7.1. Materials.

Chloramine T and sodium metabisulphite AR grade were purchased from Sigma Aldrich, USA. EDTA coated tubes for blood collection and 1 ml sterile syringes were procured from BD, India. I^{131} was obtained from Radiochemicals section, Bhabha Atomic Research Centre, India.

7.2. Protocol for *in vivo* biodistribution studies.

The protocol for the study was approved by the Institutional Animal Ethics Committee, Bhabha Atomic Research Centre, Mumbai bearing protocol no. BAEC/02/19. The Wistar rats of either sex were divided into two groups as shown in table 7.1. The animals were administered radiolabelled theranostic nanoparticles by intranasal and intravenous routes and at time points of 1, 4, 24, 48 hours the animals were sacrificed humanely by using CO_2 saturated chamber. The blood and major organs of animals were excised and radioactivity was measured individually using flatbed gamma counter, NaI (Tl) detector (ECIL, India) to determine the distribution of theranostic nanoparticles *in vivo*. The plasma and brain samples of the animals were preserved in -20°C for estimation of drug content (1, 2).

Table 7.1: Group of animals for *in vivo* biodistribution.

Sr.no.	**Group**	**No of animals (n=3/time point)**
1	Radiolabelled theranostic nanoparticles loaded with lenalidomide (intranasal)	12
2	Radiolabelled theranostic nanoparticles loaded with lenalidomide (intravenous)	12
Total		**24**

7.3. Dose calculation for biodistribution.

The dose calculation was done as per the guidelines entitled “Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers”, U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), July 2005, Pharmacology and Toxicology (3, 4).

Dose of lenalidomide in humans=5 mg

Average human weight= 60kg (as per the guidelines)

Human Km=37(as per the guidelines)

Rat Km=6(as per the guidelines)

Human equivalent dose (mg/kg) = animal dose (mg/kg)*(animal Km/Human Km)

Dose in rats=0.5118 mg/kg

7.4. Radiolabelling of theranostic nanoparticles.

The I^{131} was obtained from Radiochemicals division, BARC having half-life of 7 days. For radiolabelling, chloramine T oxidation method was used (5). 5 mg of carboxylated metallic core was taken in 1 ml of centrifuge tube and I^{131} was added in sufficient quantity so that the final labelling was 1MBq for 100 µl of the formulation. To the mixture, Chloramine T solution 100µl (5mg/ml) followed by 5 minutes of incubation after which 200µl of 5mg/ml solution of sodium metabisulphite was added to quench the reaction. The radiolabelled core was washed with deionised water after centrifugation at 15000 rpm for 10 minutes to remove unbound radioactivity (I^{131}) and reagents. After washing, the radiolabelled core was dispersed into the aqueous phase for coating of as per the procedure described in chapter 4.

7.5. Results and discussion.

The biodistribution study was performed to determine the favourable route of administration by comparison of intranasal route and intravenous route of administration. The results of the biodistribution study are depicted in table 7.2 and figure 7.1.

7.5.1. Intransal administration.

In case of the intranasal route of the administration of the radiolabelled nanoparticles, the transfer of contents from the nasal cavity into the stomach by the means of oropharynx was observed (6). Thus, high radioactivity was observed in the stomach after 1 hour of administration of the radiolabelled nanoparticles. The presence of radioactivity was observed in brain which is due to the nose to brain delivery of the nanoparticles while the radioactivity in kidneys was the result of the elimination of the nanoparticles by renal route (7).

The time point of 4 hours shows highest radioactivity in the intestinal region due to emptying of contents of the stomach into the intestinal region. There was an increase in the radioactivity in the brain due to the slow transit of theranostic nanoparticles from nose to brain pathway with an increase in radioactivity in the urine by renal clearance as the gastric emptying time in rats was estimated to be 6-12 hours (8).

At 24 hours post administration there was a reduction in the radioactivity from the intestines and kidneys due to the elimination of the radiolabelled nanoparticles. At 48 hours, highest activity was observed in case of the stool which indicated the elimination of the theranostic nanoparticles. There was increase in the brain radioactivity due to the absorption of the theranostic nanoparticles from the large vascularised region of the intestines and entry into the systemic circulation (9).

7.5.2. Intravenous administration.

The intravenous route showed the high radioactivity in the liver and intestine post 1 hr administration due to the fact that liver plays a key role in the uptake of the iron oxide nanoparticles by the means of mononuclear phagocytic system (MPS). The macrophages and the kupffer cells in the liver accumulate the iron oxide nanoparticles. The MPS system serves as a major pathway of elimination of the iron oxide based nanoparticles when administered intravenously (7).

At all time points, high radioactivity was observed in kidneys which are responsible for the renal clearance of the nanoparticles as the nanoparticles enter the nephrons which are the functional unit of kidneys from the systemic circulation.

High activity was observed in urine at time points of 24 and 48 hours due to the prolonged circulation of the theranostic nanoparticles administered by intravenous route owing to the polymeric coating of the nanoparticles as the non coated iron oxide nanoparticles have shorter elimination half life while the polymeric coating enhances the half life of the iron oxide nanoparticles (7). At 48 hours highest radioactivity was observed in the brain due to uptake of the prolonged circulating nanoparticles while highest elimination was observed by the renal pathway.

7.5.3. Comparative analysis

The drug concentration profile shows that the drug was present in the brain and plasma at all-time points. In case of the intranasal route there was decline in the concentration of drug after 1 hour due to the elimination of the nanoparticles while the plasma concentration was higher as compared to brain due to the absorption of the nanoparticles through the gastrointestinal tract while in case of the intravenous route the brain and plasma concentration were found to increase with time due to the prolonged circulation of nanoparticles. There was no significant difference between the plasma concentrations of drug after both of the routes of administration, while the drug concentration was found to decrease in brain by intranasal route due to elimination of the nanoparticles.

Table 7.2: Bio distribution of radiolabelled theranostic nanoparticles in Wistar rats.

	Intranasal ID/gm (n=3)				**Intravenous ID/gm (n=3)**			
Organs	1hr	4hr	24hr	48hr	1hr	4hr	24 hr	48 hr
Liver	1.563±0.023	1.960±0.589	0.715±0.282	0.305±0.007	28.280±9.720	33.050±13.918	21.533±11.127	15.903±3.305
Stomach	41.987±0.508	16.100±3.752	2.367±1.734	1.455±1.379	4.500±0.9157	6.890±2.131	2.903±0.432	0.707±0.250
Intestine	13.620±5.352	67.213±33.680	16.438±3.393	5.520±3.097	20.530±9.332	14.990±9.928	15.733±4.627	5.153±1.080
Kidney	0.423±0.219	0.397±0.197	0.192±0.097	0.115±0.021	1.220±0.709	1.230±0.199	1.327±0.236	1.347±0.182
Heart	0.030±0.017	0.147±0.083	0.040±0.020	0.030±0.0042	0.370±0.137	0.330±0.030	0.087±0.051	0.367±0.050
Lungs	0.233±0.098	0.447±0.276	0.094±0.030	0.105±0.021	0.880±0.425	0.760±1.519	0.547±0.246	0.620±0.096
Spleen	0.040±0.017	0.103±0.078	0.043±0.0045	0.075±0.021	0.630±0.437	0.640±0.275	0.553±0.212	0.747±0.067
Blood	**0.193±0.029**	**0.753±0.215**	**0.144±0.135**	**0.100±0.071**	**1.240±0.523**	**1.250±0.208**	**0.620±0.260**	**0.450±0.053**
Brain	**0.023±0.006**	**0.053±0.0068**	**0.017±0.0029**	**0.060±0.014**	**0.160±0.0227**	**0.140±0.01**	**0.057±0.055**	**0.323±0.006**
Urine / stool	2.820± 1.057	8.640±3.602	61.808±14.409	85.905±6.187	1.010±0.596	0.130±0.1099	46.307±21.606	57.407±6.055
Carcass	22.513±19.855	20.363±8.378	9.857±5.541	3.800±2.234	38.060±4.0781	35.250±6.188	16.197±3.958	14.687±3.972
***ID=%internalised dose**								

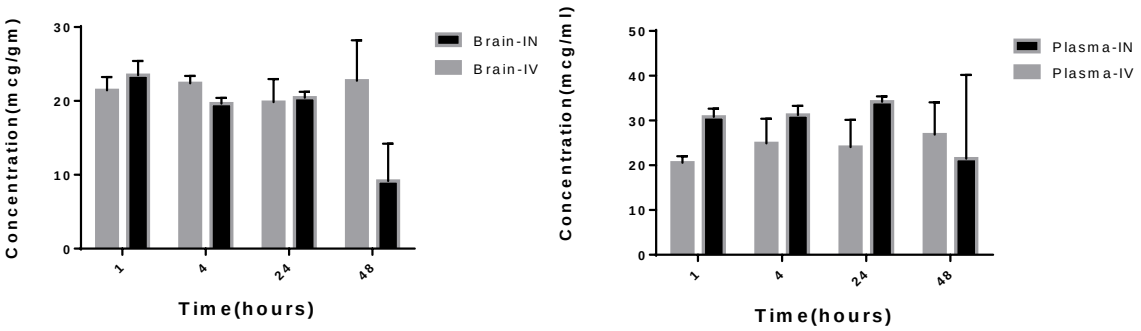

Figure 7.1: Estimation of drug in brain and plasma of samples from bio distribution study (n=3).

The ratio of % internalised dose (ID) of brain/blood for all time points for both the routes is shown in figure 7.2. It can be observed that the brain/blood ratio of % ID was found to decrease with time for intranasal route of administration but at 48 hours a drastic increase was observed due to the absorption of the theranostic nanoparticles from the gastrointestinal tract. In case of the intravenous route the brain/blood ratio of % ID was found to increase with time due to prolonged circulation of theranostic nanoparticles.

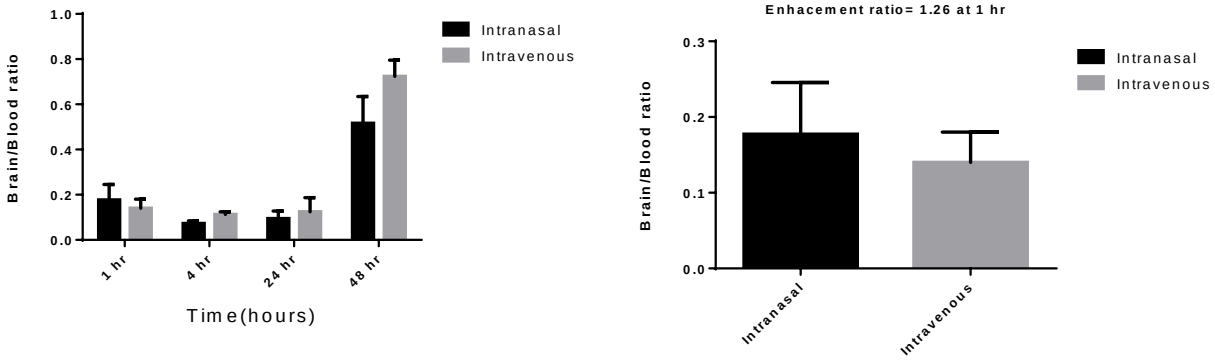

Figure 7.2: Comparison of %ID Brain/blood ratio for all time points.

The enhancement ratio was found to be 1.26 for intranasal route at the time point of 1 hour from the following equation.

$$Enhancement\ ratio = \frac{\%ID\ Brain/blood(intranasal)}{\%ID\ Brain/blood(intravenous)}$$

It can be concluded that both routes were able to deliver the nanoparticles to the brain but intranasal route showed highest enhancement ratio at the time period of 1 hour as compared to intravenous route due to the rapid delivery by nose to brain pathway. Thus intranasal route was found to be appropriate for targeting the brain with theranostic nanoparticles.

7.6. References.

1. Chertok B, David AE, Yang VC. Polyethyleneimine-modified iron oxide nanoparticles for brain tumor drug delivery using magnetic targeting and intra-carotid administration. Biomaterials. 2010;31(24):6317-24.
2. Gadhave D, Gorain B, Tagalpallewar A, Kokare C. Intranasal teriflunomide microemulsion: An improved chemotherapeutic approach in glioblastoma. Journal of Drug Delivery Science and Technology. 2019;51:276-89.
3. Shin J-W, Seol I-C, Son C-G. Interpretation of animal dose and human equivalent dose for drug development. The Journal of Korean Medicine. 2010;31(3):1-7.
4. FDA U. Guidance for industry estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. FDA, ed. 2005.
5. Wang H, Sheng W. 131 I-traced PLGA-lipid nanoparticles as drug delivery carriers for the targeted chemotherapeutic treatment of melanoma. Nanoscale research letters. 2017;12(1):1-8.
6. Patel S, Chavhan S, Soni H, Babbar A, Mathur R, Mishra A, et al. Brain targeting of risperidone-loaded solid lipid nanoparticles by intranasal route. Journal of drug targeting. 2011;19(6):468-74.
7. Arami H, Khandhar A, Liggitt D, Krishnan KM. In vivo delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles. Chemical Society Reviews. 2015;44(23):8576-607.
8. Van der Velde P, Koslowsky I, Koopmans H. Measurement of gastric emptying during and between meal intake in free-feeding Lewis rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1999;276(2):R597-R605.
9. Alphandéry E. Biodistribution and targeting properties of iron oxide nanoparticles for treatments of cancer and iron anemia disease. Nanotoxicology. 2019;13(5):573-96.