CHAPTER 7

RADIOLABELING OF FORMULATIONS

7 RADIOLABELING OF FORMULATIONS

7.1 Introduction

Radiolabeling of drugs and drug delivery systems has been widely applied to study biological distribution patterns. Particularly, the radiolabeling with short lived radionuclides has been preferred due to their rapid decay and 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 P et al., 1999). In the typical radiopharmaceutical formulation, the quantities of radionuclides and pharmaceutical agents used are normally quite less. 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 they 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 AK and Sharma RK, 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 (Sorensen JA and Phelps ME, 1980; Budinger TF, 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 to the compound is determined by the quality control tests such as labeling efficiency, stability of radiolabeled complexes, challenge tests using substances having high affinity to the radiolabel and serum stability.

In practice, the radiopharmaceutical preparation is administered to the species of interest, 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 (Ramamoorthy N and Desai CN, 1997).

Various reports are available where ^{99m}Tc (^{99m} Technetium) 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)

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

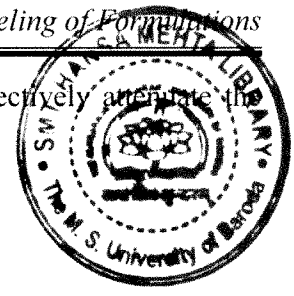
Common methods of separation of ^{99m} Technetium and ^{99} Molybdenum

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}Technetium

^{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 140.5 keV. The specific gamma ray constant for ^{99m}Tc is 0.8R/mCi-h at 1cm (5.58



μCi/kg/h/MBq at 1cm). The use of 2.5 mm 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.

7.2 Methods

7.2.1 Radiolabeling of Drug solution and Nanoparticles

The labeling of drug solution (DS) and nanoparticles (NPs) was performed by direct labeling as per the reported method with some modifications (Richardson VJ et al., 1977; Babbar A et al., 1991). For labeling of DS, required volume of Tramadol solutions (TMDS) & Lamotrigine solution (LTGS) were mixed with sufficient stannous chloride solution (5 mg/ml) to get highest labeling. The pH was adjusted with 0.5 M sodium bicarbonate solution. Further, the preparation was incubated with ^{99m}Tc pertechnetate solution (18.5-22.2 MBq) for required time at room temperature. The final volume was made up to 1.0 ml using sterile sodium chloride solution. For labeling of nanoparticles ^{99m}Tc pertechnetate solution was first reduced using sufficient quantity stannous chloride solution (5 mg/ml). 0.75 ml of NPs dispersion was mixed with reduced ^{99m}Tc pertechnetate. The pH was adjusted to 6.5 with 0.5 M sodium bicarbonate solution. Further, the preparation was incubated for required time at room temperature. The final volume was made up to 1.0 ml using sodium chloride solution.

The quality control (percentage labeling efficiency and stability of the labeled complexes) was performed as described (Theobald AE, 1990).

Labeling efficiency

The labeling efficiency of ^{99m}Tc-DS and ^{99m}Tc-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 radio-labeled complex was applied at a point 1 cm from one end of an ITLC-SG strip. The strip was eluted in acetone and solvent front was allowed to reach 7-8 cm from the point of application. The strip was cut horizontally into two 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 labeled 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 (Saha GB, 1993; Saha GB, 2005). The radiocolloids remained at the point of application, while both the free pertechnetate and the labeled complex moved away with the solvent front. The activity migrated using pyridine: acetic acid: water as a mixture was subtracted from that with the solvent front using acetone, the net amount of ^{99m}Tc-DS, ^{99m}Tc-NPs was calculated.

The radiolabeling was optimized for incubation time and the concentration of $SnCl_2.2H_2O$. The pH of the formulations was maintained at around 6.5. The influence of the incubation time on labeling efficiency of TMD and LTG loaded NPs are given in Table 7.1 and 7.4 respectively. The influence of concentration of $SnCl_2.2H_2O$ on labeling efficiency of TMD and LTG loaded NPs are given in Table 7.2 and 7.5 respectively.

In-vitro Stability of labeled complex

The stability study of radiolabeled formulations was determined *in vitro* using rat serum by ascending thin layer chromatography (Garron JY et al., 1991). The complex (0.1 ml) was mixed with 1.9 ml of rat serum and incubated at 37 °C. The samples at different time points upto 48 h were subjected to ITLC using acetone solvent systems. The % labeling efficiency for TMD solution and NPs was determined. The results for stability in rat serum for TMD and LTG loaded NPs are tabulated in Table 7.3 and 7.6 respectively. Summary of radiolabeling study of TMD and LTG loaded NPs is tabulated in table 7.7.

7.2.2 Radiolabeling of Microemulsion and Nanoemulsion

The labeling of drug solution (DS), microemulsion (ME) and Nanoemulsion (NE) was performed by direct labeling as per the reported method with some modifications (Richardson VJ et al., 1977; Babbar A et al., 1991). Briefly, 0.75 ml of drug solutions, ME and NE were mixed with sufficient stannous chloride solution (5 mg/ml) to get highest labeling. The pH was adjusted with 0.5 M sodium bicarbonate solution. Further, the preparation was incubated with ^{99m}Tc pertechnetate solution (111-131.3 MBq) for required time at room temperature. The final volume was made up to 1.0 ml using sterile sodium chloride solution.

Labeling efficiency

The labeling efficiency of ^{99m}Tc-DS, ^{99m}Tc-ME and ^{99m}Tc-NE was determined using ascending instant thin layer chromatography (ITLC) in similar manner described for NPs. The radiolabeling 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 influence of the incubation time on labeling efficiency of TMD and LTG loaded MEs and NEs are given in Table 8.8 and 8.11 respectively. The influence of concentration of $SnCl_2.2H_2O$ on labeling efficiency of TMD and LTG loaded MEs and NEs is given in Table 7.9 and 7.12 respectively.

In-vitro Stability of labeled complex

The stability study of radiolabeled formulations was determined *in vitro* using rat serum by ascending thin layer chromatography (Garron JY et al., 1991). The complex (0.1 ml) was mixed with 1.9 ml of rat serum and incubated at 37 °C. The samples at different time point up to 48 h were subjected to ITLC using acetone solvent systems. The % labeling efficiency was determined. The results for stability in rat serum for TMD and LTG loaded MEs and NEs are tabulated in Table 7.10 and 7.13 respectively. Summary of radiolabeling study of TMD and LTG loaded MEs and NEs is tabulated in Table 7.14.

7.3 Results and Discussion

7.3.1 Nanoparticles

Table 7.1: Influence of incubation time on the labeling efficiency of TMDS and TMD-NP formulations

Incubation time (min)	% Radiolabeling			
	^{99m}Tc-TMDS	^{99m}Tc-TMD-NP	^{99m}Tc-Tf-TMD-NP	^{99m}Tc-Lf-TMD-NP
5	96.43 ±1.04	91.67 ± 0.85	90.24 ± 1.12	91.58 ± 0.97
10	**98.06 ±1.35**	94.68 ± 1.23	94.13 ± 1.51	93.33 ± 1.42
15	97.72 ±1.56	95.53 ± 1.64	96.47 ± 2.13	95.09 ± 1.15
30	96.85 ±1.83	**96.65 ± 1.26**	**97.27 ± 1.86**	**96.76 ± 1.06**
60	96.56 ±2.01	96.07 ± 1.29	96.83 ± 1.76	96.45 ± 2.11

Values are represented as mean ± SD, n=3.

Table 7.2: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of TMDS and TMD-NPs formulations

$SnCl_2.2H_2O$ (µg) →	50	75	100	**150**	200
TMDS					
% labeling (mean ± SD)	86.41 ± 1.01	91.89 ± 0.87	95.68 ± 1.57	98.09 ± 1.19	96.35 ± 1.82
% colloids (mean ± SD)	0.43 ± 0.11	0.93 ± 0.31	0.97±0.23	1.12 ± 0.18	3.03 ± 0.27
% Free (mean ± SD)	13.16 ± 0.64	7.18 ±0.35	3.35 ± 0.46	0.79 ± 0.19	0.62 ± 0.31
TMD-NP					
% labeling (mean ± SD)	65.46 ± 1.87	83.75 ± 1.39	93.85 ± 1.72	96.67 ± 0.95	95.12 ± 1.36
% colloids (mean ± SD)	0.95 ± 0.12	1.12 ± 0.29	1.54 ± 0.31	2.09 ± 0.52	4.01 ± 0.79
% Free (mean ± SD)	33.59 ± 0.58	15.13 ± 0.28	4.61 ± 0.25	1.24 ± 0.35	0.87 ± 0.14
Tf-TMD-NP					
% labeling (mean ± SD)	59.76 ± 1.28	89.85 ± 1.78	92.78 ± 1.97	97.28 ± 1.37	94.12 ± 0.97
% colloids (mean ± SD)	0.69 ± 0.22	0.58 ± 0.17	1.08 ± 0.07	1.29 ± 0.16	4.52 ± 0.56
% Free (mean ± SD)	39.55 ± 0.57	9.57 ± 0.39	6.14 ± 0.36	1.43 ± 0.18	1.36 ± 0.29
Lf-TMD-NP					
% labeling (mean ± SD)	61.32 ± 1.19	91.64 ± 1.57	94.44 ± 1.08	96.82 ± 1.29	95.33 ± 1.25
% colloids (mean ± SD)	0.63 ± 0.17	0.87 ± 0.10	1.24 ± 0.13	1.78 ± 0.35	3.34 ± 1.18
% Free (mean ± SD)	38.05 ± 0.82	7.49 ± 0.32	4.32 ± 0.25	1.40 ± 0.15	1.33 ± 0.32

Values are represented as mean ± SD, n=3.

Figure 7.1: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of TMDS and TMD-NPs formulations

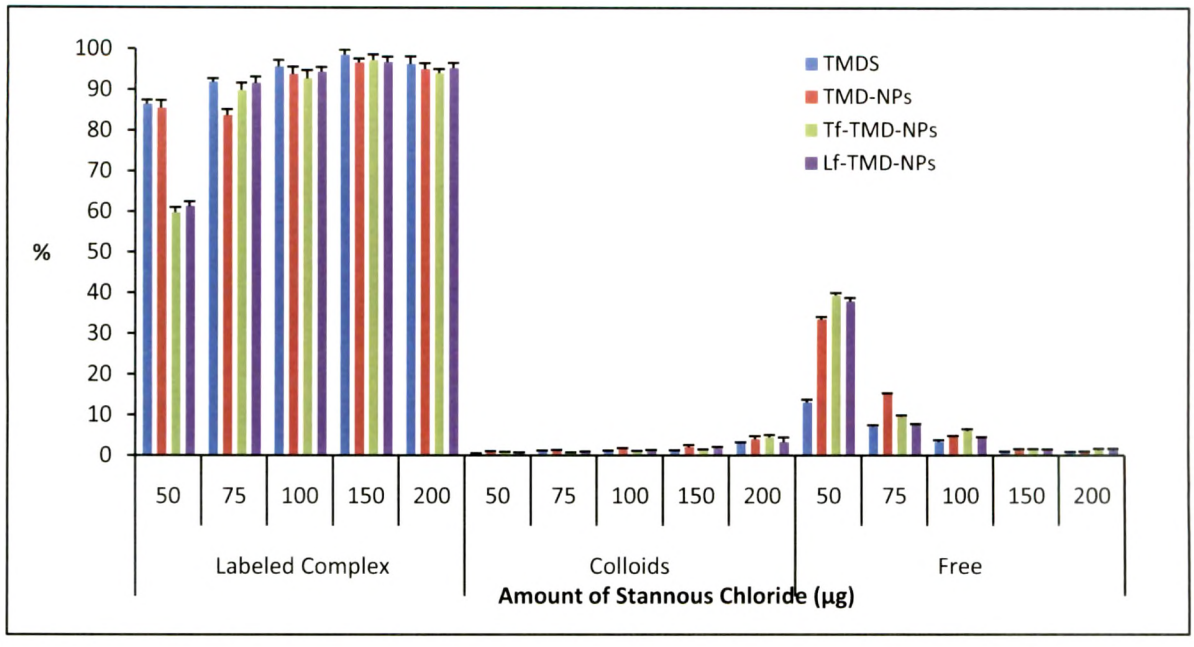


Table 7.3: *In-vitro* stability of ^{99m}Tc -labeled complex of TMDS and TMD-NPs formulations in rat serum

	% Labeling Efficiency			
Time (h)	**TMDS**	**TMD-NP**	**Tf-TMD-NP**	**Lf-TMD-NP**
0	98.09 ± 1.19	96.67 ± 0.95	97.28 ± 1.37	96.82 ± 1.29
0.5	97.89 ± 2.02	96.64 ± 1.34	97.12 ± 1.87	96.56 ± 1.15
1	97.72 ± 0.86	95.96 ± 1.81	96.55 ± 1.69	96.48 ± 1.43
2	97.06 ± 0.91	95.57 ± 2.59	96.26 ± 1.63	96.00 ± 0.75
4	96.82 ± 1.14	94.92 ± 0.97	95.84 ± 1.83	95.53 ± 2.12
6	96.36 ± 0.79	94.16 ± 1.78	95.59 ± 1.20	95.28 ± 1.92
24	95.84 ± 1.27	93.44 ± 0.61	94.26 ± 1.68	93.33 ± 1.76
48	93.11 ± 2.17	92.08 ± 1.44	91.12 ± 2.31	90.97 ± 1.65

Values are represented as mean ± SD, n=3.

Table 7.4: Influence of incubation time on the labeling efficiency of LTGS and LTG-NPs formulations

Incubation time (min)	% Radiolabeling			
	^{99m}Tc-TMDS	^{99m}Tc- LTG –NP	^{99m}Tc-Tf- LTG –NP	^{99m}Tc-Lf- LTG –NP
5	92.48 ±1.20	90.45 ± 1.73	90.89 ± 1.05	89.88 ± 0.63
10	96.74 ±1.63	93.67 ± 1.38	94.82 ± 1.41	92.25 ± 1.17
15	**98.16 ±1.69**	96.35 ± 2.15	96.09 ± 1.26	95.04 ± 1.14
30	97.85 ±2.21	**97.10 ± 1.03**	**96.88 ± 0.97**	**97.24 ± 1.43**
60	97.12 ±1.57	96.45 ± 1.23	96.47 ± 1.79	97.16 ± 2.14

Values are represented as mean ± SD, n=3.

Table 7.5: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of LTGS and LTG-NPs formulations

$SnCl_2.2H_2O$ (µg) →	50	75	100	**150**	200
LTGS					
% labeling (mean ± SD)	88.87 ± 0.68	91.76 ± 1.09	97.42 ± 1.25	98.16 ± 1.32	96.75 ± 1.34
% colloids (mean ± SD)	0.56 ± 0.21	0.98 ± 0.41	1.15±0.23	1.32 ± 0.18	2.78 ± 0.32
% Free (mean ± SD)	10.57 ± 0.43	7.26 ±0.26	1.43 ± 0.21	0.52 ± 0.11	0.47 ± 0.31
LTG-NP					
% labeling (mean ± SD)	73.32 ± 1.51	87.53 ± 1.27	92.72 ± 1.72	97.14 ± 0.83	95.72 ± 1.28
% colloids (mean ± SD)	0.97 ± 0.11	1.43 ± 0.29	1.68 ± 0.31	2.11 ± 0.52	3.67 ± 0.79
% Free (mean ± SD)	25.71 ± 0.42	11.04 ± 0.31	5.60 ± 0.12	0.75 ± 0.21	0.61 ± 0.09
Tf-LTG-NP					
% labeling (mean ± SD)	68.29 ± 1.11	78.92 ± 1.52	91.57 ± 1.43	96.87 ± 1.13	94.97 ± 0.97
% colloids (mean ± SD)	0.82 ± 0.12	1.24 ± 0.31	1.72 ± 0.23	2.07 ± 0.22	4.06 ± 0.56
% Free (mean ± SD)	30.89 ± 0.43	19.84 ± 0.25	6.71 ± 0.14	1.06 ± 0.08	0.97 ± 0.11
Lf-LTG-NP					
% labeling (mean ± SD)	70.55 ± 1.42	89.26 ± 1.18	93.32 ± 1.20	97.53 ± 1.65	96.68 ± 1.25
% colloids (mean ± SD)	0.76 ± 0.15	0.92 ± 0.10	1.42 ± 0.13	1.76 ± 0.35	2.86 ± 1.23
% Free (mean ± SD)	28.69 ± 0.56	9.82 ± 0.26	5.26 ± 0.17	0.71 ± 0.08	0.46 ± 0.41

Values are represented as mean ± SD, n=3.

Figure 7.2: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of LTGS and LTG-NPs formulations

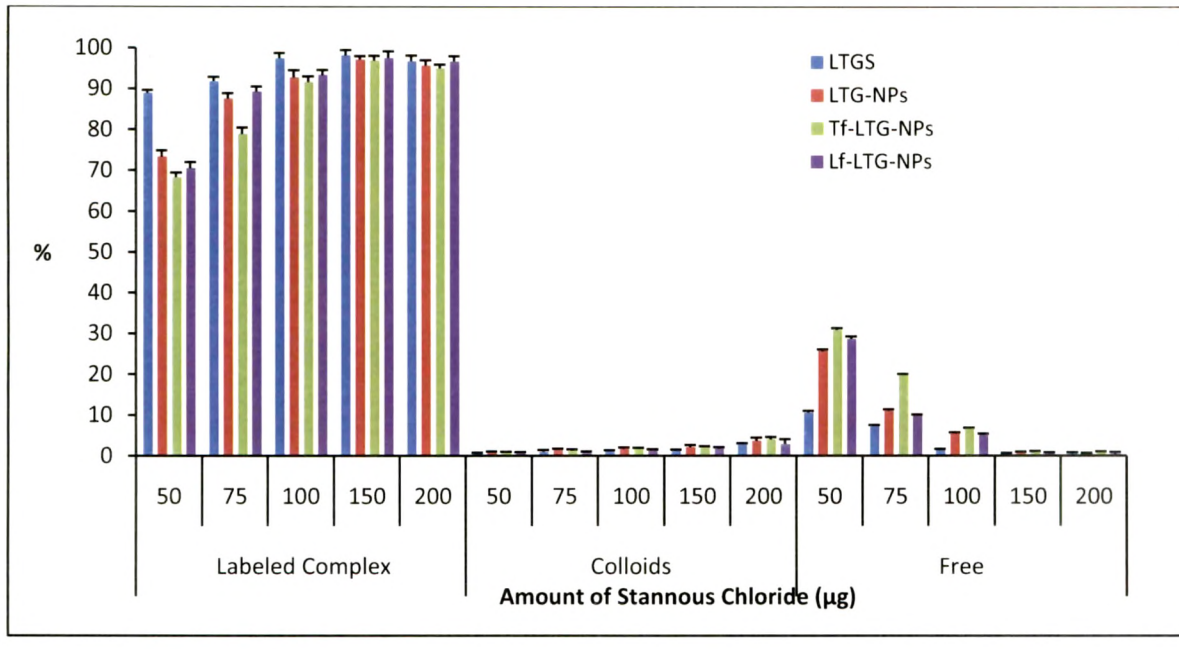


Table 7.6: *In-vitro* stability of ^{99m}Tc labeled complex of LTGS and LTG-NPs formulations in rat serum

	% Labeling Efficiency			
Time (h)	**LTGS**	**LTG-NP**	**Tf-LTG-NP**	**Lf-LTG-NP**
0	98.16 ± 1.32	97.14 ± 0.83	96.87 ± 1.13	97.53 ± 1.65
0.5	98.11 ± 1.28	96.99 ± 1.54	96.68 ± 1.94	97.51 ± 1.72
1	97.94 ± 0.95	96.47 ± 0.93	96.55 ± 1.24	97.06 ± 1.27
2	97.54 ± 1.03	96.69 ± 1.23	95.94 ± 1.56	96.74 ± 1.13
4	96.92 ± 0.86	95.74 ± 0.96	95.48 ± 1.33	96.66 ± 2.04
6	96.64 ± 0.75	95.08 ± 2.11	94.89 ± 1.47	96.02 ± 0.83
24	95.79 ± 1.10	93.88 ± 0.84	93.67 ± 2.21	93.69 ± 1.52
48	93.54 ± 1.28	90.92 ± 1.27	90.08 ± 1.68	91.75 ± 1.41

Values are represented as mean ± SD, n=3.

Table 7.7: Radiolabeling summary of DS and NPs formulations

	TMDS	**TMD-NPs**	**Tf-TMD-NPs**	**Lf-TMD-NPs**
Method	Direct Labeling	Direct Labeling with reduced technetium	Direct Labeling with reduced technetium	Direct Labeling with reduced technetium
Amt. of $SnCl_2$ (μg)	150	150	150	150
pH	6.5	6.5	6.5	6.5
Incubation duration (min)	10	30	30	30
Labeling efficiency (%)	98.09	96.67	97.28	96.82
Activity added (MBq)	18.5-22.2	18.5-22.2	18.5-22.2	18.5-22.2
	LTGS	**LTG-NPs**	**Tf-LTG-NPs**	**Lf-LTG-NPs**
Method	Direct Labeling	Direct Labeling with reduced technetium	Direct Labeling with reduced technetium	Direct Labeling with reduced technetium
Amt. of $SnCl_2$ (μg)	150	150	150	150
pH	6.5	6.5	6.5	6.5
Incubation duration (min)	15	30	30	30
Labeling efficiency (%)	98.16	97.14	96.87	97.53
Activity added (MBq)	18.5-22.2	18.5-22.2	18.5-22.2	18.5-22.2

Unconjugated and conjugated NPs of TMD and LTG were labeled with ^{99m}Tc with high labeling efficiency using direct labeling method. Generally technetium is reduced in the presence of formulations, which enable the formulations tagged with technetium. Otherwise, the previously reduced technetium is used for tagging of the formulations. Drug solutions (TMDS & LTGS) were labeled with direct labeling method while NPs were labeled with direct labeling with reduced ^{99m}Tc. The quantity of stannous chloride to reduce ^{99m}Tc plays an important role in the labeling efficiency. Lower quantity of stannous chloride leads to low labeling efficiency where as higher amount of stannous chloride leads to formation of undesirable radiocolloids. The optimum quantity of stannous chloride for high labeling efficiency and low free and reduced/hydrolyzed ^{99m}Tc, was found to be 150μg for all NPs and drug solutions. The incubation time was optimized at 30 min for NPs formulations while TMDS and LTGS require 10 to 15 min incubation time respectively. The pH for all the formulations was kept at around 6.5.

The labeling efficiency and the stability of labeled complex were ascertained by ascending TLC using ITLC strips. The labeling efficiency for TMDS, TMD-NPs, Tf-TMD-NPs and Lf-TMD-NPs was found to be 98.09%, 96.67%, 97.28 and 96.82 % respectively. The labeling

efficiency for LTGS, LTG-NPs, Tf-LTG-NPs and Lf-LTG-NPs was found to be 98.16%, 97.14%, 96.87% and 97.53 respectively. The *in vitro* stability of radiolabeled preparations was checked in presence of rat serum and 0.9 %w/v sodium chloride. Rat serum was selected to mimic the experiment *in-vivo* conditions related to serum proteins and physiological pH. The labeling efficiency of ^{99m}Tc labeled formulation at all the time points is found to be greater than 90%.

7.3.2 Microemulsion and Nanoemulsion

Table 7.8: Influence of incubation time on the labeling efficiency of TS, TME and TNE

Incubation time (min)	% Radiolabeling		
	^{99m}Tc-TS	^{99m}Tc-TME	^{99m}Tc-TNE
5	95.57 ± 0.82	89.68 ± 0.93	90.26 ± 0.91
10	**98.06 ± 1.18**	92.26 ± 1.26	93.78 ± 1.32
15	97.67 ± 1.46	94.43 ± 1.43	95.54 ± 1.14
30	97.03 ± 2.14	**96.17 ± 1.25**	**96.33 ± 1.56**
60	96.75 ± 1.31	95.86 ± 1.32	96.06 ± 2.07

Value are represented as mean ± SD, n=3.

Table 7.9: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of TS, TME and TNE

$SnCl_2.2H_2O$ (µg)→	100	150	200	250	300
TS					
% labeling (mean ± SD)	92.27 ± 1.12	**98.18 ± 1.87**	95.93 ± 1.32	93.76 ± 1.34	90.99 ± 1.23
colloids (mean ± SD)	0.41 ± 0.10	**0.60 ± 0.24**	2.89±0.13	5.23 ± 0.09	8.24 ± 0.17
% Free (mean ± SD)	7.32 ± 0.44	**1.22 ±0.12**	1.18 ± 0.21	1.01 ± 0.10	0.77 ± 0.32
TME					
% labeling (mean ± SD)	63.92 ± 1.71	79.82 ± 1.42	91.65 ± 1.37	**97.32 ± 0.85**	94.78 ± 1.27
% colloids (mean ± SD)	0.83 ± 0.11	1.08 ± 0.12	1.72 ± 0.28	**2.13 ± 0.25**	4.84 ± 0.18
% Free (mean ± SD)	35.25 ± 0.62	19.1 ± 0.22	6.63 ± 0.32	**0.55 ± 0.15**	0.38 ± 0.12
TNE					
% labeling (mean ± SD)	67.7 ± 1.41	85.34 ± 1.46	**96.28 ± 1.72**	93.88 ± 1.16	91.24 ± 0.83
% colloids (mean ± SD)	0.59 ± 0.18	0.94 ± 0.09	**1.68 ± 0.26**	4.15 ± 0.16	7.43 ± 0.25
% Free (mean ± SD)	31.71 ± 0.43	13.72 ± 0.25	**2.04 ± 0.17**	1.97 ± 0.12	1.33 ± 0.12

Values are represented as mean ± SD, n=3.

Figure 7.3: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of TS, TME and TNE

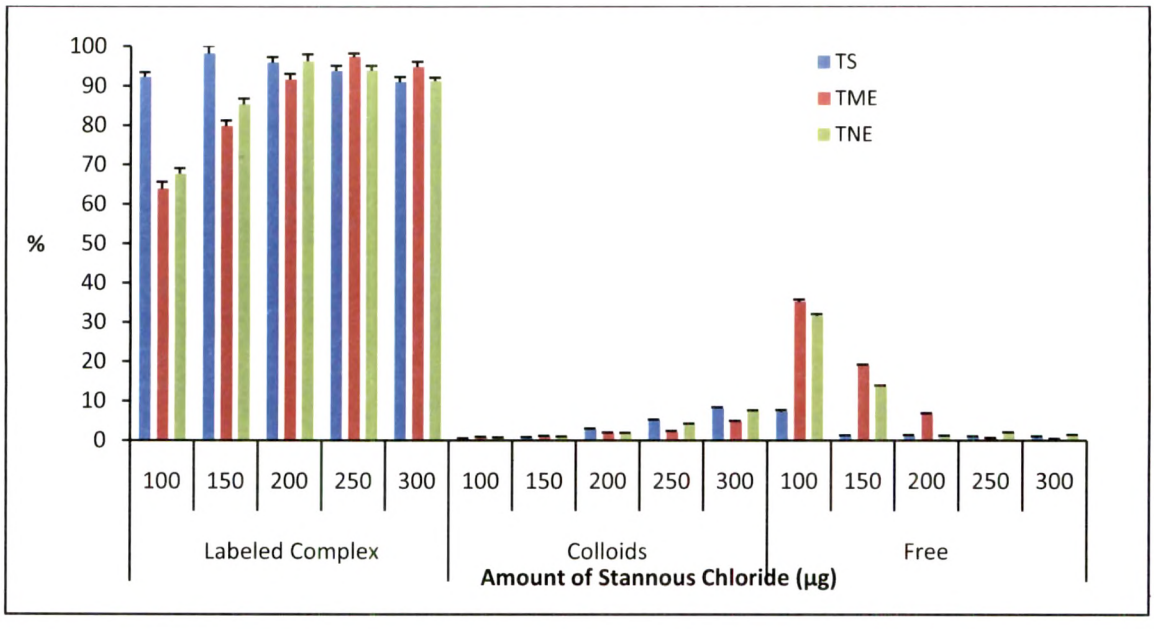


Table 7.10: *In-vitro* stability of ^{99m}Tc -labeled complex of TS, TME and TNE

	% Labeling Efficiency		
Time (h)	**TS**	**TME**	**TNE**
0	98.18 ± 1.87	97.32 ± 0.85	96.28 ± 1.72
0.5	98.04 ± 0.82	97.13 ± 1.84	96.23 ± 1.26
1	97.84 ± 0.79	96.75 ± 1.64	96.02 ± 1.57
2	97.54 ± 1.21	96.56 ± 0.95	95.85 ± 0.86
4	96.92 ± 1.34	96.06 ± 1.13	95.39 ± 1.35
6	96.00 ± 1.12	95.53 ± 1.65	95.02 ± 1.17
24	95.44 ± 2.01	94.75 ± 1.06	93.86 ± 2.11
48	92.05 ± 1.43	91.47 ± 1.32	90.83 ± 1.43

Values are represented as mean ± SD, n=3.

Table 7.11: Influence of incubation time on the labeling efficiency of LS, LME and LNE

Incubation time (min)	% Radiolabeling		
	^{99m}Tc-LS	^{99m}Tc-LME	^{99m}Tc-LNE
5	95.84 ± 1.56	88.97 ± 1.17	90.12 ± 1.25
10	96.52 ± 1.24	91.36 ± 1.34	92.79 ± 1.53
15	**97.16 ± 2.08**	95.65 ± 1.28	95.88 ± 0.89
30	97.08 ± 1.73	**98.20 ± 1.45**	**97.15 ± 1.06**
60	96.38 ± 1.38	97.41 ± 1.46	98.36 ± 2.10

Values are represented as mean ± SD, n=3.

Table 7.12: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of LS, LME and LNE

$SnCl_2.2H_2O$ (µg)→	100	150	200	250	300
LS					
% labeling (mean ± SD)	93.87 ± 1.27	**97.22 ± 0.67**	94.91 ± 1.32	92.68 ± 1.18	90.75 ± 1.37
% colloids (mean ± SD)	0.54 ± 0.10	**0.75 ± 0.11**	3.42±0.21	6.55 ± 0.29	8.89 ± 0.32
% Free (mean ± SD)	5.59 ± 0.34	**2.03 ±0.31**	1.67 ± 0.21	0.77 ± 0.10	0.36 ± 0.07
LME					
% labeling (mean ± SD)	71.46 ± 1.67	86.55 ± 1.23	92.76 ± 1.54	**98.13 ± 0.78**	96.72 ± 1.41
% colloids (mean ± SD)	0.39 ± 0.08	0.85 ± 0.29	1.08 ± 0.31	**1.23 ± 0.52**	2.84 ± 0.12
% Free (mean ± SD)	28.15 ± 0.47	12.6 ± 0.18	6.16 ± 0.43	**0.64 ± 0.15**	0.44 ± 0.23
LNE					
% labeling (mean ± SD)	73.86 ± 1.46	90.75 ± 1.57	**97.19 ± 1.97**	93.74 ± 1.42	91.86 ± 0.79
% colloids (mean ± SD)	0.65 ± 0.08	0.82 ± 0.14	**1.29 ± 0.21**	5.09 ± 0.14	7.35 ± 0.48
% Free (mean ± SD)	25.49 ± 0.46	8.43 ± 0.25	**1.52 ± 0.25**	1.17 ± 0.06	0.79 ± 0.12

Values are represented as mean ± SD, n=3.

Figure 7.4: Influence of the Amount of Stannous Chloride on the Labeling Efficiency of LS, LME and LNE

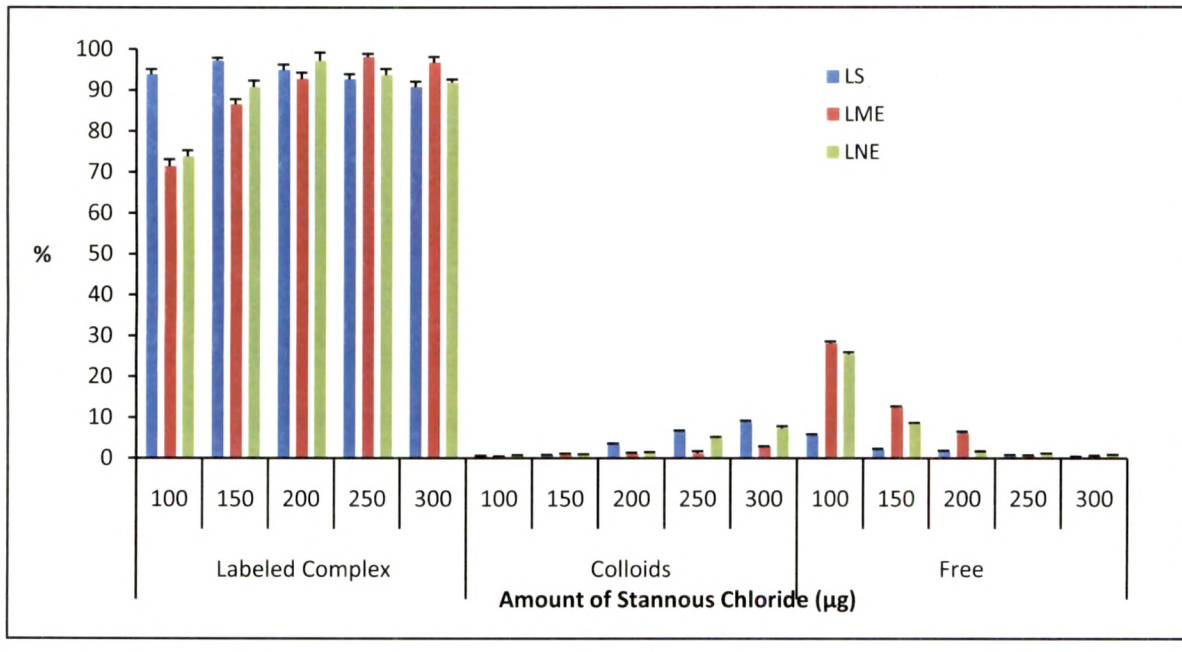


Table 7.13: Stability of ^{99m}Tc -labeled complex of TS, TME and TNE

Time	% Labeling Efficiency		
(h)	LS	LME	LNE
0	97.22 ± 0.67	98.13 ± 0.78	97.19 ± 1.97
0.5	97.19 ± 0.58	98.02 ± 0.85	97.07 ± 1.22
1	96.86 ± 1.04	97.88 ± 1.45	96.79 ± 1.35
2	97.52 ± 0.87	97.62 ± 1.28	96.45 ± 0.79
4	96.75 ± 1.08	96.87 ± 1.36	95.87 ± 1.43
6	95.36 ± 1.62	95.53 ± 2.05	95.06 ± 1.57
24	93.53 ± 2.17	93.45 ± 1.56	93.18 ± 1.33
48	91.47 ± 1.14	90.39 ± 1.24	90.76 ± 1.64

Values are represented as mean ± SD, n=3.

Table 7.14: Radiolabeling summary of DS, Microemulsion and Nanoemulsion

	TS	**TME**	**TNE**
Method	Direct Labeling	Direct Labeling	Direct Labeling
Amt. of $SnCl_2$ (μg)	150	250	200
pH	6.5	6.5	6.5
Incubation duration (min)	10	30	30
Labeling efficiency (%)	98.18	97.32	96.28
Activity added (MBq)	111-131.3	138.75-166.5	111-131.3
	LS	**LME**	**LNE**
Method	Direct Labeling	Direct Labeling	Direct Labeling
Amt. of $SnCl_2$ (μg)	150	250	200
pH	6.5	6.5	6.5
Incubation duration (min)	15	30	30
Labeling efficiency (%)	97.22	98.13	97.19
Activity added (MBq)	111-131.3	138.75-166.5	111-131.3

The optimum quantity of stannous chloride for high labeling efficiency and low free and reduced/hydrolyzed ^{99m}Tc, was found to be 250 μg, 200 μg and 150 μg for ME, NE formulations and drug solutions (TS and LS) respectively. The incubation time was optimized at 30mins for ME and NE formulations. TS and LS require incubation of 10 and 15 min respectively. The pH of all the formulations was kept at around 6.5. The labeling efficiency for TS, TME and TNE was found to be 98.18%, 97.32% and 96.28 % respectively. The labeling efficiency for LS, LME and LNE was found to be 97.22%, 98.13% and 97.19 % respectively. The radiolabeled complex show high stability in rat serum with radiolabeling efficiencies measured, greater than 90%.

7.4 Conclusion

Direct radiolabeling was found to be useful tool to study biodistribution. Radiolabeling of nanoparticles, emulsion and solution preparations of LTG and TMD were successfully performed and the results indicated good stability and bonding strength of the radiolabeled complex. Hence, these formulations were found stable and suitable to study biodistribution and to study gamma scintigraphy imaging of these formulations on animals.

7.5 References

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