±0.078	0.925±0.098	1.09727165
2	0.967±0.084	0.814±0.089	0.8417787
4	0.869±0.067	0.720±0.065	0.82853855
8	0.808±0.065	0.648±0.049	0.8019802
Intranasal administration of ^{99m}TC –LMTC- CBZ NPs			
0.5	0.645±0.047	0.888±0.078	1.37674419
1	0.899±0.099	2.103±0.212	2.33926585
2	0.959±0.076	1.89±0.231	1.97080292
4	0.798±0.065	1.77±0.236	2.21804511
8	0.765±0.034	1.55±0.121	2.02614379
Intranasal administration of ^{99m}TC – MMTC- CBZ NPs			
0.5	0.678±0.044	0.891±0.064	1.31415929
1	0.887±0.073	1.76±0.267	1.98421646
2	0.941±0.081	1.71±0.234	1.81721573
4	0.785±0.069	1.65±0.217	2.10191083
8	0.768±0.054	1.41±0.157	1.8359375
Intranasal administration of ^{99m}TC – LMTMC- CBZ NPs			
0.5	0.656±0.047	0.865±0.067	1.31859756
1	0.888±0.078	1.98±0.201	2.22972973
2	0.956±0.097	1.55±0.167	1.62133891
4	0.776±0.081	1.41±0.145	1.81701031
8	0.745±0.068	1.25±0.115	1.67785235
Intranasal administration of ^{99m}TC –MMTMC-CBZ NPs			
0.5	0.632±0.051	0.845±0.091	1.33702532
1	0.865±0.073	1.87±0.113	2.16184971
2	0.949±0.082	1.59±0.187	1.67544784
4	0.775±0.089	1.45±0.154	1.87096774
8	0.732±0.034	1.29±0.131	1.76229508

(Mean ± S.D., n = 3)

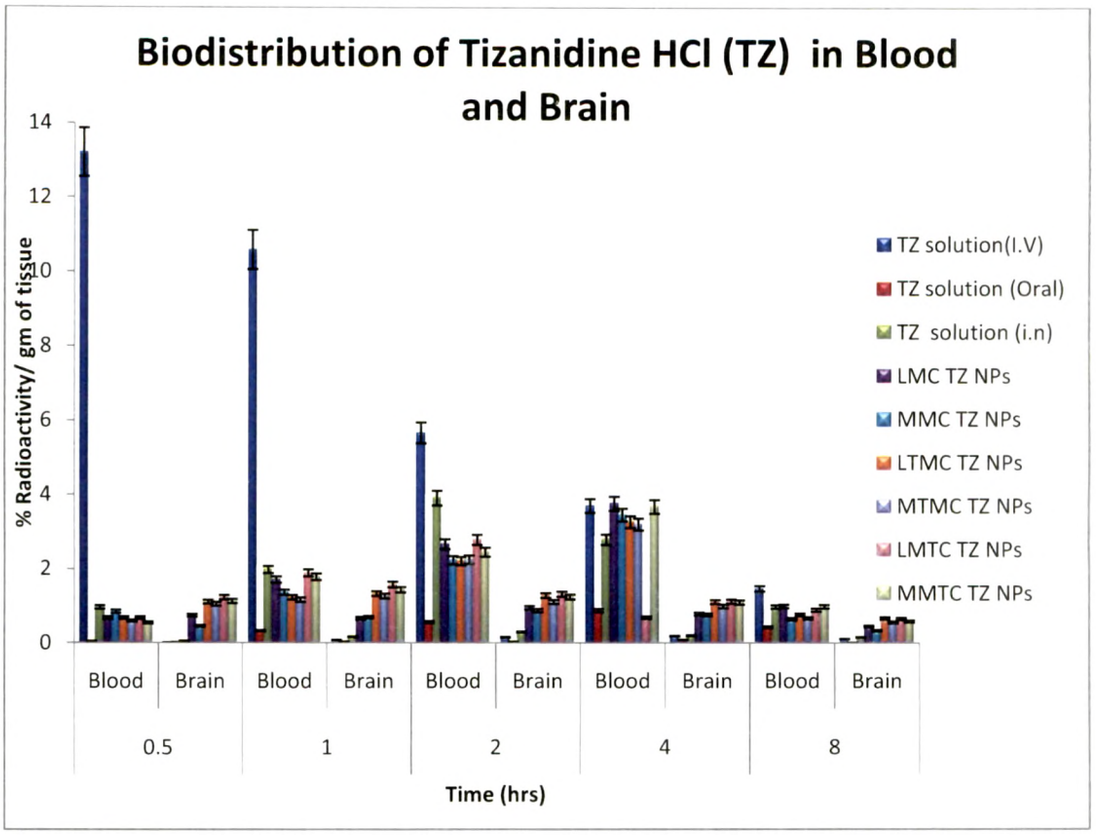


Figure 9.1: Blood, brain concentration versus time plot following administration of ^{99m}TC-Tizanidine HCl formulations

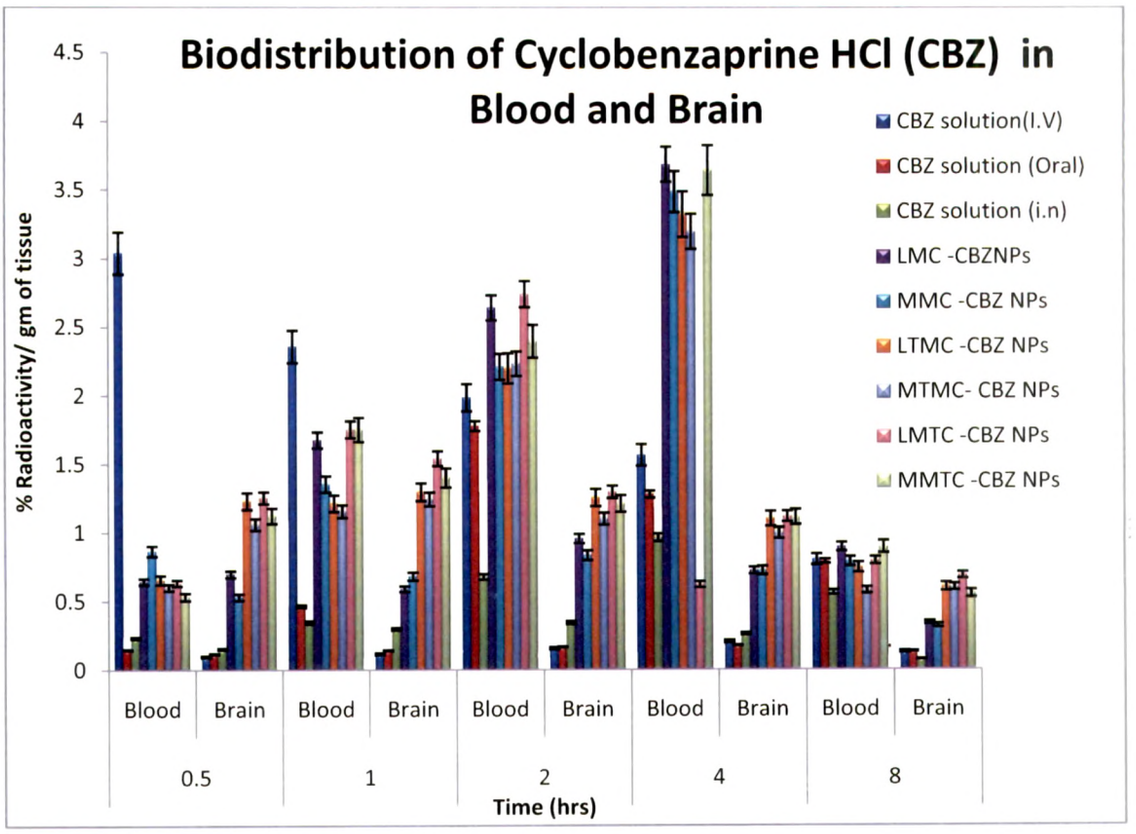


Figure 9.2: Blood, brain concentration versus time plot following administration of ^{99m}TC-Cyclobenzaprine HCl formulations

Table 9.17: Pharmacokinetics parameters of ^{99m}TC-TZ solution/CBZ solution/ LMC-TZ NPs/MMC-TZ NPs/LMTC-TZ NPs/MMTC-TZ NPs/LMTMC-TZ NPs/MMTMC-TZ NPs/ LMC-CBZ NPs/MMC- CBZ NPs/LMTC- CBZ NPs/MMTC- CBZ NPs/LMTMC-CBZ NPs/MMTMC- CBZ NPs

Formulation	Organ/ Tissue	C_{max} (%radio activity/g)	T_{max} (Hours)	$AUC_{0\rightarrow 8hrs}$ (Hours x % radioactivity/g)	$T_{1/2}$ (Hours)	MRT
TZ solution (i.v.)	Blood	10.59	0.5	44.26	3.06	4.06
	Brain	0.189	2	1.05	3.9	6.9
TZ solution (oral)	Blood	0.569	2	2.4	2.25	4.3
	Brain	0.088	2	0.301	1.95	3.95
TZ solution (i.n.)	Blood	3.9	2	21.61	2.9	5.32
	Brain	0.589	1	1.755	1.75	3.14
LMC-TZ NPs	Blood	3.76	2	21.18	3.08	5.56
	Brain	0.957	1	6.75	5.36	8.07
MMC-TZ NPs	Blood	3.45	2	16.093	2.44	4.72
	Brain	0.876	1	6.59	5.75	8.62
LMTC-TZ NPs	Blood	1.32	2	6.96	2.47	4.46
	Brain	1.72	1	14.08	6.38	9.48
MMTC-TZ NPs	Blood	1.26	2	6.64	2.41	4.42
	Brain	1.67	1	12.91	5.49	8.45
LMTMC-TZ NPs	Blood	1.34	2	6.62	2.32	4.32
	Brain	1.89	1	12.625	5.88	8.69
MMTMC-TZ NPs	Blood	1.22	2	6.21	2.37	4.36
	Brain	1.67	1	12.16	5.83	8.65
CBZ solution (i.v.)	Blood	3.04	0.5	77.3	22.18	31.96
	Brain	0.256	2	6.61	16.89	25.06
CBZ solution (oral)	Blood	1.78	2	49.01	18.66	28.04
	Brain	0.178	2	4.94	18.44	27.25
CBZ solution (i.n.)	Blood	1.78	2	49.01	18.66	28.04
	Brain	0.178	2	4.94	18.44	27.25
LMC-CBZ NPs	Blood	0.987	2	32.44	21.44	31.09
	Brain	0.936	1	24.97	19.13	27.48
MMC-CBZ NPs	Blood	0.967	2	35.56	24.52	35.64
	Brain	0.925	1	23.91	19.07	27.42
LMTC-CBZ NPs	Blood	0.959	2	28.98	20.5	30.23
	Brain	2.103	1	61.25	20.95	30.14
MMTC-CBZ NPs	Blood	0.941	2	32.07	23.25	34.18
	Brain	1.76	1	57.62	21.94	31.59
LMTMC-CBZ NPs	Blood	0.956	2	26.33	18.69	27.63
	Brain	1.98	1	47.64	19.78	28.29
MMTMC-CBZ NPs	Blood	0.949	2	24.87	17.71	26.23
	Brain	1.87	1	49.97	20.35	29.23

Table 9.18: Brain targeting efficiency and Direct nose to brain transport percentage following intranasal administration of ^{99m}TC-TZ solution/Cyclobenzaprine solution/ LMC-TZ NPs/MMC-TZ NPs/LMTC-TZ NPs/MMTC-TZ NPs/LMTMC-TZ NPs/MMTMC-TZ NPs/ LMC-CBZ NPs/MMC-CBZ NPs/LMTC-CBZ NPs/MMTC-CBZ NPs/LMTMC- CBZ NPs/MMTMC- CBZ NPs

Formulation	Route of administration	Brain targeting index (DTI)	Direct nose to brain transport percentage [DTP (%)]
Tizanidine HCl solution	Intranasal	3.42	70.82
Cyclobenzaprine HCl solution	Intranasal	6.00	83.41
LMC-TZ NPs	Intranasal	13.43	92.25
MMC-TZ NPs	Intranasal	17.26	94.21
LMTC-TZ NPs	Intranasal	85.23	98.97
MMTC-TZ NPs	Intranasal	81.95	98.78
LMTMC-TZ NPs	Intranasal	80.38	98.75
MMTMC-TZ NPs	Intranasal	82.53	98.79
LMC-CBZ NPs	Intranasal	9.00	88.90
MMC- CBZ NPs	Intranasal	9.09	87.28
LMTC- CBZ NPs	Intranasal	24.71	95.96
MMTC- CBZ NPs	Intranasal	21.01	95.24
LMTMC- CBZ NPs	Intranasal	21.15	95.27
MMTMC- CBZ NPs	Intranasal	23.49	95.75

9.7.3. Gamma scintigraphy imaging

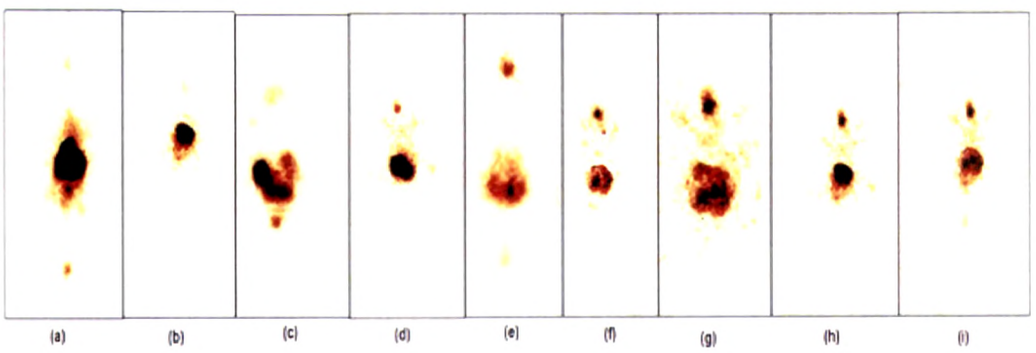


Figure 9.3: Gamma scintigraphy images of (a) ^{99m}TC –TZ solution (i.v.) (b) ^{99m}TC –TZ solution (oral) (c) ^{99m}TC –TZ solution (i.n.) (d) ^{99m}TC-LMC-TZ NPs (e) ^{99m}TC-MMC-TZ NPs (f) ^{99m}TC-LMTC-TZ NPs (g) ^{99m}TC-MMTC-TZ NPs (h) ^{99m}TC-LMTMC-TZ NPs (i) ^{99m}TC-MMTMC-TZ NPs in swiss albino mice

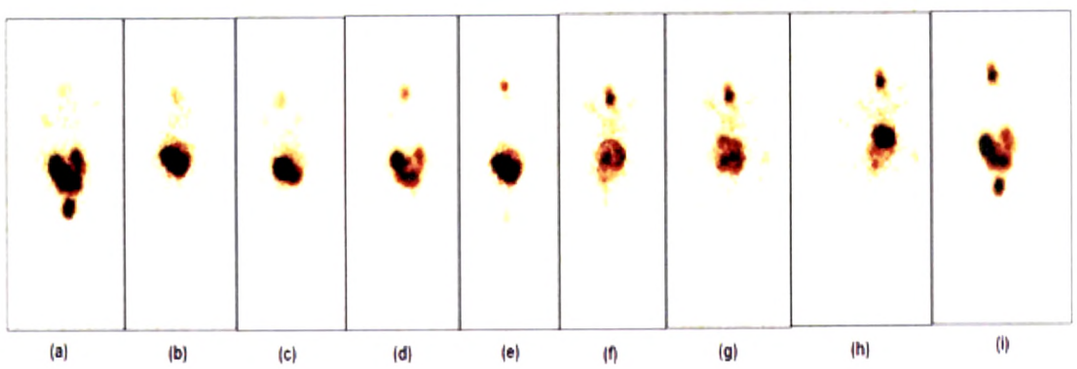


Figure 9.4: Gamma scintigraphy images of (a) ^{99m}TC –CBZ solution (i.v.) (b) ^{99m}TC – CBZ solution (oral) (c) ^{99m}TC – CBZ solution (i.n.) (d) ^{99m}TC-LMC- CBZ NPs (e) ^{99m}TC-MMC- CBZ NPs (f) ^{99m}TC-LMTC- CBZ NPs (g) ^{99m}TC-MMTC- CBZ NPs (h) ^{99m}TC-LMTMC- CBZ NPs (i) ^{99m}TC-MMTMC- CBZ NPs in swiss albino mice

9.8. Discussion

9.8.1. Radiolabeling of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) formulations and Optimization

Tizanidine HCl and cyclobenzaprine HCl formulations were effectively radiolabeled with Technetium-99m (^{99m}Tc), optimized for maximum labelling efficiency and stability. The quantity of stannous chloride to reduce ^{99m}Tc plays an important role in the labelling efficiency. Lower quantity of stannous chloride leads to low labelling efficiency where as higher amount of stannous chloride leads to formation of undesirable radiocolloids. The optimum quantity of stannous chloride for high labelling efficiency and low free and reduced/hydrolyzed ^{99m}Tc, was found to be 250µg for all preparations (Table 9.3 & Table 9.4). The incubation time was optimized at 30minutes. The pH for all the formulations was kept at around 6.5. The labelling efficiency and the stability of labelled complex were ascertained by ascending TLC using ITLC strips. Radiochemical purity of ^{99m}Tc labeled TZ solution, LMC-TZ NPs, MMC-TZ NPs, LMTC-TZ NPs, MMTC-TZ NPs, LMTMC-TZ NPs, MMTMC-TZ achieved was 97%, 97.5%, 97.9%, 99.2%, 98.5%, 99.1%, 98.89% respectively (Table 9.1). Radiochemical purity of ^{99m}Tc labelled CBZ solution, LMC- CBZ NPs, MMC-CBZ NPs, LMTC- CBZ NPs, MMTC- CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ achieved was 98.12%, 98.89%, 99.32%, 99.59%, 97.99 %, 99.21%, 98.99 % respectively (Table 9.2). The results suggested high stability of ^{99m}Tc labelled TZ and CBZ formulations. The stability studies of ^{99m}Tc labelled TZ and CBZ were carried out *in-vitro* using normal saline and mice serum by ascending ITLC. The stability of complexes for 24 hrs was assessed and results were recorded in (Table 9.5 to Table 9.8). The bonding strength of ^{99m}Tc labelled tizanidine HCl and Cyclobenzaprine HCl formulations was assessed by DTPA (Diethylene triamine penta acetic acid) challenging test. The effect of different molar concentration of DTPA on ^{99m}Tc labelled TZ and CBZ formulations and percentage transchelation were studied and results were recorded in Table 9.9 and Table 9.10. The percent transchelation of the labeled complex was below 4%w/w at highest concentration tested (50mM). The results suggested high bonding strength and stability of TZ and CBZ formulations, thus these formulations were found suitable for biodistribution studies of the drug in mice.

9.8.2. Biodistribution studies

Biodistribution studies of ^{99m}Tc-TZ/CBZ following intravenous, oral and intranasal (^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ

CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs) administration on Swiss albino mice were performed and the radioactivity was estimated at predetermined time intervals up to 8 h. The results obtained are recorded in Table 9.11 to Table 9.13. The brain/blood ratio of the drug at all time points for different formulations were also calculated and recorded in Table 9.14 to 9.16. The pharmacokinetic parameters of all formulations were calculated from Figure 9.1& Figure 9.2 are recorded in Table 9.17. After nasal administration of ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs, lower the T_{max} values in brain (around 1 h) compared to blood (around 2 h) were observed (Figure 9.1 and Figure 9.2). The brain/blood ratios of the drug were found to be higher for formulations when administered intranasally (Table 9.14 to 9.16). This result further confirms direct nose-to-brain transport. Concentration of TZ/CBZ (solution/ NPs formulation) in brain following intranasal administration were found to be significantly higher at all sampling time points compared to TZ/CBZ solution (intravenous) and TZ/CBZ solution (oral) up to 8 h. This results may recognized by the higher mucoadhesion capacity of chitosan, thiolated chitosan and trimethyl chitosan NPs on nasal mucosa that significantly improves the drug transport by enhancing the residence time of formulations on nasal mucosa compared to the TZ solution (intravenous) and TZ solution (oral). Small size of NPs also improves the penetration of formulations through the mucosa barrier. Oral administered TZ/CBZ solution found to have drug transport to the brain was significantly less than the intranasal administered TZ/CBZ solution, chitosan NPs, thiolated chitosan NPs and trimethyl chitosan NPs. These results may be due to the high first pass metabolism of both drugs by oral route. Higher brain uptake of TZ/CBZ was found in thiolated chitosan NPs/Trimethyl chitosan NPs via intranasal route than the chitosan NPs. These results may be due to higher mucoadhesive strength of thiolated chitosan than the chitosan. These results may be associated with increase in solubility at physiological pH leads quicker absorption at site of administration. The substantially higher uptake of nanoparticles formulations in the brain with intranasal administration suggests a larger extent of selective transport of TZ/CBZ from nose-to brain (Illum L et al., 2000, Thorne et al., 2004). ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs formulations were observed $T_{1/2}$ of 2.25–3.08 h (blood), 1.75–6.38 h (brain) irrespective of the routes of administration and the type of the formulations. ^{99m}Tc-CBZ solution/LMC-CBZ NPs/ MMC-

TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs formulations were observed $T_{1/2}$ of 17.71–24.52 h (blood), 16.89–21.94 h (brain) irrespective of the routes of administration and the type of the formulations. Lower C_{max} and AUC values were observed with chitosan NPs and TZ/CBZ drug solution. The mucociliary clearance under normal circumstances rapidly clears the instilled formulation. However, the drug was incorporated in higher mucoadhesive thiolated chitosan NPs/trimethyl chitosan NPs significantly improve the C_{max} and AUC. This demonstrates the value obtained with thiolated chitosan NPs/trimethyl chitosan NPs in prolonging the contact time of the formulation with the nasal mucosa. The drug targeting Index (DTI) and brain drug direct transport percentage (DTP (%)) were also calculated for nasally administered formulations and are shown in Table 9.18. Both the drug loaded thiolated chitosan /trimethyl chitosan NPs showed the highest DTI and DTP (%) values than the chitosan NPs and TZ/CBZ solution. These higher values of DTI and DTP (%) show the benefit of the thiolated chitosan/trimethyl chitosan NPs for intranasal administration. The higher DTI and DTP (%) advocate that improved brain targeting efficiency of thiolated chitosan/trimethyl chitosan NPs mainly because of considerable direct nose-to-brain transport.

9.8.3. Gamma scintigraphy imaging

Gamma scintigraphy studies were performed on swiss albino mice for ^{99m}Tc-TZ solution/LMC-TZ NPs/ MMC-TZ NPs / LMTC-TZ NPs / /MMTC-TZ NPs/ LMTMC-TZ NPs/MMTMC-TZ NPs /CBZ solution/LMC-CBZ NPs/ MMC-TZ CBZ NPs / LMTC-TZ CBZ NPs / /MMTC-TZ CBZ NPs, LMTMC- CBZ NPs, MMTMC- CBZ NPs formulations in order to visualize the drug localization in the Brain. In order to visualize the brain uptake following intravenous, intranasal and oral administrations of ^{99m}Tc-TZ/CBZ solution/chitosan NPs/thiolated chitosan NPs/trimethyl chitosan NPs, we used a gamma scintigraphy camera for to derive complete biodistribution of TZ/CBZ. The gamma scintigraphy images in swiss albino mice were taken after 15 minutes post intravenous injection, oral and intranasal administrations are shown in Figure 9.3 & Figure 9.4. The figures shows the presence of some radioactivity in the esophagus following intranasal administration could lead to absorption of a part of the formulations from gastrointestinal tract. The scintigraphy images were found consistent with the results shown in Table 9.11 to Table 9.13 and high brain uptake of TZ/CBZ was observed with the thiolated chitosan NPs and trimethyl chitosan NPs into the brain than the chitosan NPs and TZ/CBZ solution when administered via intranasal

route. The scintigraphy images were consistent with the findings of the biodistribution studies.

9.9. Conclusions

Biodistribution studies of radiolabel drug in swiss albino mice confirmed the rapid and enhanced delivery of TZ and CBZ in brain after intranasal administration of thiolated chitosan and trimethyl chitosan NPs. Gamma scintigraphy studies confirmed the high drug localization of both the drugs in to the brain for thiolated chitosan and trimethyl chitosan NPs.

9.10. References

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