

Selected radiotracers as imaging tools for the investigation of nano-sized delivery systems

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LIST OF ABBREVIATIONS

$[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$	[Samarium-153] Samarium oxide
μl	Microliter
^{111}In	Indium-111
^{123}I	Iodine-123
^{152}m	Samarium-152
$^{152}\text{SmCl}_3$	Samarium chloride
^{153}Sm	Samarium-153
^{235}U	Uranium
^{99}Mo	Molybdenum
$^{99}\text{MoO}_4^{2-}$	Molybdenum oxide
$^{99\text{m}}\text{Tc}$	Technetium-99m
$^{99\text{m}}\text{Tc-MDP}$	$^{99\text{m}}\text{Technetium-methylene diphosphonate}$
$^{99\text{m}}\text{TcO}_4^-$	Technetium oxide
aq	Aqueous
CSIR	Council for Scientific and Industrial Research
DSC	Differential scanning calorimetry
EoI	End of Irradiation
FTIR	Fourier-transform infrared spectroscopy
GI	Gastrointestinal
HCl	Hydrochloric acid
IAEA	International Atomic Energy Agency
ICP	Inductively coupled plasma
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
ID/g	Injected dose per gram
ITLC	Instant thin layer chromatographic
IV	Intravenous
keV	Kilovolts
km/h	Kilometer per hour
MBq	Megabecquerel
MDP	Methylene diphosphonate
MeV	Megavolts
mg	Milligrams
mg/kg	Milligrams per kilogram
ml	Millilitre
MPS	Mononuclear phagocytic system

mV	Millivolts
MW	Megawatt
M_w	Molecular weight
Necsa	South African Nuclear Energy Corporation
nm	Nanometer
o/w	Oil-in-water
PCL	Poly(ε-caprolactone)
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PECA	Poly(ethylcyanoacrylate)
PEG	Polyethylene glycol
PET	Positron emitter tomography
PLA	Polylactic acid
PLGA	Poly(D,L-lactide-co-glycolide)
PMMA	Poly(methylmethacrylate)
PVA	Polyvinyl alcohol
RESOLV	Rapid expansion of supercritical solution into liquid solvent
RESS	Rapid expansion of supercritical solution
ROI	Region of interest
rpm	Rotations per minute
SAFARI-1	South Africa Fundamental Atomic Research Installation-1
SEM	Scanning electron microscopy
SLN	Solid lipid nanoparticles
Sm	Samarium
SPECT	Single photon emission computed tomography
SPECT/CT	Single photon emission tomography/computed tomography
TB	Tuberculosis
Tc	Technetium
TEM	Transmission electron microscopy
UV	Ultraviolet
w/o/w	Water-in-oil-in-water
w/v	Weight per volume
XRD	X-ray diffractometry
β	Beta
γ	Gamma
ζ	Zeta potential

ABSTRACT

Developing nanoparticulate delivery systems that will allow easy movement and localisation of a drug to the target tissue and provide more controlled release of the drug *in vivo* is a challenge for researchers in nanomedicine. The aim of this study was to evaluate the biodistribution of two nano-delivery systems namely, poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles containing samarium-153 oxide ($[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$) as radiotracer and solid lipid nanoparticles (SLNs) containing technetium-99m-methylene diphosphonate ($^{99\text{m}}\text{Tc-MDP}$), after oral and intravenous administration to rats to prove that orally administered nanoparticles indeed alter the biodistribution of a drug as compared to the drug on its own.

Stable samarium-152 oxide ($[^{152}\text{Sm}]\text{Sm}_2\text{O}_3$) was encapsulated in polymeric PLGA nanoparticles. These were then activated in a nuclear reactor to produce radioactive $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ loaded-PLGA nanoparticles. Both the stable nanoparticles as well as the fully decayed activated nanoparticles, were characterized for size, Zeta potential and morphology using dynamic light scattering and scanning electron microscopy (SEM) or transmission electron microscopy (TEM), respectively. SLNs were a form of delivery system which was used to encapsulate the radiotracer, $^{99\text{m}}\text{Tc-MDP}$. $^{99\text{m}}\text{Tc-MDP}$ SLNs were characterized before and after encapsulation for size and Zeta potential. Both nanoparticle compounds were orally and intravenously (IV) administered to rats in order to trace their uptake and biodistribution through imaging and *ex vivo* biodistribution studies.

The PLGA nanoparticles containing $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ were spherical in morphology and smaller than 500 nm, therefore meeting the objective of producing radiolabelled nanoparticles smaller than 500 nm. Various parameters were optimized to obtain an average particle size ranging between 250 and 300 nm, with an average polydispersity index (PDI) ≤ 0.3 after spray drying. The particles had a Zeta potential ranging between 5 and 20 mV. The Sm_2O_3 -PLGA nanoparticles had an average size of 281 ± 6.3 nm and a PDI average of 0.22. The orally administered $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ -PLGA nanoparticles were deposited in various organs which includes bone with a total of 0.3% of the Injected Dose (ID) per gram vs the control of $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ which showed no uptake in any organs except the GI-tract. The IV injected $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ -PLGA nanoparticles exhibit the highest localisation of nanoparticles in the spleen (8.63%ID/g) and liver (3.07%ID/g).

The $^{99\text{m}}\text{Tc-MDP}$ -labelled SLN were spherical and smaller than 500 nm. Optimization of the MDP-loaded SLN emulsions yielded a slightly higher PDI of ≥ 0.5 and a size range between 150 and 450 nm. The Zeta potential was between -30 and -2 mV. The MDP-loaded SLN had an

average size of 256 ± 5.27 and an average PDI of 0.245. The orally administered ^{99m}Tc -MDP SLN had the highest localisation of nanoparticles in the kidneys (8.50%ID/g) and stomach (8.04%ID/g) while the control, ^{99m}Tc -MDP had no uptake in any organs except the GI-tract. The IV injected ^{99m}Tc -MDP SLN also exhibited a high localisation of particles in the kidneys (3.87%ID/g) followed by bone (2.66%ID/g). Both the IV and oral ^{99m}Tc -MDP SLN reported significantly low deposition values in the heart, liver and spleen.

Based on the imaging and the biodistribution studies, it can be concluded that there was a significant transfer of the orally administered radiolabelled nanoparticles from the stomach to other organs vs the controls. Furthermore, this biodistribution of the nano carriers warrants surface modification and optimisation of the nanoparticles to avoid higher particle localisation in the stomach.

Keywords: *Imaging, Nanoparticles, PLGA, Radiotracers, Samarium oxide, SLN, ^{99m}Tc -MDP*

Uittreksel

Die ontwikkeling van nanopartikel afleweringstelsse, wat gemaklike beweging en lokalisering van 'n geneesmiddel by die teikenweefsel fasiliteer en die gekontroleerde vrystelling daarvan *in vivo* bewerkstellig, is 'n uitdaging vir navorsers in die nanomedisyne veld. Die doelwit van die studie was om die bio-verspreiding van twee nano-afleweringstelsse naamlik: poli(D,L-laktied-ko-glikolied) (PLGA) nanopartikels wat samarium-153 oksied ($[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$) as radiomerker bevat en soliede lipied nanopartikels (SLNs) wat tegnesium-99-metileendifosfonaat ($^{99\text{m}}\text{Tc-MDP}$) bevat, na afloop van orale en binnearse toediening aan rotte te bestudeer. Daarmee sal gepoog word om te bewys dat oraal toegediende nanopartikels wel die bio-verspreiding van 'n geneesmiddel verander wanneer dit vergelyk word met die geneesmiddel alleen.

Die aktivering van die nanopartikels is gedoen in 'n kernreaktor om radioaktiewe $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ gelaai-PLGA nanopartikels te vervaardig. Beide die stabiele nanopartikels, sowel as die volledig afgebreekte geaktiveerde nanopartikels, is gekarakteriseer vir grootte, Zeta potensiaal en morfologie m.b.v. dinamiese lig verstrooiing en skanderende elektron mikroskopie (SEM) of transmissie elektron mikroskopie (TEM), onderskeidelik. SLN was 'n vorm van 'n afleweringstelsse wat gebruik is om die radiomerker $^{99\text{m}}\text{Tc-MDP}$ te enkapsuleer. $^{99\text{m}}\text{Tc-MDP.SLN}$ is voor en na enkapsulering vir grootte en Zeta potensiaal gekarakteriseer. Beide die geënkapsuleerde radiomerkers is gekwantifiseer met induktiewe gekoppelde spektrometrie (ICP) na afbreking. Beide nanopartikel verbindings was oraal en binnears (IV) toegedien aan rotte om hulle opname en bio-verspreiding na te spoor deur beeldvorming en *ex vivo* bio-verspreiding studies.

Die PLGA nanopartikels, wat $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ bevat, was sferies in morfologie en kleiner as 500 nm. Dit het dus die doelwit van die vervaardiging van radiogemerkte nanopartikels kleiner as 500 nm bereik. Verskeie parameters is geoptimaliseer om 'n gemiddelde deeltjie grootte tussen 250 en 300 nm te verkry, met 'n gemiddelde polidispersiteitsindeks (PDI) ≤ 0.3 na sproei-droging. Die deeltjies het 'n Zeta potensiaal van tussen 5 mV en 20 mV gehad. Die Sm_2O_3 -PLGA nanopartikels het 'n gemiddelde grootte van 281 ± 6.3 nm en 'n gemiddelde PDI van 0.22 gehad. Die oraaltoegediende $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ -PLGA nanopartikels het versamel in verskeie organe, insluitende die been met 'n totaal van 0.3% van die Ingespuite Dosis (ID) per gram, teenoor die kontrole van $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ wat geen opname in enige organe buiten die spysverteringskanaal getoon het nie.

Die IV toegediende $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ -PLGA nanopartikels toon die hoogste lokalisering van die nanopartikels in die milt (8.63% ID/g) en die lewer (3.07% ID/g).

Die ^{99m}Tc -MDP-gemerkte SLN was sferies en kleiner as 500 nm. Optimalisering van die MDP-gelaaide SLN emulsies het 'n effens hoër PDI van ≥ 0.5 en 'n grootte reeks tussen 150 en 450 nm gehad. Die Zeta potensiaal was tussen -30 en -2 mV. Die MDP-gelaaide SLN het 'n gemiddelde grootte van 256 ± 5.27 nm en 'n gemiddelde PDI van 0.245 gehad. Die oraaltoegediende ^{99m}Tc -MDP SLN het die hoogste lokalisasie van nanopartikels in die niere (8.50%ID/g) en die maag (8.04%ID/g) gehad, terwyl die kontrole, ^{99m}Tc -MDP, geen opname in enige organe buiten die spysverteringskanaal gehad het nie. Die IV toegediende ^{99m}Tc -MDP SLNs het ook 'n hoë lokalisering van deeltjies in die niere getoon (3.87%ID/g) gevolg deur die been (2.66%ID/g). Beide die IV en die orale ^{99m}Tc -MDP SLN het noemenswaardige lae deponeringswaardes in die hart, lewer en milt gehad.

Gebaseer op die beelding en die bio-verspreiding studies, kan die gevolgtrekking gemaak word dat daar 'n noemenswaardige oordrag van die radiogemerkte nanodeeltjies van die maag na die ander organe was, in vergelyking met die kontroles. Verder, regverdig hierdie bio-verspreiding van die nano-draers oppervlaksmodifisering en optimalisering van die nanopartikels om hoër partikel versameling in die maag te vermy.

Sleutelwoorde: *Beelding, Nanopartikels, PLGA, Radiomerkers, Samarium oksied, SLN, ^{99m}Tc -MDP*

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Some biological drugs exhibit poor biodistribution, clearance and biopharmaceutical properties due to poor bioavailability, often resulting in high toxicity (Brayden, 2003; Koo *et al.*, 2005a). This is the case for some drugs which are taken orally, which is the most commonly used and accepted form of drug administration. Oral formulations, particularly peptides and proteins are often unstable in the gastric environment, aggregation of drug molecules occur due to poor solubility, nonspecific delivery, *in vivo* degradation and relatively short drug half-lives leading to high doses and dose frequency which subsequently leads to high toxicity and reduced efficacy (Ensign *et al.*, 2012). These challenges are even more evident in poverty related diseases such as tuberculosis (TB) and malaria. There is thus a need to develop suitable drug delivery systems that distribute therapeutic drugs to the target without affecting non-infected organs and tissues. Drug delivery is the process of releasing a bioactive agent at a specific rate at the target site (Orive *et al.*, 2003). A more targeted delivery system can help reduce or eliminate significant drug side effects by limiting the exposure of the drug to organs or tissue and reducing the drug concentration for treatment.

Nanoparticles can be used as a drug delivery system by encapsulating a therapeutic agent within their polymeric matrix, either adsorbed or conjugated onto the surface. Nanoparticles are submicron sized colloidal particles less than 1000 nm in diameter (Mohanraj and Chen, 2006; Parveen *et al.*, 2012; Reis *et al.*, 2006).

Nanotechnology focuses on formulating therapeutic agents in biocompatible nano-scale delivery systems for drug delivery and imaging (Caruthers *et al.*, 2007; Koo *et al.*, 2005b; Parveen *et al.*, 2012). Drug delivery nanoparticulate systems may include polymeric nanoparticles (Parveen *et al.*, 2012; Smola *et al.*, 2008), solid lipid nanoparticles (SLNs) (Koo *et al.*, 2005b), polymeric micelles (Parveen *et al.*, 2012; Smola *et al.*, 2008), liposomes (Ali *et al.*, 2009; Drummond *et al.*, 1999; Koo *et al.*, 2005b) and dendrimers (Ali *et al.*, 2009; Parveen *et al.*, 2012). "Polymeric nanoparticle" is a collective term for any type of polymer nanoparticle, including nanospheres (with the therapeutic drug dispersed in the polymer matrix) or nanocapsules (encapsulated in the polymer) (Parveen *et al.*, 2012). SLNs are made from lipids which are in the solid state at room temperature and where the drug can be encapsulated in the shell or in the core of the nanoparticle (Mehnert and Mäder, 2001; Parveen *et al.*, 2012). Polymeric micelles are formed from block copolymers which assemble in aqueous solution as an inner hydrophobic core and

an outer hydrophilic layer (Parveen *et al.*, 2012). Liposomes are spherical vesicles composed of an inner aqueous core surrounded by one or more phospholipid membranes (Koo *et al.*, 2005). Dendrimers are dispersed symmetric macromolecules structured around a small molecule with an internal cavity surrounded by numerous reactive end groups (Koo *et al.*, 2005; Parveen *et al.*, 2012).

Using nanotechnology, an anti-TB drug can be encapsulated in polymeric nanosized capsules such as poly(D,L-lactide-co-glycolide) (PLGA), that will allow easy movement and localisation of the drug to the target tissue and provide more controlled release of the drug to the bloodstream *in vivo*. Polylactic acid (PLA) and its copolymers with glycolic acid such as PLGA are biodegradable and biocompatible polymers which have been widely studied and used for sustained drug delivery (Parveen *et al.*, 2012; Vergoni *et al.*, 2009) *in vivo* and to promote selective and specific targeted therapy. This has made them widely employed for the preparation of sustained release preparations and PLGA nanoparticles have successfully been employed as anti-TB drug carriers (Tripathi *et al.*, 2010). The advantages of PLGA nanoparticles and SLNs include the encapsulation of extremely hydrophobic and hydrophilic drugs and controlled drug release rates. The size and loading of the nanoparticles can easily be manipulated to provide enhanced control over drug delivery. SLNs provide enhanced bioavailability of drugs via modification of the dissolution rate and/or improved tissue distribution and targeting. Nanoparticulate delivery systems such as SLNs and PLGA nanoparticles have gained attention because of their biodegradability properties as well as low toxicity. To improve on the current inadequate control of TB, the team at the Council for Scientific and Industrial Research (CSIR) is developing polymeric anti-TB nanosized delivery systems containing anti-TB drugs that will enable entry, targeting, slow release and retention of the drugs in the cells for longer periods. This will hence reduce the dose frequency of the drug from daily intake to once a week. The team aims to individually encapsulate four of the first line anti-TB drugs in nanosized particles or capsules that will allow easy pharmacokinetics.

Enhanced *in vivo* drug biodistribution is important in improving the efficacy of these therapeutic drugs and in limiting their potential side effects. Monitoring the accumulation of a therapeutic formulation in specific organs or tissue in real-time can allow scientists to optimize the formulations to enhance their biodistribution properties. The most common method of tracking particle uptake or biodistribution involves the use of radiolabelled nanoparticles. In these studies, the animals used are normally divided into several groups and administered with the radiolabelled nanoparticles. Each group is then sacrificed at different predetermined time points followed by the preparation and examination of histology slices of each animal using autoradiography. The intensity of radiation from sections of the histology slice are then

correlated to the final distribution and rate of particle clearance. The most commonly used animal models for such studies are rats, guinea pigs and rabbits. The biodistribution data which is obtained gives an accurate representation of the tissues specifically targeted by a drug formulation and provide information on the main organs of clearance.

A powerful feature of nuclear molecular imaging is the evaluation of drug delivery systems *in vivo*. It is a technique which uses external detectors such as gamma (γ) cameras to capture and form images from the radiation emitted by radiopharmaceuticals after they are administered orally or intravenously. The use of techniques adapted from clinical radiopharmacy and nuclear medicine facilities allow drug molecules and carrier systems to be radiolabelled and their release, biodistribution and uptake may be visualized *in vivo* (Perkins and Frier, 2004). For example, by tagging a drug molecule, peptide, protein or a cell with a radiotracer, its site of release, distribution and metabolism can be studied. Imaging technology uses suitable γ emitting radionuclides, commonly technetium-99m (^{99m}Tc), indium-111 (^{111}In), iodine-123 (^{123}I) and samarium-153 (^{153}Sm) which may be imaged with a γ camera. An advantage of these techniques is that the *in vivo* distribution and kinetics of a radiolabelled pharmaceutical formulation may be quantified, as a result a correlation between the observed pharmacological effects and the specific site of delivery may be made.

This study forms part of a long term study whose aim is to establish the *in vivo* mechanism of uptake, tissue distribution and degradation of [^{153}Sm]Samarium oxide ([^{153}Sm]Sm₂O₃) loaded-nanoparticles and that of ^{99m}Tc -methylene diphosphonate (^{99m}Tc -MDP)-labelled SLNs and their release profiles in animal models (i.e. rats or mice). The aim of this study was to determine the biodistribution of PLGA nanoparticles containing [^{153}Sm]Sm₂O₃ and of ^{99m}Tc -MDP-labelled SLNs post oral and intravenous administration to healthy rats over a period of two days considering that [^{153}Sm]Sm₂O₃ has a half-life of 48 h and ^{99m}Tc a half-life of 6 h. The objectives include designing and producing radiolabelled PLGA nanoparticles containing [^{153}Sm]Sm₂O₃ and ^{99m}Tc -MDP-labelled SLNs smaller than 500 nm.

[^{153}Sm]Sm₂O₃ is the compound of choice due to its ability to allow visualization of the *in vivo* localisation of the PLGA nanoparticles without having to euthanize a large number of animals for each time point of assay. Secondly, it is a more sensitive method for detection of particle biodistribution. SmCl₃ will be encapsulated into PLGA nanoparticles as a substitute for an anti-TB drug for imaging purposes only and not as a drug compound. Although ^{153}Sm has been used as an imaging agent, PLGA nanoparticles produced via a spray drying technique have not yet been marked with radiotracers for biodistribution and imaging studies.

Technetium-99m is a radioactive γ emitting compound used as an imaging agent. ^{99m}Tc -MDP is a commonly used bone-imaging agent because it accumulates in bone. Due to the sensitivity of [^{153}Sm] Sm_2O_3 and ^{99m}Tc -MDP, it is assumed that the biodistribution will be confirmed with higher accuracy.

The objectives of this study are to:

- Encapsulate stable $^{152}\text{Sm}_2\text{O}_3$ initially in the PLGA nanoparticles and then activate them in the SAFARI-1 (South Africa Fundamental Atomic Research Installation) nuclear reactor followed by quality control to ensure that the particles are still intact.
- Label ^{99m}Tc -MDP onto a SLN formulation during preparation. This is an emulsion-based nanoparticle formulation in which no activation in the reactor is possible as these nanoparticles will not withstand the irradiation heat.
- Determine the biodistribution of these particles post oral and intravenous administration in laboratory rats over a period of one week.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Drug delivery has become a field of great interest to researchers mainly because delivering therapeutic agents or medicine to its site of action is one of the main limitations in pharmaceutical and biotechnology industries (Parveen *et al.*, 2012). Drug delivery is a process of releasing a bioactive agent at a controlled rate at a target site (Orive *et al.*, 2003; Parveen *et al.*, 2012). A more targeted delivery system can help reduce or eliminate significant drug side effects by limiting the exposure of the drug to specific organs or tissue and reducing the drug concentration for treatment. Safe and targeted drug delivery could improve the performance of therapeutic drugs which are already in the market and influence the development and success of new therapeutic drugs.

Research in the field of nanotechnology has led to the development of nanoscale drug delivery systems, which focus on formulating biocompatible nanoscale delivery systems for drug delivery and imaging (Caruthers *et al.*, 2007; Koo *et al.*, 2005(b); Parveen *et al.*, 2012). Nanoscale delivery systems can in this case be referred to as nanoparticles. Nanoparticles can be used to deliver drugs, recombinant proteins, vaccines and nucleotides (Parveen *et al.*, 2012). Nanoparticulate delivery systems may include polymeric nanoparticles (Mohammad and Reineke, 2012; Parveen *et al.*, 2012; Smola *et al.*, 2008), solid lipid nanoparticles (SLNs) (Parhi and Suresh, 2012), polymeric micelles (Parveen *et al.*, 2012; Smola *et al.*, 2008), liposomes (Ali *et al.*, 2009; Koo *et al.*, 2005(b)) and dendrimers (Ali *et al.*, 2009; Parveen *et al.*, 2012).

In this study, polymeric poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles and SLNs as delivery systems will be discussed as well as their applications in diagnostics and imaging. The advantages of PLGA nanoparticles and SLN include the encapsulation of extremely hydrophobic and hydrophilic drugs and controlled drug release rates. These delivery systems were intended to be radiolabelled by encapsulation with a radiotracer (samarium or technetium) instead of an anti-tuberculosis (TB) or anti-malaria drug compound. The conventional method of nanoparticle formulation involves encapsulating a drug into the nanoparticle shell. This was done with the aim of tracking the uptake and biodistribution of these nanoparticulate delivery systems in Sprague Dawley Rats. Thus these radiolabelled nanoparticle vectors will in this study be referred to as delivery systems because they do not contain any drug.

2.2 Polymeric nanoparticulate delivery systems

Polymeric nanoparticles is a collective term for any type of nanosphere or nanocapsule that is made from a polymer (Parhi and Suresh, 2012); examples include PLGA and Polylactic acid (PLA). Nanoparticles can be defined as solid colloidal particles smaller than 1000 nm in diameter (Koo *et al.*, 2005(a); Parveen *et al.* 2012; Rao and Geckeler, 2011; Reis *et al.*, 2006). Nanospheres are particles made up of solid mass on which molecules can be adsorbed on the sphere surface or encapsulated within the particle (Couvreur *et al.*, 1995; Reis *et al.*, 2006), Figure 2.1(a). Nanocapsules are vesicular systems which act as a kind of reservoir in which the entrapped compounds are confined to a cavity consisting of a liquid core, either oil or water, and surrounded by a solid material shell (Figure 2.1(b) and (c)) (Rao and Geckeler, 2011). An advantage of polymeric delivery systems is that they allow for chemical modifications including the synthesis of block and co-polymers (Parhi and Suresh, 2012).



Figure 2.1: An illustration of polymeric delivery systems, (a) nanosphere, and nanocapsules containing (b) oil and (c) water (Rao and Geckeler, 2011).

2.2.1 Preparation of polymeric nanoparticles

Polymeric nanoparticles can be prepared either from preformed polymers or by direct polymerization of monomers using polymerization or polyreactions (Rao and Geckeler, 2011; Reis *et al.*, 2006) using a variety of synthetic and natural polymers. Synthetic polymers such as polyacrylates, polycaprolactones, polylactides and its copolymers with polyglycolides are commonly used (Hans and Lowman, 2002; Rao and Geckeler, 2011; Reis *et al.*, 2006; Soppimath *et al.*, 2001). Natural polymers include albumin, alginate, gelatine and chitosan (Rao and Geckeler, 2011). When synthetic polymers are used, they are dissolved in a suitable solvent followed by precipitation in a liquid medium eventually leading to nanoparticle formation.

The drug intended to be encapsulated in the nanoparticle is usually incorporated during polymer salvation and precipitation.

Methods for the preparation of polymeric nanoparticles from synthetic polymers include solvent evaporation, salting-out, dialysis and supercritical fluid technology (Rao and Geckeler, 2011; Reis *et al.*, 2006).

Methods of directly synthesizing polymeric nanoparticles by the polymerization of monomers using various polymerization techniques include micro-emulsion, mini-emulsion, surfactant-free emulsion and interfacial polymerization as shown in Figure 2.2. The choice of the method of preparation is made on a basis of factors such as the type of polymeric system, area of application and size requirement (Rao and Geckeler, 2011).

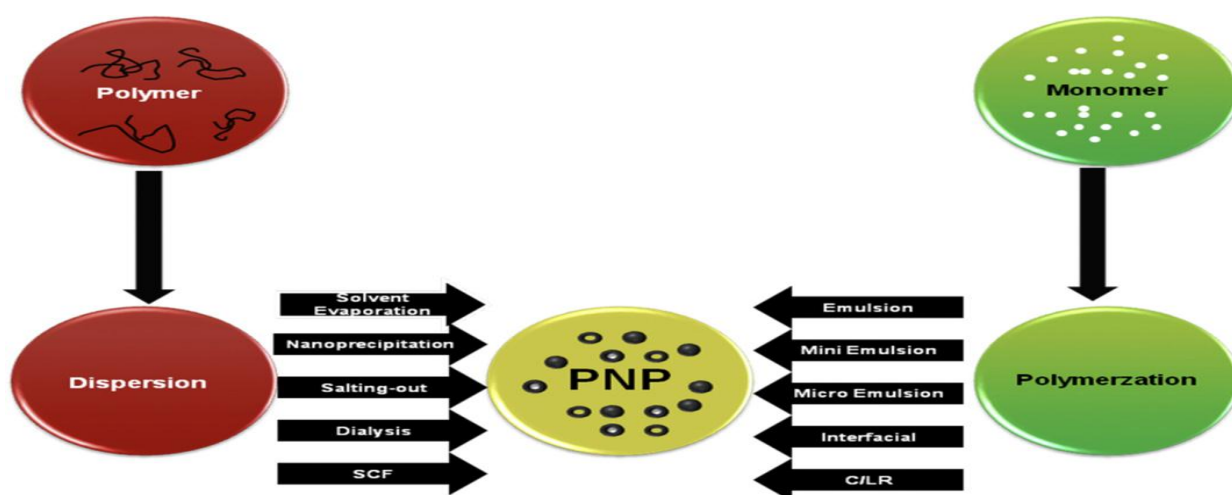


Figure 2.2: A schematic representation of the different techniques for the preparation of polymeric nanoparticles. SCF: supercritical fluid; C/LR: controlled/living radical (Rao and Geckeler, 2011)

Nanoparticles are usually in aqueous form after preparation. This may cause degradation of the polymer and leakage of the drug into the medium. Stability, easy handling and readiness for further processing such as the formulation of tablets, capsules and powders, and the conservation of the particle structure of the nanoparticulate system are achieved by drying. Spray drying and freeze-drying in appropriate conditions are techniques which conserve the structure of the particles efficiently.

2.2.1.1 Nanoparticles obtained from preformed polymers

2.2.1.1.1 Solvent evaporation/ Emulsification

The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion (Rao and Geckeler, 2011). In conventional methods, two main strategies have been developed for the formation of emulsions: preparation of single emulsions, example oil-in-water (o/w) or double emulsions, example water-in-oil-in-water (w/o/w). Solvent evaporation uses high-speed homogenization or ultrasonication, followed by evaporation of the solvent either by continuous stirring or under reduced pressure. Thereafter the solidified nanoparticles are collected by freeze-drying or spray drying (Rao and Geckeler, 2011). This method was used in this study to obtain PLGA nanoparticles encapsulated with samarium oxide ($[^{152}\text{Sm}]\text{Sm}_2\text{O}_3$) as the radiotracer, followed by powder collection through spray drying.

The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature (Rao and Geckeler, 2011; Reis *et al.*, 2006). However, this method is best applied to lipophilic drugs such as isoniazid, ethambutol, pyrazinamide. The most commonly used polymers are PLGA, PLA, ethyl cellulose and cellulose acetate phthalate (Reis *et al.*, 2006; Soppimath *et al.*, 2001).

2.2.1.1.2 Nanoprecipitation

Nanoprecipitation systems generally consist of four basic components, the polymer, the polymer solvent, the active compound and the non-solvent of the polymer. An organic solvent, for example ethanol, acetone, hexane, methylene chloride dioxane, which is miscible in water and easy to remove by evaporation, is chosen as the polymer solvent (Rao and Geckeler, 2011). The polymer, generally PLA, PLGA or poly(ϵ -caprolactone) (PCL) and the hydrophobic drug are co-dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres (Rao and Geckeler, 2011). This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer drug deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension (Reis *et al.*, 2006). This technique is often used for hydrophobic active compounds.

2.2.1.1.3 Salting out with synthetic polymers

Salting out is based on the separation of a water miscible solvent from an aqueous solution via a salting out effect. The polymer and drug are initially dissolved in a solvent such as acetone (Rao and Geckeler, 2011), which is subsequently emulsified into an aqueous gel containing the salting out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as polyvinyl pyrrolidone or hydroxyethyl cellulose (Kostog *et al.*, 2010; Rao and Geckeler, 2011). This o/w emulsion is diluted with a sufficient volume of water to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres.

2.2.1.1.4 Solvent diffusion (Dialysis)

The encapsulating polymer is dissolved in an organic solvent that is partially water soluble such as propylene carbonate and placed in a dialysis tube with proper molecular weight cut-off and saturated with water to ensure the initial thermodynamic equilibrium of both liquids (Reis *et al.*, 2006). To produce the precipitation of the polymer and the formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partially miscible with water or with another organic solvent in the opposite case (Rao and Geckeler, 2011; Reis *et al.*, 2006). Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing a stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point.

2.2.1.1.5 Techniques based on supercritical or compressed fluids

For the production of nanoparticles using supercritical fluids, there are two processes which have been developed namely, rapid expansion of supercritical solution (RESS) and rapid expansion of supercritical solution into liquid solvent (RESOLV) (Rao and Geckeler, 2011). In general, supercritical fluid technology allows for the drug and the polymer to be solubilised in a supercritical fluid (e.g. carbon dioxide), and the solution then expanded through a nozzle. The supercritical fluid is evaporated in the spraying process and the solute particles precipitate. This is a clean technique because the precipitated solute is solvent free (Rao and Geckeler, 2011).

2.2.1.2 Nanoparticles obtained by polymerization of a monomer

In the polymerization methods, monomers are polymerized to form nanoparticles in an aqueous solution. The drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after completion of polymerization. The particle suspension is then purified to remove various surfactants and stabilizers by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium (Rao and Geckeler, 2011). The formation of nanoparticles and their particle size depends on the concentration of the surfactants and stabilizers used (Mohanraj and Chen, 2006).

2.2.1.2.1 Emulsion polymerization

This method is classified into two categories, based on the use of an organic or aqueous continuous phase. Emulsion polymerization uses surfactants or protective soluble polymers to prevent aggregation in the early stages of polymerization (Kreuter, 1991; Reis *et al.*, 2006). Later, poly(methylmethacrylate) (PMMA), poly(ethylcyanoacrylate) (PECA) and poly(butylcyanoacrylate) nanoparticles are produced by dispersion via surfactants into solvents such as cyclohexane, n-pentane, and toluene as the organic phase (Rao and Geckeler, 2011; Reis *et al.*, 2006).

2.2.1.2.2 Interfacial polymerization

Interfacial polymerization involves stepwise polymerization of two reactive monomers which are dissolved respectively in two phases (continuous and dispersed) and then the reaction takes place at the interface of the two liquids (Rao and Geckeler, 2011). The advantage of obtaining nanoparticles using this method is that the polymer is formed *in situ*, which allows the polymer membrane to follow the contours of the inner phase of an o/w or water-in-oil (w/o) emulsion (Couvreur *et al.*, 2002). The main disadvantage of this technique would be the use of organic solvents which are required for the external phase.

2.3 Biodegradable polymers

2.3.1 Poly(D,L-lactide-co-glycolide)

PLGA is a polymer which is synthesised by means of random ring-opening co-polymerization of two different monomers; the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid (Vergoni *et al.*, 2009). Common catalysts used in the preparation of PLGA include tin(II) 2-

ethylhexanoate, tin(II) alkoxides and oraaluminum isopropoxide (Rao and Geckeler, 2001). During polymerization, successive monomeric units of glycolic acid or lactic acid are linked together into PLGA by ester linkages, giving the aliphatic polyester as a product (Mohammad and Reineke, 2012; Vergoni *et al.*, 2009). Different forms of PLGA can be obtained depending on the ratio of lactide to glycolide used for polymerization and identified in regard to the monomers' ratio used, (e.g. PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid) (Rao and Geckeler, 2011; Reis *et al.*, 2006). PLGA is amorphous and shows a glass transition temperature in the range of 40-60 °C (Mohammad and Reineke, 2012). It can be dissolved by various solvents such as acetone, ethyl acetate, tetrahydrofuran and chlorinated solvents (Rao and Geckeler, 2011).

PLGA degrades by hydrolysis of its ester linkages in the presence of water (Mohammad and Reineke, 2012). The time required for degradation of PLGA is related to the monomers' ratio in production; the higher the content of glycolide units, the lower the time required for degradation (Rao and Geckeler, 2011; Reis *et al.*, 2006). An exception to the rule is the copolymer with a 50:50 ratio of the monomers, which exhibits faster degradation properties (about two months) (Rao and Geckeler, 2011; Reis *et al.*, 2006). PLGA successfully undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid (Reis *et al.*, 2006; Semete *et al.*, 2010(b); Vergoni *et al.*, 2009). These monomers under normal physiological conditions are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications (Rao and Geckeler, 2011; Semete *et al.*, 2010(a), (b)).

2.4 Characterisation of nanoparticles

Nanoparticles differ from macroscopic objects because of submicron properties such as high surface area, high energy and random movement of the particles by diffusion or Brownian motion. Characterisation of a nanoparticle system is important in understanding and predicting the performance of that system in the body. The size, morphology and physical state of the encapsulated compound including the molecular weight (M_w) and crystallinity of the polymer influence the drug release and degradation of the nanoparticles. Size, surface charge and hydrophobicity or hydrophilicity of the nanoparticles affects distribution in the body and interactions with the biological environment.

2.4.1 Size

Sub-micron size nanoparticles have been shown to be more advantageous over micro-particles as drug delivery systems (Mohanraj and Chen, 2006; Panyam and Labhasetwar, 2003). They generally have higher intracellular uptake compared to micro-particles and available to a wider range of biological targets due to their small size and relative mobility. Smaller particles have a larger surface area, therefore most of the associated drug would be near or at the particle surface, leading to fast drug release. Larger particles have large cores which allow more of the drug to be encapsulated and diffuse out slowly (Redhead *et al.*, 2001). Smaller particles pose a greater risk of aggregation during storage and transportation of the nanoparticle dispersion, thus it is important to synthesize particles that have the appropriate size for drug delivery or imaging.

Photon correlation spectroscopy (PCS) is a technique that is based on dynamic (laser) light scattering and is widely used to determine the size of nanoparticles (Bivas-Benita *et al.*, 2004; Chorny *et al.*, 2002; Galindo-Rodriguez *et al.*, 2004; Govender *et al.*, 1999; Redhead *et al.*, 2001). PCS measures the intensity variation (because of the Brownian motion of nanoparticles) of scattered light and relates it to the particle size with the assistance of an autocorrelation function (Pecora, 2000). PCS presumes all particles as being spherical and as a result, a hydrodynamic diameter is obtained. This technique is fast, sensitive to nanoscale particles and provides information about the whole nanoparticulate population. The sample dispersion has to be diluted, filtered and the results are based on mathematical calculations.

2.4.2 Morphology

Nanoparticle size and morphology can also be characterised with scanning electron microscopy (SEM) (Bilati *et al.*, 2005; Bivas-Benita *et al.*, 2004; Galindo-Rodriguez *et al.*, 2004; Leroueil-Le Verger *et al.*, 1998) and transmission electron microscopy (TEM) (Chorny *et al.*, 2002; Govender *et al.*, 1999; Ren *et al.*, 2005; Texeira *et al.*, 2005; Tobìo *et al.*, 1998) because conventional light microscopy is not suitable for nanoparticle characterisation since its resolution is limited to about 1 μm . In SEM, the nanoparticulate sample which is coated for example with carbon or gold to be conductive, is scanned in a high vacuum chamber with a focused electron beam (Newman and Brittain, 1995; Parhi and Suresh, 2012), where secondary electrons which are emitted from the sample are then detected and an image is formed. In TEM, the sample which is between the electron gun and the detector, scatters electrons which are detected and an image is formed (Banerjee *et al.*, 2001; Ekambaram *et al.*, 2012). SEM and TEM provide visual and descriptive information about the nanoparticle population (Ekambaram *et al.*, 2012).

As a result, electron microscopy and light scattering techniques should be used together in determining the size of the nanoparticles.

2.4.3 Surface charge

The surface charge of nanoparticles determines the performance of the nanoparticle system in the body, such as interactions with cell membranes (Parhi and Suresh, 2012). The information about the particle surface charge is provided by Zeta (ζ) potential measurements (Ensign *et al.*, 2012; Webb and Orr, 1997). Zeta potential is the charge at the electrical double layer, which exists around each particle and is created by ions of the liquid. The mobility of charged particles is determined with the help of electric potential and transferred to Zeta potential with the help of Smoluchowski's equation (Ishikawa *et al.*, 2005; Parhi and Suresh, 2012). Zeta potential is influenced by the conditions of the dispersing medium such as pH and electrolyte concentration (Ishikawa *et al.*, 2005) and can be altered by surface modification (Ensign *et al.*, 2012; Sukhorukov *et al.*, 1998) and/or stabilizer concentration (Popovic *et al.*, 2010). Zeta potential represents a measure of an electrostatically stabilized colloidal dispersion where an adequately high Zeta potential absolute value would provide or indicate stability for a nanoparticle dispersion. Values which are above ± 30 mV are considered characteristic for a stable colloidal dispersion (Benita and Levy, 1993; Ensign *et al.*, 2012).

2.4.3.1 Surface modification

The coating or modification of nanoparticle surfaces with biocompatible, hydrophilic polymers helps to protect nanoparticles against uptake by the mononuclear phagocytic system (MPS) and enhances stability (Ensign *et al.*, 2012). Examples of polymers for this purpose are polyethylene glycol (PEG) and ethylene oxide or propylene oxide block copolymers, poloxamers and poloxamines (De Campos *et al.*, 2003; Gref *et al.*, 1994; Redhead *et al.*, 2001). Mucoadhesive coating by polymers like chitosan, poly (acrylic acid) sodium alginate and poloxamers improve the bioavailability of the encapsulated drug by prolonging the circulation or residence time of nanoparticles at the site of absorption (De Campos *et al.*, 2003; Ensign *et al.*, 2012; Hu *et al.*, 2002; Kawashima *et al.*, 2000).

2.4.3.2 Stability

Nanoparticles dispersed in aqueous solutions can be stabilized either by surface charge (electrostatic stabilization) or by surfactants or other molecules at the particle surface (steric stabilization) or by a combination of both (Overbeek, 1977; Popovic *et al.*, 2010). Loss of

stability leads to an increase in polydispersity, which can be detected by photon correlation spectroscopy and visual turbidity (Parhi and Suresh, 2012). Turbidity measurement usually indicates aggregation, where increasing turbidity indicates decreasing stability (Ekambaram *et al.*, 2012; Trimaille *et al.*, 2003).

2.4.4 Drug-polymer interactions

Drug loading can be done during the preparation of nanoparticles within the nanoparticle or by adsorption on the nanoparticle surface in preformed particles. Within the polymer, the drug can be present as a solid solution (individual drug molecules) or as a solid dispersion (amorphous or crystalline drug). The preparation process can modify the structure of the drug. The polymer is usually amorphous or semi-crystalline.

To reveal the physicochemical state and possible interactions of the drug and the polymer in nanoparticles, techniques such as differential scanning calorimetry (DSC), x-ray diffractometry (XRD) and Fourier-transform infrared spectroscopy (FTIR) are commonly used.

DSC detects phase transitions such as crystallization (exothermic), melting (endothermic) and glass transition. The nanoparticle sample is heated and the changes in heat flow compared to a reference sample are registered (Dubernet, 1995; Ekambaram *et al.*, 2012). XRD analysis provides crystallinity or amorphicity properties when the diffraction pattern of the x-ray from the sample is determined as a function of scattering angle (Ekambaram *et al.*, 2012; Suryanarayanan, 1995). In FTIR, a vibrational spectrum, characteristic for a given crystal structure is obtained (Brittain *et al.*, 1991).

2.5 Drug-loading and release

Drug encapsulation efficiency is a percentage value that describes the amount of the drug in the nanoparticle out of the total amount of drug used in the process. The encapsulation efficiency is determined by separating the nanoparticle from the dispersion medium by ultracentrifugation. Drug loading is the drug amount compared to the nanoparticle mass. It is quantified from the supernatant or after solvation of the nanoparticle pellet (Bivas-Benita *et al.*, 2004; De Campos *et al.*, 2003).

To formulate a successful nanoparticulate system, both the drug release and polymer biodegradation should be considered. Drug release from nanoparticles can occur by diffusion through the particle by desorption from the surface or after degradation. The drug release

environment *in vivo* is complex and may be difficult to simulate. A successful nanoparticulate system should ideally have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug-loading and encapsulation efficiency depends a lot on the solid-state drug solubility in the polymer which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end-functional groups such as ester or carboxyl groups (Govender *et al.*, 1999; Govender *et al.*, 2000; Panyam and Labhasetwar 2003). The drug release rate generally depends on the solubility of the drug, the polymer degradation, diffusion of the drug through the polymer and a combination of erosion or diffusion processes. Thus diffusion, solubility and biodegradation of the polymer influence the release process.

In nanospheres, the drug is uniformly distributed and the release occurs by diffusion or degradation of the polymer (Rao and Geckeler, 2011). If the diffusion of the drug is faster than the degradation of the polymer, the mechanism of release is largely controlled by a diffusion process. The rapid initial release or burst is mainly attributed to weakly bounded drug molecules on the large surface of the nanoparticle (Mohanraj and Chen, 2006). The method of incorporation has an effect on the release profile. If the drug is encapsulated, the system has a relatively small burst effect and better sustained release characteristics (Mohanraj and Chen, 2006).

2.6 Lipid-based nanoparticulate delivery systems

2.6.1 Nanoemulsions

Nanoemulsions are colloidal dispersions of oil in water or water in oil, where the dispersed droplets are of nanosize range and stabilised with a surface active film composed of a surfactant and sometimes a co-surfactant (Brime *et al.*, 2003; Martins *et al.*, 2012; Podlogar *et al.*, 2004; Seki *et al.*, 2004). Nanoemulsions are generally transparent or translucent systems that have a dispersed-phase droplet size range between 50 and 200 nm (Tadros *et al.*, 2004). They are becoming more popular as pharmaceutical formulations because they are generally easy to prepare, are thermodynamically stable and transparent (Koo *et al.*, 2005(a)). The size range of the droplets prevents sedimentation or creaming from occurring on storage (Koo *et al.*, 2005(a); Tadros *et al.*, 2004). Nanoemulsions can be prepared by sonication, high speed homogenisation and low energy emulsification whereby water is added to an oil solution of the surfactant. The lipid phase of the nanoemulsion is composed of fatty vegetable oils or middle chain triglycerides, which make up typically 10-20% of the emulsion. Other ingredients include phospholipids (stabilizers) and glycerol (osmolarity regulation) (Jaspreet *et al.*, 2012). The advantages of nanoemulsions are their toxicity safety and high content of the lipid phase as well as the possibility of large scale production by high pressure homogenization.

2.6.2 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are an alternative delivery system to emulsions, microparticles, liposomes and other polymeric counterparts for various application routes. SLNs are colloidal delivery systems (Castelli *et al.*, 2005; Martins *et al.*, 2012; Wang *et al.*, 2013) which are like nanoemulsions but differ in the nature of the lipid. The liquid lipid used in nanoemulsions is replaced by a solid lipid at room temperature and high melting point glycerides or waxes in SLNs (Müller and Keck, 2004; Üner and Yener, 2007).

SLNs can be used in different application routes such as oral (Pandey *et al.*, 2005; Üner and Yener, 2007), ophthalmic (Friedrich *et al.*, 2005; Üner and Yener, 2007), parenteral (Martins *et al.*, 2012; Wissing *et al.*, 2004), rectal and topical (Üner and Yener, 2007).

SLNs exhibit the following advantages (Martins *et al.*, 2012; Üner and Yener, 2007):

- Possibility of controlled drug release and drug targeting
- Increased drug stability
- A high drug load

- The avoidance of organic solvents
- The incorporation of lipophilic and hydrophilic drugs
- No biotoxicity of the carrier and
- No problems with respect to large scale production and sterilization

SLNs offer more advantages compared to other delivery systems. Although little attention and investigation has been given to the limitations of SLNs, a few points to consider include: the coexistence of different lipid modifications and different colloidal systems, low drug-loading capacity, high pressure-induced drug degradation and the kinetics of the distribution processes (Üner and Yener, 2007).

2.7 Preparation methods of SLNs

SLNs are carriers which are composed of physiological lipids that are dispersed in water or an aqueous surfactant solution such as polyvinyl alcohol (PVA) (Jaspreet *et al.*, 2012; Mehnert and Mäder, 2001). The general ingredients for the production of SLNs are solid lipid(s), emulsifier(s) and water. Lipids can refer to triglycerides (e.g. tristearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes (e.g. bees wax) (Ekambaram *et al.*, 2012; Parhi and Suresh, 2012). All classes of emulsifiers have been used to stabilize the lipid dispersion.

A combination of emulsifiers, with respect to charge and molecular weight, may prevent agglomeration more effectively. The choice of the emulsifier may also depend on the route of administration of the SLN dispersion.

SLNs consist of 0.1 to 30% solid lipid dispersed in an aqueous solution and stabilized with 0.5 to 5% surfactant (Jaspreet *et al.*, 2012). Recent technologies in lipid production use blends of solid lipids and liquid lipids (oils), preferably in a ratio of 70:30 to 99.9:0.1 (Jaspreet *et al.*, 2012). An advantage of SLNs are that they offer properties such as small particle size, large surface area, high drug loading and the interaction of phases at the interface (Wang *et al.*, 2013).

There are several methods used to produce SLNs, namely; high speed homogenization (Gardouh *et al.*, 2012; Jaspreet *et al.*, 2012), high pressure homogenization (Jaspreet *et al.*, 2012; Jores *et al.*, 2004), solvent emulsification/evaporation (Üner and Yener, 2007), and breaking of oil in water microemulsions (Üner and Yener, 2007). These methods will be discussed in the following sections.

2.7.1 High speed homogenization

High speed homogenization was initially used for the production of solid lipid nanodispersions (Jaspreet *et al.*, 2012; Üner and Yener, 2007). This method is easy to handle and simple to perform, however, the quality of the dispersions is often compromised by the presence of microparticles. High speed homogenization uses a rotor-stator homogenizer to break the shear forces between particles to reduce the particle size. The emulsion is placed in the homogenizer and mixed into a homogenous dispersion at very high speeds. The homogenizing speed and time have an effect on the resulting particle size, where higher stirring speeds are expected to reduce the size of the nanoparticles significantly.

Most homogenizers have rotor speeds ranging between 2000 and 35 000 rpm and can take volumes of up to 2 l, depending on the type of homogenizer.

This method was used in this study to obtain SLNs encapsulated with ^{99m}Tc -methylene diphosphonate (^{99m}Tc -MDP) via a w/o/w double emulsion.

2.7.2 High pressure homogenization

High pressure homogenizers work by pushing a liquid with high pressure (100-2000 bar) through a narrow gap (in the range of a few microns) (Jaspreet *et al.*, 2012). The liquid accelerates at a very short distance to a very high velocity of over 1000 km/h. High shear stress and cavitation forces break down or disrupt the particles to the submicron range. Two general approaches, hot and cold homogenization techniques (Figure 2.3) can be used for the production of SLNs using high pressure homogenization (Jaspreet *et al.*, 2012; Mehnert and Mäder, 2001; Üner and Yener, 2007). In both cases, preparation involves the incorporation of the drug into the bulk lipid by dissolving or dispersing the drug in the lipid melt. The cold homogenization technique is employed for hydrophilic drugs in order to reach the highest payload and to prevent drug partition to the aqueous phase during SLN production, whereas the hot homogenization technique is more suitable for lipophilic drugs.

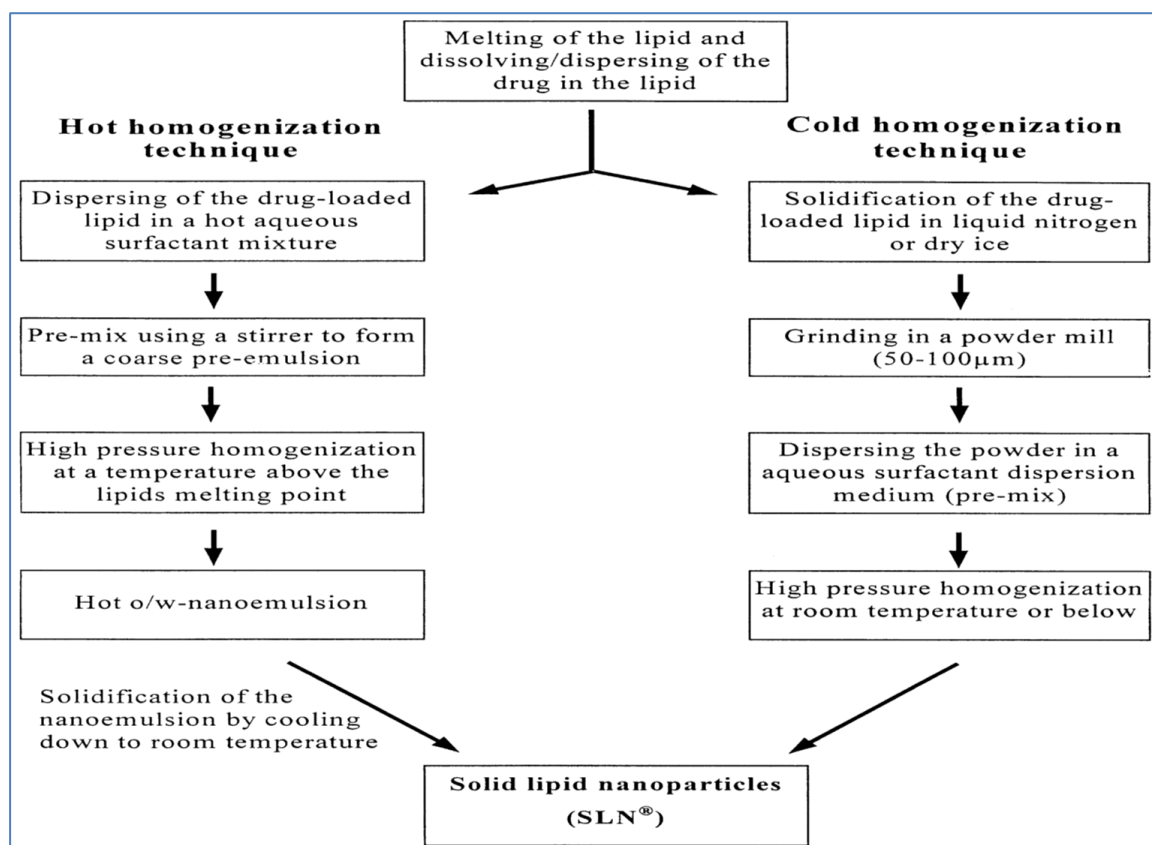


Figure 2.3: A schematic illustration of the hot and cold homogenization techniques for the production of SLN (Mehnert and Mäder, 2001).

2.7.2.1 Hot homogenization

Hot homogenization (Figure 2.3) can be regarded as the homogenization of an emulsion as it is carried out at temperatures above that of the melting point of the lipid (Jaspreet *et al.*, 2012). A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase at the same temperature is obtained by high-shear mixing. The quality of the pre-emulsion affects the quality of the final product and it is desirable to obtain droplets in the size range of a few micrometers.

The primary product of hot homogenization is a nanoemulsion due to the liquid state of the lipid (Jaspreet *et al.*, 2012). Solid nanoparticles are expected to form after cooling the sample to room temperature or below.

2.7.2.2 Cold homogenization

Cold homogenization (Figure 2.3) is carried out with a solid lipid and represents a high pressure milling of a suspension. The temperature in this case needs to be effectively controlled and

regulated in order to ensure the unmolten state of the lipid due to the increase in temperature during homogenization (Mehnert and Mäder, 2001).

2.7.3 SLNs prepared by solvent emulsification/ evaporation

A production method to prepare nanoparticle dispersions by precipitation in o/w emulsions was described by Sjöström and Bergenståhl (1992). The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. A nanoparticle dispersion is then formed by precipitation of the lipid in the aqueous medium after evaporation of the solvent.

2.7.4 Microemulsion based SLN preparations

Microemulsions are prepared by stirring a transparent mixture, which is typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water, at 65- 70 °C (Üner and Yener, 2007). The hot emulsion is dispersed in cold water (at 2-3 °C) under stirring (Ekambaram *et al.*, 2012).

2.8 Influence of ingredient composition on product quality

2.8.1 Influence of the lipid

Small differences in the lipid composition, such as impurities, may have a considerable effect on the quality of the SLN dispersion (e.g. by changing the Zeta potential, retarding the crystallization process, etc.). Lipid nanodispersions made with cetyl palmitate from different suppliers for instance, may have different particle size and storage stabilities (Ekambaram *et al.*, 2012; Mehnert and Mäder, 2001).

It has been shown that when hot homogenization has been used, the average particle size of SLN dispersions increases with higher melting lipids (Üner and Yener, 2007). These results can be explained by the higher viscosity of the dispersed phase. However, critical parameters for nanoparticle formation will differ for different lipids. Examples include the shape and surface area of the lipid crystal, the velocity of lipid crystallization and the hydrophilicity of the lipid (influence on self-emulsifying properties) (Üner and Yener, 2007).

2.8.2 Influence of the emulsifier

The choice of the emulsifiers and their concentrations has an impact on the quality of the SLN dispersion (Jaspreet *et al.*, 2012). High concentrations of the emulsifier reduce the surface tension and facilitate the particle partitioning during homogenization. The decrease in particle size is associated with a rapid increase in surface area during high pressure homogenization (Ekambaram *et al.*, 2012). The process of a primary coverage of the new surfaces competes with the agglomeration of uncovered lipid surfaces (Ekambaram *et al.*, 2012; Müller *et al.*, 1998). The primary dispersion should thus contain excessive emulsifier molecules, which should rapidly cover the new surfaces. The excessive emulsifier molecules may be present in different forms as micelles, liposomes or in the form of molecular solubilised emulsifier monomers.

2.9 Characterisation of SLNs

Adequate characterisation of the solid lipid nanodispersion is necessary for the control of the quality of the product. The methods for characterisation should be sensitive to the key parameters of SLN performance and should avoid artefacts. In characterising SLNs, the particle size, surface morphology and surface charge are studied.

2.9.1 Particle size and Zeta potential

The most powerful techniques used for routine measurements of particle size are PCS and laser diffraction. PCS measures particle size of a few nanometers to about 3 microns.

The measurement of the Zeta potential helps predict the storage stability of a colloidal dispersion (Üner and Yener, 2007). Particle aggregation is less likely to occur in charged particles with a high Zeta potential due to electric repulsion.

Certain features of SLNs, such as supercooled melts, particle shape and different modifications contribute to or determine the stability of the colloidal lipid suspension. Gelation, drug expulsion from the lipid carrier and an increase in particle size pose as major problems of storage stability. The conversion of a lipid melt to lipid crystals results in an increase in the particle surface, a decrease in the loading capacity of the lipid and eventually lead to increased problems in stability of the SLNs (Jaspreet *et al.*, 2012). The stability of lipid dispersions decreases as the stability of the lipid modification increases. To improve the stability of these suspensions the aqueous phase is reduced or removed. This can also be achieved by converting the

suspensions to solids. However, factors such as temperature and light influence the long term stability of SLNs. The Zeta potential should generally be greater than -60 mV for the suspension to remain physically stable (Ekambaram *et al.*, 2012). The most favourable storage temperature ranges from 4 to 20 °C, with 4 °C the optimum temperature (Ekambaram *et al.*, 2012). A rapid growth of particle size has been observed at 50 °C (Ekambaram *et al.*, 2012).

2.10 Surface modification of SLNs

SLNs are hydrophobic and exposed to phagocytic uptake by macrophages. There is thus a need to improve the blood circulation time of SLNs since macrophages in the reticulo-endothelial system recognize them as foreign substances and remove them due to their physicochemical properties, mainly surface hydrophobicity, surface charge and particle size. To avoid phagocytic uptake, the surface properties of SLNs can be modified by using various techniques such as surface coating with PEG (Üner and Yener, 2007). PEG has been shown to favourably modify the surface hydrophobicity of particles and sterically stabilize them, therefore suppressing the binding of serum proteins (e.g. apoproteins) and other opsonic factors (Üner and Yener, 2007).

2.11 Administration routes and *in vivo* aspects

The fate of the SLNs *in vivo* will depend mainly on the following factors:

- Administration route
- Interactions of the SLN with the biological environment including;
- Enzymatic processes such as lipid degradation by lipases and esterases
- Biodistribution processes

SLNs are composed of physiologically related lipids and/or waxes. Therefore, pathways for transportation and metabolism which are present in the body may contribute to the *in vivo* fate of the delivery system. The toxicity of emulsifiers has to be considered with regard to relevant routes of administration. No toxicity problems should be observed for oral or transdermal administration and intramuscular or subcutaneous injection if appropriate surfactants are used. The size of the particles is not very significant for these administration routes because a low content of microparticles may decrease the performance of the SLN system but will not cause toxicity.

2.12 Oral administration of SLNs

Controlled release behaviour of particular systems in oral drug delivery is reported to enable the bypass of gastric and intestinal degradation of the encapsulated drug and their possible uptake and transport through the intestinal mucosa (Tobio *et al.*, 1998 Üner and Yener, 2007). The assessment of the stability of colloidal delivery systems in gastrointestinal fluids is essential in order to predict their suitability for oral administration. In the design of new and efficient colloidal drug delivery systems for oral administration, critical parameters have to be considered: firstly, their stability on contact with gastrointestinal (GI) fluids because they consist of biodegradable materials, and particle sizes in the nano range maximise the surface area for enzymatic degradation.

Secondly, particle aggregation due to environmental conditions of the GI tract has to be considered. This leads to a decrease in the interaction capability of particles with the intestinal mucosa (Üner and Yener, 2007). The microclimate of the stomach favours particle aggregation due to the acidity and high ionic strength. The adhesive properties of nanoparticles increase bioavailability and reduce erratic absorption (Üner and Yener, 2007). Absorption of nanoparticles occurs through the mucosa of the intestine by several mechanisms, namely through the Peyer's patches by intracellular uptake or by the paracellular pathway.

2.13 Radiopharmaceuticals

2.13.1 Introduction

Radiopharmaceuticals are dosage forms consisting of a delivery system and a trace amount of a radionuclide with a defined radiation type and are used routinely in nuclear medicine for diagnosis or imaging (Banerjee *et al.*, 2001; Liu, 2004, 2008). The aim of this study is to encapsulate stable [^{152}Sm] Sm_2O_3 into PLGA nanoparticulate delivery systems, followed by irradiation to activate the γ -emitting radionuclide and also to encapsulate $^{99\text{m}}\text{Tc}$ -MDP into SLNs and evaluate their biodistribution profile through imaging and *ex vivo* studies in rats. Researchers in nanomedicine aim to use this technology to develop novel approaches in targeted therapy, molecular diagnosis and cancer imaging (Hamoudeh *et al.*, 2008).

Gamma or positron emitter tomography (PET) images of the *in vivo* distribution of radiolabelled pharmaceutical formulations can be used to monitor the release, biodistribution and kinetics of a pharmaceutical agent. These imaging techniques provide quantitative information and correlate between the observed pharmacological effects and the exact site of delivery, which demonstrates the concept for targeted drug delivery. The use of radiotracers to monitor the biodistribution, transit and pharmacokinetics of drug formulations is therefore an attractive concept.

2.14 Scintigraphic imaging

Scintigraphic imaging is a tool which produces two-dimensional images of the distribution of radioactivity in tissues and is performed after the internal administration of a non-absorbable radiotracer such as ^{153}Sm , $^{99\text{m}}\text{Tc}$ or ^{111}In . The gamma radiation properties of these isotopes are used to obtain images using a scintillation camera (Yeong *et al.*, 2011(b), 2012). Pharmacoscintigraphy is the combination of scintigraphy with pharmacokinetic studies. Pharmacoscintigraphic studies which are designed and performed correctly in conjunction with *in vitro* testing are proving to be valuable for assessing the *in vivo* performance of intravenous and oral dosage formulations under normal physiological conditions in humans (Perkins and Frier, 2004; Wilding *et al.*, 2001; Yeong *et al.*, 2011(b)). It has become an important means of providing information about the transit and release behaviour of oral dosage forms and drug absorption.

2.14.1 Single photon emission tomography

Single photon emission computed tomography (SPECT) is an imaging modality used in nuclear medicine to provide clinicians with data about the turnover of radioisotopes in different body compartments and organs (Hamoudeh *et al.*, 2008). Typical imaging studies include dynamic and or static imaging and *in vivo* function tests. SPECT is commonly performed using a rotating gamma camera system (Perkins and Frier, 2004). SPECT detects one gamma photon at any time to produce an image data, unlike PET in which two photon events arise from a positron emitting radionuclide. In SPECT, images can be displayed as axial, orthogonal, coronal and sagittal slices or as oblique cuts through any chosen plane (Perkins and Frier, 2004; Wallis and Miller, 1991). SPECT can increase the image contrast and visualization of data slice by slice. It also provides accurate quantification of volume and of organ uptake of single photon tracers (Liu, 2008; Perkins and Frier, 2004). Scintigraphic imaging has the ability to quantify the image data. Each pixel in an image represents the number of detected gamma rays from that site. Therefore, it is possible to quantify regional uptake over specific portions of the image. This uptake can be expressed as a percentage of the administered dose or directly in units of activity (MBq). In this work, SPECT will be used for imaging purposes.

2.15 Gamma radiation

The nuclei of atoms consist of protons and neutrons packed closely together. The nuclei stay together when nucleic forces overcome electromagnetic repulsion forces.

Due to the superposition of different forces, only the nuclei with certain numbers of protons and neutrons are stable. Labile nuclei are radioactive and are gradually transformed toward a stable state by emitting radiation. Radiation can be the emission of α or β particles or electromagnetic γ radiation.

γ radiation originates when the unstable nuclei transit from an excited state to a lower excited state or to the ground state. The amount of energy emitted (energy of γ photons or quanta) depends on the emitting nucleus. γ radiation that is utilised in clinical or pharmaceutical studies should have relatively low energy levels generally in the range of 40 to 400 keV which is higher than the energy of x-rays. This γ radiation penetrates well into tissue, or from inside the tissue to the outside to be detected by a gamma camera. In this work, γ radiation was used for detection of images using a SPECT gamma camera.

2.16 Gamma emitting radiotracers

2.16.1 Properties of radiotracers

The physical characteristics of a radionuclide will determine how suitable it is for imaging. The radionuclide should be suitable for incorporation into the compound of interest and have a half-life long enough to allow radiolabelling, administration and imaging. The γ photon energy should be in the range of detection of the gamma camera, generally 75–360 keV (Perkins and Frier, 2004). Commonly used γ emitters for drug delivery scintigraphic studies are ^{99m}Tc , lutetium-177 (^{177}Lu), ^{153}Sm , ^{111}In , ^{123}I and gallium-67 (^{67}Ga) (Table 2.1) (Hamoudeh *et al.*, 2008; Perkins and Frier, 2004; Yeong *et al.*, 2011(b)).

Table 2.1: Suitable gamma emitting radionuclides for scintigraphic studies of drug delivery

Radionuclide	Physical half-life (hours)	Gamma Energy (keV)
^{153}Sm *	47	103
^{99m}Tc	6	141
^{111}In	67	171,245
^{123}I	13,2	159
^{177}Lu *	161	208
^{131}I	192.48	971

* Radionuclides suitable for radiolabelling dosages formed by neutron activation

The most important properties of stable tracer nuclides are (Perkins and Frier, 2004):

- Gamma emission energies suitable for gamma imaging
- Cross-section for neutron capture that is large enough and
- Neutron irradiation that does not produce significant amounts of α and β emitting daughter radionuclides

This study aims to establish the *in vivo* mechanism of uptake, tissue distribution and degradation of [^{153}Sm]Sm₂O₃ loaded nanoparticles and that of ^{99m}Tc -MDP-labelled solid lipid nanoparticles and their release profiles in animal models.

2.16.2 Samarium

Samarium (Sm) is a rare earth lanthanide. It is a hard, silvery-white metal. Most of its salts are pale yellow in colour. Samarium has thirty isotopes, with mass numbers from 131 to 160. Naturally occurring samarium is a mixture of seven isotopes with the following abundance: ^{152}Sm (26,7%), ^{154}Sm (22,8%), ^{147}Sm (15%), ^{149}Sm (13,8%), ^{148}Sm (11,2%), ^{150}Sm (7,4%) and ^{144}Sm (3,1%).

^{153}Sm has a relatively long half-life of 46,3 h and emits γ radiation at 103 keV, which makes it ideal for scintigraphic imaging (Yeong *et al.*, 2011(a)(b), 2012). It has one γ -emission (28% 103 keV) and three β -emissions (30% 0,64 MeV, 50% 0,71 MeV and 20% 0,81 MeV) (Fani *et al.*, 2002; Liu, 2008; Thunus and Lejeune, 1999). ^{152}Sm , unlike $^{99\text{m}}\text{Tc}$ is non-radioactive and allows formulations to be produced in pharmaceutical facilities and reduces the radiation exposure to workers. Samarium is non-absorbable within the gastrointestinal tract and suitable for studies of slow release oral drug formulations. In this work ^{153}Sm will be used as a radiotracer encapsulated in PLGA nanoparticles for scintigraphic imaging in rats.

2.16.3 Technetium

Technetium (Tc) is a transition metal which belongs to group VIIB of the periodic table and has eight different oxidation states ranging from -1 to +7 (Banerjee *et al.*, 2001). The stability of these oxidation states depends on the type of ligands coordinated and their chemical environment. Technetium-99m is obtained as $\text{Na}^{99\text{m}}\text{TcO}_4$, in which technetium is in the oxidation state of +7 (Banerjee *et al.*, 2001). TcO_4^- is chemically non-reactive and in this oxidation state does not react with any ligand. A number of reducing agents (e.g. concentrated hydrochloric acid (HCl), ascorbic acid or sodium dithionite) reduce this species into the desired oxidation state, thereby making it possible to produce a plethora of $^{99\text{m}}\text{Tc}$ complexes with a wide spectrum of ligands (Banerjee *et al.*, 2001).

$^{99\text{m}}\text{Tc}$ is a short-lived metastable radionuclide ('m' indicates that it is a metastable isomer and mass number 99) (Banerjee *et al.*, 2001; Hamoudeh *et al.*, 2008). It is the most commonly used radionuclide in nuclear imaging. $^{99\text{m}}\text{Tc}$ has a short half-life of 6 h and a γ photon emission of 140 keV, which is an advantage for effective imaging and patient safety perspectives (Banerjee *et al.*, 2001; Hamoudeh *et al.*, 2008; Perkins and Frier, 2000, 2004; Wilson *et al.*, 1997; Yeong *et al.*, 2011(b), 2012). $^{99\text{m}}\text{Tc}$ has the disadvantage of being radioactively harmful; the radiolabelling requires dedicated radiation protection facilities at the premises where

investigations will be undertaken. Another limitation of ^{99m}Tc is its short half-life with respect to the time needed for manufacturing and imaging, especially for drug delivery to the colon which may take between 12 and 24 h.

^{99m}Tc can be derived as a column elute from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator which makes it readily available. It is produced by the decay of ^{99}Mo , when molybdenum oxide ($^{99}\text{MoO}_4^{2-}$) is absorbed through an alumina column. ^{99}Mo has a half-life of 2, 78 days (Liu, 2008). Technetium oxide ($^{99m}\text{TcO}_4^-$) is eluted from the column with saline. The ^{99m}Tc that is produced by the $^{99}\text{Mo}/^{99m}\text{Tc}$ generator is never carrier-free because 13% of ^{99}Mo decays directly to the isotope ^{99}Tc (Banerjee *et al.*, 2001; Liu, 2008). In this work, ^{99m}Tc was encapsulated as a radiotracer in solid lipid nanoparticles for scintigraphic imaging in rats.

2.17 Radiolabelling of radiopharmaceuticals

2.17.1 Introduction

Once a suitable radionuclide has been chosen, an appropriate agent must be chosen to carry the isotope, such as colloidal systems (nanoparticles), macromolecules, antibodies or cells (Wilding *et al.*, 2001). In drug delivery studies, it is important that the radiolabelling procedure does not affect the integrity of the formulation and interfere with the fate of drug delivery (Hardy, 1990; Perkins and Frier, 2004).

In this study, there are two possible methods of radiolabelling which will be employed. The one is conventional radiolabelling, which involves conjugating radioactive technetium to methylene diphosphonate (MDP) to form the standard radiopharmaceutical ^{99m}Tc -MDP that will then be encapsulated in a solid lipid nanoparticle colloidal system. The other method is irradiation after incorporation; in which samarium that is labelled to polymeric nanoparticles will be irradiated to produce radioactive samarium.

2.17.2 Conventional radiolabelling

Conventional radiolabelling involves linking a radioactive isotope to a molecule to form a complex which allows application in nanoparticle liquid formulations. There are two basic approaches to conventional labelling of oral and intravenous dosage forms. One method is to incorporate a non-absorbable chelate of the radioactive isotope, for example ^{99m}Tc -MDP (Wilding *et al.*, 2001). The ^{99m}Tc species is chemically reactive and combines with various chelating agents. The chelating agent usually donates lone pairs of electrons to form co-

ordinate covalent bonds with ^{99m}Tc . ^{99m}Tc is radiolabelled to MDP, a bone seeking agent, to give an organ-specific complex. This is done by combining ^{99m}Tc with the MDP and gently mixing it to dissolve the lyophilized MDP powder. The contents are left to stand for 5 min at room temperature to form a ^{99m}Tc -MDP complex. The ^{99m}Tc -MDP complex which forms is then incorporated into the nanoparticle formulation by high speed mixing into the emulsion.

2.17.3 Irradiation after incorporation

The incorporation of stable isotopes such as ^{152}Sm into the nanoparticles during the formulation is an indirect labelling method. The isotope is then irradiated to form the radioactive isotope inside the nanoparticle. The radioactive samarium isotopes are produced by irradiation with neutrons that in turn are generated from the fission of uranium and plutonium in a nuclear reactor.

The use of stable isotopes and neutron activation methods helps in minimising the exposure to radiation levels, maintaining quality assurance and helps in labelling complicated delivery systems easily and efficiently (Wilding *et al.*, 2001). Stable isotopes such as ^{152}Sm or ^{177}Lu can be incorporated into the dosage form at low levels and then the product irradiated in a neutron source to convert the isotope into a gamma emitting radionuclide (e.g. ^{153}Sm or ^{177}Lu) (Wilding *et al.*, 2001). These radionuclides possess large neutron capture cross-sections and can be obtained in highly enriched forms, which improves the radionuclidic purity of the final dosage form. The exposure of a subject to radiation after dosing with these radionuclides is comparable to that received using conventional radiolabelling with ^{99m}Tc or ^{111}In .

^{153}Sm is a radiotracer which is neutron activated and is used in scintigraphic studies of drug delivery and pharmacology applications (Marvola *et al.*, 2004, 2008; Perkins and Frier, 2004; Yeong *et al.*, 2011(b), 2012). ^{153}Sm is derived via neutron activation from a rare earth element ^{152}Sm , which is commercially available as the oxide, samarium oxide ($^{152}\text{Sm}_2\text{O}_3$) and an acidic aqueous chloride, samarium chloride ($^{152}\text{SmCl}_3$). Sm_2O_3 in micro particle form is recommended for *in vivo* oral drug delivery studies because it is poorly soluble in water and chemically stable, therefore it will not be absorbed in the GI tract. After ^{152}Sm is irradiated with a thermal neutron flux in the range of 1×10^{12} to $5 \times 10^{12} \text{ n.cm}^{-2}.\text{s}^{-1}$, it produces ^{153}Sm (Wilson *et al.*, 1997; Yeong *et al.*, 2011(a)(b)). The radioactivity produced from ^{153}Sm depends on the amount of SmCl_3 or Sm_2O_3 (mg) incorporated, the dimension of the irradiated product, the neutron flux ($\text{cm}^{-2}.\text{s}^{-1}$) and the irradiation time (seconds) (Yeong *et al.*, 2011(b)). In this study, ^{153}Sm will be produced by thermal neutron activation in the SAFARI-1 nuclear reactor.

2.18 Nuclear reactor

Neutron activation requires a thermal nuclear reactor facility. Current statistics from the International Atomic Energy Agency (IAEA) state that there are currently 24 nuclear research reactors in operation in more than 58 countries (IAEA, 2011; Yeong *et al.*, 2012). Although some of these nuclear research reactors are capable of producing clinical radionuclides, only a few of them have the potential to supply ^{153}Sm (Yeong *et al.*, 2012).

SAFARI-1 (South African Fundamental Atomic Research Installation) is a 20 MW tank in pool-type nuclear reactor with a neutron flux of $2,8 \times 10^{14} \text{ s}^{-1} \cdot \text{cm}^{-2}$, licensed and operated by the South African Nuclear Energy Corporation (Necsa), located at Pelindaba, 30 km west of Pretoria. It is the only research nuclear reactor in South Africa (IAEA, 2011).

The utilization of SAFARI-1 is focused on research and commercialization, which includes neutron radiography, neutron diffraction and neutron activation analysis, providing industrial and academic links; all receiving a high profile in the day-to-day operations of the reactor. NTP, a subsidiary of Necsa, is Africa's largest producer of various medical isotopes that are used for diagnostic, therapeutic and research purposes, all originating from SAFARI-1. The Necsa group together with SAFARI-1 is a reliable and leading provider of radiochemicals to the global healthcare market. It has become one of four leading producers of molybdenum-99 (^{99}Mo) (IAEA, 2011). ^{99}Mo is the radioisotope extensively used as a raw material for ^{99}Tc , the most important diagnostic radioisotope in nuclear medicine.

SAFARI-1 received ISO 14001 Environmental Management (2003) and OHSAS 18001 Occupational Health and Safety Management System (2011) certification. SAFARI-1 utilizes an overall Integrated Management System which encompasses environmental controls and also includes operational safety, product quality, radiological and conventional safety and security systems which ensure that a good safety culture is established (Necsa, 2012).

2.19 Conclusion

Particle size and size distribution are the most important characteristics of nanoparticles. These characteristics determine the *in vivo* distribution, biological fate, toxicity and the targeting ability of nanoparticle delivery systems (Mohanraj and Chen, 2006). Nanoparticle size can also influence the drug loading, drug release and stability of nanoparticles. Radiolabelling these nanoparticulate delivery systems can allow them to be imaged and evaluated *in vivo*. These *in*

vivo biodistribution studies could help improve the efficacy of current therapeutic formulations and in limiting their potential side effects. Monitoring the accumulation of a therapeutic formulation in specific organs or tissue in real-time can allow scientists to optimize the formulations to enhance their biodistribution properties.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This chapter describes the experimental procedures which were followed to prepare nano-sized polymeric nanoparticles encapsulated with samarium-152 oxide ($[^{152}\text{Sm}]\text{Sm}_2\text{O}_3$) and solid lipid nanoparticles (SLNs) encapsulated with technetium-99m–methylene diphosphonate ($^{99\text{m}}\text{Tc}$ -MDP) with a size range smaller than 500 nm. These delivery systems were intended to be radiolabelled by encapsulation of a radiotracer (samarium or technetium) instead of an anti-tuberculosis (TB) or anti-malaria drug compound. The conventional method of nanoparticle formulation involves encapsulating a drug into the nanoparticle shell instead of a radionuclide. This was done with the aim of tracking the uptake, biodistribution, localisation and metabolism of these nanoparticulate delivery systems in Sprague Dawley rats.

In this study, polymeric poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were encapsulated with stable $[^{152}\text{Sm}]\text{Sm}_2\text{O}_3$. These were then activated to induce radioactive samarium-153 oxide ($[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$) loaded PLGA nanoparticles via neutron activation in a nuclear reactor. The stable nanoparticles were characterized for size and Zeta potential using a Malvern Zetasizer Nano ZS and their morphology were established via scanning electron microscopy (SEM) and transmission electron microscopy (TEM). After irradiation and sufficient decay this was repeated to ensure that neutron activation had no detrimental effect on the nanoparticles. Thereafter the radioactive $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ loaded PLGA nanoparticles were orally and intravenously administered to rats in order to trace their uptake and biodistribution through imaging and biodistribution studies.

SLNs were another form of delivery system which was used to encapsulate a radionuclide. $^{99\text{m}}\text{Tc}$ -MDP was encapsulated in this SLN emulsion. For optimising and characterisation purposes, SLNs were encapsulated with methylene diphosphonate (MDP) without technetium-99m ($^{99\text{m}}\text{Tc}$). These were characterized for size and Zeta potential (Malvern Zetasizer Nano ZS). Instant thin layer chromatographic (ITLC) assays were performed to determine whether the $^{99\text{m}}\text{Tc}$ and MDP had formed a complex before encapsulation in the SLNs. The radioactive $^{99\text{m}}\text{Tc}$ -MDP loaded SLNs were orally and intravenously administered to rats in order to trace their uptake and biodistribution through imaging and *ex vivo* biodistribution studies.

3.2 Materials

The materials used for the production of nanoparticles are listed in Table 3.1.

Table 3.1: Materials used in the preparation of nanoparticles

Materials	Manufacturer/ Distributer
Samarium oxide (Sm_2O_3) 99% enriched	Johnson Matthey, Alfa, Ward Hill, MA, USA
Samarium oxide (Sm_2O_3)	Sigma Aldrich
Polyvinyl alcohol (PVA), hydrolysed 87-89%, Mw 13 000-23 000	Sigma-Aldrich
Poly(D,L-lactide-co-glycolide) (PLGA), Mw 30 000-60 000, 50:50	Sigma-Aldrich, Johannesburg, South Africa
Ethyl acetate, 99.5%	Sigma-Aldrich
Stearic acid, Mw 284.48 g/mol	Merck
Surfynol 104 PG 50 surfactant	Air Productions and Chemicals
Polyethylene glycol (PEG), Pluriol E 9000	BASF
Chitosan-De-Ac, Low viscous, 2-amino-2-deoxy-(1 $\rightarrow$ 4) B-D-glucopyranan	Sigma-Aldrich
Tween 20-polyethylene glycol sorbitan monolaurate	Sigma-Aldrich
Biogapress Vegetal BM 297ATO, glyceryl distearate	Gattefossé
Methylene diphosphonate (MDP)	NTP, Pelindaba, Necsa
Technetium-99m ($^{99\text{m}}\text{Tc}$)	NTP, Pelindaba, Necsa
Dichloromethane (DCM), ACS reagent $\geq 99.5\%$	Sigma-Aldrich
Methanol 99.5%	Sigma-Aldrich
Ketamine/ Xylazine	Phoenix Pharmaceutical

Both distilled and deionized water was used for the preparation of all solutions and emulsions.

3.3 Preparation of nanoparticles

3.3.1.1 Preparation of Sm₂O₃

A small quantity of the raw samarium oxide (Sm₂O₃) 99% enriched (Johnson Matthey, Alfa, Ward Hill, MA, USA) powder was milled with a Stuart SHM1 high speed homogenizer (Bibby Scientific Ltd. Stone, Staffordshire, ST15 OSA, United Kingdom) to reach the desired nanometric size range (i.e. <500 nm) prior to emulsification. (Due to the cost of enriched material this procedure was first optimised with high purity natural Sm₂O₃). The milled Sm₂O₃ (50 mg) was dispersed in 2.5 ml of an aqueous solution of 87-89% partially hydrolysed PVA (2 %w/v used as polymeric stabiliser) and the resulting dispersion was milled for 2 min at 35 000 rpm using the high speed homogenizer. Thereafter the coarse particles were separated by centrifuging at 3000 rpm for 15 min. The supernatant was collected to be added into the PLGA solution for the preparation of nanoparticles.

3.3.1.2 Preparation of Sm₂O₃ loaded PLGA nanoparticles

PLGA coated nanoparticles were prepared with PLGA 50:50; Mw 30 000-60 000 purchased from Sigma-Aldrich, as the encapsulating polymer by using a modified double emulsion solvent evaporation technique (Semete *et al.*, (b), 2010; Lamprecht *et al.*, 1999). Briefly, 100 mg of PLGA was dissolved in 10 ml of ethyl acetate containing stearic acid 0.2 %w/v and one drop of surfynol 104 PG 50. A 2 ml suspension of Sm₂O₃ supernatant was added into the dissolved PLGA solution. This mixture was emulsified by means of a Silverson L4R high speed homogenizer (Silverson Machines Limited, Buckinghamshire, United Kingdom) at 8 000 rpm for 10 min in an ice bucket. The resulting water-in-oil (w/o) emulsion was then transferred into a water phase of surface modifying excipients consisting of 15 ml PVA 2 %w/v, 5 ml PEG 1 %w/v, 10 ml chitosan 0.3 %w/v and 10 ml lactose 5 %w/v solution. The mixture was further emulsified using the homogenizer set at 8 000 rpm for 20 min. The resulting water-in-oil-in-water (w/o/w) double emulsion was spray dried in a Buchi mini spray dryer (BUCHI Labortechnik AG, Flawil, Switzerland) at 96 °C, an atomisation pressure of 7 bar, with a pump feeding rate of 2 and 100% aspiration to obtain a dry nanoparticle powder.

3.3.2.1 Preparation of MDP loaded SLNs

To produce an SLN formulation in non-radioactive laboratories, SLNs were formulated with only the encapsulation of MDP dissolved in distilled water. This was also done to ensure analytical characterisation of a non-radioactive emulsion. This method was optimised for the application

of the ^{99m}Tc -MDP method described below. MDP was dissolved in 5 ml distilled water and gently mixed to enhance dissolution. The lipid glyceryl distearate; Biogapress Vegetal was dissolved in dichloromethane at 50 °C to make up the oil phase. Once the powder lipid glyceryl distearate had dissolved, Tween 20, 2 ml MDP solution and 10 drops of surfynol were added to the oil phase while stirring. This entire solution was then homogenized by means of a Stuart SHM1 homogenizer set at 10 000 rpm for 5 min. The homogenized emulsion was transferred into a beaker containing 20 ml PVA 2% w/v aqueous solution and further homogenized at 35 000 rpm for 10 minutes using the same Stuart SHM1 homogenizer. The resulting w/o/w double emulsion was left to stand for 1 h to allow the dichloromethane solvent to evaporate.

3.3.2.2 Preparation of ^{99m}Tc -MDP loaded SLNs

Using aseptic technique working in a laminar flow cabinet, ^{99m}Tc contained in a 10 cm³ vial was added to a MDP vial and mixed gently to dissolve the lyophilized powder. Contents were allowed to stand for 5 min at room temperature to form a ^{99m}Tc -MDP complex.

An aqueous solution of 20 ml of PVA 2% w/v was prepared in an 80 ml beaker then set aside.

Using a spatula, 250 mg of glyceryl distearate lipid was weighed into a polytop tube and dissolved in 5 ml dichloromethane 99% by stirring on a stirrer plate at 50 °C. The polytop was covered with foil to prevent the solvent from evaporating. Once the glyceryl distearate had dissolved 10 drops of Tween 20 were added to the glyceryl distearate solution while stirring. Thereafter, 2ml of ^{99m}Tc -MDP complex solution plus one drop of the surfactant surfynol 104 PG 50 was emulsified into the glyceryl distearate solution by means of a Stuart SHM1 homogenizer set at 10 000 rpm for 5 min. The homogenized emulsion was transferred into a PVA 2 %w/v aq. solution and further homogenized at 35 000 rpm for 10 min using the Stuart SHM1 homogenizer. The resulting w/o/w double emulsion was left to stand for 1 h to allow the dichloromethane solvent to evaporate.

3.4 Neutron activation of Sm_2O_3 - PLGA nanoparticles

The nanoparticles were sent to the reactor at SAFARI-1 (South African Fundamental Atomic Research Installation) operated by the South African Nuclear Energy Corporation (Necsa), located at Pelindaba, South Africa, for neutron activation. SAFARI-1 is a 20 MW tank in pool-type nuclear reactor which utilises uranium zirconium hydride assembly with low enriched uranium (20% ^{235}U) fuel source. The PLGA nanoparticle sample and a [^{152}Sm] Sm_2O_3 control were weighed out to 160 mg and 10 mg respectively and heat-sealed into individual

polyethylene vials and packed into a polyethylene ampoule (commonly known as a rabbit). The ampoule was then delivered to the reactor core by a pneumatic transport system. The nanoparticles were then irradiated in a neutron flux of $2.8 \times 10^{14} \text{ s}^{-1} \cdot \text{cm}^{-2}$ for 60 s as set by preliminary test protocol to achieve a nominal radioactivity of 1 MBq at 48 h after End of Irradiation (EoI). The nanoparticle sample was kept for at least 24 h after EoI to allow for the decay of unwanted irradiation by-products (Yeong *et al.*, 2011(a) (b)). To allow for characterisation in non-radioactive laboratories as described below, the sample(s) was kept for 30 days before release as non-radioactive material.

3.5 Characterisation

3.5.1 Particle size and surface morphology

Particle size and size distribution indicated as the polydispersity index (PDI) were measured by dynamic laser scattering or photon correlation spectroscopy using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom). For each sample, 1-3 mg of Sm_2O_3 -PLGA nanoparticles or one drop of MDP SLNs respectively, were suspended in distilled water, and then sonicated for a few minutes. Each sample was measured in triplicate.

3.5.2 Zeta potential

The Zeta potential was determined using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom) at a pH of 6.8. The instrument calculates the nanoparticle net charge at the surface by determining the electrophoretic mobility using the Laser Doppler Velocity principle. For each sample, 1-3 mg of Sm_2O_3 -PLGA nanoparticles or one drop of MDP SLNs respectively, were suspended in distilled water, then sonicated for a few minutes and introduced into a U-shape Zeta cell. Each sample was measured in triplicate to determine the Zeta potential.

3.5.3 Scanning Electron Microscopy

SEM uses a focused beam of high-energy electrons to bombard a solid surface of a conductive sample to generate a variety of signals from the interaction volume. These electrons interact with the atoms of the sample producing signals which reveal visible information about the sample, relating to its morphology while size measurements of the structures are also possible. The morphology of the Sm_2O_3 -PLGA nanoparticles was analysed using a SEM (Carl Zeiss Microscopy, Oberkochen) operating at an accelerated voltage of 8 kV. The nanoparticle

samples were initially thinly spread on a specimen holder using a spatula and adhered to the surface containing adhesive carbon tape. The nanoparticle samples were coated under argon atmosphere with an ultrathin 10 nm cobalt layer (Emitech K550 Super Coated; Emitech Ltd, South Stour Avenue Ashford, Kent, United Kingdom) to increase electrical conductivity. This is essential so as for samples to be imaged to prevent the accumulation of electrostatic charge build up at non-conductive areas on the sample surface. The stubs were then placed in a SEM high vacuum chamber for analysis. The nanoparticle images were analysed at different magnifications (5000 to 50 000 x).

3.5.4 Transmission Electron Microscopy

The morphology and size of the Sm_2O_3 -PLGA nanoparticles was established using high resolution TEM. The powder nanoparticle samples were initially dispersed in ethanol and briefly sonicated for 2 min. A carbon coated, mesh copper grid was dipped into the nanoparticle suspension and allowed to air dry. Once the suspension had dried out, the grid was then analysed under a JEOL JEM 2100 (JEOL Inc., USA) high resolution TEM at an accelerating voltage of 200 kV. The images were taken using Digital Micrograph Software.

3.5.5 Inductively Coupled Plasma Spectrometry

To quantify the amount of metal in each nanoparticle sample, ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) was performed on a SPECTRO ARCOS (SPECTRO Analytical Instruments, Kleve, Germany). Each nanoparticle sample was diluted by acid digestion. The instrument was calibrated using the standard solutions. The samples were then run to generate a report. The optics are hermetically sealed and filled with argon, to achieve high optical transmission in the vacuum ultraviolet, allowing the determination of non-metals and interference free lines in this region. A wavelength region of between 130 and 770 nm can be simultaneously analysed, allowing capturing of a complete spectrum within 2 s.

3.6 Quality control of compounded radiopharmaceuticals

The $^{99\text{m}}\text{Tc}$ becomes attached to a MDP ligand (a process referred to as labelling), designed to localize in bones. Most of the $^{99\text{m}}\text{Tc}$ should be labelled to the ligand for the radiopharmaceutical to be effective. Very little free $^{99\text{m}}\text{Tc}$ should be present in the final product. Hydrolysed reduced $^{99\text{m}}\text{Tc}$, another by-product of the labelling process, should also be present in low levels.

3.6.1 Instant thin layer chromatography

Instant thin layer chromatographic (ITLC) assays of ^{99m}Tc -MDP complex, ^{99m}Tc -MDP solid lipid nanoparticles and technetium free MDP solid lipid nanoparticle compounds were run on Non-ultraviolet (UV) ITLC silica gel paper, UV ITLC silica gel paper and fibreglass ITLC paper strips. These compounds were spotted at the origin of each ITLC paper and run in a 10 ml solution of methanol 10%. The resulting compounds were then viewed under a UV light. Each strip was then cut in half, placed in plastic pockets and labelled to quantify the radioactivity. Strips were placed and read individually for radioactivity in a (Capintec, Inc, Ramsey, NJ, USA) dose calibrator as well as a MCA scintispec well counter.

3.7 *In vivo* biodistribution studies

3.7.1 Animals

Eight healthy female, inbred Sprague Dawley Rats, weighing between 280 and 390 g were selected and housed under standard environmental conditions at an ambient temperature of 25 °C. The rats were cared for and supplied with food and water ad libitum. The rats were allowed one week to acclimatize before the commencement of the study. The rats also received a unique ear mark; left ear for individual mark and right ear for group mark. Ethics approval was obtained for this study from the Ethics Committee for Research on Animals (ECRA), Tygerberg, Cape Town, South Africa.

3.7.2 Imaging and Biodistribution assays of nanoparticles

To obtain scintigraphic images of orally and intravenously administered radiolabelled particles, the rats were anaesthetised with Ketamine/ Xylazine (90/10 mg/kg). Eight rats were assigned at random into two groups of 4 rats each. Further grouping included assigning 2 rats to each nanoparticle test compound of Sm_2O_3 labelled PLGA nanoparticles (1), Sm_2O_3 labelled PLGA nanoparticles (2), ^{99m}Tc -MDP labelled SLN (3) and ^{99m}Tc -MDP control (4), one rat for oral and the other for intravenous administration (IV). Immediately after receiving anaesthesia and radiolabelled nanoparticles, the rats were positioned under the gamma camera collimator (Infinia SPECT/CT, GE Healthcare, Salt Lake City, Utah and Lawrence, Massachusetts, USA) in ventral decubitus. The rats were injected IV with an average volume of 200 μl or orally administered 500 μl dose of radioactive nanoparticles with an activity of 0.23-7.85 MBq for Sm_2O_3 labelled PLGA nanoparticles and an activity of 13.50-37.74 MBq for the ^{99m}Tc -MDP compounds. Acquisition of dynamic images (120 s per frame) was recorded for the first 45 min.

Acquisition of static images were performed at 1, 2, 4, 6, 24 and 48 h after administration for the Sm_2O_3 labelled PLGA nanoparticles and static images at 1, 2, 3 and 4 h for the $^{99\text{m}}\text{Tc}$ -MDP compounds. Regions of interest (ROIs) were drawn on the images of the liver, stomach, bladder, intestines and tail to obtain time activity curves from the dynamic imaging study. Data for the ROIs, which were decay corrected, were obtained from the static images. After acquisition of images, the rats were sacrificed via 100 mg sodium pentobarbital/ kg intravenously. Several organs including the heart, lungs, liver, spleen, pancreas, stomach, intestines, kidneys, bladder, muscle, skin, bone, tail and blood were immediately removed after sacrificing the rats. Each organ was then weighed and the radioactivity was measured using a Capintec well type counter (Capintec, Inc, Ramsey, NJ, USA) as well as a MCA scintispec well counter.

To determine the biodistribution of the nanoparticles, radioactively labelled particles were orally and intravenously administered to rats. Thirty rats were assigned at random into six groups of four rats per group and two groups of three rats per group representing a Sm_2O_3 control group. These were further grouped into two groups per nanoparticle sample for the nanoparticle sample of Sm_2O_3 labelled PLGA nanoparticles and the Sm_2O_3 control, $^{99\text{m}}\text{Tc}$ -MDP labelled SLN and $^{99\text{m}}\text{Tc}$ -MDP control. The ventral tail artery of all rats was catheterized under Ketamine/ Xylazine (90/10 mg/kg) anaesthesia immediately before the study. Additionally, lateral tail veins were catheterized for all the rats which were administered radiolabelled nanoparticle compounds intravenously. The catheter was then removed after intravenous administration. One dose of each radiolabelled nanoparticle compound was administered either via oral route (500 μl in saline) using a 22G feeding needle attached to a 1 ml syringe or via intravenous route (200 μl in saline) through a catheterized tail vein. Blood was sampled from the catheterized tail vein in volumes of 200 μl at time of dissection per time point after single oral and intravenous administration of the Sm_2O_3 -labelled PLGA nanoparticles and of $^{99\text{m}}\text{Tc}$ -MDP labelled SLN compounds. The radioactivity of the blood was determined by means of a dose calibrator immediately after blood collection. One rat was sacrificed per time point via 100 mg sodium pentobarbital/ kg intravenously. The heart, lungs, liver, spleen, pancreas, stomach, intestines, kidneys, bladder, muscle, skin, bone, tail and blood were immediately removed after killing the rats. Each organ was then contained in a test tube, weighed and the radioactivity was measured using a Capintec well type counter (Capintec, Inc, Ramsey, NJ, USA) as well as a MCA scintispec well counter. Three dilutions of the radioactive solution were made to correlate counts with activity measured in MBq.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

This chapter provides the results to all the experimental procedures which were followed as well as the different characterisation data as obtained. The samarium oxide-152-loaded poly(D,L-lactide-co-glycolide) ($[^{152}\text{Sm}]\text{Sm}_2\text{O}_3\text{-PLGA}$) nanoparticles and the technetium-99m–methylene diphosphonate-loaded solid lipid nanoparticles ($^{99\text{m}}\text{Tc-MDP SLN}$) were characterized for size and dispersity (PDI) by means of dynamic laser scattering and quantified for metal content using inductively coupled spectroscopy (ICP). The morphology of $^{152}\text{Sm}_2\text{O}_3$ -loaded PLGA nanoparticles was additionally visualized with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Furthermore, $^{152}\text{Sm}_2\text{O}_3$ -loaded PLGA nanoparticles were irradiated using neutron activation to induce radioactivity. The $^{99\text{m}}\text{Tc-MDP}$ -loaded SLN compounds were analysed using instant thin layer chromatography (ITLC) assays to assess the labelling and stability of $^{99\text{m}}\text{Tc-MDP}$ complexes. These nanoparticles were then used to obtain data for imaging and biodistribution studies after intravenous and oral administration.

4.2 Sm_2O_3 -PLGA nanoparticles

4.2.1 Neutron activation of Sm_2O_3 -PLGA nanoparticles

The $[^{152}\text{Sm}]\text{Sm}_2\text{O}_3\text{-PLGA}$ nanoparticle test compounds were irradiated via neutron activation and activity reported in megabecquerel (MBq). The activity measured in two $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3\text{-PLGA}$ nanoparticle samples after neutron activation was 11 (sample 1) and 1.2 MBq (sample 2). The radioactivity energy values differed greatly due to the irradiation method employed (non-cadmium and cadmium), in which the non-cadmium method yielded higher radioactivity. The mean activity at time of administration was 8.90 (sample 1) and 0.90 MBq (sample 2) respectively. The activity of the oral doses which were given to the respective rats were 7.85 and 0.23 MBq in a 500 μl saline suspension (Yeong *et al.*, 2011). The activity of the $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3\text{-PLGA}$ nanoparticles' injected doses were 4.49 and 0.49 MBq each in a 200 μl saline suspension.

4.2.2 Characterisation of Sm₂O₃-PLGA nanoparticles

4.2.2.1 Particle size and Zeta potential

The characterisation of nanoparticles is required to control the quality of the manufactured nanoparticles. The particle size and surface charge are some of the parameters which need to be assessed because of their direct impact on the stability and release kinetics of the radionuclides which have been incorporated (Parhi and Suresh, 2012).

The nanoparticles were developed and the size measured by dynamic laser scattering. Three homogenization cycles of 800 bars were used and it was found that all nanoparticle formulations had a particle size less than 500 nm. Various parameters were optimized to obtain an average particle size ranging between 250 and 300 nm, with an average polydispersity index ≤ 0.3 after spray drying. The particles had a Zeta potential ranging between 5 and 20 mV.

There were no significant differences between the two Sm₂O₃-PLGA nanoparticles in terms of size, size dispersion and surface charge. The nanoparticles portrayed a size range below 300 nm, an average size distribution of 0.2 and a Zeta potential between 5 and 8 mV (Table 4.1). The bigger the Zeta potential of the suspension, the more likely it is to be stable because the charged particles repel each other and therefore overcome the natural tendency to aggregate (Parhi and Suresh, 2012).

Table 4.1: Size and Zeta potential of Sm₂O₃ loaded PLGA nanoparticles

Sample	Size (nm)	PDI	Zeta potential (mV)
1 (before irradiation)	270.70 $\pm$ 6.80	0.21 $\pm$ 0.18	7.45 $\pm$ 0.49
2 (before irradiation)	292.10 $\pm$ 5.80	0.24 $\pm$ 0.02	5.40 $\pm$ 1.01
1 (after irradiation)	286.70 $\pm$ 8.56	0.23 $\pm$ 0.01	17.00 $\pm$ 0.36
2 (after irradiation)	333.90 $\pm$ 181.4	0.59 $\pm$ 0.13	5.52 $\pm$ 2.97

PDI: polydispersity index, nm: nanometres, mV: millivolts

The Sm₂O₃-PLGA nanoparticles were irradiated via neutron activation to achieve a nominal radioactivity of 1 MBq at 48 h after End of Irradiation (Eol) (Yeong *et al.*, 2011). To allow for further size and morphological characterisation in non-radioactive laboratories, the sample was kept for 30 days before released as non-radioactive material. The nanoparticles were larger in

size, with an increased PDI and Zeta potential after the irradiation process (Table 4.1). As depicted by size analysis, the nanoparticles could have aggregated and increased in size as a result of the increase in Zeta potential during the irradiation process. It is accepted that Zeta potentials more than 30 mV are sufficient for good electrostatic stabilization (Parhi and Suresh, 2012).

4.2.2.2 Morphology of Sm_2O_3 - PLGA nanoparticles

4.2.2.2.1 Scanning electron microscopy

SEM images of the unirradiated Sm_2O_3 -PLGA nanoparticles (Figure 4.1) showed spherical particles with a smooth surface. The particle sizes ranged between 200 and 600 nm, with a few particles in the 1 μm range. These images give a visual profile of the size and size distribution results obtained from the dynamic laser scattering using a Malvern Zetasizer Nano ZS.

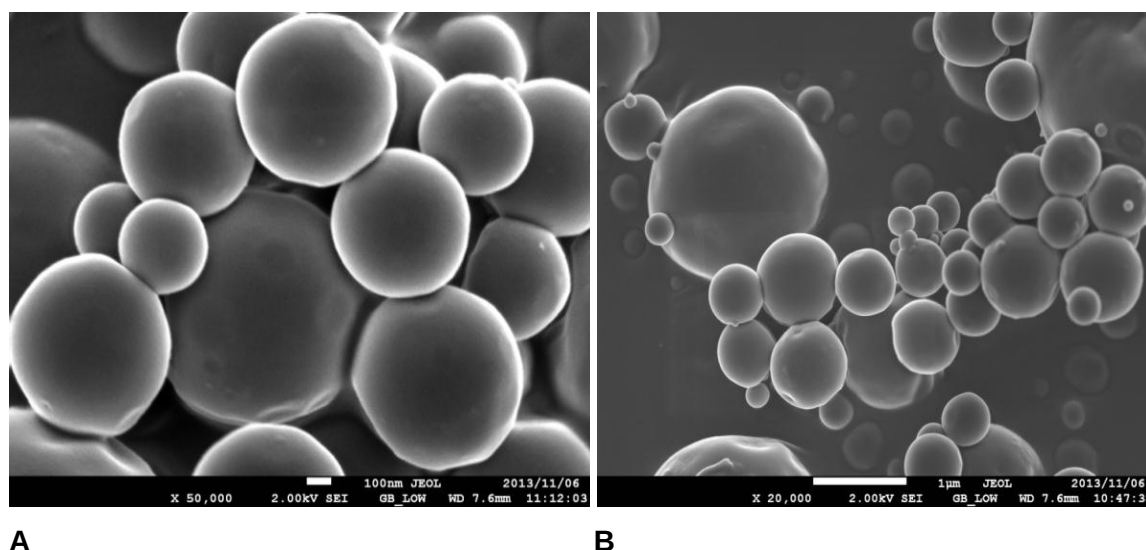


Figure 4.1: Scanning electron microscopic images of spray-dried Sm_2O_3 -PLGA nanoparticles at a high (A) (x50000) and low (B) (x20000) magnification, before neutron activation.

The images of the irradiated nanoparticles show crystalline and aggregated nanoparticles which do not look as spherical and as smooth as they did before neutron activation (Figure 4.2). The relatively larger nanoparticle size after neutron activation could be a result of particle aggregation due to fusion of particles at a specific temperature reached in the reactor or to moisture in the air which occurs during the 30 day period waiting for the ^{153}Sm to decay.

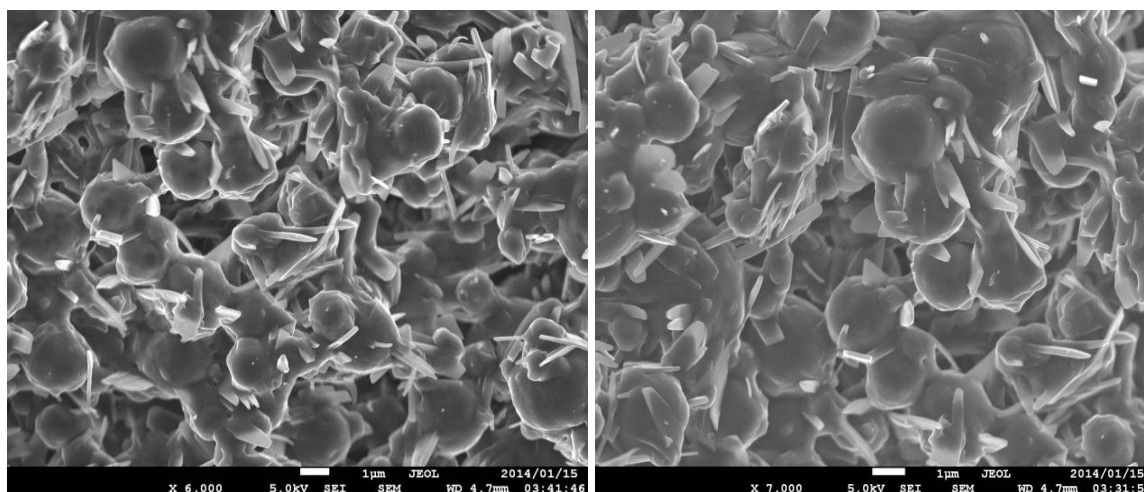


Figure 4.2: Scanning electron microscopic images of Sm_2O_3 -PLGA nanoparticles after neutron activation in a nuclear reactor.

4.2.2.2. Transmission electron microscopy

TEM images confirmed the SEM observations. The Sm_2O_3 -PLGA nanoparticles before irradiation were observed to be spherical, smooth and significantly smaller than 500 nm (Figure 4.3). This technique further satisfies the observation of the desired morphology and size of nanoparticles as they should be for *in vivo* administration purposes.

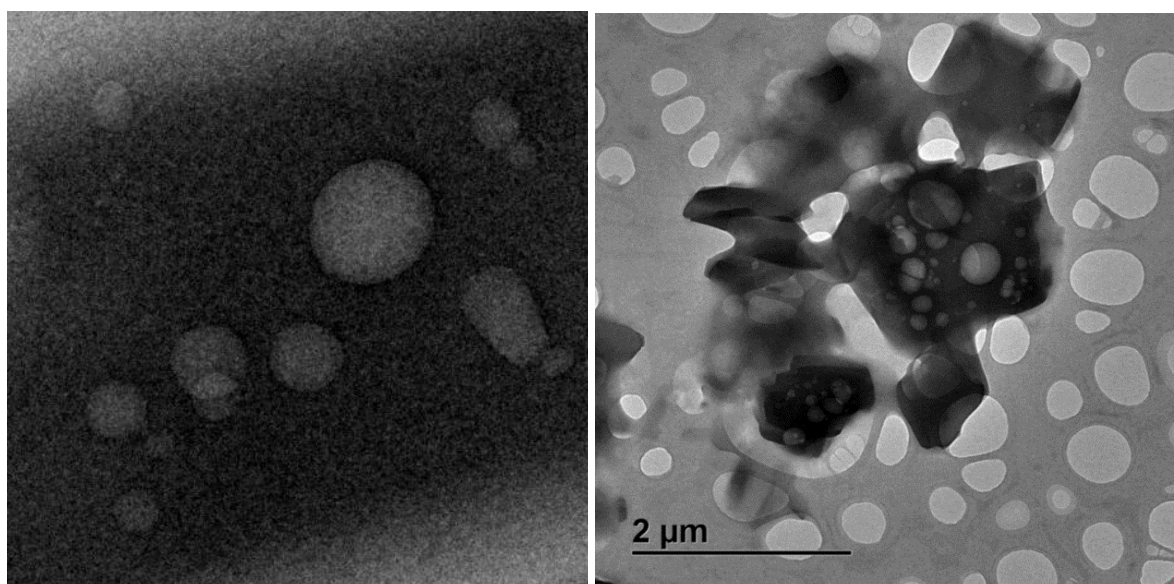


Figure 4.3: Transmission electron microscopic images of spray-dried Sm_2O_3 -PLGA nanoparticles before neutron activation.

4.2.2.3 Inductively Coupled Plasma Spectrometry

To quantify the amount of metal in each nanoparticle sample, ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) was performed. PLGA nanoparticles containing Sm_2O_3 contained an average amount of 20 621 mg/kg (2%) samarium-152. ICP confirmed the presence of the tracer elements but does not however inform if the tracers are encapsulated within the nanoparticles or on the surface.

Table 4.2: ICP spectrometry results Sm_2O_3 loaded PLGA nanoparticles before neutron activation

Sample	Result (mg/kg)	Percentage (%)
1	20 918	2
2	20 324	2

4.2.3 *In vivo* biodistribution studies

4.2.3.1 Imaging and Biodistribution assays of Sm_2O_3 -PLGA nanoparticles

4.2.3.1.1 Scintigraphic images: Sm_2O_3 -PLGA nanoparticles

The imaging procedures for [^{153}Sm] Sm_2O_3 -PLGA nanoparticles were employed on four rats. The test compounds included two [^{153}Sm] Sm_2O_3 radiolabelled PLGA nanoparticles which were each administered once orally and once intravenously in the rats for imaging purposes. There was unfortunately no control group for imaging, due to unforeseen technical circumstances. The nanoparticle test compounds which were used for imaging were formulated using the same method but differed in size and radioactivity. The ^{153}Sm -PLGA nanoparticle formulation which gave the best static scintigraphic images initially had a nanoparticle size of 270 nm, a size distribution of 0.21 and a Zeta potential of 7.45 mV. The activity measured after neutron activation was 11 MBq. The mean activity at time of administration was 8.9 MBq. The gamma spectroscopic analysis demonstrated that the main photopeak measured from the organs after imaging was in the expected energy region of 94 to 125 keV. Additional peaks were detected in

the region of 97 and 103 keV, which were recognized as the associated gamma energies emitted from the ^{153}Sm (Yeong *et al.*, 2012).

Ingestion of the ^{153}Sm -PLGA nanoparticles and the imaging procedures were well tolerated by all the rat subjects with no adverse effects reported. The ^{153}Sm -PLGA nanoparticles were clearly visualised in all the scintigraphic images as a hot spot where the nanoparticles localized dominantly and the nanoparticles were mostly still intact. The activity was observed to disperse when the $^{153}\text{Sm}_2\text{O}_3$ -PLGA nanoparticles continued to distribute and the PLGA polymer disintegrate. The activity of the oral dose which was given to the specific rat was 7.85 MBq in a 500 μl saline suspension. Figure 4.4 shows the regions of interest (ROI's) in the rat subject at 1, 6, 24 and 48 h after oral ingestion of radiolabelled ^{153}Sm -PLGA nanoparticles. In these images, nanoparticles were observed to have moved from the oral route of administration and in the process the radioactive formulation sample got stuck in the oesophagus. The activity was initially a hot spot in the stomach and gradually moved to the liver and small intestines within the first hour post-ingestion. There was no significant change between 1 and 6 h. Between 24 and 48 h, the activity in the stomach moved to the liver thus showing a slightly higher degree of fluorescence in the liver as compared to 1 h post-ingestion. This indicated that the nanoparticles were distributed throughout the rat. However, the biodistribution of the ^{153}Sm -PLGA nanoparticles was slow as the images showed no major difference between 1 and 48 h post-ingestion and there was no visual demonstration of distribution of the particles from the stomach to major organs such as the lungs and the kidneys.

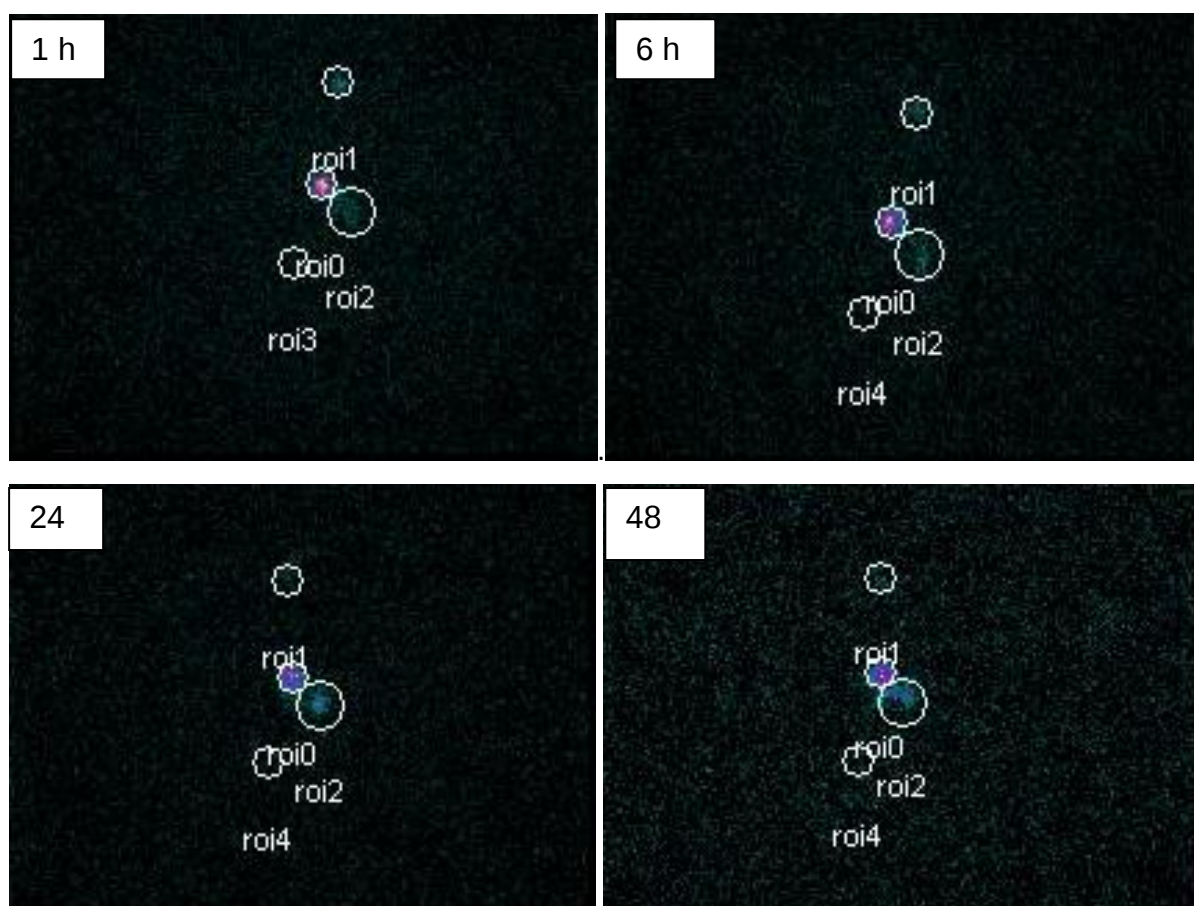


Figure 4.4: Static scintigraphic images of orally administered [^{153}Sm] Sm_2O_3 -PLGA nanoparticles at 1, 6, 24 h and a final image at 48 h post-ingestion.

Figure 4.5 demonstrates what happened between 1 and 48 h after oral ingestion of ^{153}Sm -PLGA nanoparticles. The images demonstrate the oral route of the particles, localisation in the stomach and the gradual movement of the nanoparticles from the stomach to the liver and further slow biodistribution to the small and large intestines. To obtain scintigraphic images of orally administered radio-labelled nanoparticles, the rats were anaesthetised with Ketamine/Xylazine (90/10 mg/kg). Immediately after receiving anaesthesia and radio-labelled nanoparticles, the rats were positioned under the Infinia single-photon emission computed tomography (SPECT/CT) gamma camera in ventral decubitus. The rats were orally administered 500 μl dose of radioactive nanoparticles with an activity of 7.85 MBq. The rats were put under heavy anaesthesia resulting in them not eating or drinking for long periods during the 48 h duration of the study. This could have resulted in the rats having a dormant or slowed down metabolism and could therefore explain the slow and limited biodistribution of the ^{153}Sm nanoparticles *in vivo*.

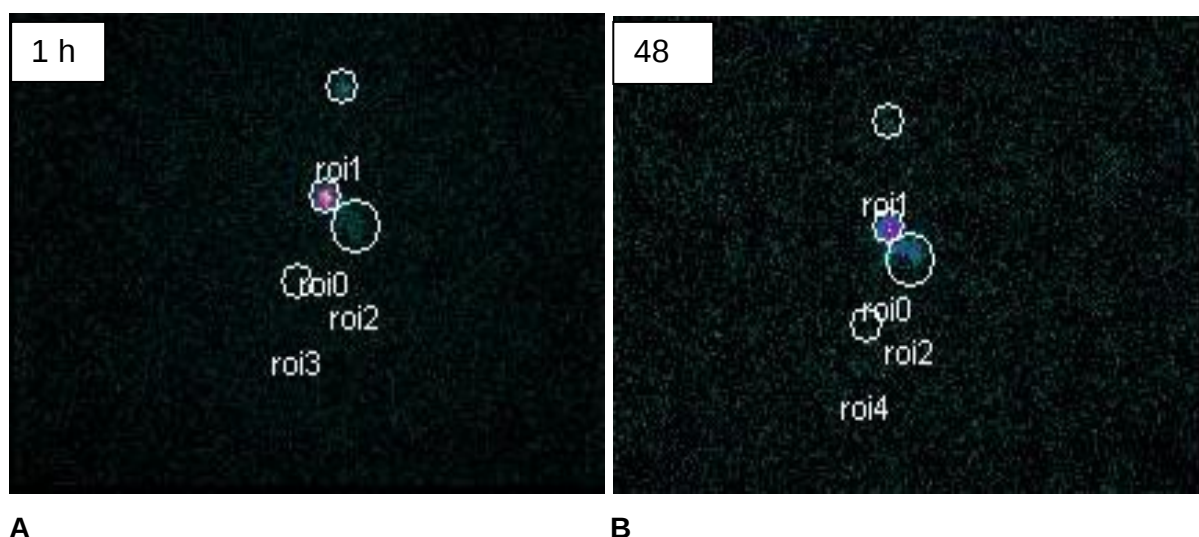


Figure 4.5: Images comparing orally administered radioactive [^{153}Sm]Sm₂O₃-PLGA nanoparticles after 1 (A) and 48 h (B).

The intravenous study was conducted using a 10 ml suspension of [^{153}Sm]Sm₂O₃-PLGA nanoparticles with a mean activity of 8.9 MBq at time of administration. The injected dose was 4.49 MBq. Sequential static images from a rat at 1, 6, 24 and 48 hours post-injection of radiolabelled ^{153}Sm -PLGA nanoparticles were obtained (Figure 4.6). According to the images, the nanoparticles moved via the bloodstream to the liver and localized there over a 48 h period. The activity was initially very high in the liver and gradually moved to the small intestines within the first hour post-injection. The ROIs in the images (Figure 4.6) are the liver and the site of injection (tail). The small intestines represented a very small amount of radioactivity, which indicated that the ^{153}Sm -PLGA nanoparticles were still being distributed. There was once again no significant change between 1 and 6 h. Between 24 and 48 h, the activity in the liver has moved to the small intestines thus showing a reduced amount of fluorescence as compared to the 1 h period after injection. The biodistribution of these ^{153}Sm nanoparticles was significantly slow as the images showed no major difference between 1 and 48 h post-injection and there was no visual demonstration of movement of the particles from the liver to other organs.

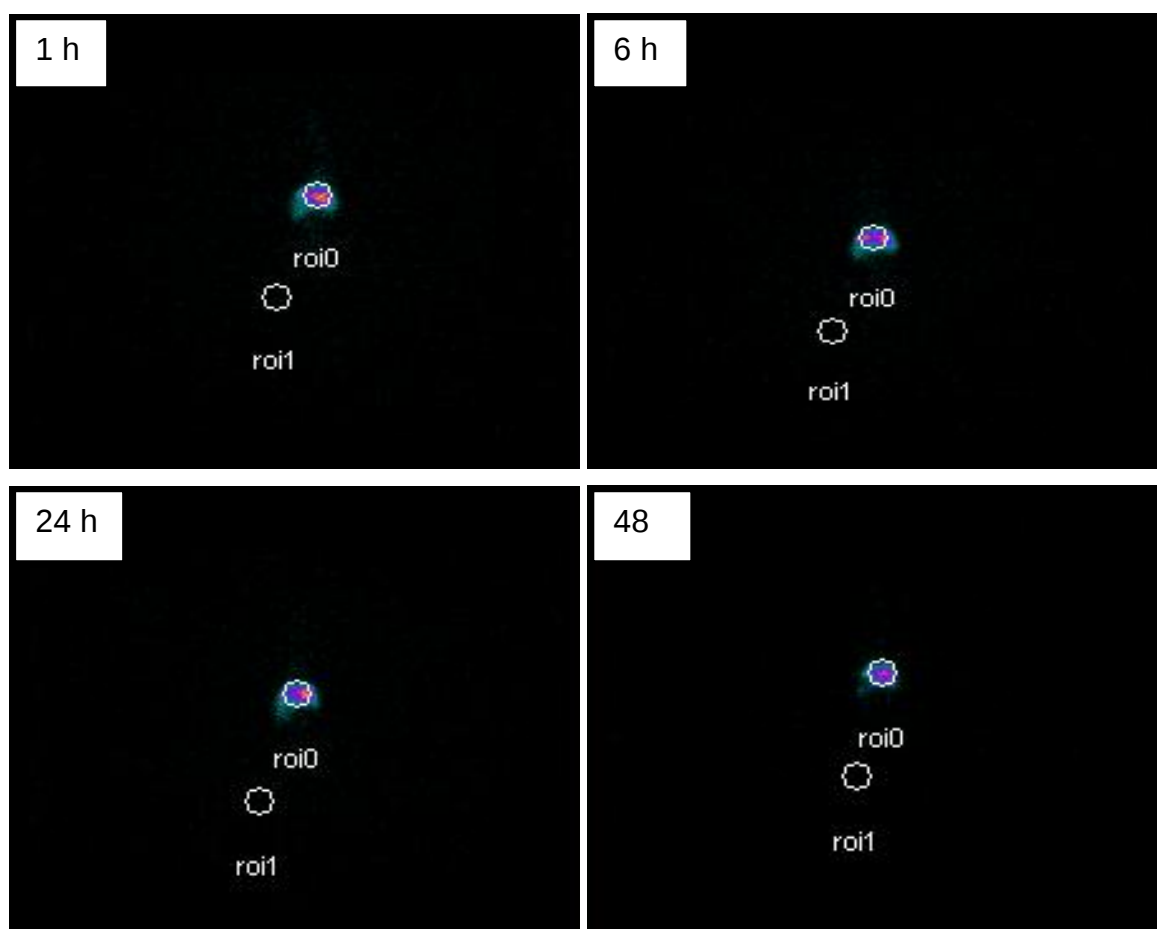


Figure 4.6: Static scintigraphic images of intravenously administered [^{153}Sm] Sm_2O_3 - PLGA nanoparticles at 1, 6 and 24 h and a final image at 48 h post-injection.

Figure 4.7 demonstrates what happened between 1 and 48 h after intravenous administration of ^{153}Sm -PLGA nanoparticles. The images demonstrate the injection route of the particles, localisation in the liver and the slow biodistribution of the ^{153}Sm nanoparticles from the liver to the intestines. To obtain scintigraphic images of injected radio-labelled ^{153}Sm nanoparticles, the rats were anaesthetised with Ketamine/Xylazine (90/10 mg/kg). Immediately after receiving anaesthesia and radio-labelled ^{153}Sm -PLGA nanoparticles, the rats were positioned under a SPECT/CT gamma camera in ventral decubitus. The rats were injected a 200 μl dose of radioactive nanoparticles with an activity between 0.23 and 7.85 MBq. Once again the rats were put under heavy anaesthesia resulting in them not eating or drinking for long periods during the 48 h duration of the study. This could have resulted in the rats having decreased metabolism and could therefore explain the slow and limited biodistribution of the ^{153}Sm -PLGA nanoparticles in the rats.

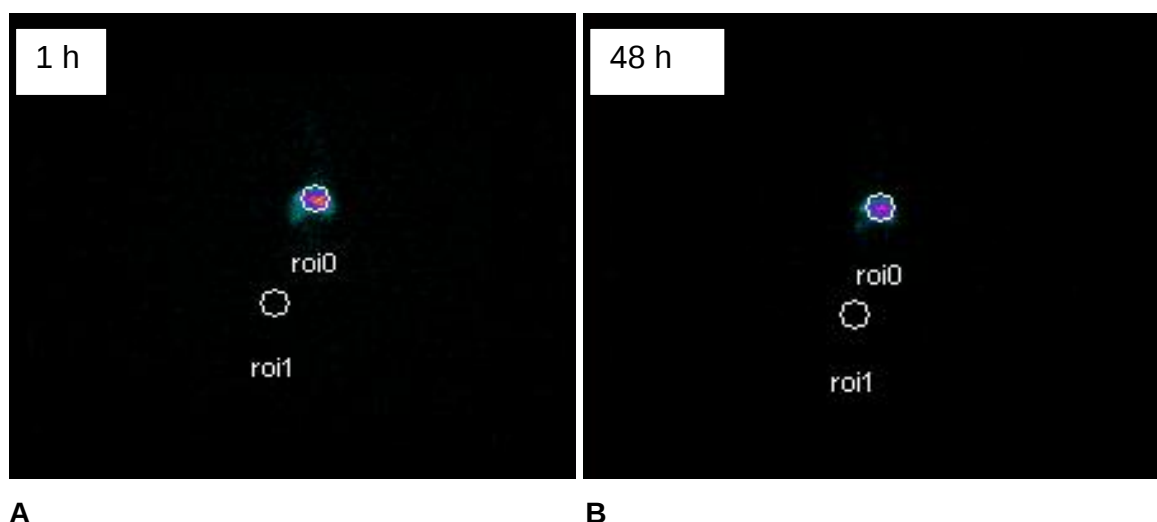


Figure 4.7: Images of [^{153}Sm] Sm_2O_3 -PLGA nanoparticles at 1 (A) and 48 hours (B) post-injection in rats.

Once the ^{153}Sm -PLGA nanoparticles were administered, a trace amount of the nanoparticles was trapped in the site of administration (oesophagus or tail) (Figure 4.8). The stomach was the main organ of particle absorption for orally administered ^{153}Sm -PLGA nanoparticles. The liver was the main organ of particle deposition following intravenous administration. In both circumstances, the ^{153}Sm -PLGA nanoparticles distributed from the latter organs to the intestines. The scintigraphic images obtained with ^{153}Sm were of acceptable quality and were comparable to those commonly acquired using $^{99\text{m}}\text{Tc}$ in other clinical patients. After 48 h the nanoparticles had optimally distributed to other organs, but remained prominent in the stomach, liver and small intestines.

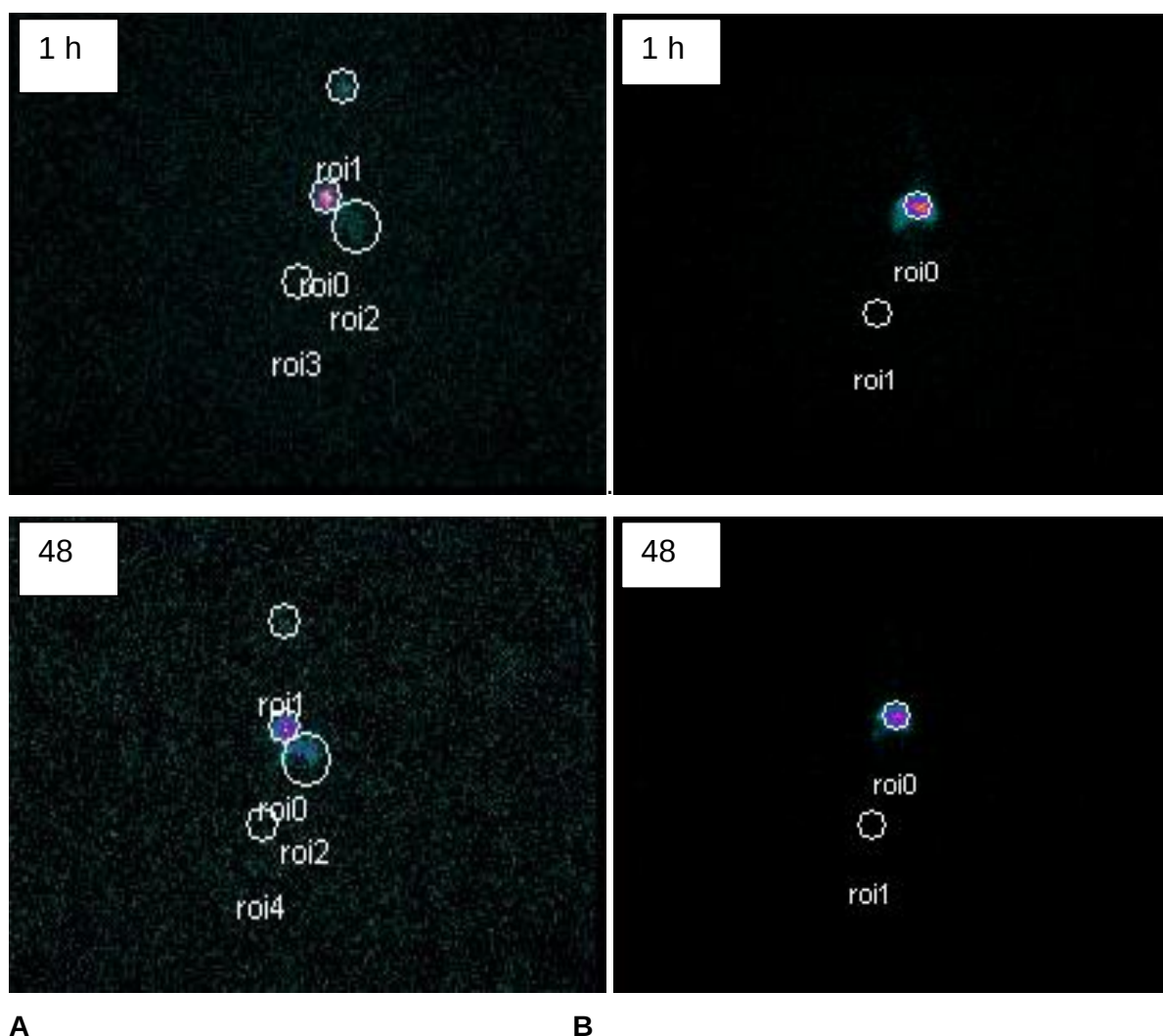


Figure 4.8: Static scintigraphic images of orally (A) and intravenously (B) administered radioactive [^{153}Sm] Sm_2O_3 -PLGA nanoparticles at 1 and 48 h showing the regions of interest in circles.

After IV administration of ^{153}Sm nanoparticles, the first distribution point was the liver. There was a difference observed between the oral and IV biodistribution over 48 h. Forty eight hours was the end point of the study considering the 47 h half-life of ^{153}Sm and leaving enough time to quantify the radioactivity of the organs.

4.2.3.1.2 Biodistribution graphs of Sm_2O_3 -PLGA nanoparticles

For the Sm_2O_3 control compound, the large intestine (1.06%) was the organ with the highest amount of deposition, followed by the oesophagus (site of administration) (0.14%) and the skin (0.03%) (Figure 4.9). The transition of the compound was observed to be slow. A high amount of Sm_2O_3 was composed in the large intestine which facilitated the absorption from the gut into bone. Sm_2O_3 was most probably not absorbed in the stomach and small intestines; therefore it

accumulated in the large intestine and eliminated via the large intestines. More than 90% of the Sm_2O_3 were excreted in 48 h.

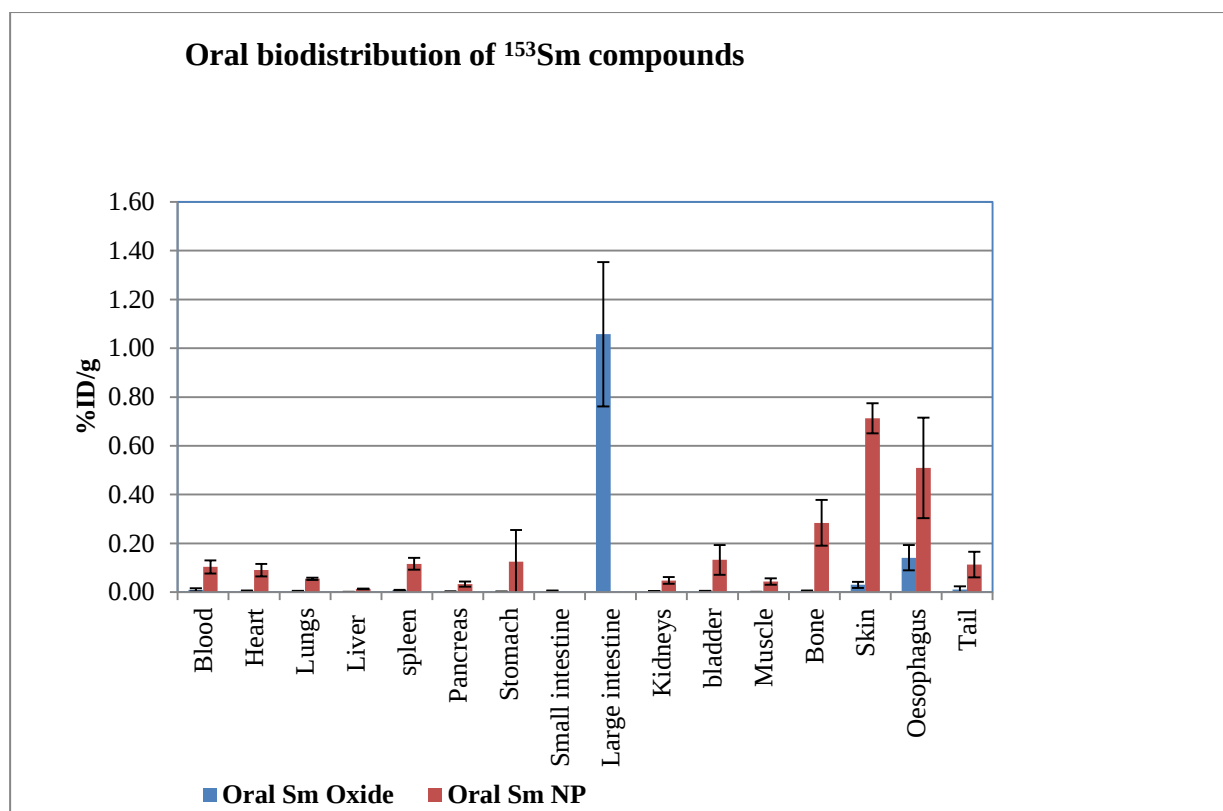


Figure 4.9: The biodistribution of orally administered $^{153}\text{Sm}_2\text{O}_3$ - PLGA nanoparticles and Sm_2O_3 after 48 h.

The $[^{153}\text{Sm}]\text{Sm}_2\text{O}_3$ -PLGA nanoparticles were distributed everywhere (Figure 4.9), which suggested that there was absorption of nanoparticles. From this distribution, we can assume that the nanoparticles facilitated the transport or distribution of the ^{153}Sm into the various organs. The nanoparticles also showed slower distribution and longer residence time in the organs than the Sm_2O_3 control (Mohammad and Reineke, 2013; Semete *et al.*, 2010). This could possibly be due to the positive charge of the particles which cause adhesion to mucus layers in the gastrointestinal (GI) tract (Semete *et al.*, 2010). Nanoparticles enhance absorption of the encapsulated compound (^{153}Sm). The ^{153}Sm -PLGA nanoparticles were mostly deposited in the skin (0.71%) probably as a result of the rats licking themselves and contamination through handling and contact, followed by the oesophagus (0.51%), bone (0.28%) and bladder (0.13%). Both the control and the nanoparticle samarium compounds were detected in the skin and oesophagus and absent in the small and intestines.

Studying the *in vivo* distribution of the intravenously injected compounds showed that the highest trace amount of the control compound was reported in the tail (the site of injection) (0.63%). The respective deposition of $^{153}\text{Sm}_2\text{O}_3$ was followed by the liver (0.12%) and skin (0.11%). The stomach, spleen, lungs, small intestines, large intestines and kidneys presented no traces of $^{153}\text{Sm}_2\text{O}_3$ (Figure 4.10). PLGA nanoparticles accumulated in the liver and spleen (Figure 4.10), which is the reported and the expected fate of intravenously administered PLGA nanoparticles (Semete *et al.*, 2010). Nanoparticles with hydrophobic surfaces are rapidly sequestered from the circulation by organs of the mononuclear phagocyte system (i.e. liver, spleen, lungs) (Mohammad and Reineke, 2013). The ^{153}Sm -PLGA nanoparticles were deposited mostly in the spleen (8.63%) followed by the liver (3.07%). From this data, we know that the radiotracer was encapsulated because the encapsulated compound followed the distribution of PLGA nanoparticles. Reports show that particles bigger than 250 nm deposit and accumulate to a greater extent in the liver and spleen than smaller ones (Mohammad and Reineke, 2013). The skin (0.28%), bladder (0.19%), blood and kidneys (0.15%) and lungs (0.13%) also showed trace amounts of deposited $^{153}\text{Sm}_2\text{O}_3$ -PLGA nanoparticles. Due to the Sm_2O_3 having a faster clearance than the ^{153}Sm -PLGA nanoparticles; it can be assumed that PLGA nanoparticles as a delivery system slow down clearance of ^{153}Sm and assisted with the biodistribution in various organs. In both ^{153}Sm compounds, injected doses per gram of organ of less than 0.04% were reported in the heart, muscles, pancreas, stomach, small and large intestines.

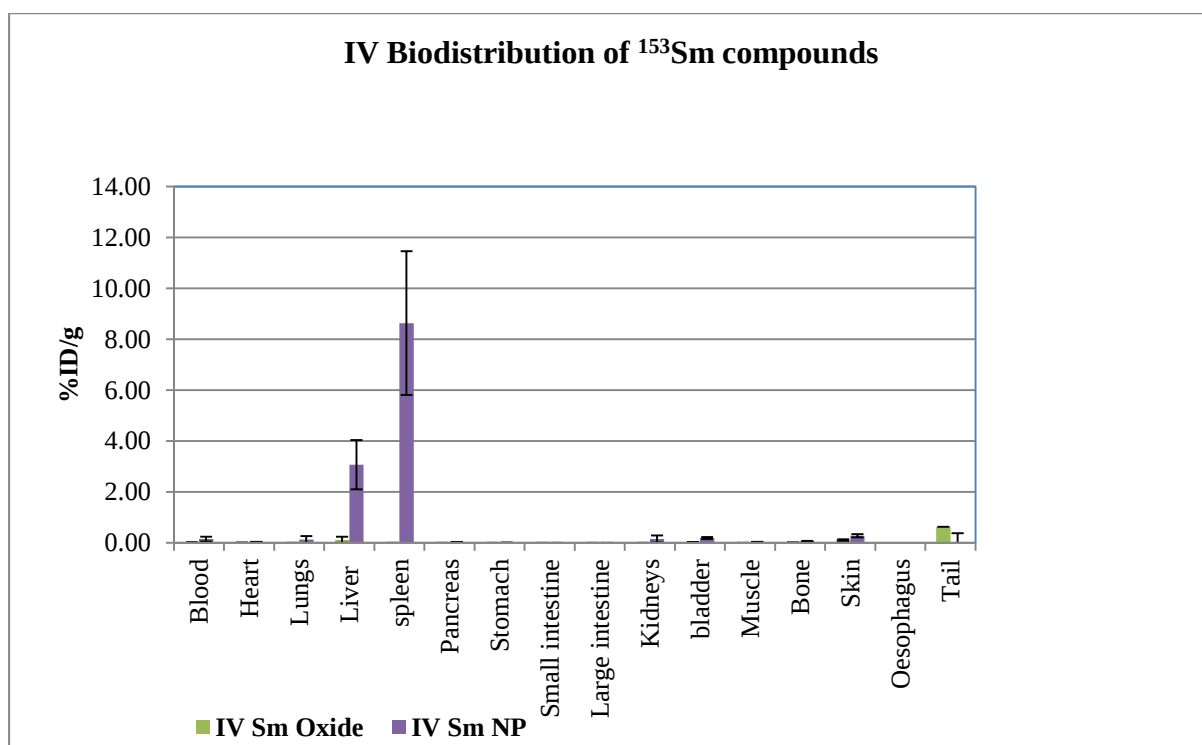


Figure 4.10: The *in vivo* biodistribution of intravenously injected $^{153}\text{Sm}_2\text{O}_3$ - PLGA nanoparticles and $^{153}\text{Sm}_2\text{O}_3$ after 48 h.

Figure 4.11 gives a comparative illustration of the organs which had the highest and lowest deposition of ^{153}Sm nanoparticles and $^{153}\text{Sm}_2\text{O}_3$. The injected ^{153}Sm PLGA nanoparticles exhibit the highest localisation of nanoparticles in the spleen (8.63%) and liver (3.07%). The lowest amount of deposition was in the stomach (0.01%), small intestines (0.01%), large intestines (0.01%) and pancreas (0.02%). The orally administered ^{153}Sm nanoparticles were highly deposited in the skin (0.71%), oesophagus (0.51%) and bone (0.28%). The least deposition of oral nanoparticles was in the liver (0.01%) and pancreas (0.03%). The Sm_2O_3 control groups had a faster rate of clearance and limited biodistribution *in vivo*.

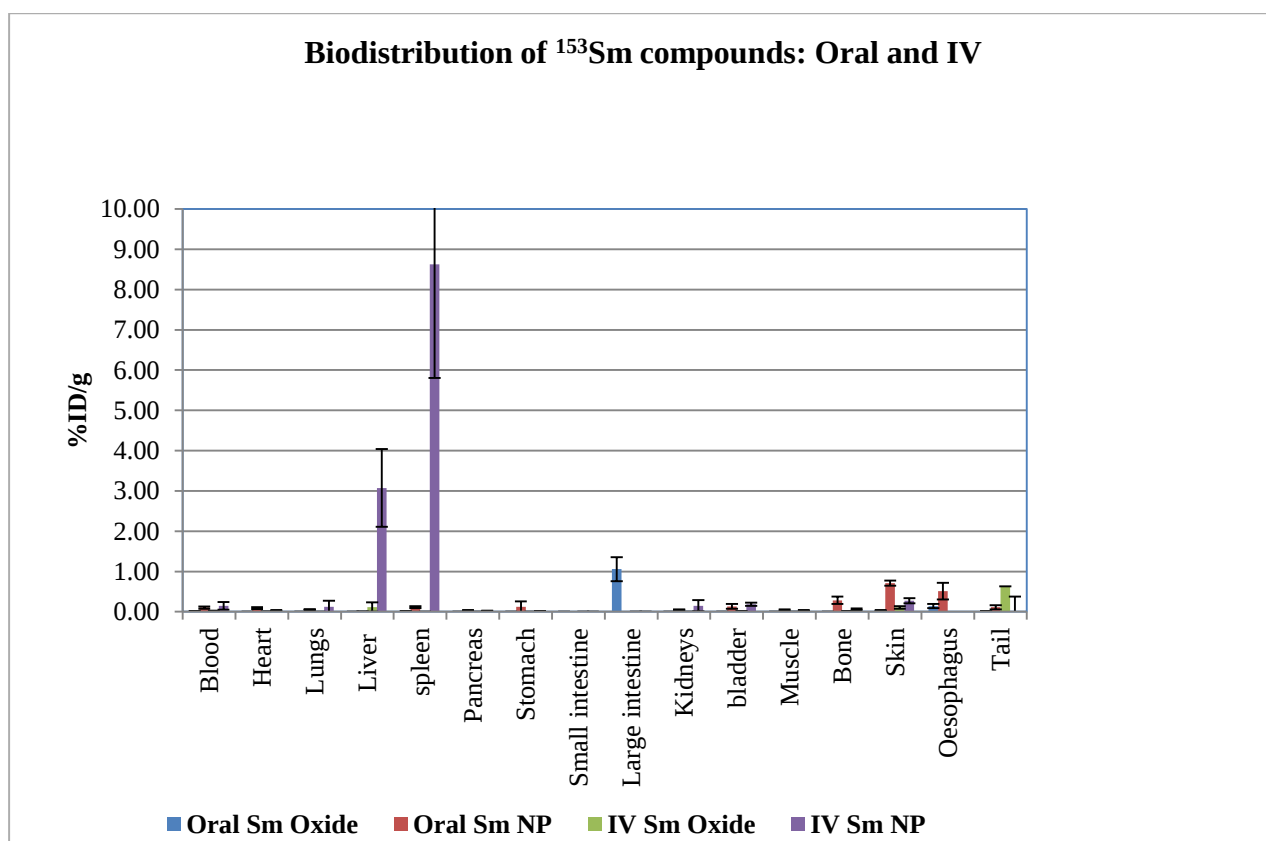


Figure 4.11: *In vivo* biodistribution comparing the oral and intravenous profiles of $^{153}\text{Sm}_2\text{O}_3$ -PLGA nanoparticles and Sm_2O_3 after 48 h.

The orally administered $^{153}\text{Sm}_2\text{O}_3$ were highly deposited in the large intestines (1.06%), oesophagus (0.14%) and skin (0.03%). There was no deposition of $^{153}\text{Sm}_2\text{O}_3$ reported after 48 h in the following organs: heart, lungs, liver, pancreas, stomach, intestines and kidneys. A similar profile was observed in intravenously administered $^{153}\text{Sm}_2\text{O}_3$ seeing there was no absorption of the tracer in the stomach, spleen, lungs, intestines and kidneys.

4.3 $^{99\text{m}}\text{Tc}$ -MDP solid lipid nanoparticles

4.3.1 Characterisation of $^{99\text{m}}\text{Tc}$ -MDP solid lipid nanoparticles

4.3.1.1 Particle size and Zeta potential

Optimization of the MDP-loaded SLNs emulsions yielded a slightly higher polydispersity index of ≥ 0.5 and a size range between 150 and 450 nm. The Zeta potential was between -2 and -30 mV.

The MDP-loaded SLNs had an average PDI of 0.245 and particles sizes of 260 ± 2.66 nm and 252 ± 7.89 with a Zeta potential of -15 and -12.36 (Table 4.3). Zeta potentials between -5 and -15 mV are in a region where a limited degree of particle aggregation could occur (Parhi and Suresh, 2012). Thus particle aggregation is less likely to occur for nanoparticles with a high Zeta potential due to electric repulsion (Parhi and Suresh, 2012). The Zeta potential of these emulsions may be negatively charged as a result of the ingredients added. Unlike with the Sm_2O_3 -PLGA nanoparticles, no chitosan was added to the emulsion. Chitosan contains cationic groups which would alter the surface charge of an emulsion to a positive value when added.

Table 4.3: Size and Zeta potential of $^{99\text{m}}\text{Tc}$ -MDP solid lipid nanoparticles

No.	Size (nm)	PDI	Zeta potential (mV)
1	260.70 ± 2.66	0.26 ± 0.10	-15.00 ± 0.55
2	252.10 ± 7.89	0.23 ± 0.08	-12.30 ± 0.36

PDI: polydispersity index, nm: nanometres, mV: millivolts

4.3.1.2 Inductively Coupled Plasma Spectrometry

To quantify the amount of metal in each nanoparticle sample, ICP-OES was performed. SLN containing a $^{99\text{m}}\text{Tc}$ -MDP tracer were quantified to determine the amount of phosphate in each sample. The protocols available allowed phosphate to be quantified rather than the total amount of MDP. Each sample contained an average amount of 23 mg/L (1%) of phosphate. ICP confirmed the presence of the tracer elements but does not however inform of whether the tracers are encapsulated within the nanoparticles or on the surface.

4.3.1.3 Quality control of compounded radiopharmaceuticals

4.3.1.3.1 Instant thin layer chromatography

A high value in radioactivity at the front is a good indication that a $^{99\text{m}}\text{Tc}$ -MDP complex was formed during the conjugating process of the two compounds. Any value of radioactivity at the origin represents the quantity of unbound or unlabelled radiopharmaceutical compound (technetium). According to the results in Table 4.4, a complex was formed between the technetium-99m and the MDP ligand. The $^{99\text{m}}\text{Tc}$ -MDP complex reported activity values of 0.1 (2.20%) and 0.4 (5.42%) MBq at the origin of the fiberglass and UV sheets respectively. The

activities at the front were 4.53 (99.88%) and 7.34 (99.46%) MBq on the fiberglass and UV sheets respectively. Labelling efficiency and stability of the ^{99m}Tc -MDP nanoparticle complex was also assessed using fiberglass, non-UV and UV sheets. Values of 0, 0.1 (3.50%) and 0 MBq were recorded at the origin of the fiberglass, non-UV and UV sheets respectively. The activities at the front were 0.9 (100%), 2.85 (99.65%) and 3.86 (100%) MBq on the fiberglass, non-UV and UV sheets respectively. A zero value at the origin could imply that a 100% complex was formed between the two compounds (Geskovski *et al.*, 2013). The radioactivity of nanoparticulate complexes was generally lower than that of free ^{99m}Tc -MDP complexes. These results don't however prove that the ^{99m}Tc -MDP complex is encapsulated in the ^{99m}Tc -MDP solid lipid nanoparticle formulation but rather just an indication of the ^{99m}Tc -MDP complex that was formed.

Table 4.4: QC results of compounded radiopharmaceuticals

	Radioactivity (MBq)		
	Paper type		
	Fibreglass	Al: Non-UV	Al: UV
^{99m}Tc -MDP			
Origin	0.1		0.4
Front	4.53		7.34
^{99m}Tc -MDP SLN			
Origin	0	0.1	0
Front	0.9	2.85	3.86
MDP SLN			

Al: aluminium, Non-UV: non-ultraviolet, UV: ultraviolet

4.3.2 *In vivo* biodistribution studies

4.3.2.1 Imaging and Biodistribution assays of ^{99m}Tc -MDP solid lipid nanoparticles

4.3.2.1.1 Scintigraphic Images: ^{99m}Tc -MDP solid lipid nanoparticles

The imaging procedures for the ^{99m}Tc -MDP solid lipid nanoparticles were employed on four rats. The test compounds included one ^{99m}Tc -MDP SLNs and a control compound ^{99m}Tc -MDP which were each administered orally and intravenously in rats for imaging purposes. The gamma spectroscopic analysis demonstrated that the main photopeak measured from the organs after imaging was in the energy region of 14 to- 200 keV. Additional peaks were detected in the

regions of 100 and 170 keV. The rats were administered doses of radioactive ^{99m}Tc -MDP compounds with an activity of 13.50 to 37.74 MBq. The end point of the study was at 6 h considering the 6 h half-life of ^{99m}Tc and leaving enough time to quantify the radioactivity of the organs.

Ingestion of ^{99m}Tc -MDP SLNs and the imaging procedures were well tolerated by all the rats with no adverse effects reported. The rat was administered with SLNs with a radioactivity of 19.61 MBq. The ^{99m}Tc -MDP SLNs were clearly visualised in all the scintigraphic images as a hot spot where the nanoparticles have mainly localized and the distribution was observed to occur significantly fast. Figure 4.12 shows the ROIs from a rat which were captured at 1, 2, 3 and 4 h after oral ingestion of radiolabelled ^{99m}Tc -MDP SLNs. In the images, the nanoparticles were observed to have moved from the oral route and in the process radioactivity got stuck in the oesophagus. The images illustrate the stomach, liver, spleen, heart and the bladder as the main regions of interest. High activity in the stomach was quickly distributed to the liver, spleen, heart, small intestines and bowels within a few minutes after oral administration. There was a significant change in biodistribution between 1 and 4 h. Between 2 and 4 h, the activity in the stomach had moved to the bladder and throughout the body thus showing a slightly higher degree of fluorescence in the bladder as compared to the first hour after ingestion. This indicated that the nanoparticles were distributed throughout the rat. The biodistribution of the ^{99m}Tc -MDP SLNs was significantly fast and demonstrated movement of the nanoparticles from the stomach to other organs. The presence and distribution of the ^{99m}Tc -MDP SLNs in the bladder and bowels after 1 h could be an indication of excretion, good metabolism and easy movement of the nanoparticulate emulsion.

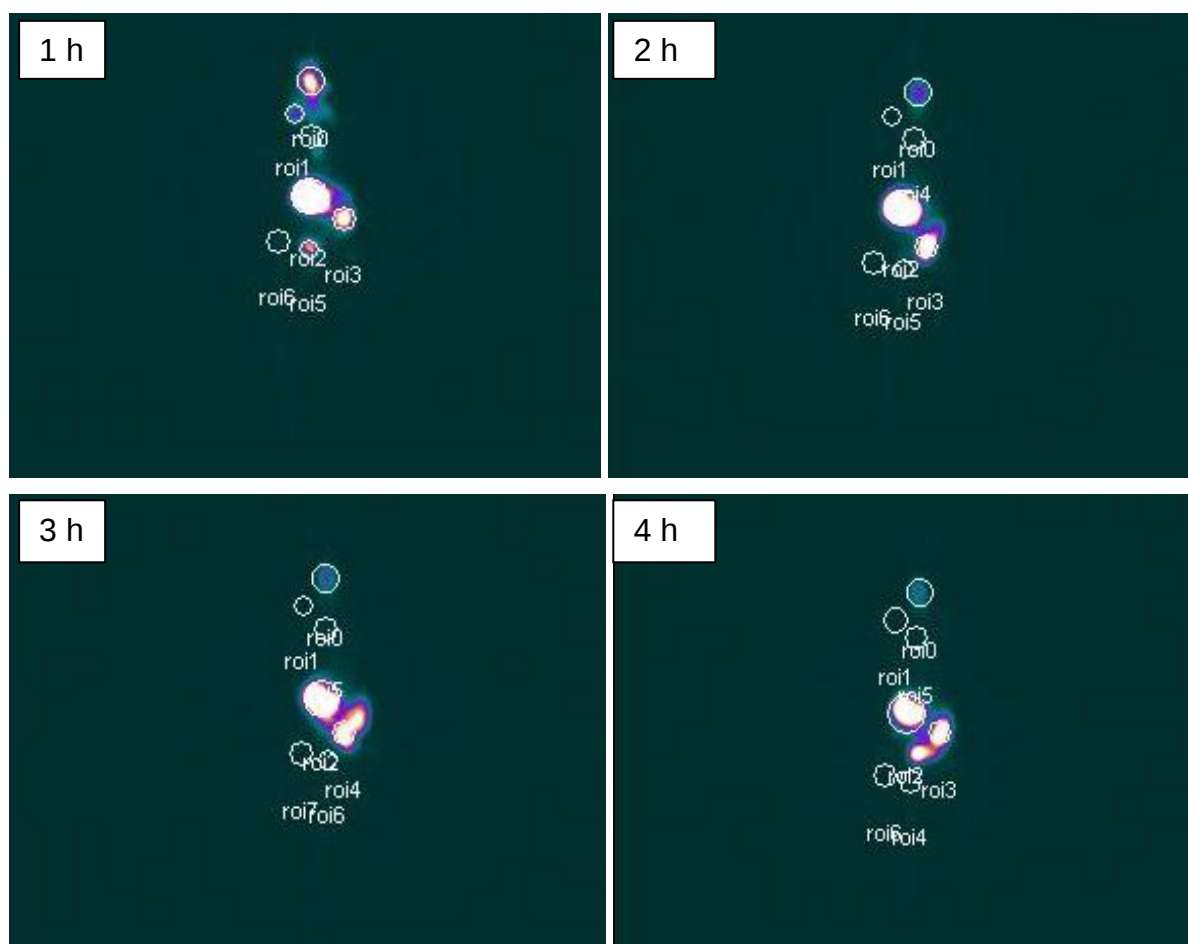


Figure 4.12: Static scintigraphic images of orally administered ^{99m}Tc -MDP solid lipid nanoparticles at 1, 2, 3 and 4 hours post-ingestion.

The ^{99m}Tc -MDP was clearly visualised in all the scintigraphic images as a hot spot where the nanoparticles dominantly localized and distributed fairly quickly. Figure 4.13 shows the ROIs which were captured at 1, 2, 3 and 4 h after oral ingestion of radiolabelled ^{99m}Tc -MDP. The ingested radioactivity of the ^{99m}Tc -MDP was 35.52 MBq. The ^{99m}Tc -MDP exhibits similar biodistribution characteristics to ^{99m}Tc -MDP solid lipid nanoparticles. The ^{99m}Tc -MDP images illustrate the oesophagus (site of administration) stomach, liver, spleen, heart and the bladder as the main regions of interest. High activity was observed in the stomach and was quickly distributed to the liver, spleen, heart, small intestines and the bladder and bowels within a few minutes after oral administration. The biodistribution was highly fluorescent from the stomach to the bladder between 1 and 4 h. The activity in the bladder started to decrease after 4 h. The biodistribution of the ^{99m}Tc -MDP was fast and demonstrated movement of the nanoparticles from the stomach to other organs. The presence and distribution of the ^{99m}Tc -MDP in the bladder and bowels after 1 h could be an indication of excretion, good metabolism and easy movement of the nanoparticulate emulsion.

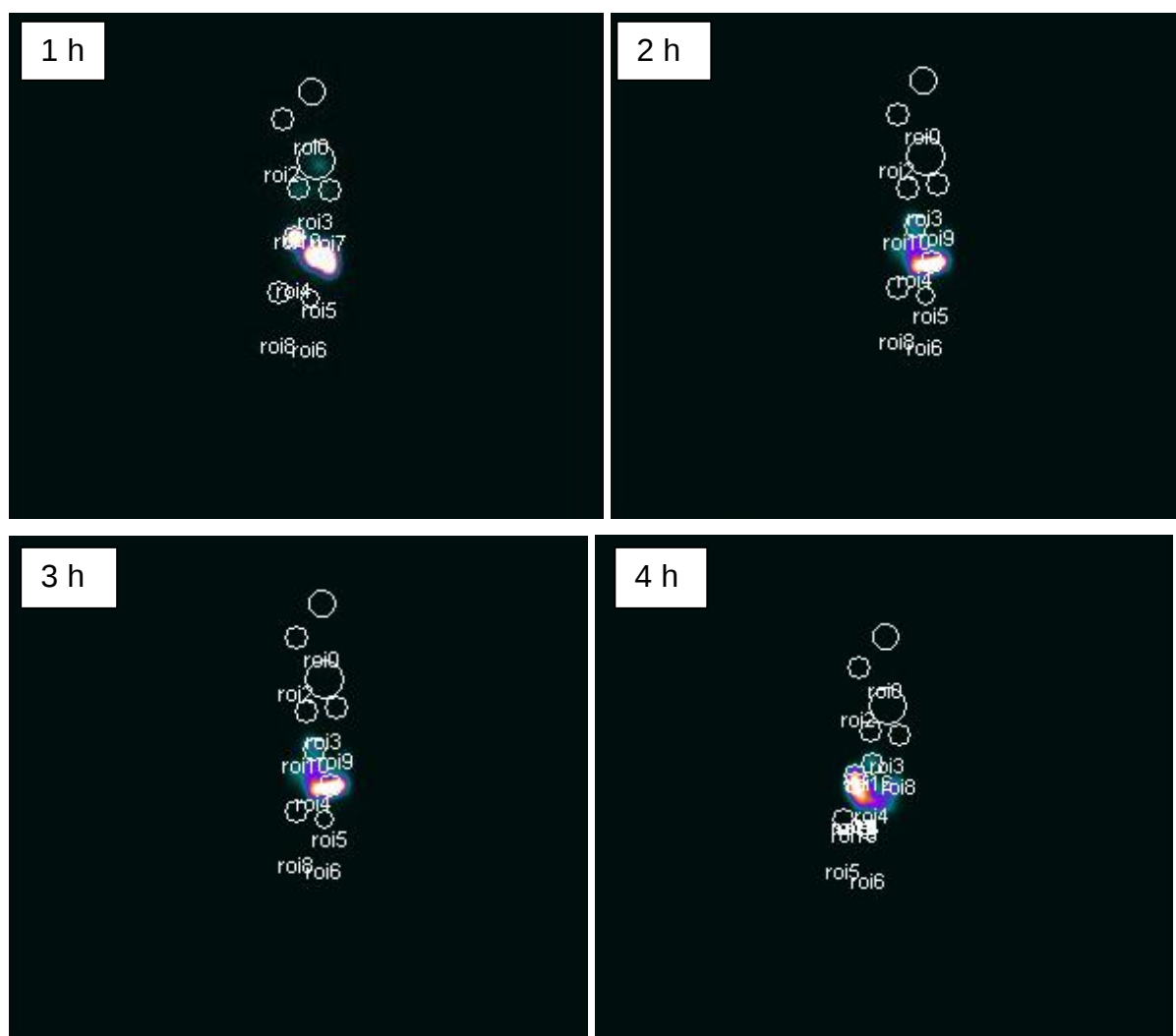


Figure 4.13: Images illustrating the regions of interest of orally administered ^{99m}Tc -MDP at 1, 2, 3 and 4 h post-ingestion.

The ^{99m}Tc -MDP and the ^{99m}Tc -MDP solid lipid nanoparticles portray similar clearance and biodistribution characteristics. Judging from the images in Figure 4.14, ^{99m}Tc -MDP SLNs has a wider distribution and a more distinct distribution. Both the ^{99m}Tc -MDP control and the radioactive SLN emulsion were observed primarily at the site of ingestion (oesophagus), stomach, intestines and the bladder. This does not eliminate the possibility that the nanoparticles were also present in the blood, skin and other organs in trace amounts. Both ^{99m}Tc -MDP compounds were observed in the stomach, liver, spleen, heart, small intestines, bladder and bowels. The ^{99m}Tc -MDP showed a higher clearance and dominated the bladder and bowels.

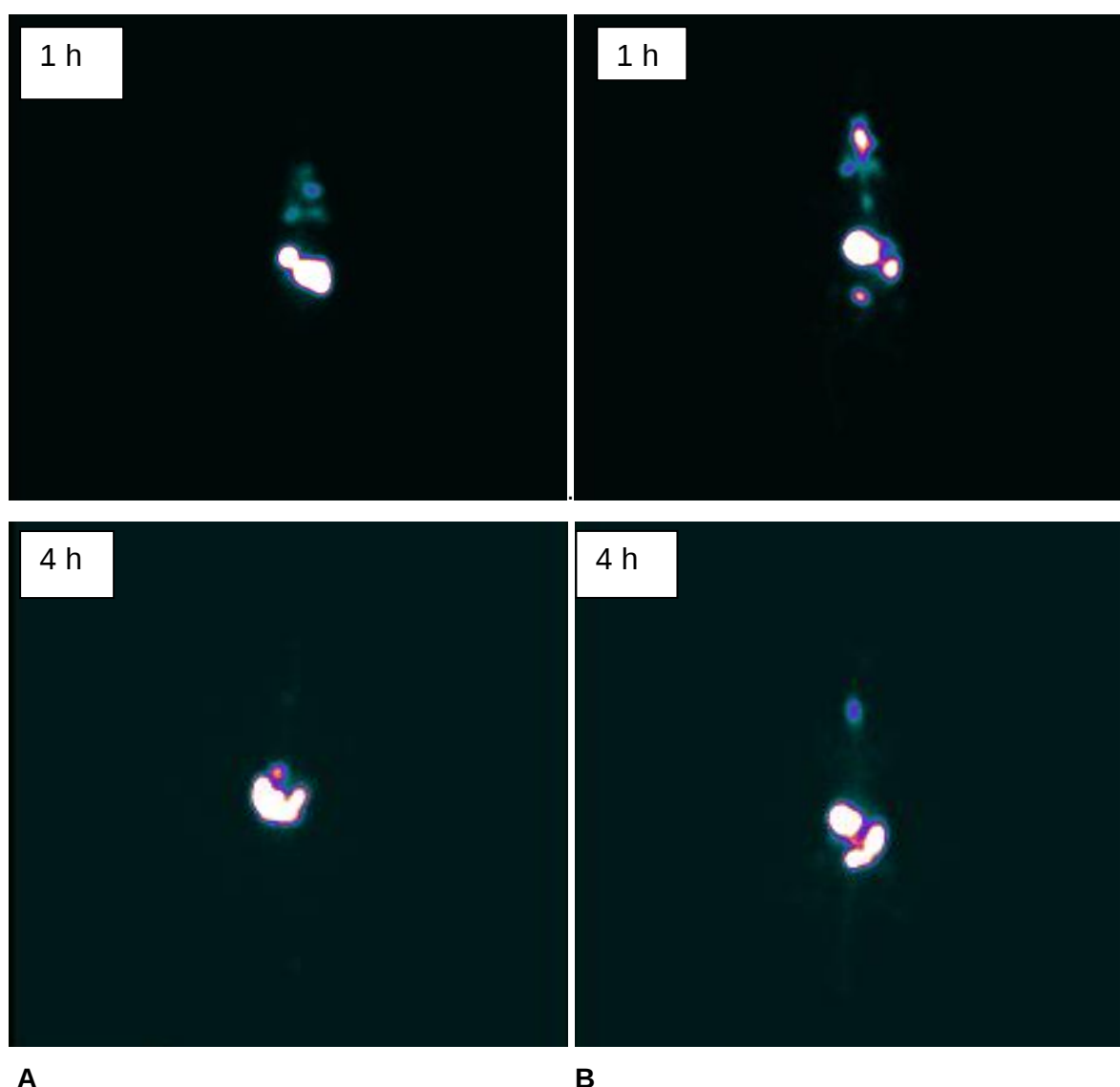


Figure 4.14: Static scintigraphic images illustrating orally administered ^{99m}Tc -MDP solid lipid nanoparticles (A) and ^{99m}Tc -MDP (B), 1 and 4 h post-ingestion.

Static images indicating the ROIs at 1, 2, 3 and 4 h after injection of radiolabelled ^{99m}Tc -MDP SLNs depicted in Figure 4.15 illustrate the biodistribution of ^{99m}Tc -MDP SLNs via the bloodstream over a 4 h period. The injected dose had a radioactivity of 13.50 MBq. High radioactivity was observed in the liver, stomach and bladder and also had distributed to the intestines, spleen, bowels and trace amounts in bone within the first hour post-injection. The ROIs as depicted in the images are the liver, the site of injection (tail), stomach, spleen, bladder and bowels and bone. The small intestines represented a very small amount of radioactivity, which indicated that the ^{99m}Tc -MDP SLNs were still being distributed. There was a significant difference in clearance between 1 and 4 h post intravenous administration of ^{99m}Tc -MDP SLNs. The biodistribution of the ^{99m}Tc -MDP SLNs was significantly fast and demonstrated movement of the nanoparticles from the bloodstream to other organs. The presence and distribution of the

^{99m}Tc -MDP SLNs in the bladder and bowels after 1 h could be an indication of excretion, good metabolism and easy movement of the nanoparticulate emulsion.

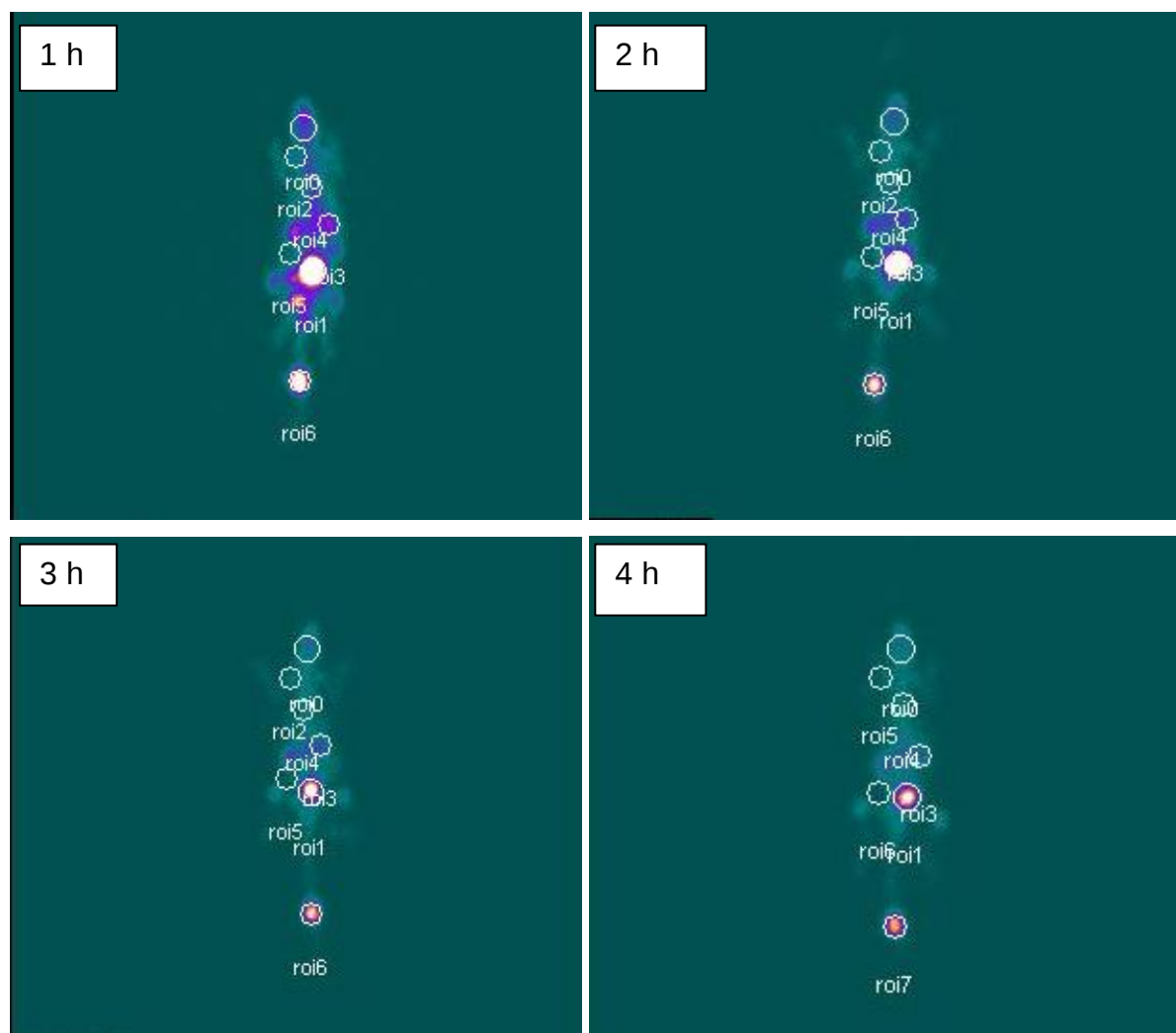


Figure 4.15: Images illustrating the regions of interest of injected ^{99m}Tc -MDP solid lipid nanoparticles at 1, 2, 3 and 4 h post-intravenous administration.

Figure 4.16 illustrates the biodistribution of ^{99m}Tc -MDP via the bloodstream over a 4 h period. After administration of ^{99m}Tc -MDP with an activity of 37.74 MBq to rats, the control compound distributed throughout the bloodstream and concentrated in the skeletal system of the rat, namely the skull, shoulders and the joints of the limbs. Small amounts of radioactivity were also evident in soft tissue such as the kidney and bladder. The main ROIs were the site of injection (tail), bladder and bowels, kidneys and bone. There was a significant difference in clearance between 1 and 4 h post intravenous administration of ^{99m}Tc -MDP. The biodistribution of the ^{99m}Tc -MDP was significantly fast and demonstrated movement of the nanoparticles from the

bloodstream to mainly bone. The presence of the ^{99m}Tc -MDP in the kidneys, bladder and bowels was an indication of excretion of ^{99m}Tc -MDP from the body.

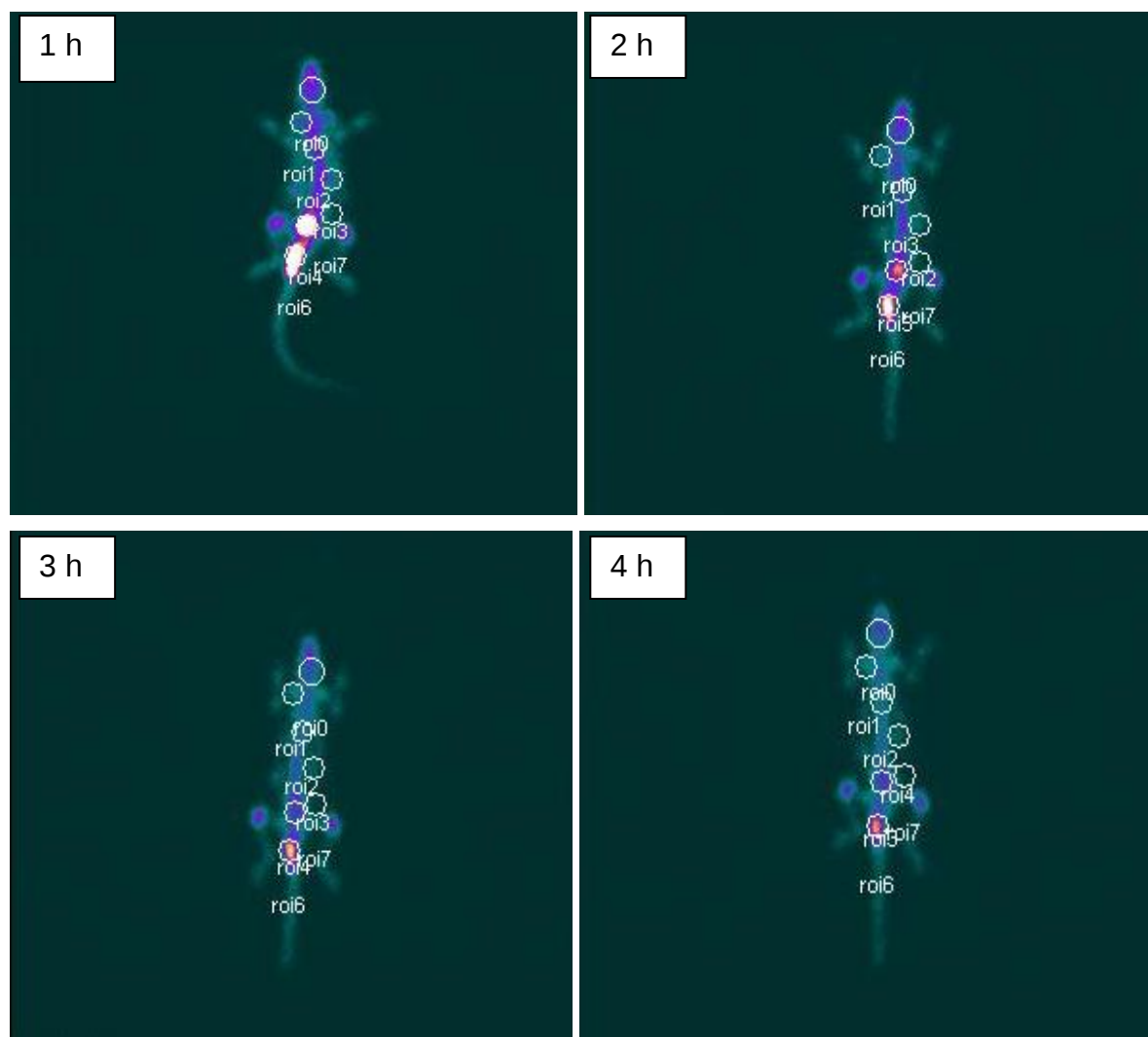


Figure 4.16: Images illustrating the regions of interest of injected ^{99m}Tc -MDP at 1, 2, 3 and 4 h post-intravenous administration.

The ^{99m}Tc -MDP SLNs and the ^{99m}Tc -MDP portray similar clearance and biodistribution characteristics. Observations of the images in Figure 4.17 demonstrate that ^{99m}Tc -MDP SLNs has a wider distribution and localisation in organs like the liver, stomach, spleen, heart and the bladder. The intravenous administration of the ^{99m}Tc -MDP lead to distribution from the bloodstream to the skeletal frame of the rat. Trace amounts of ^{99m}Tc -MDP were also observed in the kidneys and bladder. Both the ^{99m}Tc -MDP control and the radioactive SLN emulsion were reported in the site of injection (tail), liver and the bladder.

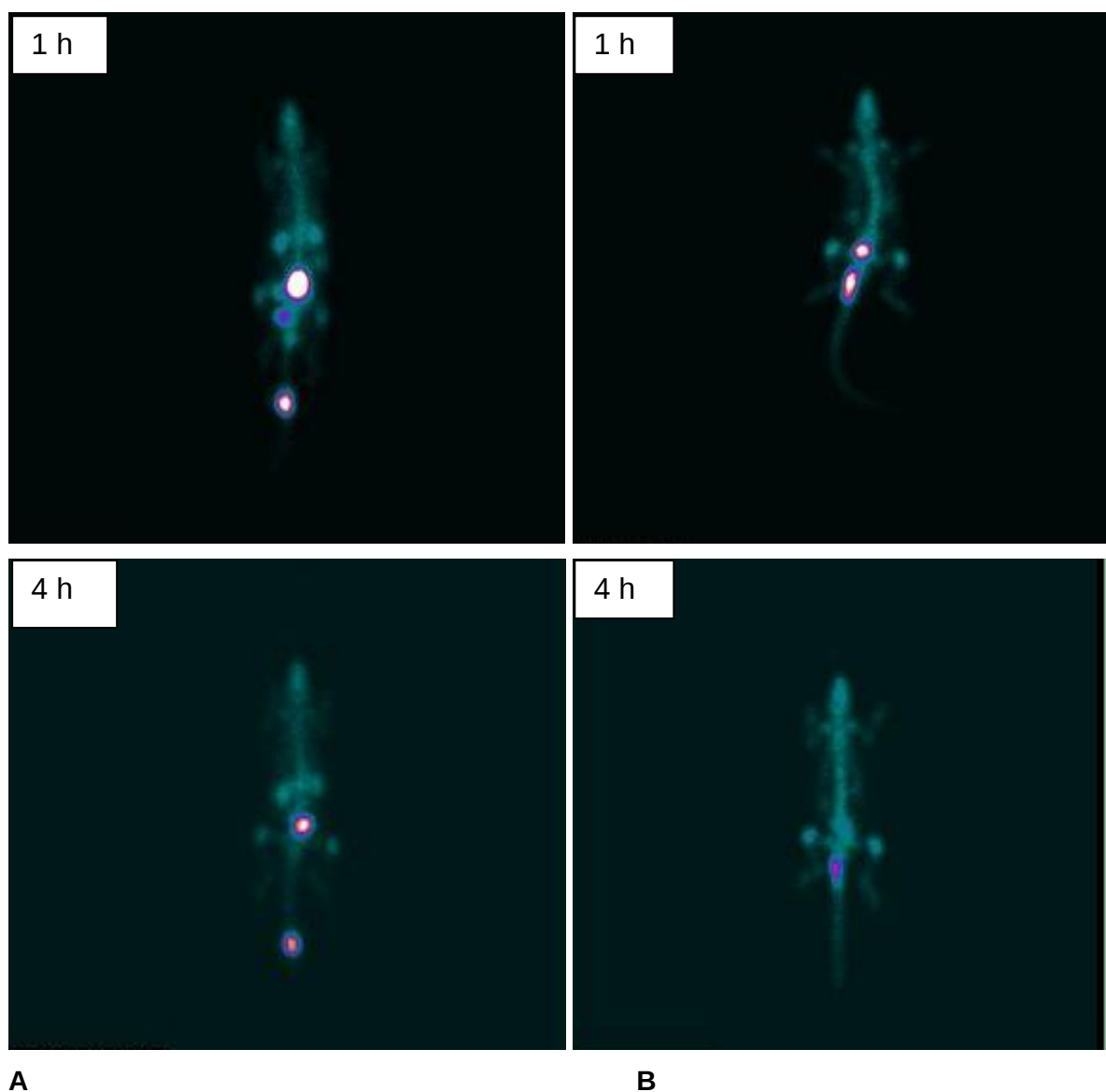


Figure 4.17: Images illustrating injected ^{99m}Tc -MDP solid lipid nanoparticles (A) and ^{99m}Tc -MDP (B), 1 and 4 h post-intravenous administration.

Orally and injected ^{99m}Tc -MDP SLNs had a wider and a more distinct distribution as compared to ^{99m}Tc -MDP. After oral administration, both the ^{99m}Tc -MDP control and the radioactive ^{99m}Tc -MDP SLN emulsion were observed at the site of ingestion (oesophagus), stomach, intestines and the bladder. The orally administered ^{99m}Tc -MDP SLNs and ^{99m}Tc -MDP portrayed similar clearance and biodistribution characteristics after 1 h.

Intravenously administrated ^{99m}Tc -MDP SLNs and ^{99m}Tc -MDP portrayed similar clearance and biodistribution characteristics. Injected ^{99m}Tc -MDP SLNs had a wider biodistribution and localisation in the liver, stomach, spleen, heart and the bladder (Figure 4.18). The intravenous administration of ^{99m}Tc -MDP lead to distribution from the bloodstream to the skeletal frame of the rat. Trace amounts of ^{99m}Tc -MDP were also observed in the kidneys and bladder. Both the

^{99m}Tc -MDP control and the ^{99m}Tc -MDP SLNs were reported at the site of injection (tail), liver and the bladder.

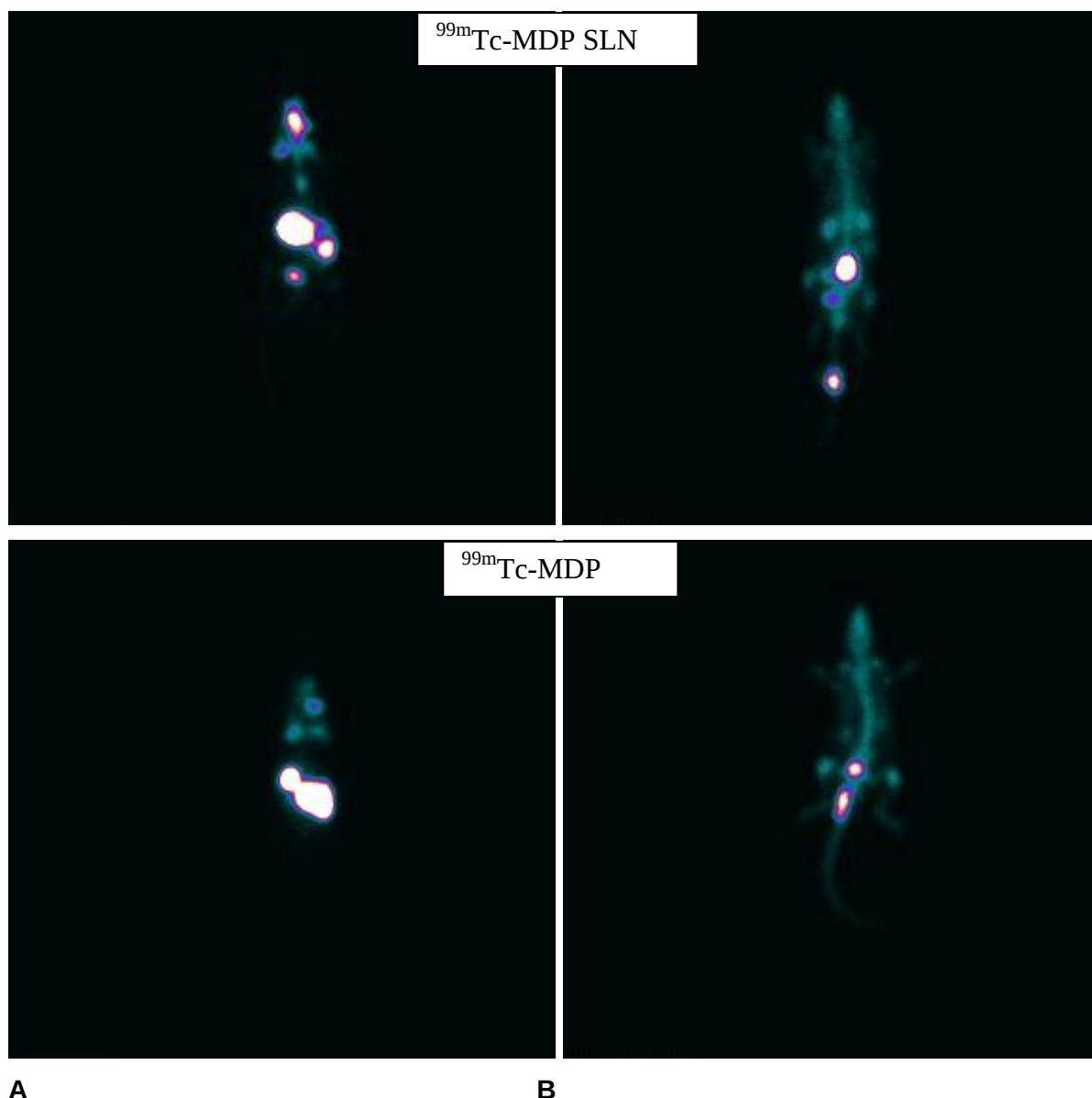


Figure 4.18: A comparison between oral (A) and intravenous (B) administration of radioactive ^{99m}Tc -MDP labelled SLN and the control ^{99m}Tc -MDP at 1 h.

Figure 4.19 shows a comparative distribution of ^{99m}Tc -MDP and SLN observed 1 h post oral and intravenous administration. There was however, a difference between the oral and intravenous biodistribution observed. The main difference was the amount of radioactivity (fluorescence) and the wider biodistribution of the nanoparticles as compared to the control compounds. Looking at the images, it's seems that SLNs change the distribution of ^{99m}Tc -MDP.

Both ^{99m}Tc -MDP and SLNs (oral) showed a similar biodistribution where both compounds were absorbed in the stomach, spleen and liver. Rapid excretion was observed via the bladder, although the ^{99m}Tc -MDP clearance was faster. Therefore we can conclude that the SLNs delivery system slows down the movement of the encapsulated ^{99m}Tc -MDP.

^{99m}Tc -MDP is attracted to bone and will therefore accumulate in bone when administered intravenously, leaving the remaining unbound ^{99m}Tc -MDP to be excreted via the kidneys and bladder (Truluck, 2007; Geskovski *et al.*, 2013).

When ^{99m}Tc -MDP is injected intravenously, its biodistribution is dependent on the blood flow and the uptake reflects the rate of new bone formation (Truluck, 2007). This radiotracer accumulates in the inorganic hydroxyapatite crystal component of bone. It is therefore normal to see the uptake of the radiotracer throughout the entire skeleton (Truluck, 2007). The radiotracer ^{99m}Tc -MDP undergoes urinary excretion and activity can be seen in the kidneys, ureters and bladder (Truluck, 2007). Symmetric uptake of ^{99m}Tc -MDP throughout the skeleton is the expected appearance of a normal bone scan of the injected radiotracer. The observation of ^{99m}Tc -MDP SLNs in the organs, especially for oral administration, and not much in the bone is a good indication that the ^{99m}Tc -MDP was encapsulated in the SLN emulsion. Therefore it indicated that the SLNs served as a reliable delivery system.

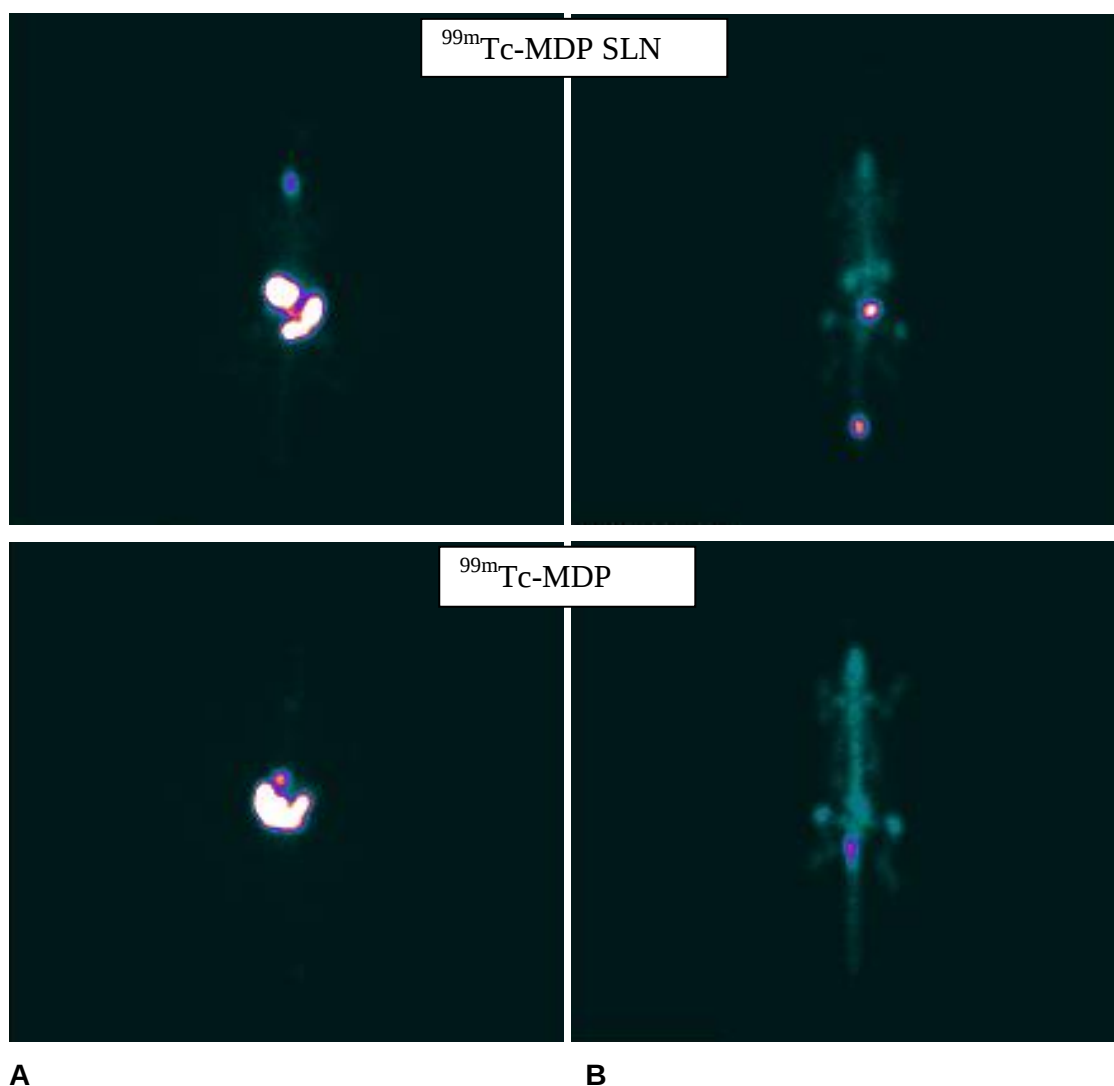


Figure 4.19: A comparison between oral (A) and intravenous (B) administration of radioactive ^{99m}Tc -MDP labelled SLNs and the control ^{99m}Tc -MDP at 4 h.

4.3.2.1.2 Biodistribution graphs of ^{99m}Tc -MDP solid lipid nanoparticles

After oral ingestion of ^{99m}Tc -MDP SLNs, the emulsion distributed to all major organs as depicted in Figure 4.20. The highest deposition of the ^{99m}Tc -MDP SLNs was observed in the kidneys (8.50%), stomach (8.04%), bone (3.51%) and small intestines (3.39%). The least deposition of ingested nanoparticles was reported in the heart (0.12%) and spleen (0.13%). The oral control group ^{99m}Tc -MDP reported great localisation in the large intestines (7.08%), kidneys (1.71%), small intestines (1.46%) and stomach (1.10%). The liver (0.24%) and spleen (0.42%) are the organs which composed of the least amount of ^{99m}Tc -MDP deposition. A large amount of orally administered ^{99m}Tc -MDP was not absorbed; therefore a large amount of it accumulated and was observed in the large intestine after the 6 h duration of the study. Therefore, orally administered ^{99m}Tc -MDP was excreted via the large intestine. SLN appeared to enhance intestinal absorption

of ^{99m}Tc -MDP. SLNs were observed in bone as well because there was more absorption from the small intestine which also facilitated more absorption in bone.

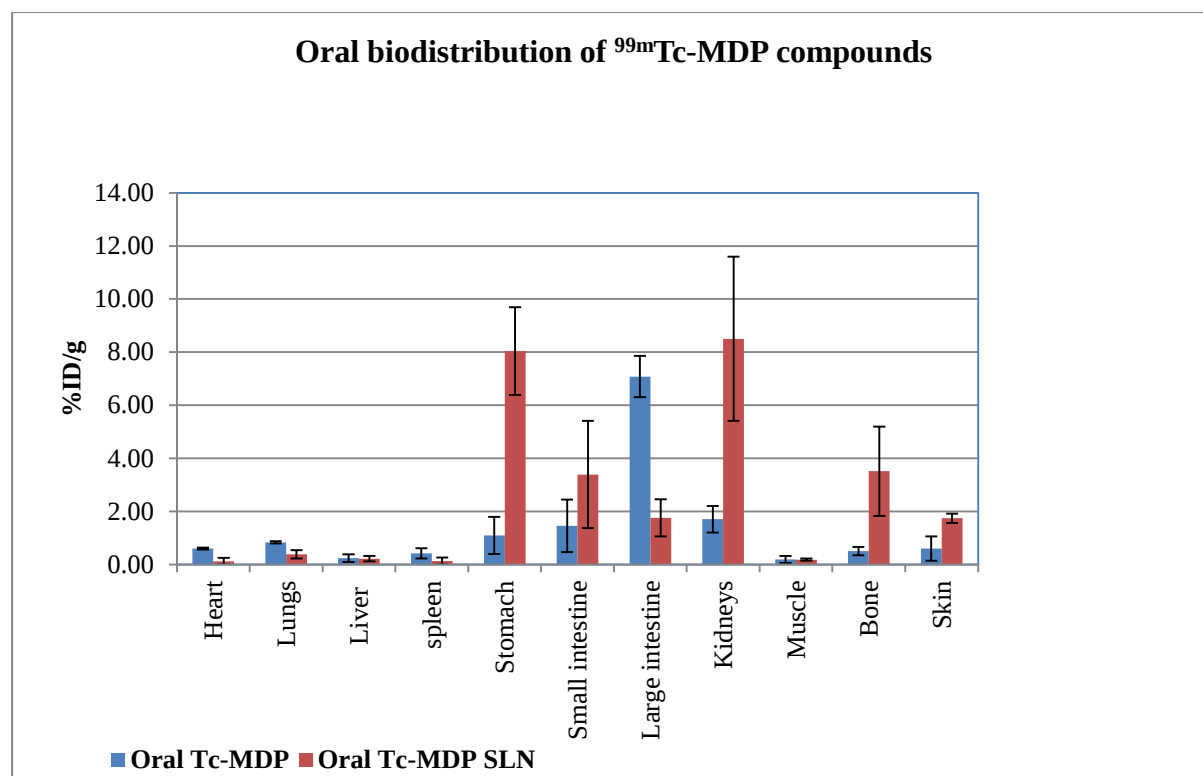


Figure 4.20: The biodistribution of orally administered ^{99m}Tc -MDP solid lipid nanoparticles and ^{99m}Tc -MDP after 4 h.

Post-injection scans of ^{99m}Tc -MDP SLNs in rats showed that, the emulsion moved via the blood circulation and deposited the highest in the following organs; kidneys (3.87%), bone (2.66%), small intestines (0.59%) and stomach (0.55%). The least amount of deposition was in the heart (0.18%) and liver (0.14%). The biodistribution and clearance profile of ^{99m}Tc -MDP was similar to that displayed by the SLN formulation (Figure 4.21). Similarly a high deposition of the ^{99m}Tc -MDP compound was observed in the kidneys (3.07%) and bone (1.95%). The following organs reported the least amounts of deposition; liver (0.05%), muscle (0.05%), heart (0.10%), spleen (0.12%), lungs (0.17%) and stomach (0.17%). These compounds localized in the bone due to the fact that ^{99m}Tc -MDP is a bone seeking agent and will advertently accumulate in bone and soft tissue. Thereafter they are excreted via the kidneys and bladder. Intravenously administered ^{99m}Tc -MDP is expected to rapidly clear from the blood and accumulate in bone (Geskovski *et al.*, 2013). The remaining ^{99m}Tc -MDP is excreted via the kidney in urine (Geskovski *et al.*, 2013).

High absorption of the SLNs was reported in the kidneys, bone and GI tract followed by the spleen. Although high absorption was observed in the kidneys and bone, trace amounts in the stomach, spleen, small and large intestines prove that sufficient amounts of ^{99m}Tc -MDP was encapsulated. Rapid excretion was observed via the bladder, although ^{99m}Tc -MDP clearance was faster. Therefore we can conclude that the SLN delivery system slows down the movement of the encapsulated ^{99m}Tc -MDP. There was however, no great difference in biodistribution between the ^{99m}Tc -MDP and SLNs post intravenous administration. Therefore, it's unclear whether what was observed was the SLNs or ^{99m}Tc -MDP and ^{99m}Tc -MDP probably influences the distribution of SLNs and possibly influences its own release rate. It is not clear whether SLNs (intravenous) distributed to organs or whether it released the ^{99m}Tc -MDP.

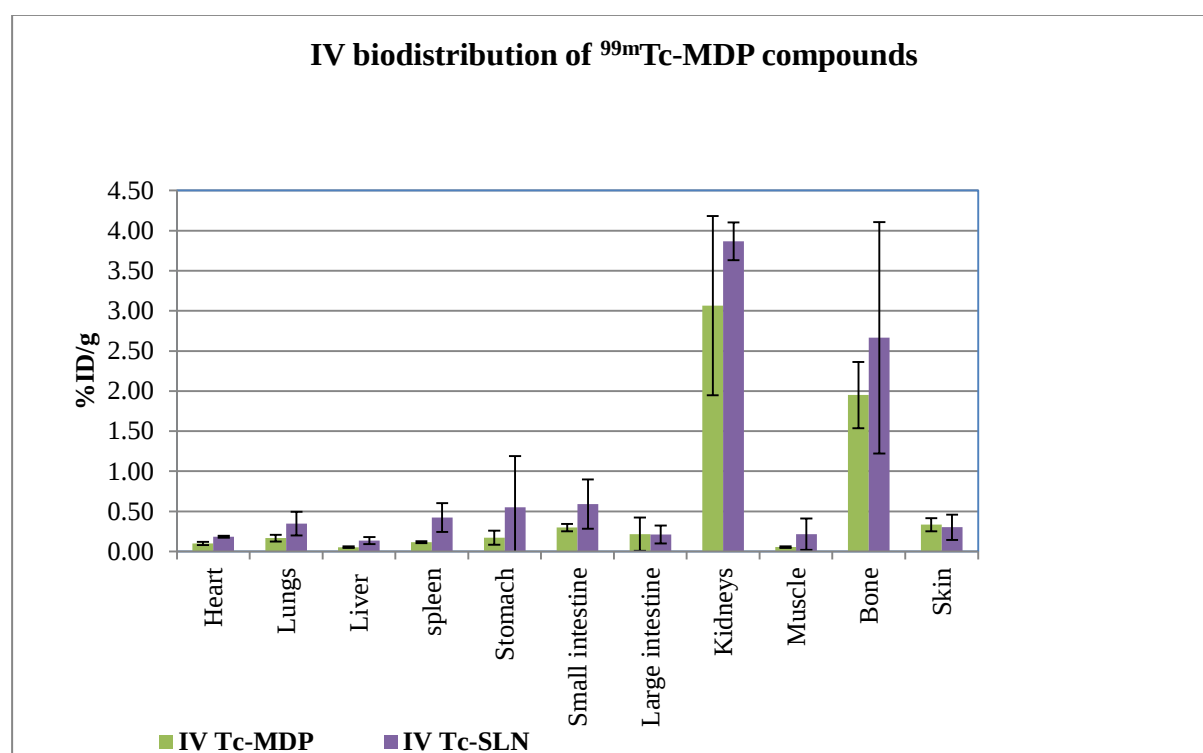


Figure 4.21: The *in vivo* biodistribution of intravenously injected ^{99m}Tc -MDP solid lipid nanoparticles and ^{99m}Tc -MDP after 4 h.

A comparative illustration of the organs which had the highest and lowest deposition of ^{99m}Tc -MDP SLNs and ^{99m}Tc -MDP is shown in Figure 4.22. The orally ingested ^{99m}Tc -MDP SLNs reported the highest localisation of nanoparticles in the kidneys (8.50%) and stomach (8.04%). The same ingested SLNs incurred the least amount of deposition in the heart (0.12%), spleen (0.13%) and muscle (0.18%). Similarly, ingested ^{99m}Tc -MDP had great localisation in the large

intestines (7.08%), kidneys (1.71%), small intestines (1.46%) and stomach (1.10%). The liver (0.24%) and spleen (0.42%) had the least amount of ^{99m}Tc -MDP deposition.

The injected ^{99m}Tc -MDP SLNs also showed a high localisation of particles in the kidneys (3.87%) followed by bone (2.66%) accumulation. The intravenous control group ^{99m}Tc -MDP also exhibited the highest deposition in the kidneys (3.07%) and bone (1.95%). Both these compounds reported significantly low deposition values in the heart, liver and spleen.

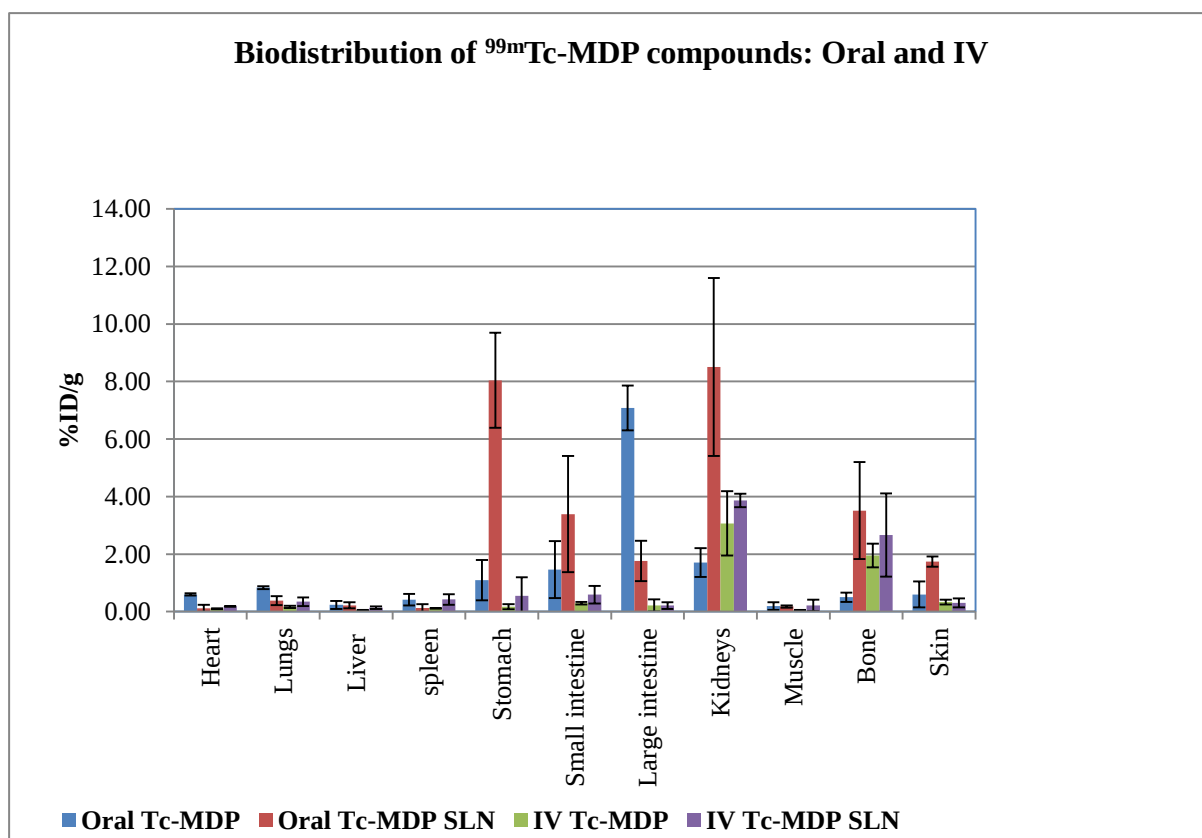


Figure 4.22: A comparative biodistribution of orally ingested and injected ^{99m}Tc -MDP SLNs and ^{99m}Tc -MDP after 4 h.

CHAPTER 5: SUMMATION, CONCLUSION AND FUTURE RECOMMENDATIONS

5.1 Conclusion

5.1.1 Sm₂O₃ loaded PLGA nanoparticles

The encapsulation of samarium oxide-153 [¹⁵³Sm]Sm₂O₃ into poly(D,L-lactide-co-glycolide) (PLGA) was successfully carried out followed by brief activation of the Sm₂O₃ loaded PLGA nanoparticles in a nuclear reactor. The irradiation time of the Sm₂O₃ loaded PLGA nanoparticles in the nuclear reactor had no negative effect on the physicochemical properties of the nanoparticles and yielded enough radioactivity for scintigraphic imaging. Based on the observed biodistribution profiles of the radiolabelled delivery systems, it can be concluded that the radiolabelling process was successful. Therefore, the distribution of the encapsulated radioactive Sm₂O₃ in nanoscale delivery systems could be followed *in vivo*, where distribution of fluorescence was observed under a gamma camera and quantified by measuring the radioactivity in each organ. This work successfully showed the *in vivo* biodistribution of radiolabelled Sm₂O₃-PLGA in tissue at several time points after intravenous and oral administration; this was confirmed by observation of the radiolabelled nanoparticle compounds under a single photon emission tomography/computed tomography (SPECT/CT) gamma camera. The data obtained also showed that there were differences between the oral and intravenous distribution profiles.

Nanoparticles aim to provide sustained release of the drug or encapsulated compound from the matrix or shell. Thus, these properties could help improve drug bioavailability and dose frequency and hence could resolve the common problem of noncompliance with prescribed therapy. The use of the nanoparticles as drug delivery systems may also reduce the toxicity of the incorporated drug. Specific importance should also be accorded to the toxicity of the empty non-drug-loaded nanoparticles. This was successfully demonstrated by the encapsulation of [¹⁵³Sm]Sm₂O₃ in PLGA nanoparticles where both orally and intravenously administered PLGA nanoparticles change the biodistribution of encapsulated ¹⁵³Sm₂O₃. PLGA nanoparticles and it also appears to enhance the intestinal absorption of ¹⁵³Sm *in vivo*.

Oral and intravenous administration of PLGA nanoparticles for drug delivery purposes indicate that surface-modification of these particles with hydrophilic molecules such as polyethylene glycol (PEG) minimises opsonisation of the nanoparticles, thus increasing the circulation time in the blood and subsequently minimising the number of nanoparticles that reach the liver. In this

instance, another polymer, chitosan was also used for this purpose. The use of chitosan in turn minimises the first-pass metabolism of the encapsulated compound or drug. Delivery systems such as PLGA nanoparticles provide an ideal transport system to various tissues that are generally difficult to target even with oral administration (Semete *et al.*, 2010). PLGA nanoparticles have the ability to cross the blood brain barrier, therefore they could provide a means to effectively treat neurological and psychiatric disorders (Vergoni *et al.*, 2009) including tuberculosis, by administering the encapsulated compound orally or via other non-invasive modes.

According to this study conducted with PLGA nanoparticles, it was evident from the *ex vivo* animal experiments, the inductively coupled spectrometry and scintigraphic imaging results that PLGA nanoparticles are safe to use as delivery systems when administered both orally and intravenously in the mentioned doses, although many concerns exist regarding the safety of nanoparticles in general. This does not, however, rule out that more studies need to be conducted to evaluate the safety or toxicity of nanoscale PLGA and other polymeric delivery systems. From the biodistribution data obtained it is clear that polymeric nanoscale delivery systems would be suitable for improving permeability and thus the bioavailability of therapeutic compounds. With this approach, delivery of poorly permeable and soluble drugs can be enhanced with effective drug delivery systems.

Polymeric materials and other additives used in the nanoformulations were carefully selected in order to minimize the *in vivo* toxicity. The encapsulating polymers or fatty acids are biodegradable, biocompatible and non-immunogenic in compliance with US-FDA recommendations for orally administered formulations. In case of PLGA, it degrades over time into lactic acid and glycolic acid that are easily eliminated from the system.

5.1.2 ^{99m}Tc -MDP loaded SLN

The distribution of the encapsulated technetium-99m–methylene diphosphonate (^{99m}Tc-MDP) in solid lipid nanoparticles (SLN) was successful. The *in vivo* biodistribution of radiolabelled ^{99m}Tc-MDP SLN in tissue at several time points after intravenous and oral administration, was confirmed by observation of the radiolabelled nanoparticle compounds under a single photon emission tomography/computed tomography (SPECT/CT) gamma camera.

Orally administered SLN delivery systems change the biodistribution of encapsulated ^{99m}Tc-MDP. These also appear to enhance the intestinal absorption of ^{99m}Tc-MDP *in vivo*. There was however, no significant difference in biodistribution between intravenously injected ^{99m}Tc-MDP

and SLN. Based on these studies at the Steve Biko Academic Hospital using ^{99m}Tc as an imaging radionuclide, SLN have been identified as a formulation that might enable oral administration of radiopharmaceuticals as opposed to the standard intravenous form of administration.

In summary, it was observed that encapsulation of radiopharmaceuticals in nanoparticles significantly reduced the apparent clearance of the radiopharmaceutical from the blood, thereby enhancing the circulation time and potential cumulative delivery to tissue. Furthermore, positively charged nanoparticles (PLGA nanoparticles) appeared to have a longer circulation time and reduced plasma clearance as compared to negatively charged nanoparticles (SLNs), resulting in slower delivery. In addition to reduced clearance from the gastrointestinal tract and liver, it is anticipated that reduced clearance from tissue at the site of administration or absorption may contribute to enhanced radiopharmaceutical (or drug) exposure with nanoparticles.

5.2 Future work and recommendations

Future plans include optimising the current nanoparticulate delivery system formulations, with the hope and aim of commercialising these products and to determine the release rate of ^{153}Sm and ^{99m}Tc -MDP from the respective nanoparticles. Optimisation could include changing some parameters in the development of nanoparticles, to achieve smaller and more spherical nanoparticles. Determining the *in vivo* release kinetics could enable targeted drug therapy using nanoparticles to be more effective and is a prospective research focus area.

Further work may also need to be done to understand the interaction of SLN with the biological surrounding such as the absorption process, agglomeration and interaction with lipid delivery systems. There is a need to improve the methodologies for nanoparticle synthesis and characterisation such that meaningful structure versus activity relationships of nanoparticles can be defined in terms of safety and efficacy. Characterisation of solid lipid dispersions requires several analytical methods. However, there are limited techniques which have protocols for the characterisation of lipid dispersions, such as scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Further research would need to be done to characterise SLN on a molecular level to understand the structure and dynamics of SLN in *in vivo* and *in vitro* studies.

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