PREPARATION, QUALITY CONTROL AND BIO-EVALUATION OF TECHNETIUM-99m LABELED COMPOUNDS FOR INFECTION AND TUMOR IMAGING

by
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DEPARTMENT OF BIOINFORMATCS AND BIOTECHNOLOGY GC UNIVERSITY, FAISALABAD.

August 2015
In the name of Allah, the most beneficent, the most merciful, 
Who has the knowledge of each and every thing
DEDICATED TO,

MY PARENTS

My HUSBAND
(Dr. Syed Tanveer Hussain Bokhari)

&

My daughters
DECLARATION

The work reported in this thesis was carried out by me under the supervision of Dr. Muhammad Ibrahim Rajoka, Department of Bioinformatics and Biotechnology GC University, Faisalabad, Pakistan.

I hereby declare that the title of thesis “Preparation, Quality Control and Bio-evaluation of Technetium-99m labeled compounds for Infection and Tumor Imaging” and the content of thesis are the product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models / equations / formulas / protocols etc. I further declare that this work has not been submitted for award of any other degree/diploma. The university may take action if the information provided is found inaccurate at any stage.

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SAIRA HINA
ABSTRACT

The aim of the proposed research work was to label some drugs/compounds with medically interesting Tc-99m. For this purpose antibiotics clarithromycin, clindamycin, vibramycin and peptide cecropin A were labeled with Tc-99m as infection imaging agents using animal models whereas epirubicin, vincristine and lanreotide peptide were chosen for tumor study. In the present investigation, synthesis of the 99mTc-clarithromycin and its biological evaluation in mice artificially infected with Staphylococcus aureus was evaluated. A good labeling efficiency (More than 99%) with 99mTcO₄⁻ was achieved at pH 6–7 while 25 μg using stannous chloride as reducing agent and 500 μg of clarithromycin at room temperature. Electrophoresis indicates the neutral behavior of 99mTc-clarithromycin. HPLC analysis confirms the single specie of the labeled compound. Biodistribution and SPECT imaging of 99mTc-clarithromycin was performed in infection induced Swiss Albino mice and rabbits respectively which revealed high uptake of 99mTc-clarithromycin at Staphylococcus aureus infected sites in model animals.

Clindamycin, a lincosamide antibiotic was labelled with technetium-99m (~380 MBq). Clindamycin has proved to be efficient for treating serious infections caused by bacteria such as staphylococcus aureus. More than 95% labeling efficiency with 99mTc was achieved at pH 6–7 while using 2.5–3 μg SnCl₂.H₂O as reducing agent and 100 μg of ligand at room temperature. The characterization of the compound was performed by using electrophoresis, HPLC and shake flask assay. Electrophoresis indicates the neutral behavior of 99mTc-clindamycin. HPLC analysis confirms the single specie of the labeled compound, while shake flask assay confirms high lipophilicity. The biodistribution studies of 99mTc-clindamycin were performed Sprague-Dawley rats bearing bacterial infection. Scintigraphy and biodistribution studies showed high uptake of
$^{99m}$Tc-clindamycin in the liver, heart, lung, and stomach as well as at *S. aureus* infected sites in rabbits.

A new technetium-$^{99m}$ labeled vibramycin radiopharmaceutical, labeled with technetium-$^{99m}$ using SnCl$_2$.2H$_2$O as a reducing agent is also prepared. The stability of $^{99m}$Tc-vibramycin was evaluated in human serum at 37 $^\circ$C. Biodistribution studies of $^{99m}$Tc-vibramycin were performed in a model of bacterial infected Sprague-Dawley rats. *In vitro* studies were performed to determine the binding interaction of the labeled antibiotic with bacteria and its stability. Scintigraphic study was done with a $\gamma$-camera at 1, 4 and 24 hours after radiotracer injection in rats having infectious intramuscular lesions. It was confirmed through this study that $^{99m}$Tc-vibramycin possessed high radiolabeling yield (95%) as determined by instant thin-layer chromatography. The binding assay shows good binding with *S. aureus*. Scintigraphy showed uptake of prepared $^{99m}$Tc-vibramycin in the infectious lesions at 1 hour, 4 hours and 24 h after injection. Biodistribution studies of $^{99m}$Tc-vibramycin revealed that the radiopharmaceutical accumulated significantly at infection sites and showed the renal route of excretion. Target-to-non target ratio for $^{99m}$Tc-vibramycin was found to be significantly different for the infectious lesion from control muscle. The study demonstrated that $^{99m}$Tc-vibramycin shows preferential binding to living bacteria. The biological activity (*in vitro*) of $^{99m}$Tc-vibramycin was studied with the help of optimized parameters and the $^{99m}$Tc-vibramycin was found to be a good infection imaging agent.

*In vivo* study of peptides/receptor systems with medical radiotracers have great potential across the whole range of nuclear medicine investigations, their initial focus was in oncology and the present interest has focused especially on the field of inflammation and infection. $^{99m}$Tc-labeled antimicrobial peptide cecropin A was evaluated as a bacterial infection seeking agent in
*Escherichia coli* induced infections. $^{99m}$Tc-cecropin A was tested for stability at room temperature, stability in human serum, cysteine challenge test and bacterial binding study. Experimental thigh muscle infection was induced by injecting $2 \times 10^8$ cfu of live *E. coli* bacteria into the right thigh muscle in mice and rabbits. Heat-killed *E. coli* and turpentine oil were used for inducing sterile thigh muscle inflammation. In Scintigraphic imaging, regions of interest were drawn over infected (T) and non-infected (NT) thigh, and accumulation of $^{99m}$Tc-cecropin A at sites of infection was expressed as a target to non-target ratio.

Direct radiolabeling of epirubicin with $^{99m}$Tc, quality control, biological characterization and scintigraphic evaluation in tumor bearing mice was done. The optimum conditions ensuring $^{99m}$Tc-epirubicin labeling yield as high as 99% by adding 35μg SnCl$_2$.2H$_2$O, 200μg of ligand at pH 6 for 30 minutes reaction time at room temperature (25°C±2°C). The radiochemical purity of $^{99m}$Tc-epirubicin was evaluated by chromatographic techniques. HPLC of $^{99m}$Tc-epirubicin shows about 99% binding of the compound with technetium-99m. Electrophoresis study indicates the neutral nature of $^{99m}$Tc-epirubicin. Biodistribution data and scintigraphic results showed that $^{99m}$Tc-epirubicin accumulated in the tumor with significant uptake and excellent retention. $^{99m}$Tc-epirubicin shows good stability in human serum. *In vitro* and *in vivo* studies showed significantly selective uptake of $^{99m}$Tc-epirubicin in the tumor, indicating efficiency of $^{99m}$Tc-epirubicin as a tumor diagnostic agent.

Methodology was developed for the preparation of DOTA-lanreotide and labeling with $^{99m}$Tc. The radiochemical purity of $^{99m}$Tc-DOTA-lanreotide was evaluated by chromatographic techniques. Labeling efficiency of 96% was obtained using 5 μg of ligand (DOTA-lanreotide), with 4 μg SnCl$_2$.2H$_2$O as a reducing agent at pH 7 at room temperature for 30 minutes. The
stability of $^{99m}$Tc-DOTA-lanreotide was studied up to 4 h. Electrophoresis indicated that $^{99m}$Tc-DOTA-lanreotide has no charge and HPLC shows a single species of labeled compound. Biodistribution studies of $^{99m}$Tc-DOTA-lanreotide were performed in normal and tumor induced Swiss Webster mice at various time intervals after intravenous administration. The biodistribution and scintigraphic results in tumor bearing mice show accumulation of $^{99m}$Tc-DOTA-lanreotide in tumor sites. These results suggest that $^{99m}$Tc-DOTA-lanreotide may be useful as a selective imaging agent for diagnosis and visualization of tumors.

The study was also performed for the radiolabeling and biological testing of vincristine labeled with $^{99m}$Tc. The optimum conditions required to obtain ~100% yield of $^{99m}$Tc-vincristine($^{99m}$Tc-vinc) were as follows: pH 4, 5 µg of vincristine sulphate , 6 µg SnCl$_2$.2H$_2$O as reducing agent and 10 min incubation time at room temperature. The labeling yield was confirmed by HPLC using radioactive and UV detector operating at 230 nm. $^{99m}$Tc-vinc was stable in vitro for 5 h. Biodistribution and scintigraphy of $^{99m}$Tc-vinc was performed in normal mice (Swiss Albino mice) and rabbits respectively and that showed high uptake of it in liver and spleen. Biodistribution of $^{99m}$Tc-vinc in solid tumor bearing mice showed accumulation of major activity in tumors. Therefore $^{99m}$Tc-vinc can be important radiopharmaceutical in the detection and follow up of tumor in patients simultaneously with chemotherapy.
CHAPTER 1

INTRODUCTION

Cancer and infectious diseases persist to be a major problem and cause of death worldwide, especially in poor and developing countries. For diagnosis of cancer and infections, nuclear medicine imaging provides an attractive option. This needs a reliable radiopharmaceutical that can selectively concentrate in sites of infection or tumor. Over the year’s various medical radiotracers that localize in inflammation associated with infection sites, also known as “non-specific agents” have been used for infection imaging. However, experience has shown that an “infection specific agent” that concentrates selectively at sites of infection and not inflammation would have several advantages. The first such agent developed more than three decades ago was indium-111 ($^{111}$In)-leucocyte which is still considered a “gold standard”. Considerations of cost, availability, and superior properties for imaging make technetium-$^{99m}$Tc a better label than $^{111}$In. $^{99m}$Tc white blood cell (WBC) was developed subsequently and used for infection imaging. However, both $^{111}$In and $^{99m}$Tc WBCs have a number of drawbacks, in particular: each patient’s blood sample has to be collected and individually radiolabelled; well-trained staff and suitable facilities for separating and labeling the patient’s blood are needed; the risk of infection and cross-contamination associated with potential blood-borne microorganisms; and cost of materials. Because of these, efforts have been continuously made towards developing convenient replacements for $^{99m}$Tc WBCs with limited success, $^{99m}$Tc-antigranulocyte antibody being a good example. However, these radiopharmaceuticals still have many disadvantages, related to either their cost and availability or performance. The progress in development of new and better $^{99m}$Tc labeled infection specific imaging agents was considered as a very valuable aim for scientific
research due to their great potential in patients. Cancer is most widespread and life threatening disease in the world. A major challenge is to synthesize and design tumour specific biomolecule based new radiopharmaceuticals for the diagnosis and treatment of cancer. Medical Biotechnology is estimated to have a very great impact on medicine. The use of radiations in medical biochemistry and biotechnology for diagnosis, therapy, and biological systems control is stated as nuclear medicine. The application of different radioisotopes in pharmaceutics which achieved particular attention is for delivery systems for drugs, DNA and imaging agents (Das et al., 2002; Strula et al., 2007).

1.1. Nuclear Medicine in Biosciences

Nuclear medicine is started with the discoveries of many scientific materials, including the x-rays discovery by Wilhelm. C. Roentgen in 1895. Radiopharmaceuticals are molecules labeled with radionuclides that are used in the field of nuclear medicine, for the diagnosis or therapy of various disorders and diseases. Radiopharmacy is contributing a lot due to its great potentials to fundamental medicinal and biochemical research. In addition to radiopharmaceuticals application in hospitals, radiopharmaceutical chemistry is also contributing to the industrial progress by developing new drugs. The natural radioactivity was first discovered in the year 1896 by the scientist Henri Becquerel and in 1898 Madam Curie discovered the radioactive elements such as radium and polonium. The study on the therapy of fetal diseases was published by Frederick Proescher in 1913 (Alzaaki et al., 1984). Irene Curie and Frederic Joliot introduced artificial radioactivity in 1934 (Gotteschalk et al., 1978). Now there are more than 100 successive methods are available for radioisotopes using in diagnosis and therapy. The use of radioanuclides for physiology and medical biochemistry provided first the idea into the dynamics for biochemistry and physiology reactions in living
systems. The tracer principles were used to biology adopted labeled agents and radionuclides for investigating different physiological procedures. Today uranium fission provides an important source of radionuclides for its application in diagnostics and therapeutics. In 1950s, $^{131}$I became a main radionuclide when S.A. Berson and R.S. Yalow developed the radioimmunoassay method for in vitro quantitative study of various biochemical and physiological procedures (1977 Nobel Prize to R.S. Yalow for “The development of radioimmunoassays”). This part of the route of radiopharmaceuticals and nuclear medicine was supplemented not only by biology, physics and mathematics but also by industry and technology, providing the essential equipment like detectors and tomographs, reactors and cyclotrons. In the presence of all of these advancements and developments in radioisotope and radiotracers, the major problem associated with genetic disorders can also be resolved by using the biomedical imaging techniques for targeted therapies (Krijger et al., 2013)

1.2. Bio-imaging

Imaging techniques are important for medical diagnosis: diagnosis can be crucial for health and life itself. Diagnostic techniques are essential in medicine for a range of investigations from the detection of cancer where early diagnosis is vital to the study of neurological diseases and cardiology (Golestani et al., 2010). Metabolic pathways and drug metabolism can be studied to give a greater understanding of a variety of disorders and perhaps progress towards an effective treatment of these diseased states (Willowson et al., 2010). A whole range of techniques are available, each with different advantages and abilities to gain different levels of information. All are complementary as they range from providing structural information to molecular detail. A particular technique is chosen according to the level of information required. For example, a ruler is a useful tool for measuring the breadth
of this page but quite inadequate for measuring the length of a football pitch. Different techniques and instrumentation are required to answer different medical questions. Current imaging techniques available include X-ray computed tomography (X-ray CT), magnetic resonance imaging (MRI), ultrasound and the radiopharmaceutical modalities such as, positron emission tomography (PET) and single photon emission tomography (SPECT) (Jirak et al., 2007; Kim et al., 2015; Liang et al., 2013). The real advantage of radionuclide imaging is the ability to monitor biochemical and physiological processes as they occur in living systems. SPECT and PET both use radiolabeled biomolecules to investigate the biological processes and the concerned biomolecules may be very simple as O-15-labeled water molecules (Huang et al., 1983) for blood perfusion into study or can be complex such as radiolabeled cells for studying autoimmune diseases (Dubey et al., 2003; Shah et al., 2004; Allport et al., 2001). A very small amount of molecules can be measured without disturbing the biological system due to their sensitivity providing powerful method to study pharmacokinetics and pharmacodynamics in vivo.

1.3. Small Animal Models in Biomedical Research

Medically interesting radiotracers and radiopharmaceuticals proposed for use in clinical medicine are evaluated extensively in animals prior to human use. Animal model selection plays a key role in the eventual success or failure of the animal studies in predicting in vivo selectivity of a radiotracer for a physiologic process, the biodistribution and pharmacokinetics in man. Small animals are mostly used in clinical research field as models of tumor as well as inflammation and infection. The studies by small animal imaging technologies have been important for evaluation of the immunology, pathology, physiology and other aspects of pathogenesis. Especially mice are the main choice in
radiopharmaceutical research because they are economical, may provide suitable model for different human disorders and also their rapid production (Altiparmak et al., 1978). The mouse genome sequence has already determined and the knockout mice are also available as models of different human abnormalities. Human tumor xenografted animal models have been used in the field of inflammation imaging from several years. In vitro and in vivo oncological models have been used frequently for the screening of diagnostic radiotracers. In vitro systems have gained some additional prominence during last few years, particularly for gallium and for steroid receptor studies. Therefore these small animal models are playing a critical role in the radiopharmaceutical research and biomedical sciences. Table 1.1 represent some diseases, biochemical processes, and the animal models that have been documented as useful models of human disease and biochemistry.
Table 1.1: Various animal models for the regulation of Human diseases

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<tr>
<th>Disease</th>
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<td>Ulcer</td>
<td>Rat</td>
<td>Huang et al., 2013</td>
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<tr>
<td>Rhematoid arthiritus</td>
<td>Dog</td>
<td>Allen &amp; Newton, 1975</td>
</tr>
<tr>
<td>Ocular melanoma</td>
<td>Nu/nu Mice, Syrian hamster, rabbit, rat, kitten</td>
<td>Albert, 1980</td>
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<td>Breast Cancer lung metastasis</td>
<td>Mice</td>
<td>Davison et al., 2013</td>
</tr>
<tr>
<td>Gastric Ulcer</td>
<td>African rodent</td>
<td>Andre &amp; Andre, 1981</td>
</tr>
<tr>
<td>Carcinoma of ureter and urinary bladder</td>
<td>BN/BiRij rats</td>
<td>Boorman et al. 1977</td>
</tr>
<tr>
<td>Endodermal sinus tumor (ovaries, testes)</td>
<td>Rat</td>
<td>Damjanov, 1980</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia, aplastic anemia</td>
<td>Cat</td>
<td>Cotter, 1977</td>
</tr>
<tr>
<td>arthritis</td>
<td>Rabbit</td>
<td>Turker et al., 2005</td>
</tr>
<tr>
<td>Teratoma and teratocarcinoma</td>
<td>Mice</td>
<td>Damjanov &amp; Solter, 1976</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Cat</td>
<td>Misdrop &amp; Weijer, 1980</td>
</tr>
<tr>
<td>Diseases of endocrine System</td>
<td>Rat, mice, Chinese, hamster, dog, swine, sheep, goats, cattle</td>
<td>Capen, 1980</td>
</tr>
<tr>
<td>Metastasizing mammary tumors</td>
<td>Asplenic mice</td>
<td>Mitchell et al, 1982</td>
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1.4. Chemistry and Biology of Technetium-99m

The application of technetium-99m labeled compounds and biomolecules for diagnostic purposes is a quite new area of biomedical research. The practice of nuclear technology for medical purposes began in 1950s for medical isotope production with nuclear reactors, cyclotrons and accelerators. The $^{99}$Mo/$^{99m}$Tc generator was established by Brookhaven National Laboratory in 1959 (Richards et al., 1999) and after 5 years the first Tc-99m radiotracers were developed by University of Chicago (Harper et al., 1964). The study of $^{99m}$Tc radiopharmaceuticals was the start of the research of coordination chemistry as it is linked to imaging for diagnostic purposes. Although the most extensively used radionuclide for diagnostic imaging is Tc-99m (Jurisson et al., 1993, Eckelman, 1995, Tisato et al., 1994), a number of other radionuclides have also been explored for their uses in medicine. Technetium-99m is the most common medical radioisotope used for the medical diagnostic scans throughout the world each year (Eckelman, 2009, Srivastava, 1996) expecting that $^{99m}$Tc will continue its key role in diagnostic nuclear medicine into the future (IAEA, 2008). The element technetium (Z=43) is positioned in the middle of the second-row transition series. The $^{99m}$Tc is effectively ideal for diagnostic imaging because of its nuclear properties such as emission of γ-rays of 140 keV with 89 percent abundance which is best for imaging with the help of gamma cameras established in most (Jurisson et al., 1993).

1.5. Discovery of Technetium-99m

$^{99m}$Tc was discovered by Italian physicist Emilio Segre and Glenn Seabore in 1938 (Perrier, 1937; Segre, 1986). They called the element Technetium; from the Greek word Technetos, meaning “Artificial”. The first artificial element is technetium which is not found in the
earth. Technetium-99m has since proved useful in imaging a wide variety of organ systems and has been chemically attach to various carriers, including human serum albumin, albumin microspheres, ferric hydroxide macro aggregates, iron ascorbic acid, sulphur colloid and polyphosphate (Harper et al., 1964).

1.6. $^{99m}$Tc Generator

The technetium-99m is most commonly used in nuclear medicine for a large number of diagnostic tests. The ideal physical characteristics of technetium-99m are mainly used for the applications of external detection, having a half-life of 6 hours and gamma emission of 140 keV. The $^{99m}$Tc has ideal photons energy which has penetrability in the tissues and easily detection. A technetium-99m generator is a device which is used to extract the radioisotope technetium-99m from its parent source molybdenum-99. The half-life of $^{99}$Mo is 66 hours and can be easily moved to a long distances such as hospitals and research institutes where its decaying product technetium-99m is easily extracted and can be used for a vast variety of nuclear medicine used for diagnostic treatments. PAKGEN $^{99m}$Tc-Generator is a locally manufactured radionuclide generator system for the preparation of sodium Pertechnetate ($\text{Na}^{99m}\text{TcO}_4$) injection. The IPD (Isotope Production Division) of PINSTECH (Pakistan Institute of Nuclear Science & Technology) Islamabad, Pakistan has the facility for the manufacturing of $^{99m}$Tc-Generator. Due to ideal characteristics of $^{99m}$Tc, it can be used in different chemical forms for the detection and imaging of all body internal parts in humans. In addition, very short half-life greatly lessens the internal threat by radiation and other difficulties that may rise from inadvertent $^{99m}$Tc contamination. Due to very short half-life of Tc-99m, Annual Limit of Intake (ALI) for technetium-99m is far higher than some other
commonly using radioisotopes in the laboratory. The annual dose limit recommendation for a radiation worker is:

The ALI (inhalation) $^{99m}$Tc = $2 \times 10^9$ Bq

The ALI (ingestion) $^{99m}$Tc = $1 \times 10^9$ Bq

1.7. Characteristics of Ideal Radiopharmaceutical Generator

An ideal radiopharmaceutical generator must have the following features

i. Simple to use and easy handling.

ii. Rapid elution providing a high yield repeatedly and reproducibility.

iii. Proper shielding so that radiation exposure can be minimized, and robust enough to withstand shipment.

iv. The daughter eluate must be separated from the parent radioisotope and absorbent materials.

v. The eluate should be free from other radioactive contaminants.

vi. The decay of daughter radioisotope should be stable hence the radiation dose for the patient can be kept minimum.

vii. The daughter radionuclide should be in a form ready for administration to a patient, or readily converted to a suitable radiopharmaceutical.

viii. The maximum activity should be obtainable from the generator for early morning preparation.
1.8. Labeling Mechanism of Biologically active compounds with Tc-99m

When technetium-99m is eluted with saline from the $^{99}$Mo/$^{99}$mTc generator system, it is found in the form of pertechnetate salt of sodium, which is very stable and having +7 oxidation state of technetium. Lower oxidation state of technetium can also be achieved and stabilized by the presence of chelating ligands, which form discrete technetium complexes. To produce radiopharmaceuticals for clinical purposes, it is necessary to convert $^{99m}$TcO$_4^-$ into different complexes by the good choice of ligand and by using a chemical reducing agent. The stannous ion is most commonly used as a reducing agent in radiopharmaceutical kits in the form of SnCl$_2$ 2H$_2$O, although other reducing agents are also known and may be used under certain conditions. The $^{99m}$TcO$_4^-$ ion according to the following equation are:

\[
2^{99m}TcO_4^- + 16 H^+ + 3Sn^{+2} \leftrightarrow 2^{99m}Tc^{+4} + 3Sn^{++} + 8H_2O
\]

However stabilization of the other oxidization states known for Technetium, i.e. +1, +2, +3, +5 and +6 has been also achieved and these are used in many of the common radiopharmaceuticals. The reduced forms of $^{99m}$Tc react with a range of chemicals, e.g., chelating agents, and form various radiopharmaceuticals. The structure and chemical properties of the technetium complex describes its biological behavior. For a successful $^{99m}$Tc-labelled pharmaceutical, it is essential that most of not all $^{99m}$Tc bind to the pharmaceutical. The following reaction scheme shows that the $^{99m}$Tc can exist in atleast 3 chemical forms: the unreduced pertechnnetate ion, the reduced form (unreacted with the chelate) and the chelated form. Various reducing agents with the chemical formula are incorporated in Table 1.2.

$^{99m}$Tc O$_4^-$ + Sn$^{2+}$ + ligand $\rightarrow$ reduced $^{99m}$Tc + ligand or $^{99m}$Tc – ligand complex.
Table 1.2: Various reducing agents active to convert $^{99m}$TcO$_4^-$ to a lower valence state

<table>
<thead>
<tr>
<th>Reducing agents</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stannous ion</td>
<td>SnCl$_2$. 2H$_2$O/ SnCl$_2$/ SnF</td>
</tr>
<tr>
<td>Ascorbic acid + Ferric chloride</td>
<td>C$_6$H$_8$O$_6$ + FeCl$_3$</td>
</tr>
<tr>
<td>Concentrated Hydrochloric acid</td>
<td>(Conc. HCl)</td>
</tr>
<tr>
<td>Sodium borohydride</td>
<td>NaBH$_4$</td>
</tr>
<tr>
<td>Sodium dithionite</td>
<td>Na$_2$S$_2$O$_4$</td>
</tr>
<tr>
<td>Zinc dust</td>
<td>Zn</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>FeSO$_4$. 7H$_2$O</td>
</tr>
</tbody>
</table>
1.9. Infection and Inflammation

1.9.1. Infection

The infection is a state of disease in which different viruses, crucial bacteria, and fungi or parasites may enter into the body and causes a form of disease. In a lot of clinical situations, the diagnosis of infection remains challenging particularly after surgeries. Painful and fevered patients are given a series of diagnostic procedures to optimize their treatment. These studies comprise of laboratory tests utilizing non-specific parameters and are often not adequate for differentiating the bacterial infections from sterile inflammation or tumors which are essential for further clinical examination and treatment. X-rays MRI and CT are imaging techniques that show abnormalities caused by morphologic changes and thus are not reliable for differentiation (Palestro et al., 2007). The morphological imaging does not contribute significantly to quick diagnosis because many abnormalities are detectable only at very progressive stages of disease.

Molecular imaging may play very important role in diagnostic and therapeutic response because these rely on functional changes not morphology. A variety of radiopharmaceutical markers, such as gallium-67 citrate and radiolabeled products of antigranulocyte monoclonal antibodies, human IgG and autologous leukocytes (Becker, 1995), are employed for scintigraphic detection of bacterial infections and inflammation. Research has been ongoing to develop infection specific markers as widely used tracers cannot discriminate between infection and inflammation (Alberto et al., 2003). One marker that shows potential is Technetium-99m ($^{99m}$Tc) labeled anti-infective agent which has been recently introduced as infection seeking tracers (Antonella et al., 2003).
1.9.1.1. Pathophysiology of infection

An infection is caused by a pathogenic microorganism, while inflammation is caused by the response of the organism to invading pathogen/tissue damage. After a foreign particle invasion from a pathogen, the host produces an inflammatory response for removal of pathogen. Additionally the process of inflammation attempts for removal of injured tissue and motivate reconstruction of normal tissue. Subsequently immune system causes memory for effectively dealing with reinfections from the same micro-organism (Weiner & Thakur, 1999). The main types of infections by different agents are given below.

1.9.1.2. Bacterial Infections

They are the most common cause of infection in people. Types of Bacteria that causes infection includes Staphylococcus, Streptococcus, Pseudomonas and Escherichia coli

1.9.1.3. Viral Infections

The most common types of viruses that cause infections are Common cold viruses, Herpes Simplex and Varicella Zoster.

1.9.3.4. Fungal Infections

Fungal Infection occurs when the immune system is weak. The most common fungal infection is “Candida albicans”.

1.9.3.5. Protozoa Infections

The disease “Toxoplasmosis” is by an infection caused by protozoa.

1.9.2. Inflammation

Inflammation is complex biological response of body against harmful stimuli such as pathogens, damaged cells, and irritants. Inflammatory diseases may be classified into acute or chronic forms. Both these forms of inflammation are characterized by a number of
histopathological immunological and procedures. Infection caused by a pathogenic microorganism is generally followed by acute inflammatory response. Acute inflammation results in vascular changes including increased vascular permeability and vasodilatation, Exudate formation, cellular actions such as diapedesis (the accumulation of leukocytes at site of tissue injury by migration from small blood vessels) and phagocytosis by ingesting foreign objects by PMNs and macrophages. Chronic inflammation is usually the result of acute inflammation. Under this situation, PMNs number is reduced and proliferation of fibroblasts and infiltration by lymphocytes macrophages, and plasma cells occurs. During this period, immature monocytes found in blood and bone marrow are transformed into powerful phagocytic macrophages, resulting in PMN and monocyte production. Acute inflammation lasts hours or days and is resolved without lasting lesions while chronic inflammation can last from a few weeks to many years and causes late complications generally. During last two decades, large numbers of antimicrobial peptides are found in all types of living organisms which play a very important role in innate immunity to microbial invasion. Their selective behaviour is significant in understanding their future role in molecular imaging and monitoring therapeutic response.

1.10. Biologically active compounds for infection and inflammation

Antibiotics for Infection

An antibiotic is a chemical substance produced by a microorganism that has the capacity to inhibit the growth and even destroy bacteria and other microorganism. Since Flemming’s discovery of penicillin, many antibiotics have been found that can damage pathogens in several ways (Table 1.3). There are mainly two major types of antibiotics
- Bactericidal: - These types of antibiotics directly kill the bacteria cells.
- Bacteriostatic: - These types of antibiotics prevent bacterial cells from dividing.

Macrolides

The macrolide antibiotics contain 12-22 carbon lactone rings linked to one or more sugars. In 1950 picromycin, the first of this group to be identified as macrolide compound was reported. Erythromycin and carbomycin were reported in 1952 as new antibiotics and these were followed in subsequent years by other macrolides. Macrolides are also products of actinomycetes (soil bacteria) or semi-synthetic derivatives of them. The commonly prescribed macrolides are erythromycin, azithromycin, and clarithromycin. Erythromycin is a relatively broad-spectrum antibiotic effective against gram positive bacteria, mycoplasmas and a few gram negative bacteria, but is usually only bacteriostatic. Azithromycin is particularly effective against many bacteria, including chlamydia trachomatis.

Clarithromycin

Clarithromycin (6-O-methylethromycin) is synthesized by substitution of a methoxy group for the C-6 hydroxyl group of erythromycin. Clarithromycin is an advance antibiotic characterized by a macrocyclic lactone ring with attached deoxy sugars. This substitution of methoxy group forms a more acid-stable antimicrobial agent which results in better oral bioavailability and gastrointestinal tolerance.

Microbiology: Clarithromycin exhibits equal or better *in vitro* activity against gram-positive organisms as compared to erythromycin. It is used extensively for treating a large number of bacterial infections such as pneumonia, respiratory infections, sinusitis, tonsillitis, pharyngitis, acute exacerbation of chronic obstructive pulmonary disease, skin infections and Lyme disease (Wormser *et al.*, 2006).

**Lincosamide**

These are sulfur-containing antibiotics isolated from *Streptomyces lincolnensis*. Lincomycin is most active and medically useful obtained from fermentation. Extensive efforts to modify lincomycin structure in order to improve its antibacterial and pharmacological properties resulted in the preparation of 7-chloro-7-deoxy derivative, clindamycin which appears to have greater antibacterial potency and better pharmacokinetics properties as well. These resemble the macrolides in antibacterial spectrum and biochemical mechanism of action.

**Clindamycin**

Clindamycin is a bactericidal antibiotic of lincosamide family approved for treating respiratory tract, dental, skin, acne, vaginosis, peritonitis, and toxic shock syndrome infections. Clindamycin are of great interest in treating infections because of its high intracellular levels in phagocytic cells, bones, and have an antitoxin effect against toxin-elaborating strains of staphylococci and streptococci.

Mode of action: Clindamycin acts mainly by its binding to 50s ribosomal subunit of bacteria and disrupts protein synthesis by interfering transpeptidation reaction which ultimately inhibits chain elongation. Macrolides and Chloramphenicol also act at the 50s ribosomal subunit and thus can compete for specific site binding. Clindamycin and lincomycin are frequently discussed along with the macrolides but are not related chemically.
Microbiology: Clindamycin can cause phagocytosis of bacteria even at sub inhibitory concentrations. By disrupting protein synthesis, clindamycin results changes in the bacterial cell wall surface that decreases attachment of bacteria to host cells and thus increases intracellular killing of organisms (Verina & Verhoef, 1986).

Tetracyclines

The tetracyclines are a class of antibiotics having a common four ring structure with a variety of side chains attachment. These are obtained by fermentation procedures from Streptomycetes species or by chemical transformations of the natural products. Oxytetracycline and chlortetracycline are produced naturally by Streptomycetes species, whereas others are semisynthetic drugs. Tetracyclines are broad spectrum antibiotics that are active against most of the bacteria.

Vibramycin (Doxycycline)

Vibramycin is a member of wide-spectrum antibiotic of the family tetracycline synthesized from oxytetracycline controls the ability of bacteria to produce proteins crucial to them.

Mode of action: The antimicrobial activity of tetracyclines lies in its binding to bacterial 30S ribosomal subunit interference in binding of aminoacyl-tRNA molecules to A site of ribosome resulting protein inhibition. Vibramycin go into microorganisms partly by diffusion and to some extent by an energy-dependent, carrier-mediated system that is responsible for the high concentrations in susceptible bacteria.

Microbiology: All tetracyclines including vibramycin are equally active and usually have almost the same broad spectrum against both aerobic and anaerobic gram-positive and gram-negative bacteria. Vibramycin is used to treat infections of urinary tract, genitals, lungs, or
eyes caused by infecting bacteria (Quarterman et al., 1997). Although there is over-all cross-resistance among tetracyclines, vibramycin is more effective against staphylococci.

Biomolecules such as antibodies, peptides have been considered attractive as selective carriers of therapeutic agents because of their unique \textit{in vitro} specificity and high affinity for their antigen.

**Antimicrobial Peptides**

Antimicrobial peptides are small cationic peptides having ability to protect host organism from invasion of bacteria, fungi and viruses. Presently, about one thousand antimicrobial peptides are being reported from various natural resources such as microbes, plants, insects and amphibians for battle against invading micro-organisms (Wang \textit{et al.}, 2009). Antimicrobial peptides from natural resources have 15-45 amino acid residues in sequence having a net positive charge (from +2 to +9) because of an extra lysine, histidine and arginine residues (Hancock, 2000). The activities of antimicrobial peptides are usually due to their interaction with cell membranes of bacteria (Dawson & Liu, 2008). AMPs interact selectively with phospholipid bilayers of negatively charged bacterial membrane surface (Splith & Neundorf, 2011; van't Hof \textit{et al.}, 2001; Matsuzaki \textit{et al.}, 1997; Huang, 2000). As compare to traditional antibiotics, most AMPs target the bacterial cell membrane without specific receptors requirement hence may be an ideal agents to overcome resistance as a result of bacterial mutations (Huang \textit{et al.}, 2010). Peptides with antimicrobial activity have received particular attentions because they are found to exist in nature as part of a natural defence system (Lai \textit{et al.}, 2008; Zasloff, 1992; Marr \textit{et al.}, 2006).
Cecropin A

Currently peptide known to exhibit potent antimicrobial activity are cecropin A which is highly potent in lysing bacteria membranes. This mechanism involves outer membrane permeation in bacteria and, thus, the possible reduced likelihood of the emergence of resistance (Calandra, 2001; David et al., 2000; Guerra et al., 2003). Cecropin A is a linear 37-residue antimicrobial polypeptide isolated from cecropia moth as part of its defense against bacterial infection (Hancock & Chapple, 1999). Its 37-amino acid sequence contains seven Lys, one Arg, one Glu, and one Asp for a net charge of +7 at neutral pH.

1.11. $^{99m}$Tc-pharmaceuticals for Imaging of Infection/Inflammation

Several $^{99m}$Tc-labeled compounds such as $^{99m}$Tc-erythromycin (Ercan et al., 1992), $^{99m}$Tc-ceflizoxime (Gomes et al., 2005), $^{99m}$Tc-enrofloxacin (Siaens et al., 2004) $^{99m}$Tc-ciprofloxacin (Oh et al., 2002; Larikka et al., 2002; Sarda et al., 2003; Appelboom et al., 2003), $^{99m}$Tc-lomefloxacin & $^{99m}$Tc-ofloxacin complexes (Motaleb, 2007), $^{99m}$Tc-pefloxacin (El Ghany et al., 2005), $^{99m}$Tc-Kanamycin (Roohi et al., 2006), $^{99m}$Tc-ethumbutol (Verma et al., 2005), $^{99m}$Tc-flucanazole (Lupetti et al., 2002), $^{99m}$Tc-vancomycin (Roohi et al., 2005), $^{99m}$Tc-dextran (Bhatnagar et al., 1995), Tc-99m-Hynic labeled peg-liposomes (Laverman et al., 2000), $^{99m}$Tc-labeled therapeutic inhaled amikacin loaded liposomes (Lee et al., 2013) $^{99m}$Tc-tetrofosmin (Degirmenci et al., 1998, Wang et al., 2002), Technetium-99m Labeled Oligonucleotides (Wagner et al., 1997) $^{99m}$Tc(CO)$_3$ Mannosylated Dextran Bearing S-Derivatized Cysteine Chelator (Pirmettis et al., 2012), $^{99m}$Tc-cefuroxime (Lumbrecht et al., 2008) and $^{99m}$Tc-piroxicam (El Ghany et al., 2005) have been developed for imaging purposes and some of them are routinely employed in diagnostic nuclear medicine (Welling et al., 2009; Seyedeh et al., 2010; Lupetti et al., 2003). Radiolabeled antibiotics express a
promising approach for the precise diagnosis of infectious foci. These can bind specifically to bacterial components making possible the discrimination of bacterial infection from sterile inflammation (Britton et al., 1997; Ugur et al., 2006; Laken et al., 2000; Oyen et al., 2001; Asikoglu et al., 2000). The target interaction mechanism of radiopharmaceuticals are diverse i.e. passive diffusion into body cavities, active excretion or secretion by different body organs, trapping in blood or lymphatic vessels and binding to receptors on surface of cells. Each radiopharmaceutical has its specific advantages and disadvantages. Although we have precise radiopharmaceuticals for imaging infection and inflammation, still there is need for more sensitive and specific radiotracers that can identify the receptors, adhesion molecules and levels of different activation markers. The design of the radiotracer must be with its optimized interaction with the preferred biological target. The properties critical for radionuclides to incorporate into radiopharmaceuticals are summarized in Table 1.4. The improvement in nuclear medicine imaging stands on the progress of these two aspects; radiopharmaceutical tracers and devices. The information provided by radiopharmaceuticals is useful not only for mapping receptors of the infectious or inflammatory sites but also for the therapeutic decisions (Gnanasegaran & Ballinger, 2014). Various substances which have been successfully labeled with $^{99m}$Tc are listed in Table 1.5.
Table 1.3: Antibacterial drugs and their mode of action (Willey et al., 2010)

<table>
<thead>
<tr>
<th>Drugs by their mode of action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitors of cell wall synthesis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Natural Penicillins</strong></td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Against gram-positive bacteria</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>Against gram-positive bacteria</td>
</tr>
<tr>
<td><strong>Semisynthetic Penicillin</strong></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>Resistant to penicillinase</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Broad spectrum</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Broad spectrum, combined with inhibitor of penicillinase</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>A monobactam, effective for gram-negative bacteria</td>
</tr>
<tr>
<td>Imipenem</td>
<td>A carbapenem, very broad spectrum</td>
</tr>
<tr>
<td><strong>Cephalosporins</strong></td>
<td>First generation cephalosporin, activity similar to penicillin</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>Third generation cephalosporin</td>
</tr>
<tr>
<td>Cefixime</td>
<td></td>
</tr>
<tr>
<td><strong>Antimycobacterial Antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Inhibits synthesis of mycolic acid of cell wall of <em>mycobacteriums sp</em></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Inhibits incorporation of mycolic acid into cell wall of <em>Mycobacterium sp.</em></td>
</tr>
<tr>
<td><strong>Competitive Inhibitors of the Synthesis of Essential Metabolites: Sulphonamides</strong></td>
<td>Broad spectrum, Combination is widely used.</td>
</tr>
<tr>
<td>Trimethoprin-sulfamethoxazole</td>
<td></td>
</tr>
<tr>
<td><strong>Inhibitors of Protein synthesis</strong></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Broad spectrum, potentially toxic</td>
</tr>
<tr>
<td><strong>Polypeptide Antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Against gram positive bacteria, topical application</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>A glycopeptide type</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Broad spectrum, including mycobacteria</td>
</tr>
<tr>
<td>Neomycin</td>
<td>Topical use, broad spectrum</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>Broad spectrum, including <em>Pseudomonas sp.</em></td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
</tr>
<tr>
<td>Tetracycline, vibramycin</td>
<td>Broad spectrum, including chlamydias and Rickettsias, animal feed additives</td>
</tr>
<tr>
<td><strong>Macrolides</strong></td>
<td>Alternative to Penicillin</td>
</tr>
<tr>
<td>Erythromycin, clarithromycin</td>
<td></td>
</tr>
<tr>
<td><strong>Injury to Plasma membrane</strong></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Topical use, gram negative bacteria, including <em>Pseudomonas sp.</em></td>
</tr>
<tr>
<td><strong>Inhibitors of Nucleic acid synthesis: Rifamycins</strong></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>Inhibition of mRNA, treatment of tuberculosis.</td>
</tr>
</tbody>
</table>
Table 1.4: Technetium-99m Complexes with different ligands

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Insulin, gluconate, sorbitol, phytate, lactobionate, mannitol, glucoheptonate</td>
</tr>
<tr>
<td>Proteins</td>
<td>Serum albumin, microagregated albumin, ribonuclease, albumin microspheres, low molecular weight proteins, lysozyme</td>
</tr>
<tr>
<td>Colloids</td>
<td>Stannous hydroxide, Sulfur</td>
</tr>
<tr>
<td>Phosphates</td>
<td>Polyphosphates, pyrophosphate, diphosphonates</td>
</tr>
<tr>
<td>Metallic chelates</td>
<td>Sn-DTPA, Fe-ascorbate complex</td>
</tr>
<tr>
<td>Mercaptides</td>
<td>Pancillamine, dihydrothioptic acid, dimercaptosuccinate</td>
</tr>
</tbody>
</table>
Table 1.5: History of technetium-99m labeled antibiotics applied for infection imaging

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Antimicrobial group</th>
<th>Medical Radiotracer</th>
<th>Micro-organisms</th>
<th>T/NT ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftizoxime</td>
<td>Cephalosporine-3rd generation</td>
<td>Tc-99m</td>
<td>Escherichia coli</td>
<td>3.24</td>
<td>Gomes et al., 2005</td>
</tr>
<tr>
<td>Floclunazol</td>
<td>Antifungal</td>
<td>Tc-99m</td>
<td>Candida albicans Aspergillus fumigates</td>
<td>3.6</td>
<td>Lupetti et al., 2002</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Antituberculosis</td>
<td>Tc-99m</td>
<td>Mycobacterium Tuberculosis</td>
<td>-</td>
<td>Singh et al., 2003</td>
</tr>
<tr>
<td>Enrofloxaclin</td>
<td>Fluoroquinolones-2nd generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus and Candida albicans</td>
<td>4.25</td>
<td>Siens et al., 2004</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Glycopeptide antibiotic</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus 25923</td>
<td>5</td>
<td>Roohi et al., 2005</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>Fluoroquinolones-2nd generation</td>
<td>Tc-99m</td>
<td>Escherichia coli</td>
<td>5.6</td>
<td>El-Ghany et al., 2005</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Antituberculosis</td>
<td>Tc-99m</td>
<td>Mycobacterium Tuberculosis</td>
<td>-</td>
<td>Verma et al., 2005</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>Aminoglycoside</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus 25923</td>
<td>2.5</td>
<td>Roohi et al., 2006</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>Cephalosporine-4th generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>4.5</td>
<td>Motaleb et al., 2007a</td>
</tr>
<tr>
<td>Lomefloxaclin</td>
<td>Fluoroquinolones-2nd generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>6.5</td>
<td>Motaleb et al., 2007b</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Fluoroquinolones-2nd generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>4.3</td>
<td>Motaleb et al., 2007b</td>
</tr>
<tr>
<td>Sparfloxaclin</td>
<td>Fluoroquinolones-3rd generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>5.9</td>
<td>Motaleb et al., 2009</td>
</tr>
<tr>
<td>Cefuroxime axetil</td>
<td>Cephalosporine-2nd generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>2.5</td>
<td>Lumbrecht et al., 2008</td>
</tr>
<tr>
<td>Moxifloxaclin</td>
<td>Fluoroquinolones-4th generation</td>
<td>Tc-99m</td>
<td>Escherichia coli</td>
<td>6.8</td>
<td>Chattopadhyay et al., 2010</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Rifamycin group</td>
<td>Tc-99m</td>
<td>Methicillin resistant S. aureus</td>
<td>7.34</td>
<td>Shah et al., 2010</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Cephalosporin-3rd generation</td>
<td>Tc-99m</td>
<td>Escherichia coli</td>
<td>5.6</td>
<td>Mostafa et al., 2010</td>
</tr>
<tr>
<td>Gemifloxaclin</td>
<td>Fluoroquinolones-4th generation</td>
<td>Tc-99m</td>
<td>Streptococcus Pneumoniae</td>
<td>4.88</td>
<td>Shah &amp; Khan., 2011a</td>
</tr>
<tr>
<td>Rufloxaclin</td>
<td>Fluoroquinolones-4th generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>6.04</td>
<td>Shah &amp; Khan., 2011b</td>
</tr>
<tr>
<td>Clinafloxaclin</td>
<td>Fluoroquinolones-4th generation</td>
<td>Tc-99m</td>
<td>Staphylococcus aureus</td>
<td>4.96</td>
<td>Shah &amp; Khan., 2011c</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Nitrofuran derivatives DNA inhibitors</td>
<td>Tc-99m</td>
<td>Escherichia coli</td>
<td>4.84</td>
<td>Shah et al., 2011a</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Radionuclide</th>
<th>Pathogen</th>
<th>BID</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garenoxacin</td>
<td>Fluoroquinolones-4(^{th}) generation</td>
<td>Tc-99m</td>
<td>Multi-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>5.2</td>
<td>Shah <em>et al.</em>, 2011b</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>Fluoroquinolones-4(^{th}) generation</td>
<td>Tc-99m</td>
<td><em>Escherichia coli</em></td>
<td>4.5</td>
<td>Motaleb <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Cefepime</td>
<td>Cephalosporin- 4(^{th}) generation</td>
<td>Tc-99m</td>
<td><em>Escherichia coli</em></td>
<td>8.4</td>
<td>Motaleb <em>et al.</em>, 2011</td>
</tr>
</tbody>
</table>

### 1.12. Tumor

Just as the growth can be considered the normal characteristic of living systems, orderly or regulated growth can be considered the Hallmark of normal multicellular organisms. The influence of pathological growth may result in the alteration of such growth, and consequently growth may become characterized by malformation, excessive or diminished growth, disturbance in cellular differentiation, disturbance in organ growth, or autonomous growth. Tumor is a collection of cells that do not respond to the mechanisms controlling the body’s systems. The reason for the cause, growth and cure of tumor cells has preoccupied the researchers throughout the world for many years. Tumor cells are capable of undergoing changes not suggested as normal behavior and grow, multiply and invade surrounding healthy tissues in a way that normal cells does not. Some malignant cells metasitize in the body and tumors appear in secondary sites. It is metastatic disease that accounts for majority of cancer-related deaths, partly because of the difficulties encountered in the early detection and delineation of metastatic deposits (Britton, 2002). Tumors develop through a sequence of stages by progression. The research studies by applying carcinogens in animals distinguished initiation as starting the first developmental stages of tumor that by initiator chemicals promotes cell division (Lawley, 1994). The progression is generally restricted to the final stages of tumor development that leads to promotion. Cancer may also progress by addition...
of heritable changes in cell lineages. Beginning from the initial cell, the carcinogenic process develops through successive accumulation of genetic changes that ultimately gives rise to the tumor (Panyutin & Neumann, 2005). Historically tumors have been detected using histochemical techniques for the identification of particular tissue components such as lipids, glycoproteins, enzymes, glycogen etc. Such histochemical detection has been improved by the introduction of special strains that are taken up by specific molecules because of the ability of antibodies to recognize specific antigens (Marcos-Silva et al., 2014). Technetium-99m based diagnostic radiopharmaceuticals are given in Table 1.6. In most of the cases the cancer is not diagnosed until its cells already occupy in surrounding areas and metastasized all over the body. In most of patients with ovarian, breast and colon cancers, there is limitation in success by therapeutic treatment because the tumor has spread away from the site of origin. Therefore the timely detection of cancer is essential for its proper control and prevention. By the advancement in molecular biology based medicine, the diagnosis can be made possible by information from biochemical perturbations linked with disease (Phan et al., 2014; Khalkhali et al., 1994). In vivo functional imaging can assist for tumor diagnosis and staging, optimizing drug planning, and predicting the response to a therapeutic treatment, advantageous to both patient and oncologists (Chen et al., 2006., Conti et al., 1996).
Table 1.6: Technetium-99m based diagnostic radiopharmaceuticals

<table>
<thead>
<tr>
<th>Compound</th>
<th>Organ(s) Visualized/Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloidal dispersions based upon ( ^{99m} \text{Tc}/\text{S} ), ( ^{99m} \text{Tc}/\text{phytate(Ca)} ), ( ^{99m} \text{Tc}/\text{SnO}_2 ), ( ^{99m} \text{Tc}/\text{SbS}_3 ), ( ^{99m} \text{Tc}/\text{albumin} )</td>
<td>Liver, spleen and bone marrow, Lungs (inhalation), lymphnodes, Lung (perfusion)</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{MDP} )</td>
<td>Tumor detection</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc} ) (V)- DMSA</td>
<td>Kidneys, brain, tumors</td>
</tr>
<tr>
<td>Solution of ( ^{99m} \text{Tc}/\text{DTPA} ); ( ^{99m} \text{Tc}-\text{Gluconate (some)} ); ( ^{99m} \text{Tc}-\text{heptagluconate} ); ( ^{99m} \text{Tc}/\text{citrate} ); ( ^{99m} \text{Tc}-\text{Dimercaptosuccinic acid} )</td>
<td></td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{HM-PAO WBC} ); ( ^{99m} \text{Tc} ) colloid</td>
<td>Infection imaging</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc} ) - Iminodiacetic acid derivatives.</td>
<td>Liver, bile ducts, gall bladder</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{MIBI}, \text{Tetrofosmin, Teboroxime} )</td>
<td>Heart</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{MAG}_3 ); ( ^{99m} \text{Tc}-\text{L.LEC} )</td>
<td>Kidney</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{ECD} ); ( ^{99m} \text{Tc}/\text{HMPAO} )</td>
<td>Brain</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{SestaMIBI} )</td>
<td>Parathyroid, breast, myocardium, thyroid &amp; non-specific tumor imaging</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{human immunoglobulin} )</td>
<td>Infection and inflammation</td>
</tr>
<tr>
<td>( ^{99m} \text{Tc}-\text{Fab\textsuperscript{-}SH murine antigranulocyte IMMU-MN3} )</td>
<td>Infection and inflammation</td>
</tr>
</tbody>
</table>
1.13. Biologically active compounds for tumour imaging and therapy

Many biologically active compounds involved in diseases, such as infection, thrombosis, inflammation, neurological disorders and tumours, are well known (Karamalakova et al., 2014). The use of drugs and chemicals to kill the cancer cells in the blood is called chemotherapy. The systemic chemotherapy is passed from the bloodstream to capture the cancer cells present in the body. The Chemotherapy drugs are referred by only a medical oncologist or a hematologist. The use of chemotherapy is usually followed by a given period of time which consists on a specific number of different cycles (Rodrigues et al., 2014; Minotti et al., 2004).

1.13.1. Anti-tumor Anthracyclines

Anti-tumor anthracyclines are the antibiotics which derived from the streptomycyes bacteria and are used in cancer chemothersapy (Fujiwara et al., 1985). These antibiotics are used to treat a broad range of carcinomas, lymphomas, breast and uterine tumor as well as ovarian and lungs cancers. These anti-tumor antibiotics are the most effective against the different many other types of cancers which cannot be treated by any other chemotherapeutic agents (Peng et al., 2005, Chorawala et al., 2012). The main disadvantage of these anthracyclines is cardiotoxicity which limits its usefulness.

Epirubicin

Epirubicin is an anthracycline cytotoxic agent and is found to interfere with a number of biochemical and biological functions within eukaryotic cells. This antineoplastic agent of anthracycline family is found to have good potential for chemotherapy. The mechanism of epirubicin cytotoxicity is by DNA intercalation, topoisomerase II activity inhibition, oxygen
production and drug free radicals that end in interference with DNA or protein synthesis and resulting cytotoxic activity (Bonadonna et al., 1993). Epirubicin is used to treat stomach cancer, lung cancer, ovarian cancer, myeloma and early and metastatic breast cancer (Robert & Hong, 1984).

1.13.2. Anti-tumor vinca alkaloids

**Vincristine**

Vincristine is a naturally occurring vinca alkaloid which is extracted from the leaves of the Catharanthus *roseus* and remains to be an important anti-neoplastic drug in paediatric oncology since its introduction in the early nineteen sixties. Vincristine targets selectively against actively proliferating cells such as anti-metabolites, intercalating agents, mitotic inhibitors and DNA-alkylating agents. It is a cell cycle-dependent compound that is administered via intravenous infusion and is used to inhibit cell cycle progression at M-phase. Vincristine is active against Hodgkin's and non-Hodgkin's lymphoma, acute leukaemia, Wilm's tumour, brain tumour and neuroblastoma. Different anti-cancer agents for clinical applications are given in Table 1.7.
Table 1.7: Different anti-cancer agents with their mechanism of action and clinical uses (Chorawala et al., 2012)

<table>
<thead>
<tr>
<th>Class</th>
<th>Subclass</th>
<th>Mechanism of action</th>
<th>Clinical uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antimetabolites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate Antagonists: Methotrexate</td>
<td>Inhibits the dihydrofolate reductase and thus affect nucleoside metabolism.</td>
<td>Acute Lymphoblastic Leukaemia (ALL), Choriocarcinoma, Cancer of breast-neckhead-lungs-cervical</td>
</tr>
<tr>
<td></td>
<td>Pyrimidine antagonists:</td>
<td>Block pyrimidine nucleotide formation by incorporation into newly synthesized DNA.</td>
<td>Basal cell skin cancer, GIT adenocarcinoma, Cancers of breast-colon-stomach-rectum-pancreas, Cancer of prostate and bladder.</td>
</tr>
<tr>
<td></td>
<td>5-Flourouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemcitabine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capecitabine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alkylating Agents: Cisplatin</td>
<td>Introduce alkyl groups into DNA and create cross linking between two DNA strands and inhibit protein synthesis</td>
<td>Brain tumor, Testicular cancer, Head and Neck cancer, Hodgkin’s disease, Pancreas carcinoma, Ovarian and bladder cancer.</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temazolomide</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purine Antagonists:</td>
<td>Act as fraud substrate for biochemical reactions and inhibit the synthetic steps during S-phase of replication.</td>
<td>Acute and Chronic myelogenous leukaemia, Acute lymphocytic leukaemia, Acute myelomonocytic leukaemia</td>
</tr>
<tr>
<td></td>
<td>6-Mercaptopurine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-Thioguanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotoxic agents</strong></td>
<td></td>
<td>Drugs bind to DNA through intercalation between specific base pair thus block the DNA synthesis.</td>
<td>Breast cancer, Endometrial cancer, Thyroid cancer, Wilm’s tumor, Ewing’s sarcoma,Acute leukaemia, Rhabdomyosarcoma, Neuroblastoma</td>
</tr>
<tr>
<td></td>
<td>Intercalating Agents:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epirubicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dactinomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enzyme Inhibitors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Etoposide</td>
<td>Etoposide: Inhibits topoisomerase II thus prevent resealing of DNA which leads to cell death.</td>
<td>Small cell lung cancer, Breast cancer</td>
</tr>
<tr>
<td></td>
<td>Topotecan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irinotecan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Topotecan/Irinotecan:</td>
<td>Topotecan/Irinotecan: Inhibits topoisomerase I which allows single strands break in DNA but not affect resealing</td>
<td>Cancer of ovary, lungs, colon and small cell lung cancer.</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Taxanes Derivatives:</td>
<td>Paclitaxel Docetaxel</td>
<td>Stabilise polymerization of tubulins and inhibit the disassembly of microtubules.</td>
<td>Cancer of breast, ovary, lungs, head and neck</td>
</tr>
<tr>
<td>Epidermal Growth factor Receptor (EGFR) Inhibitors: Gefitinib Erlotinib</td>
<td>Activation of EGFR induces dimerisation and intracellular activation of protein tyrosine kinase (same as above)</td>
<td>Metastatic non-small cell lung cancer, solid tumors.</td>
<td></td>
</tr>
<tr>
<td>Proteosome Inhibitors:</td>
<td>Bortezomib</td>
<td>Prevents degradation of intracellular protein leading to activation of signaling cascade, cell cycle arrest and apoptosis</td>
<td>Refractory and relapsed multiple myeloma.</td>
</tr>
<tr>
<td>Protein tyrosine Kinase inhibitors: Imatinib</td>
<td>By inhibiting this enzyme, inhibit proliferation of myeloid cell</td>
<td>Cancer of breast, ovary, lungs, head and neck</td>
<td>Chronic myeloid leukaemia (CML), GIT stromal cell tumor.</td>
</tr>
<tr>
<td>Monoclonal Antibodies:</td>
<td>Rituximab Alemtuzumab Trastuzumab</td>
<td>This agents targets CD20, CD52, B-cell antigen which activates apoptosis. Trastuzumab targets against human epidermal growth factor receptor protein-2 (HER-2)</td>
<td>B-cell lymphoma, Chronic lymphocytic leukaemia, T-cell lymphoma and breast cancer.</td>
</tr>
<tr>
<td>Aromatase Inhibitors:</td>
<td>Anastrozole Letrozole</td>
<td>Aromatase, responsible for conversion of testosterone to estradiol</td>
<td>Estrogen Receptor (ER) positive metastatic breast cancer in post menopausal women that are resistant to tamoxifen therapy.</td>
</tr>
</tbody>
</table>
1.14. $^{99m}$Tc-labeled compounds for Tumor Imaging

Several different radiopharmaceuticals are routinely used in tumor localization and detection. The newer trends in the development of diagnostic agents of Technetium-99m have expanded the horizon of nuclear medicine procedures (Ali et al., 2012; Zhang et al., 2012). The use of $^{99m}$Tc-sestamibi (Abdel Dayem et al., 1994), Tc-99m-Labeling of Modified RNA (Hilger et al., 1999), $^{99m}$Tc-oxo Complex with Deoxyglucose Dithiocarbamate (Lin et al., 2012) and $^{99m}$Tc-tetrofosmin (Rambaldi et al., 1995) for tumor imaging is well recognized. $^{99m}$Tc-d, I-HMPAO (hexamethylpropyleneamineoxime) may be used for brain tumors (Suess et al., 1991). $^{99m}$Tc-GHA, the well-known renal agent, has been reported to be a $^{18}$F-FDG mimic for brain tumors, such as gliomas (Ravichandran et al., 1990). Block Copolymer Micelles Target Auger Electron Radiotherapy to the Nucleus of HER2-Positive Breast Cancer Cells (Hoang et al., 2012) $^{99m}$Tc labeled cartilage link protein (CLP) may be used as diagnostic agent for lung cancer (Qiang et al., 2005).

1.14.1. Antibodies

Antibodies are raised against tumor antigens which can be radiolabelled and upon injection localize with a reasonable degree of sensitivity and specificity in the tumor. The first antibody for in vivo diagnostics has been licensed in Europe. The antibody has been successfully used for the detection of rhabdomyosarcoma. Monoclonal antibodies (MoAb) raised against lesion-associated antigens are being used as carriers for drugs, radionuclides, or toxins to target sites of cancer, cardiac lesions, and other diseases for diagnosis. In recent years, efforts have been directed toward the use of $^{99m}$Tc for labeling polyclonal and monoclonal antibodies and their fragments with preservation of the immunoreactivity (Cheng et al., 2015). Several techniques have been used for labeling MoAb for use in
radioimmunoscintigraphy and have proved their diagnostic utility. The efficacy of ascorbic acid versus other reducing agents for the cleavage of disulfide bridges in antibodies has been evaluated (Thakur et al. 1991). The first approach to direct labeling of proteins with $^{99m}$Tc was introduced by Rhodes et al. (Rhodes et al., 1986) and applied in a kit of an antimelanoma tumor antibody (Siccardi et al., 1990; Dias et al., 2005). Improved radiolabeling methods have been introduced to increase the target-to-nontarget ratio.

### 1.14.2. Peptides

In oncology, major progress has been made with radiolabeled peptide analogs for in vivo localization and therapy of tumors. Peptides are molecules consisting of several amino acids linked together with peptide bonds. The action of peptides is facilitated through specific membrane-bound receptors; almost all belong to the group of G protein-coupled receptors. They can influence many intracellular effector systems; for instance, the emerging role of peptides in MAPK pathways which are known to play an important role in cell proliferation or apoptosis contributing to the interest for peptides in cancer research (Villalba et al., 1997). The radiolabeled peptides provide a class of targeting molecules appropriate for both molecular imaging and radiotherapy. Labeled peptides also play a role in targeting nonmalignant lesions, such as infection and thrombus. With the advances in organic, bioconjugate and coordination chemistry, solid phase peptide synthesis and phage display techniques radiolabeled peptides with high receptor binding affinity for a selected target have been developed (Ji et al., 2013; Flook et al., 2013; Pathuri et al., 2014). Peptides, cover many biologically important targets, have high receptor binding affinity, are of relatively low molecular weight, easy to synthesize. These are accessible to modification like conjugation with chelators for radiolabeling which allows straightforward kit-preparation of peptide
radiopharmaceuticals. provide favorable pharmacokinetics resulting in a rapid whole body clearance, good tumor penetration and reach it in high concentration and for therapeutic purposes, they are applied at doses lower than conventional drugs, and therefore cause few side effects and in addition lack immunogenicity (Fani et al., 2012; Wester et al., 2004; Zhu et al., 2014). Somatostatin is a peptide hormone consisting of 14 amino acids. It is present in the hypothalamus, the cerebral cortex, the brain stem, the gastrointestinal tract and the pancreas. Various tumours contain high numbers of somatostatin receptors, which enable *in vivo* localization of the primary tumour and its metastases by scintigraphy with radiolabelled somatostatin analogue peptides (Krenning et al., 1993; Olsen & Pozderac, 1995).

Somatostatins (SST) are a family of cyclopeptides and now five different subtypes of SSTrs (SSTr1-SSTr5) have been identified which overexpressed on a majority of tumors (neuroendocrine tumors, gliomas, breast cancer, and small cell lung cancers).

**Lanreotide**

Lanreotide [D-(Nal-Cys-Tyr-D-Trp-Lys-Val-CysThr.NH2] and octreotide [H2N-D-Phe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr (OH)] are cyclic octapeptide analogues of somatostatin used for treatment of wide range of tumors (Smith-Jones et al., 1999; Arnold et al., 2000). Somatostatin analogues are favourable target molecules because their receptors are overexpressed on most tumors but their therapeutic application has been limited by a short half-life in circulation. To overcome this shortcoming, analogues have been synthesized having more prolonged activity than somatostatin itself. Somatostatin analogue such as lanreotide were synthesized because the somatostatin peptide is susceptible to very rapid enzymatic degradation.
1.15. Quality Assurance Procedures for $^{99m}$Tc-labeled Compounds

To ensure that $^{99m}$Tc-labeled compounds are pure and satisfy the requirements for the intended use, rigorous quality control is carried out in all cases several aspects that influence the integrity of $^{99m}$Tc- labeled compounds are described below.

1.15.1. Chemical Control

Chemical control include evaluation of the radiochemical purity, estimation of certain specific chemical impurities which might be present in the preparation, in concentrations of radicals, in additives and in preservatives, and measurement of the pH and the absolute concentration of the labeled material to evaluate the chemical purity. Radiochemical purity is generally determined by paper, column, or thin layer chromatography, paper electrophoresis and in few cases, by reverse isotope dilution analysis. The pH of the labeled preparations should be carefully controlled, since, in some cases, the stability and physiological behavior of the preparations are significantly affected by the pH of the solution.

1.15.1.1. Chromatography

Various techniques are available to detect the impurities in $^{99m}$Tc-Labeled radiopharmaceuticals as discussed below (Saha, 1992, Akhter, 2004). A number of chromatographic techniques have proven useful in the preparation and characterization of $^{99}$Tc complexes, and in the quality control of $^{99m}$Tc labeled radiopharmaceuticals. These techniques include low-pressure column chromatography, thin layer and paper chromatography. In some cases, paper electrophoresis and HPLC techniques are also useful.
Thin Layer and Paper chromatography

Thin layer chromatography (TLC) and paper chromatography currently comprise the clinically used procedures for quality control of $^{99m}\text{Tc}$ labeled radiopharmaceuticals. The chromatographic papers are highly pure especially free of metal impurities. Thickness and porosity of the paper is strictly controlled during their manufacturing process. In this technique, a small drop of the radiopharmaceuticals is spotted on a paper or an ITLC stripe. The stripe is dipped into a solvent contained in a jar. The distribution depends upon the retardation factors ($R_f$ values) of the sample, which is characteristic of each substance. Its values lie between zero and one. Reversed–phase C$_{18}$ plates are particularly useful since they do not promote decomposition of metastable Tc complexes and the TLC elution conditions can often be roughly translated to the conditions necessary to effect separation on reversed-phase HPLC columns.

Low –Pressure Column Chromatography

In Biochemistry, low pressure column chromatography is a traditional synthetic tool for the purification and separation of metal complexes, and it has been effectively used as such for $^{99}\text{Tc}$ coordination complexes. In addition, $^{99m}\text{Tc}$- radiopharmaceutical preparation are often analyzed by the low-pressure column chromatography, especially using gel matrices such as Sephadex, Bio-Gel, etc.

High Performance Liquid Chromatography (HPLC)

Because of its high resolution, HPLC method has become a most important technique in analysis of radiopharmaceuticals. The system consist of HPLC column, which are heavy walled stainless steel or glass tubes, with dimension of 15-30 cm length and 2-5 mm
diameter packed with appropriate gel. The separation takes place due to packed gel (stationary phase). In liquid-solid partition chromatography, Stationary phase is a polymer chemically bonded to the surface of silica support. The components of the sample get resolved due to their interaction with mobile phase and stationary phase. The selectivity of the former depends upon the polarity. HPLC method may be normal phase or reverse phase type depending upon the type of stationary and mobile phase. Reverse phase type is more commonly used in radiopharmaceuticals. It is usually equipped with ultraviolet monitor or NaI (TI) detector.

1.15.1.2. Electrophoresis

In this technique, sample is applied on a moistened filter paper or a cellulose acetate stripe. The ends of the stripe are dipped into a buffer solution which contains electrodes. The migration of the components of sample depends upon the voltage, time, buffer, and composition of the sample. The mobility is expressed as unit per volts. After drying the stripe, the activity is measured by \( 2\pi \) scanner. Like TLC and paper chromatography, electrophoresis is regularly applied to the analysis and separation of \(^{99m}\text{Tc}\)-radiopharmaceutical mixtures. However, this technique can also give qualitative information on the charge type of the components, that is, whether they are anionic, cationic, or neutral. The presence of a neutral components cannot always be determined unequivocally since some charged complexes do not migrates either because of high adsorption to the substrate or because of low solubility in the electrophoresis medium.
1.15.1.3. Freedom from Particles

The eluate solution should be free from extraneous particles such as fibre and glass. Particle Sizing is an important part of radiopharmaceutical preparation because of the effect particle size has on biodistribution. The optimum size for aggregated albumin (MAA) particles for a lung perfusion study is 30-100 microns and for a colloid for liver imaging is approximately 0.01-1 micron. Oversize colloid particles may lodge in the capillary bed and localize in the liver.

1.15.2. Biological Controls

Biological controls consist of tests for sterility, apyrogenicity, freedom from undue toxicity and in few cases for selectivity.

1.15.2.1. Sterility testing

Sterility test are refers to the absence of any viable bacteria or microorganism in the radiopharmaceuticals kits. There are many methods to maintain sterility of radiopharmaceuticals.

- Commercially available disposable Milipore filter (um).
- Autoclave (suitable to sterilize the glass)
- $^{60}$Co irradiation

1.15.2.2. Apyrogenicity testing

Pyrogens are bacterial endotoxins which produce a febrile response. The limulus test is used to detect the presence of pyrogens. The test uses an agent derived from the blood of the
horseshoe crab (limulus polyphemus). The limulus extract forms a gel when mixed with endotoxins. The eluate is intended for parenteral administration and must be sterile and free from pyrogens. Official methods of testing for sterility and pyrogens should be used.

1.15.2.3. Organ Specificity

The organ Specificity is usually determined by the manufacturer. For a new radiopharmaceuticals it is essential to perform biodistribution studies by confirmatory biodistribution testing (using the gamma camera, organ counting) and to determine various pharmacokinetic parameters such as blood clearance, renal excretion and sometimes biliary clearance.

1.15.3. Radionuclide Purity

Molybdenum is the most important radionuclide contaminant. Each time a generator is eluted it must be evaluated for $^{99}$Mo breakthrough. When given intravenously, molybdate is phagocytized by the reticuloendothelial system. Its long half life and beta emissions result in a very high radiation dose even from only a small amount of activity. Current regulations limit $^{99}$Mo activity to 0.15µCi/mCi of $^{99m}$Tc at the time of administration (0.15kBq/1MBq). $^{99}$Mo can be produced by fission of $^{235}$U in a reactor, or by irradiation of $^{98}$Mo with neutrons. $^{99}$Mo produced in fission reactions is essentially carrier free.

1.15.4. Efficiency of the Labeling Methodology

High labeling yield is always desirable, although it may not be attainable in many cases. The better the method of labeling higher will be the yield. However a lower yield is sometimes
acceptable if the product is pure and not damaged by the labeling method, the expense involved is minimal, and no better method of labeling is available.

1.15.5. Radiochemical Purity

The $^{99m}$Tc in the eluate should be in the form of $^{99m}$TcO$_4^-$ (pertechnetate). Any other chemical form is a radiochemical impurity. The eluate can be analyzed for the impurities by chromatography. This is the percentage purity of the radionuclide-pharmaceutical complex, or in case of the $^{99m}$Tc kits, $^{99m}$Tc-ligand complex. Reduced technetium can be hydrolyzed to TcO$_2$ or can complex with tin colloids. These stannous or technetium colloids localize to the reticuloendothelial system. So free pertechnetate and hydrolyzed technetium is the radiochemical impurities in the labeled $^{99m}$Tc kits. These impurities give poor quality scans and inaccurate measurements of the parameters that depend upon the biodistribution of the radiopharmaceutical. They should not exceed 5% of the total activity. Radiochemical purity of labeled compounds is generally determined by chromatographic techniques. Of all the routinely available experimental techniques and procedures (e.g., diffraction, visible UV and IR spectrophotometry) only chromatography is the technique successfully applied to both $^{99}$Tc complexes at $10^{-1}$ to $10^{-4}$ M concentration and to $^{99m}$Tc complexes to $10^{-6}$ to $10^{-8}$ M concentration.

1.15.6. Chemical Purity

The optimum amount of active ingredient required to produce pharmaceutical effect and to form stable complex with radionuclide is called the chemical purity of the radiopharmaceutical. The undesired compounds may be labeled with the radionuclide and interfere with the diagnostic procedures. They may also be toxic for injecting into the body.
Using pure starting material can reduce such impurities. Toxic substances are checked by the spot tests. Aluminum (Al$^{+3}$) is a chemical contaminant which can be found in the Technetium elute. Aluminum can interfere with some labeling reactions. The United States Pharmacopeia (USP) limits the amount of aluminum which can be detected in elute to be less than 10 μg/mL. Most of the $^{99m}$Tc-labeled radiopharmaceuticals are clear and colorless, without particulate matter. However $^{99m}$Tc-labeled particles e.g., macro-aggregated albumin and sulphur colloid are turbid. The pH of the solution is also important because it can alter its stability and integrity. The pH should be measured with pH-meter. Chromatography is used to separate various stereoisomers of ligand in the kit. Other chemical checks includes the specific gravity and determination of Sn(II) in the kits.

1.15.7. Measurement of the pH

The stability and physiological behavior of the preparation are significantly affected by the pH of the solution. Therefore, pH of the labeled preparation should be carefully controlled.

1.15.8. Chemical stability and Denaturation.

Stability is related to the type of bond between the radionuclide and the compound. Compounds with covalent bonds are relatively stable under various physiochemical conditions. The structure and the biologic properties of a labeled compound can be altered by various physiochemical conditions during labeling procedure. For example, proteins are denatured by heating, at pH below 2 and above 10, and by excessive iodination. The red blood cells are denatured by heating. Therefore biodistribution studies are carried out in animal models prior to human use.
1.15.9. Specific activity and determination of Sn (II) in the kits.

The specific activity is defined as the activity per gram of the labeled material. In many instances high specific activity is required for the applications of radiolabeled compounds therefore, appropriate methods should be devised to attain high specific activity. In others, high specific activity can cause more radiolysis in the labeled compound and should be avoided.

1.15.10. Shelf Life of Labeled Compound

A labeled compound has a shelf life during which it can be used safely for its intended purpose. The loss of efficacy of a labeled compound over a period of time may result from radiolysis.

1.15.11. Storage Condition

Many labeled compounds are decomposed at higher temperatures. Proteins and labeled dyes are degraded by heat therefore labeled protein and dyes should stored at proper temperature e.g. albumin should be stored in refrigerator. Some labeled compound breakdown in the presence of light. These chemicals should be stored in the dark. e.g., radioiodinated rose Bengal. The loss of carrier free tracers by adsorption on the walls of the container can be prevented by the use of silicon-coated vials.

1.16. Biomedical Imaging Techniques

Nuclear medicine imaging is characterized by the use of radiopharmaceuticals (radiolabeled probes) that are ordered in pico- and nanomolar amounts take part in biochemical and physiological processes and allow visualization and assessment and quantitation of these
processes *in vivo*. A radiopharmaceutical consists of a targeting compound (the drug or probe) labeled with a radionuclide (an unstable isotope). Depending on the radionuclides used, positron emitters or single photon emitters, the tomographic equipment specifically designed according to the intrinsic physical characteristics of these radionuclides is classified as single photon emission computed tomography (SPECT) or positron emission tomography (PET) respectively. Decay of SPECT isotopes by single photon emission results in the production of γ rays, while PET isotopes emit β⁺ particles (positrons), which, upon collision with electrons, produce two collinear γ rays of 511 keV that are then detected. This signal is captured by external detectors, allowing *in vivo* assessment of a forementioned processes. The term “molecular imaging” is defined as the visualization *in vivo* biological processes at the molecular or cellular level using specific imaging probes and broadly used in conjunction with imaging modalities that provide anatomic as well as functional information. Molecular imaging may be used for early detection, characterization, and monitoring of disease as well as investigating the efficacy of drugs (Mishra et al., 2012; Medarova, 2014). Presently, there is a consensus among experts in the field that the most sensitive molecular imaging modalities in the diagnosis and follow-up of cancer patients are the radionuclide-based single photon emission computed tomography (SPECT) and positron emission tomography (PET) providing the functional and metabolic activity characteristics of the tumour (Dijkgraaf & Boerman., 2010; Raty *et al*., 2007; Li *et al*., 2012). Hybrid SPECT/CT improves the diagnostic accuracy of these well-recognized imaging techniques by precise anatomical localization and characterization of morphological findings, differentiation between foci of physiological and pathological tracer uptake, resulting in a significant impact on patient
management and more definitive interpretations (Abikhzer & Keidar., 2014; Wang et al., 2012; Duheron et al., 2014).

1.16.1. Single photon emission computed tomography (SPECT)

Single photon emission computed tomography (SPECT) is based on the molecular tracer principle and is an established tool in noninvasive imaging. SPECT uses gamma cameras and collimators to form projection data that are used to estimate (dynamic) 3-D tracer distributions in vivo (Golestani et al., 2010). A SPECT scanner uses longer-lived, more easily obtained radioisotopes. SPECT deals the possibility to widen the observational time window (owing to the longer half-life of single photon emitters) thus allowing biomedical scientists to observe biological processes in vivo several hours or days after administration of the labeled compound (Meikle et al., 2005). In SPECT, the image generated from a point source is degraded by a number of factors related to collimators and detectors in gamma cameras, thus referred to as the collimator–detector response. In recent years, a great deal of work has gone into developing methods to compensate for the collimator–detector response (Frey et al., 1998).

1.16.2. Positron emission Tomography (PET)

Positron Emission Tomography imaging have been developed and used for diagnostic clinical studies, basic human studies for understanding biochemical processes in neurobiology, and preclinical studies especially using non-human primates and rodents. PET has the sensitivity needed to visualize most interactions between physiological targets and ligands such as neurotransmitters and brain receptors. Radionuclide based imaging modalities are able to determine concentrations of specific biomolecules as low as in the
picomolar range. PET technology has been applied in various ways to assist in drug
development, whereby understanding drug action, establishing dosage regimens of central
nervous system drugs, and determining treatment strategies. Central to molecular imaging
with PET is the development of appropriate PET imaging probes. [\(^{18}\text{F}\)] FDG (2-\(^{18}\text{F}\)-fluoro-deoxy-D-glucose) is the best clinically known and the most successful commercial PET
radiopharmaceutical. All experts in the field agree that there would be no clinical PET
imaging today without [\(^{18}\text{F}\)] FDG (Li et al., 2012; Ametamey et al., 2008).

The main objectives of the proposed project was the labeling of some common
drugs/compounds with Technetium-99m having clinical significance and their biological
evaluation as infection and tumour imaging agents in animal models. Some other factors such
as appropriate reducing agents, optimum pH, optimization of incubation time and
temperature and stability of labeled complex were also evaluated. In vitro binding of Tc-99m
labeled pharmaceuticals/compounds with bacteria such as Staphylococcus aureus and
Escherichia coli was assessed.

- Selection of drugs/compounds such as clathromycin, clindamycin, vibramycin and
  cecropin A for infection study whereas epirubicin, lanreotide, and vincristine were
  chosen for tumor study.
- Development of different \(^{99}\text{mTc}\) labeling strategies.
- Techniques for the in vitro and in vivo testing of label stability.
- Biodistribution/ Biotechnological imaging strategies in model animals.
- Scintigraphic study of labeled drugs in mice, rats/rabbits.
CHAPTER 2

REVIEW OF LITERATURE

Huang et al., (2015) investigated SPECT to image Parkinson’s disease (PD) which is neurodegenerative disease characterized by progressive loss of dopaminergic neurons in the basal ganglia. Single photon emission computed tomography (SPECT) scans using $^{99m}$Tc TRODAT-1 can image dopamine transporters and provide valuable diagnostic information of PD. They optimized the parameters for scanning $^{99m}$Tc TRODAT-1/SPECT using the Taguchi analysis to improve quality of image.

Sakr et al. (2014) prepared Tc-pyrimidine-4, 5-diamine ($^{99m}$Tc-PyDA) complex with in vitro stability of 24h. The yield under the optimum conditions at 5 mg of PyDA, 25 μg of SnCl$_2$·2H$_2$O) and pH 8 was 96 ± 3%. The complex was analyzed on mice as potential marker for tumor hypoxia imaging. The complex showed high tumor hypoxia uptake with the target/nontarget (T/NT) ratio of ~3.

Amin et al. (2014) performed the evaluation of Sulfadimidine labeled with $^{99m}$Tc producing a yield of about 90%. Biodistribution study in normal mice showed high uptake of the $^{99m}$Tc complex in stomach and intestine. The ratio of the uptake of the $^{99m}$Tc complex in muscles infected with E. coli to normal mice was about 2, 1.5 and 1.4 at 15, 90, and 180 min post injection, respectively, while for the muscles inflamed with heat-killed E. coli or sterile turpentine oil, the difference from the normal muscles was insignificant.

Motaleb & Ayoub. (2013) prepared a new radiopharmaceutical ($^{99m}$Tc-rufloxacin) for infection imaging, capable to differentiate between septic and aseptic inflammations with
radiochemical yield of 93.4 ± 3%. Biodistribution studies in Albino mice bearing septic and aseptic inflammation models showed the effectiveness of $^{99m}$Tc-rufloxacin to differentiate between septic and aseptic inflammation.

Ibrahim & Sanad. (2013) developed a procedure for preparing high radiochemical purity $^{99m}$Tc-Losartan in a yield of about 90%. The optimal reaction conditions are as follows: 100 μg of Losartan, 50 μg of SnCl$_2$·2H$_2$O, 150 μL of phosphate buffer (pH 7), room temperature (25°C) with reaction time of 5 minutes. The radiochemical yield of $^{99m}$Tc-Losartan reached 98% under these conditions. The radiochemical yield and purity of the labeled product were determined by electrophoresis and paper chromatography. Biodistribution studies were carried out in normal Albino Swiss mice at different time intervals after administration of $^{99m}$Tc-Losartan. The heart uptake of $^{99m}$Tc-Losartan was sufficiently high as compared to other organs indicated it as myocardial imaging agent.

Xu et al. (2013) performed experiment for developing new tumor imaging agent $^{99m}$Tc-spermine. Spermine was labeled with $^{99m}$Tc, and its characters were also evaluated via in vitro and in vivo studies. The stability of the $^{99m}$Tc-spermine and its capacity to accumulate into 4T1 tumor cells were also evaluated. Biodistribution of $^{99m}$Tc-spermine was studied in 4T1 tumor-bearing mice.

Rizvi et al. (2013) stated that Daunorubicin, a chemotherapeutic antibiotic of the anthracycline family used for the treatment of many type of cancers can be labeled with $^{99m}$Tc, quality control, characterization, and biodistribution of radiolabeled Daunorubicin.

Sanad & Amin. (2013) done the labeling of Meloxicam with $^{99m}$Tc by addition of pertechnetate in isotonic solution to Sn-Meloxicam solution. High labeling yield (98%) was
attained in 30 min at room temperature. The labeled compound was separated and purified by HPLC. The biological distribution in infected mice demonstrates the suitability of $^{99m}$Tc-labeled Meloxicam for inflammation and tumor imaging.

Tsao et al. (2013) evaluated the synthesis of $^{99m}$Tc-N-guanine ($^{99m}$Tc-N4amG). Cellular uptake and cellular fraction studies were performed to evaluate the cell penetrating ability. Biodistribution and planar imaging were conducted in breast tumor-bearing rats. Biodistribution and scintigraphic imaging studies showed increased tumor/muscle count density ratios as a function of time. Our results demonstrate the feasibility of using $^{99m}$Tc-N4amG in tumor specific imaging.

Sanad & Ibrahim. (2013) illustrated that Pantoprazole which is an antiulcer drug can be labeled with $^{99m}$Tc to obtain an agent for ulcer imaging. Intravenous biodistribution studies of $^{99m}$Tc-Pantoprazole shown high concentration in the stomach ulcer, reaching about 27.2% of the total injected dose at 30 min post injection which make this agent promising for stomach ulcer imaging.

Abdel-Ghaney & Sanad. (2013) evaluated the Labeling of erythromycin with $^{99m}$Tc using SnCl$_2$·2H$_2$O as a reducing agent. Dependence of the yield of $^{99m}$Tc-erythromycin complex on the concentrations of erythromycin and reducing agent, on pH, and on the reaction time was studied. Biodistribution studies in Albino mice bearing septic and aseptic inflammation models showed that $^{99m}$Tc-erythromycin does not allow differentiation between septic (Staphylococcus aureus) and aseptic inflammation. The maximum accumulation of $^{99m}$Tc-erythromycin at the infection site was observed in 30 min after administration and was
followed by gradual decline. The abscess-tomuscle ratio for $^{99m}\text{Tc}$-erythromycin was 5 ± 0.6, whereas that for the commercially available $^{99m}\text{Tc}$ ciprofloxacin was 3.8 ± 0.8.

Sanad, (2013) labeled an antibiotic Azithromycin used to treat bacterial infections with $^{99m}\text{Tc}$. Biological distribution of $^{99m}\text{Tc}$-azithromycin was studied in mice infected with *Staphylococcus aureus* in the left thigh. The ratio of $^{99m}\text{Tc}$-azithromycin uptake in the bacterially infected and contralateral thighs, T/NT, for was found to be 6.20 ± 0.12 at 2 h after intravenous injection (higher than the ratio obtained with the commercially available $^{99m}\text{Tc}$-ciprofloxacin), which was followed by gradual decline. The results obtained show that $^{99m}\text{Tc}$-azithromycin can be used for infection imaging and allows differentiation between bacterial infection and sterile inflammation.

Ibrahim *et al.* (2013) demonstrated the labeling of tannic acid with $^{99m}\text{Tc}$ under the conditions: 150 μg of SnCl$_2$$\cdot$2H$_2$O, 50 μg of the substrate, 30 min, pH 7. $^{99m}\text{Tc}$-tannic was stable for 6 h. Experiments on biodistribution of orally administered $^{99m}\text{Tc}$-tannic acid showed the concentration of $^{99m}\text{Tc}$-tannic acid in the stomach ulcer (50% of the total administrated dose at 1 h post administration) presenting it to be sufficient for ulcer radioimaging.

Zdemir *et al.* (2013) demonstrated that radiolabeled antibiotics specifically bind to the bacterial components they are promising radiopharmaceuticals for the precise diagnosis and detection of infectious lesions. Doxycycline hyclate (DOX) was chosen to investigate as a new radiolabeled antibacterial agent since its bacteriostatic activity against a wide variety of microorganisms.
Sanad et al. (2013) stated that Omeprazole which was a proton pump inhibitor is labeled with $^{99m}\text{Tc}$ to obtain $^{99m}\text{Tc}$-omeprazole in $\sim$96% yield in basic media. The Intravenous biodistribution of $^{99m}\text{Tc}$-omeprazole showed that it concentrated in the stomach ulcer to reach about 22% of the total injected dose at 1 h post injection considering that $^{99m}\text{Tc}$-omeprazole in stomach ulcer may be sufficient for ulcer imaging.

Amin et al (2013) carried out the experiments on piracetam labeling with $^{99m}\text{Tc}$. In vivo biodistribution studies showed that the initial brain uptake correlated fairly well with the brain binding affinity of the compound. $^{99m}\text{Tc}$-piracetam shows promise in radioreceptor assays of neuroleptic drug levels and, in the labeled form, for brain imaging.

Dar et al. (2013) studied the 5-Fluorouracil that is a well-known drug for chemotherapy of various types of cancer. They studied Radiolabeled 5-fluorouracil with $^{99m}\text{Tc}$ is used for a diagnostic study of cancer. After successful labeling of the drug an animal study was performed to evaluate the potential of this radiopharmaceutical as a tumor diagnostic agent. The results showed 98.1 ± 1.2 % labeling efficacy of 5-fluorouracil with $^{99m}\text{Tc}$. Biodistribution study in rabbit models and Bioevaluation was performed in Swiss Webster mice having naturally developed tumor. Mice were dissected, uptake of drug in various organs was studied and results showed prominent uptake in liver and tumor and further investigation was performed by histopathological study.

Chattopadhyay et al. (2012) evaluated Technetium-99m labeled cefuroxime, a second-generation cephalosporin antibiotic and potential bacteria specific infection imaging agent was evaluated.
Motaleb et al. (2012) isolated Shikonin from Ratanjot pigment and characterized it. Biodistribution studies showed the accumulation of $^{99m}$Tc-shikonin in tumor sites with higher target to non-target ratio than other currently available $^{99m}$Tc(CO)3-VIP, $^{99m}$Tc-nitroimidazole analogues and $^{99m}$Tc-polyamine analogues indicating that shikonin deliver $^{99m}$Tc to the tumor sites with a percentage sufficient for imaging and can overcome drawbacks of other radiopharmaceuticals used for tumor imaging.

Fazli et al. (2012) determined the radiochemical purity of $^{99m}$Tc-ceftriaxone with a good stability at room temperature and human serum. The biodistribution studies showed the localization of $^{99m}$Tc-ceftriaxone at the site of infection with high sensitivity without any significant accumulation in vital organs. Due to the ease of $^{99m}$Tc-ceftriaxone conjugation method, high labeling efficiency, and high uptake in the infected muscle, it may provide a promising candidate as a targeting radiopharmaceutical for imaging infectious foci due to *Staphylococcus aureus* in nuclear medicine.

Rizvi et al. (2012) evaluated doxorubicin (DOX) which is an anthracycline anti-neoplastic and one of the most potent and widely used drug in clinical oncology.

Ibrahim & Attallah. (2012) evaluated an L-carnitine derivative labeled with $^{99m}$Tc found to be effective in tumor imaging. The labeling was done using SnCl$_2$ as a reducing agent. Biodistribution was done in normal and tumor-bearing mice. The uptake in ascites and in solid tumor was over 5% of the injected dose per gram tissue at 4 h post injection. These data revealed localization of the tracer in the tumor tissues with high percentage sufficient to use $^{99m}$Tc L-carnitine as a promising tool for diagnosis of tumor.
Sakr et al. (2012) illustrated that Meropenem can be radiolabeled with $^{99m}$Tc in high labeling yield 92±2% having stability of ~6 h. $^{99m}$Tc-meropenem showed high accumulation in tumor hypoxic tissue (4.193% injected dose/g organ). $^{99m}$Tc-meropenem showed high ability to differentiate the tumor tissue from inflamed or infected tissues in different mice models as its Target to non-target ratio of ~4 in case of tumor mice model while Target to non-target ratio of ~1 in case of inflamed mice model indicating it as a selective potential imaging agent for tumor hypoxia diagnosis.

Zhang et al. (2012) have developed ethylenedicysteine-glucosamine (ECG) as an alternative to $^{18}$F-fluoro-2-deoxy-D-glucose ($^{18}$F-FDG) for cancer imaging. Tumor could be clearly visualized with $^{99m}$Tc-ECG and $^{68}$Ga-ECG in mesothelioma-bearing rats.

Hsia et al. (2011) demonstrated that $^{99m}$Tc-BnAO-NI (3, 3, 10, 10-tetramethyl-1-(2-nitro-1H-imidazo-1-yl)-4,9-diazadodecane-2,11-dionedioxime) is a hypoxia-sensitive radio probe for monitoring hypoxic regions in a malignant neoplasm. $^{99m}$Tc-BnAO-NI, having higher lipophilicity did not achieve better specific accumulation in hypoxic tissues. $^{99m}$Tc-BnAO-NI/SPECT could be applied in clinics to noninvasively evaluate the feasibility of its use as a radiosensitizer by reducing tumor hypoxia in vivo.

Wang et al. (2011) found Technetium-99m human serum albumin ($^{99m}$Tc-HSA) as an important radiopharmaceutical required in nuclear medicine studies. The biologic half-life was determined, and all samples passed the pyrogenicity and sterility tests. Auto albumin could be extracted and radiolabeled properly in a nuclear medicine setting.

Shah et al. (2011) investigated the radiosynthesis of the $^{99m}$Tc-tricarbonyl moxifloxacin dithiocarbamate complex ($^{99m}$ Tc (CO)$_3$–MXND) and its biological evaluation
in male Wister rats (MWR) artificially infected with *Staphylococcus aureus* was assessed. Based on the elevated and stable radiochemical yield in saline, serum and saturated *in-vitro* binding with *S. aureus* and higher accumulation in the target organ of the male Wister rats indicates it a potential infection imaging agent.

Ocakoglu *et al.* (2011) studied the preparation of $^{99m}$Tc-Pheophorbide-a ($^{99m}$Tc-PH-A) complex and evaluate its efficiency as an infection imaging agent. These experiments indicated that the ratio of $^{99m}$Tc-PH-A uptake in bacterially infected muscle as compared to normal muscle and may be used as a radiopharmaceutical agent to distinguish infection from inflammation by nuclear imaging.

Wang *et al.* (2011) investigated the preparation and biodistribution of $^{99m}$Tc-labeled zoledronic acid derivative 1-hydroxy-2-(2-isopropyl-1H-imidazole-1-yl) ethylidene-1, 1-bisphosphonic ($^{99m}$Tc–i-PIDP) as a potential bone imaging agent. Bioevaluation and pharmacokinetics studies in mice and single photon emission computed tomography (SPECT) bone scan in rabbit of $^{99m}$Tc–i-PIDP were systematically studied.

Al-wabli *et al.* (2011) performed the synthesis of 7-Bromo-1,4-dihydro-4-oxo-quinolin-3-carboxylic acid (BDOQCA) with a yield of 93% and well characterized. Biodistribution studies in mice were carried out using experimentally induced infection in the left thigh using *E. coli*. The *in vitro* binding and biodistribution of $^{99m}$Tc-BDOQCA complex in the septic and aseptic inflammation bearing mice showed $^{99m}$Tc-BDOQCA complex as a promising agent for infection imaging which can differentiate between infected and inflamed muscle.
Kong et al. (2011) developed efficient synthesis of $^{99m}$Tc-O-(3-(1,4,8,11-tetraazabicyclohexadecane)-propyl)-α-methyl tyrosine ($^{99m}$Tc-N4-AMT) and evaluated its potential in cancer imaging. His Study suggested that $^{99m}$Tc-N4-AMT, a novel amino acid-based radiotracer, efficiently enters breast cancer cells, effectively distinguishes mammary tumors from normal tissues, and thus holds the promise for breast cancer imaging.

Chattopadhyay et al. (2010) evaluated of $^{99m}$Tc labeled fluroquinolone, moxifloxacin as a potential bacteria specific infection imaging agent. Rats and rabbit with infectious intramuscular lesions induced in thigh with E. Coli were used for studying biodistribution and scintigraphic imaging of the labeled product. Imaging of an infected thigh of a rabbit was performed at various time intervals.

Shah et al. (2010) stated that the $^{99m}$Tc-rifampicin ($^{99m}$Tc-RMP) a new radioantibiotic complex is synthesized specifically for the infection localization caused by methicillin-resistant Staphylococcus aureus (MRSA).

Zhang et al. (2010) synthesized and radiolabeled the N-benzyl dithiocarbamate (BZDTC) with ($^{99m}$TcN)$^{2+}$ intermediate to form the bis (N-benzyl dithiocarbamato) nitrido technetium-99m complex ($^{99m}$TcN(BZDTC)$_2$). This complex could be a potential brain perfusion imaging agent.

Ibrahim & Wally. (2010) synthesized a quinoxaline derivative, 3-Amino-2-quinoxalinecarbonitrile 1,4-dioxide (AQCD) and labeled with $^{99m}$Tc with labeling yield above 90% investigated by paper chromatography. Biodistribution study of $^{99m}$TcAQCD in tumor bearing mice reflected that its uptake in tumor sites in both ascites and solid tumor sites. This uptake of $^{99m}$Tc-AQCD in tumor sites was sufficient to radioimage the inoculated sites.
Altiparmak et al. (2010) demonstrated that Folate conjugates exhibit high affinity for folate receptor (FR) positive cells and tissues, such as those in tumors, making them attractive candidates and of interest in diagnostic tumor imaging.

Zhang et al. (2009) synthesized $^{99m}$Tc N (DMCHDTC)$_2$ complex, where DMCHDTC is 2,3-dimethylcyclohexyl dithiocarbamato through a ligand-exchange reaction. The heart/lung, heart/liver and heart/blood ratios of the complex were 1.06, 0.25 and 8.06 at 60 min post-injection, suggested its potential for use as a myocardial imaging agent.

Sinha et al. (2009) synthesized and evaluated potential radiopharmaceutical $^{99m}$Tc-Diethylene triamine pentaacetic acid-bis (amide) conjugates for tumor imaging. The synthesis of compounds was done by the condensation reaction of DTPA bis (anhydride) with different L-amino acids (methyl tryptophan, and 5-hydroxy tryptophan) and the characterization was performed. $^{99m}$Tc-labeled compounds were found to be stable for about 24 h under physiological conditions with more than 95% radiolabeling yield.

Barros et al. (2009) synthesized successfully a D-glucose-MAG$_3$ derivative and radiolabeled in high labeling yield. Biodistribution studies in Ehrlich tumor-bearing mice were performed. This compound showed high accumulation in tumor tissue with high tumor-to-muscle ratio and moderate tumor-to-blood ratio presenting D-glucose-MAG$_3$ as a potential tumor diagnostic agent.

Zhang et al. (2009) evaluated the deoxyglucose dithiocarbamate (DGDTC) as a tumor imaging agent. The tumor/blood and tumor/muscle ratios increased with time and reached 2.32 and 1.68 at 4 h post-injection, suggesting it would be a promising candidate for tumor imaging.
Zhang et al. (2009) also demonstrated the $^{99m}$TcN(PNP5)(DMCHDTC) + (DMCHDTC: 2,3-dimethylcyclohexyldithiocarbamate, NP5: bis(dimethoxypropylphosphinoethyl) ethoxyethylamine) complex synthesis through a ligand-exchange reaction. The biodistribution results in mice indicated that $^{99m}$TcN(PNP5)(DMCHDTC) + was significantly retained into the heart. The heart uptake (ID%/g) was 14.47, 12.23 and 8.76 at 5, 30 and 60 min post-injection, respectively suggesting it a potential myocardial imaging agent.

Shi et al. (2008) demonstrated the $^{99m}$Tc-labeling of AlaBN (7-14)NH$_2$ ($^{99m}$Tc(HYNIC-ABN)(tricine)(TPPTS)) in high yield and high specific activity. Its metabolic instability may contribute to its rapid clearance from the tumor and non-tumor organs, such as the blood, liver, lungs, and stomach. They clearly visualized the tumor at 30-60 min p.i. by planar imaging of the BALB/c nude mice bearing the HT-29 human colon xenografts. The combination of relatively high tumor uptake with its very favorable pharmacokinetics makes $^{99m}$Tc(HYNIC-ABN)(tricine)(TPPTS) a promising SPECT radiotracer for imaging colon cancer.

Wei et al. (2008) developed a glycopeptide to be used as a carrier for anti-cancer drug delivery. Glycopeptide was synthesized by conjugating glutamate peptide and chitosan using carbodiimide as a coupling agent with~95% purity. The Glycopeptide was labeled with sodium pertechnetate for in vitro and in vivo studies. Biodistribution and planar imaging were conducted in breast tumor-bearing rats. Biodistribution of $^{99m}$Tc-glycopeptide showed increased tumor-to-tissue and Planar images confirmed that $^{99m}$Tc-glycopeptide could assess tumor uptake changes after paclitaxel treatment. In vitro and in vivo studies indicated that
glycopeptide could target tumor cells, thus may be a useful carrier for anti-cancer drug delivery.

Kothari et al. (2007) explored radiolabeled VIP analogues for tumor imaging and therapy. They reported synthesis of three VIP analogues and their radiolabeling with $^{99m}$Tc via a novel tricarbonyl synthon. The radiolabeled product could be prepared in high yields (495%) and stability. In vitro studies showed significant uptake of $^{99m}$Tc (CO)$_3$-VP05 in human colon carcinoma cells. Biodistribution studies in animal tumor model also showed good uptake of drug in tumor tissue.

Chen et al. (2006) described the radiolabeling and preliminary biologic testing of diethylenetriaminepentaacetic acid (DTPA)-deoxyglucose (DG) labeled with $^{99m}$Tc. The tumor-to-brain and tumor-to-muscle concentration ratios for $^{99m}$Tc-DTPA-DG uptake were higher than those for fluorine-18-flurodeoxyglucose ($^{18}$F-FDG). Scintigraphic results showed the feasibility of $^{99m}$Tc-DTPA-DG in tumor imaging. The $^{99m}$Tc-DTPA-DG complex is a potential imaging agent due to the ideal physical characteristics of the radionuclide, ease of preparation, low cost, early accumulation and the preference for the renal route of excretion.

Urker et al. (2005) stated a novel approach for designing drug delivery systems for intra-articular (i.a.) treatment of rheumatoid arthritis. Retention of these systems was evaluated by radiolabeling with Tc-99m and gamma scintigraphy in arthritic rabbits. Serial scintigraphic images in rabbits were obtained to investigate the retentions of labeled drug delivery systems in the arthritic joints and choose a suitable formulation for the treatment protocol of arthritis.
Visnjic et al. (2005) studied & assessed the role of lymphatic mapping and gamma-probe guided lymph node biopsy in breast cancer patients. Thirteen women (mean age 49 years) were analysed. Invasive ductal carcinoma was found in 62%, invasive lobular carcinoma in 15%, and ductal carcinoma in situ in 23%. A total of 0.3 mL (50 MBq) of human albumine labeled by $^{99m}$Tc was injected intradermally over the tumor. Sentinel node biopsy is a highly accurate method for staging and treatment of breast cancer patients.

Akhtar et al. (2004) stated that $^{99m}$Tc-UBI (29-41) scintigraphy can be used for differentiating infection with *S. aureus* and *E. Coli* with a significantly higher scintigraphic intensity compared with that of the sterile inflammatory site. Relatively low Target to non-target ratios were observed in *E. coli* induced infections compared with those of *S. aureus* infected lesions, which may be due to a low virulence of *E. Coli* used.

Gomes et al. (2002) studied the effect of mitomycin-C on the biodistribution of the radiopharmaceutical $^{99m}$Tc phytic acid ($^{99m}$Tc -PHY) which is used in hepatic scintigraphy. The changes in the distribution of $^{99m}$Tc-PHY may be the result of metabolic processes and/or therapeutic actions produced by the administration of mitomycin-C.

Spradau et al. (1999) demonstrated the labeling of Octreotide with Tc-99m-cyclopentadienyltricarbonyltechnetium at its N-terminus and the biodistribution of the labeled conjugate was studied in adult female Sprague-Dawley rats. The $^{99m}$Tc-cyclopentadienyltricarbonyltechnetium-labeled octreotide conjugate ($^{99m}$Tc-CpTT-octreotide) showed receptor-mediated uptake in the pancreas and adrenals.
CHAPTER 3

MATERIALS AND METHODS

Preparation, quality control, characterization, and biological evaluation of $^{99m}$Tc labeled compounds for infection and tumor imaging were done in Department of Bioinformatics and Biotechnology GC university Faisalabad, Quality Control Group (QCG) of Isotope Production Division (IPD), Pakistan Institute of Nuclear Science and Technology, Islamabad while the Scintigraphic scans of normal and infected mice, rats and rabbits were performed in Department of Medical Sciences (DMS), Pakistan Institute of Engineering and Applied Sciences (PIEAS). In an effort to label Clarithromycin, clindamycin, vibramycin, Epirubicin, vincristine and Lanreotide with a medically important Technetium-99m by using various combinations of substrate and reducing agent at different pH were tried. The final preparation was then used to study the incubation time, serum stability, *in-vitro* and *in-vivo* binding, biodistribution and scintigraphy in normal, tumor bearing mice, infected and inflamed mice, rats and rabbits.

3.1. Materials

Antibiotic and Antitumor drugs (Clarithromycin, clindamycin, vibramycin, cecropin A, Epirubicin, vincristine, Lanreotide), SnCl$_2$.2H$_2$O, Distilled Water, saline (0.9% NaCl), acetone, methanol, 0.5M NaOH, Ascorbic Acid, Chloroform, Diazepam injections, Brain Heart Infusion media, Oxide Acetone, THF (Tetrahudrofuran) and Hydrochloric Acid were purchased from E.Merck (Germany). For manufacturing of fission based Mo-99/Tc-99m generator glass columns were purchased from Isotope Company, Hungary, whereas lead pig,
aluminum container, steel tubes and needles were obtained from local market. ITLS-SG (Gelman Sciences Inc, USA), Whatman paper, Millipore filter, Spikes were imported from USA. Aluminum (Al\(^{3+}\)) test (Merckoquant\(^{10} 015\)) strips were product of E. Merck.

### 3.2. Animals

- Mice (Swiss Albino mice, average body weight 30-35g) and Male rats (Sprague-Dawley, average body weight 200-270g) were purchased from the National Institute of Health (NIH), Islamabad.
- Tumor bearing mice (swiss albino mice) were obtained from Department of Zoology, Microbiology and Molecular Genetics, University of the Punjab.
- Rabbits (New Zealand white, average weight; 2.5kg) were obtained from the NIH, Islamabad. The local experimental animal ethical committee approved all the experiments correctly.

### 3.3. Bacterial Strains

*Staphylococcus aureus* (American type Culture Collection, Strain ATCC 25923) and *E.Coli* (5154552) was purchased from NIH, Islamabad and Quaid-e-Azam University, Islamabad.

### 3.4. Techniques

Following techniques were utilized for accomplishment of the present work.

(i) Chromatography

a. Paper chromatography

This technique was employed for certain radiochemical separations.

b. Thin Layer Chromatography (TLC)

TLC was used for radiochemical quality control of radiolabeled compounds.
c. High Performance liquid Chromatography (HPLC)

(ii) Electrophoresis

(iii) Gamma spectrometry

The activity of each irradiated sample was measured by γ-ray spectrometry using a 30cm$^3$ HP Ge - detector coupled with a Canberra Series 85 multichannel analyzer Nuclear Data 66 system. The detector counting efficiencies for different photon energies, required to measure the absolute disintegration rates of different radioactive products at a specific position, were determined using standard sources calibrated to < 3% error. Fig.4.1. shows the efficiency curves for a HP Ge - detector at different distances between the source and the detector.

(iv) SPECT Imaging Technology

### 3.5. Instruments and Equipments

Some important instruments/equipments used during this project includes:

- Laminar flow Cabinet- Biohazard
- Incubator-Nuaire Autoflow-IR direct Heat Co$_2$ incubator, Model No, NU 5500F
- CCD Coupled Inverted Microscope Optica, ITALY with attached DSP Colour camera(FINE)
- Gamma counter Ludlum Model 261 spectrometer, USA
- Radioisotope dose calibrator (Capintec Model CRC-5RH, USA)
- 2 π scanner Berthold coupled with NaI detector
- pH meter Hanna instruments Model 8417 attached with single electrode
- Centrifuge (WIROWKA, laboratory Jna, WE-1 220V/ 50Hz)
- Oven (RIPO - 14)
• Shaker (Griftin Flask Shaker)
• Mechanical stirrer (Thermolyne)
• Weighing balance (AND, EK – 2009 Japan)

3.6. $^{99}$Mo/$^{99m}$Tc-Generator

The commercially produced PAKGEN $^{99}$Mo/$^{99m}$Tc-generators were used to get technetium-$^{99m}$. (Fig.3.1)

3.7. Elution methodology of Technetium-$^{99m}$

The $^{99m}$Tc is eluted as follows:

i) Removed the cover-lid of generator and the protective plastic caps of the spike and the Millipore needle.

ii) Pushed the saline vial tightly onto the spike.

iii) Pushed the 30 mL evacuated vial in a lead shielded container on to the elution needle which further connected to the Millipore filter.

iv) Step away from the generator so that the radiation exposure should be reduced.

v) Observed the saline vial when it empty then removed it.

vi) Removed the 30 mL elution vial which containing the Na-$^{99m}$TcO$_4$.

vii) Then placed another 30 ml empty evacuated vial to remove the unnecessary liquid from the column and discard it.

viii) Placed the protective caps back on the spike and the Millipore needles.

ix) Closed the generator with lid.
Fig 3.1: Schematic Diagram of Pakgen Tc-99m Generator
3.8. Synthesis, Quality Control and Biological evaluation of $^{99m}$Tc-clarithromycin as a potential Bacterial Infection Imaging Agent

Clarithromycin injection was obtained from Abott Healthcare Products Limited. All reagents used were of analytical grade and purchased from E. Merck, Germany. Na$^{99m}$TcO$_4$ eluted with 0.9% saline from a locally produced fission based PAKGEN $^{99}$Mo/$^{99m}$Tc generator. 

*Staphylococcus aureus* (American Type Culture Collection, ATCC 25923) was obtained from National Institute of Health (NIH), Islamabad. The radioactivity of tissue and organs was measured with a gamma counter (Ludlum model 261). The scintigraphic imaging of rabbits was done with the help of Gamma scintillation camera.

3.8.1. Animals

Swiss Albino mice weighing 30–35 g were used for biodistribution study. All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Ethics committee. The animals were given free access to food and water.

3.8.2. Synthesis of $^{99m}$Tc-clarithromycin

The experiments were performed to determine the optimum conditions for the synthesis of $^{99m}$Tc- Clarithromycin. The different amounts of clarithromycin and reducing agent (SnCl$_2$.2H$_2$O) were used. The pH of the mixture was adjusted by using 0.5 M NaOH. After adding all reagents the mixture was stirred for few minutes and freshly eluted ~ 330 MBq Na$^{99m}$TcO$_4$- in physiological saline (0.9% NaCl) was injected into the vial. The volume of
reaction mixture was ~1.5 mL. All experiments were carried out under sterile conditions at room temperature.

3.8.3. Radiochemical analysis of $^{99m}$Tc-clarithromycin

To determine the Radiochemical purity of $^{99m}$Tc-clarithromycin the sample of 1-$\mu$L was spotted on ITLC silica gel strips (Gelman Laboratories) and developed using 0.5 M NaOH as the mobile phase. By using this system, $^{99m}$Tc-clarithromycin and free pertechnetate migrated with the solvent front of the mobile phase of the strip and the colloid was found at the origin. To determine the $^{99m}$TcO$_4^-$ content of the preparations, a strip of Whatman Paper No. 3 was developed using acetone as the mobile phase. In this system, $^{99m}$TcO$_4^-$ migrated with the solvent front of the mobile phase ($R_f = 1.0$) while labeled/reduced hydrolysed $^{99m}$Tc remained at origin. The distribution of labeled, free and hydrolyzed on chromatographic strips was measured by a 2$\pi$ Scanner (Berthold, Germany).

3.8.4. Electrophoresis study of $^{99m}$Tc-clarithromycin

A drop of $^{99m}$Tc-clarithromycin was spotted on Whatman No. 1 paper strip of 30 cm and Phosphate buffer (pH 6.8) in an electrophoresis bath (Deluxe electrophoresis chamber (Gelman) system). The voltage of 300 V was applied for 30-60 min at the room temperature. The strip was dried after completion of electrophoresis and scanned by 2$\pi$ scanner for the charge on $^{99m}$Tc-clarithromycin.

3.8.5. HPLC analysis of $^{99m}$Tc-clarithromycin

High performance liquid chromatography (HPLC), equipped with NaI crystal detector and UV detector (wavelength of 254 nm), was done on an analytical reverse phase column of C-
18 (Alltech) as stationary phase. The mixture of acetonitrile and 0.02M Sodium dihydrogen phosphate (pH adjusted up to 2-3 by using 0.5M NaOH) were used as mobile phase in the ratio 850:150 (v/v %) at a flow rate of 1mL/min.

### 3.8.6. Human Protein Binding Assay

The stability of $^{99m}$Tc-clarithromycin was studied *in vitro* by mixing of 1.8 mL of normal human serum with 0.2 mL of $^{99m}$Tc-clarithromycin and incubated at 37$^\circ$C for 24 hours. 0.2 mL aliquots were withdrawn at different time intervals and analyzed by Instant Thin-Layer Chromatography for determination of $^{99m}$Tc-clarithromycin, reduced/hydrolyzed $^{99m}$Tc and free $^{99m}$TcO$_4^-$.

### 3.8.7. Bacterial Strains

*Staphylococcus aureus* (ATCC 25923) was obtained from the American Type Culture Collection. Overnight cultures of *Staphylococcus aureus* were grown in brain heart infusion broth (BHI, Oxoid) in a shaking water bath at 37$^\circ$C. Aliquots of suspensions containing viable stationary phase bacteria were snap frozen in liquid nitrogen and stored at -70$^\circ$C. An aliquot of the suspension was rapidly thawed in a water bath at 37$^\circ$C just before use, and diluted in sodium phosphate buffer.

### 3.8.8. In vitro Bacterial Binding studies

*In vitro* binding of $^{99m}$Tc-clarithromycin can be used for accessing infection caused by *Staphylococcus aureus* using the procedure reported (Britton *et al.*, 1997). Tc-$^{99m}$ labeled clarithromycin (~6MBq) in 0.1mL of sodium phosphate buffer (Na-PB) was transferred in a sterilized test tube. After this 0.8 mL of 50% (v/v) of 0.01M acetic acid in sodium phosphate buffer
buffer containing almost $1 \times 10^8$ viable bacteria were added. The mixture was incubated at 4°C for 1 h and centrifuged for 10 minutes. The supernatant was removed and the bacterial pellet was suspended in 1mL of ice cooled Na-PB. The supernatant was removed and the radioactivity in the bacterial pellet was determined using a γ-counter. The supernatant were also counted. The radioactivity bound to bacteria was expressed in percent of the added $^{99m}$Tc activity bound to viable bacteria compared to total Tc-99m activity.

3.8.9. Induction of infections and sterile inflammations in animals

A single clinical isolation of *Staphylococcus aureus* from biological samples was used to produce focal infection. Individual colonies were diluted in order to obtain turbid suspension of *Staphylococcus aureus*. $2 \times 10^8$ colony-forming units (cfu) of *Staphylococcus aureus* in 0.2 mL of saline were intramuscularly injected into the left thigh muscle of mice (Laken *et al.*, 2000; Oyen *et al.*, 2001). Then, the mice were left for 24 h to get a visible swelling in the infected thigh. Sterile inflammation was induced by injecting 0.2 mL of turpentine oil intramuscularly in the left thigh muscle of the mice (Asikoglu *et al.*, 2000). After two days the swelling appeared. Sterile inflammation with the help of heat killed bacteria was induced by injecting 0.2 mL of heat killed *Staphylococcus aureus* intramuscularly in the left thigh muscle of the mice. The swelling appeared after 2 days. The *Staphylococcus aureus* suspension was provided the temperature of 100°C for 2 h for heat killing. Groups of three mice were used for each set of experiment.

3.8.10. Biological distribution of $^{99m}$Tc-clarithromycin in mice

The Swiss Albino mice were injected intravenously with 0.2 mL of $^{99m}$Tc-clarithromycin ($\sim 38$ MBq) via the tail vein. Then, the mice were sacrificed at 30 min, 1h, 4h and 24h post-
injection after ether anesthesia. Samples of the different organs were removed and counted for activity distribution in well type gamma counter. The average percent values of the administrated dose/organ were calculated. By cardiac puncture 1mL blood was taken and weighed. Activity in total blood was calculated by assuming blood volume 6.34% of the body weight. The study was approved and was in accordance with the guidelines set out by animal ethics committee.

3.8.11. SPECT Image study of $^{99mTc}$-clarithromycin in Rabbits

The evaluation of $^{99mTc}$-clarithromycin as potential infection imaging agent was further confirmed by scintigraphic study in rabbit models by single headed Siemens Integrated Orbiter Gamma Camera System interfaced with high-resolution parallel hole collimator. The infection was induced intramuscularly by injecting 0.4mL of S. aureus containing $2 \times 10^8$ colony-forming units (cfu) in saline in left thigh muscle of rabbit. After ethical approval, animal was placed on a flat hard surface with both hind legs spread out and all legs fixed with surgical tape. After injecting Diazepam (5 mg) into the right thigh muscle, Saline (0.2 mL) containing 74 MBq of $^{99mTc}$-clarithromycin was injected intravenously into the marginal ear vein. Immediately after injection, dynamic acquisition was done with both thighs in focus.
3.9. Preparation, Biodistribution and Scintigraphic evaluation of $^{99m}$Tc-clindamycin: An Infection Imaging Agent

Clindamycin for intravenous injection was obtained from Ameer medical and Superstores, Islamabad, Pakistan. Na$^{99m}$TcO$_4$ was eluted from a locally produced fission based PAKGEN $^{99}$Mo/$^{99m}$Tc generator, with 0.9% saline. All other reagents used were of analytical grade and purchased from E. Merck, Germany. *Staphlococcus aureus* (American Type Culture Collection, ATCC 25923) was obtained from National Institute of Health (NIH), Islamabad. All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Ethics committee. The animals were kept under standard conditions with free access to food and water. Tissue and organ radioactivity was measured with a $\gamma$-counter (Ludlum model 261). Gamma scintillation camera was used for imaging of rabbits.

3.9.1. Preparation of $^{99m}$Tc-clindamycin

For the radiolabelling of $^{99m}$Tc-Clindamycin, the optimum conditions were determined. The varying amounts of clindamycin (ligand) and SnCl$_2$.2H$_2$O (reducing agent) were used. The pH of the solution was adjusted by using 0.5 M NaOH. Ascorbic acid (3 mg) was used as stabilizer in the reaction mixture. After adding all reagents the mixture was stirred for few minutes and freshly eluted $\sim$ 380 MBq $^{99m}$TcO$_4^-$ in isotonic saline was injected into the vial and left to react at room temperature. The volume of reaction mixture was $\sim$1.5 mL.
3.9.2. Quality control of $^{99m}$Tc-clindamycin

Instant thin layer chromatography (ITLC) was used to access the radiochemical purity of $^{99m}$Tc-clindamycin. In ITLC the sample of $^{99m}$Tc-clindamycin (1-µL) was spotted on silica gel plates (Gelman Laboratories) and developed using 0.5 M NaOH as the mobile phase. In this system, $^{99m}$Tc-clindamycin and free pertechnetate migrated with the solvent front of the mobile phase and the colloid was found at the origin of the strip. To determine the $^{99m}$TcO$_4^-$ content of the preparations, a strip of Whatman Paper No. 3 was developed using acetone as the mobile phase. In this system, $^{99m}$TcO$_4^-$ migrated with the solvent front of the mobile phase (R$_f$ = 1.0) while labeled/reduced hydrolysed $^{99m}$Tc remained at origin. The distribution of labeled, free and hydrolyzed on chromatographic strips was measured by a 2π Scanner (Berthold, Germany). Otherwise, the strips were cut into segments of 1 cm and counted by a gamma-counter.

3.9.3. HPLC of $^{99m}$Tc-clindamycin

The analysis of $^{99m}$Tc-clindamycin was performed by D-200 Elite HPLC system using the analytical column of C-18 (Alltech) as stationary phase. The mixture of acetonitrile and 0.02M Sodium dihydrogen phosphate (pH adjusted up to 2-3 by using 0.5M NaOH) were used as mobile phase in the ratio 850:150 (v/v %). The flow rate of the mobile phase was adjusted up to 1 mL per minute. UV detector was used for detection purpose and work was performed at wavelength of 215 nm. Radiation detector was used for monitoring of $^{99m}$Tc activity.
3.9.4. Electrophoresis and Lipophilicity test of $^{99m}$Tc-clindamycin

Electrophoretic experiments of the $^{99m}$Tc-clindamycin were performed by using Deluxe electrophoresis chamber (Gelman) system. Whatman No. 1 paper strip of 30 cm and Phosphate buffer (pH 6.8) was used in this experiment. A drop of $^{99m}$Tc-clindamycin was placed at midpoint of the strip and voltage of 300 V was applied for 30-60 min at the room temperature. After completion of electrophoresis, the strip was dried and scanned by 2π scanner for the charge on $^{99m}$Tc-clindamycin. Shake flask method was used for determination of lipophilicity. The partition coefficient was determined by mixing the complexes with an equal volume of 1-octanol and phosphate buffer (0.025M, pH 7.0 and 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5000 rpm for 5 min. From each phase, 0.1 mL of the aliquot was pipetted and counted in a well γ-counter. The measurement was repeated three times. The partition coefficient (p) was calculated using the following equation: 

\[ p = \frac{\text{cpm in octanol} - \text{cpm in background}}{\text{cpm in buffer} - \text{cpm in background}}. \]

3.9.5. In vitro stability test

To test the serum stability of the $^{99m}$Tc-clindamycin complex, we added 1.8 mL of normal human serum with 0.2 mL of $^{99m}$Tc-clindamycin and incubated in 37°C for different time intervals up to 24 h. 0.2 mL aliquots were withdrawn and analyzed by Instant Thin-Layer Chromatography for determination of $^{99m}$Tc-clindamycin, reduced/hydrolyzed $^{99m}$Tc and free $^{99m}$TcO$_4^-$.  

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3.9.6. Culturing of *Staphylococcus aureus*

Labeled $^{99m}$Tc-clindamycin can be used for infection diagnosis caused by *Staphylococcus aureus* (ATCC 25923). Overnight cultures of *S. aureus* bacteria were prepared in brain heart infusion broth (BHI, Oxoid) in a shaking water bath at 37°C. Fresh bacterial suspensions containing viable stationary phase bacteria were snap frozen in liquid nitrogen and stored at -70°C. Just before use, an aliquot of this suspension was rapidly thawed in a water bath at 37°C and diluted in sodium phosphate buffer (Na-PB).

3.9.7. *In vitro* cell binding studies

Binding of $^{99m}$Tc-clindamycin to *S. aureus* bacteria was assessed by the method described elsewhere (Singh *et al*., 2003). Briefly, 0.1mL of sodium phosphate buffer (Na-PB) containing ~5MBq of $^{99m}$Tc-clindamycin was transferred to a sterilized test tube. 0.8 mL of 50% (v/v) of 0.01M acetic acid in Na-PB containing about $1 \times 10^8$ viable bacteria was added. The mixture was incubated for 1 hour at 4°C and then centrifuged for 10 minutes at 2000 g at 4°C. The supernatant was removed and the bacterial pellet was suspended again in 1mL of ice cooled Na-PB. The supernatant was removed and the radioactivity in the bacterial pellet was determined by a gamma-counter. The supernatant were also counted. The radioactivity related to bacteria was expressed in percent of the added $^{99m}$Tc activity bound to viable bacteria in regard to total $^{99m}$Tc activity. For comparison purposes *in vitro* binding study of $^{99m}$Tc-clindamycin with *S. aureus* and $^{99m}$Tc-ascorbic acid was also performed.
3.9.8. Labeling Ascorbic acid with Tc-99m

One milligram ascorbic acid was dissolved in 1 mL pure water, the pH adjusted to 5 with 0.1 M NH₄OH solution, and then 0.2 mL of SnCl₂.2H₂O (1 mg/1 mL 0.1 M HCl) and 0.5 mL of Na⁹⁹mTcO₄⁻ (100 MBq) in saline were added to the vial. The reaction vial was left at 25°C for 20 min. The radiochemical purity of the labeled compound was checked with TLC (Ugur et al., 2006).

3.9.9. Induction of infectious foci

A single clinical isolation of S.aureus from biological samples was used to produce focal infection. Individual colonies were diluted in order to obtain turbid suspension containing 2×10⁸ colony-forming units (cfu) of S. aureus in 0.2 mL of saline were intramuscularly injected into the left thigh of rats (Laken et al., 2000; Oyen et al., 2001). Then, the rats were left for 24 h to get a visible swelling in the infected thigh. Three rats were used for one set of experiment.

3.9.10. Induction of non-infected inflammation with heat killed S.aureus

Sterile inflammation was induced by injecting 0.2 mL of heat killed S.aureus intramuscularly in the left thigh muscle of the rats. Two days later, swelling appeared. The S.aureus suspension was given the temperature of 100°C for 2 h for heat killing.
3.9.1. Induction of non-infected inflammation

Sterile inflammation was induced by injecting 0.2 mL of turpentine oil (Asikoglu et al., 2000) in the left thigh muscle of the rats, intramuscularly. After two days the swelling appeared.

3.9.12. Biodistribution

The study was approved and was in accordance with the guidelines set out by animal ethics committee. The animals were intravenously injected with 0.2 mL of $^{99m}$Tc-clindamycin (~38 MBq) via the tail vein. After a definite time, the rats were sacrificed at 1, 4 and 24-hour post-injection after ether anesthesia. Samples of the different organs were removed and counted for activity distribution in well type gamma counter. The average percent values of the administrated dose/organ were calculated. By cardiac puncture 1mL blood was taken and weighed. Activity in total blood was calculated by assuming blood volume 6.34% of body weight.

3.9.13. Bioevaluation by Scintigraphy

The evaluation of $^{99m}$Tc-clindamycinas a potential imaging agent was done by acquisition study in rabbit models by single headed Siemens Integrated Orbiter Gamma Camera System interfaced with high-resolution parallel hole collimator. The infection was introduced by injecting 0.4mL of S. aureus containing $2 \times 10^8$ colony-forming units (cfu) in saline intramuscularly in left thigh muscle of rabbit. After ethical approval, anesthetized animal was placed on a flat hard surface with both hind legs spread out and all legs fixed with surgical tape. After injecting Diazepam (5 mg) into the right thigh muscle, Saline (0.2 mL) containing
38 MBq of $^{99m}$Tc-clindamycin was injected intravenously into the marginal ear vein. Immediately after injection, dynamic acquisition with both thighs in focus was done for 120 minutes.

3.10. Labeling, Quality Control and Biological Evaluation of $^{99m}$Tc-vibramycin for Infection Sites Imaging

Vibramycin for intravenous injection was obtained from Ameer medical and Superstores, Islamabad, Pakistan. Na$^{99m}$TcO$_4$ was eluted from a locally produced fission based PAKGEN $^{99m}$Mo/$^{99m}$Tc generator, with 0.9% saline. All other reagents used were of analytical grade and acquired from E. Merck, Germany. Staphlococcus aureus were obtained from National Institute of Health, Islamabad. All animal experiments were performed following the principles of laboratory animal care and were approved by the Institutional Animal Ethics committee. The animals were kept under standard conditions with free access to food and water. Tissue and organ radioactivity was measured with a γ-counter (Ludlum model 261). Gamma scintillation camera (Capintec Caprac-R1) was used for imaging of experimental animals.

3.10.1. Labelling of vibramycin with Technetium-99m

Vibramycin was directly labeled with $^{99m}$Tc by using 0.2 mg vibramycin. Optional amount of 2-4 µg of SnCl$_2$.2H$_2$O was used as a reducing agent (pH 3). Ascorbic acid (4 mg) was used as a stabilizer in the mixture. After addition of all reagents ~ 370 MBq $^{99m}$TcO$_4^-$ in saline was injected into the vial at room temperature.
3.10.2. Determining labeling efficiency by ITLC analysis

Stability and labeling yield of $^{99m}\text{Tc}$-vibramycin was done by Instant Thin-Layer Chromatography. One $\mu$L sample of the preparation was spotted on ITLC strips (Gelman Laboratories) using 0.5 M NaOH as the mobile phase. In this system, $^{99m}\text{Tc}$-vibramycin migrated with the solvent front of the mobile phase ($R_f = 1.0$) and the colloid was found at the origin of the strip ($R_f = 0$). To determine the pertechnetate content of the preparations, strip of Whatman Paper No. 3 was developed using acetone as the mobile phase. In this system, pertechnetate migrated with the solvent front of the mobile phase ($R_f = 1.0$).

3.10.3. Electrophoresis studies

Electrophoresis of prepared $^{99m}\text{Tc}$-vibramycin was done on Whatman No. 1 paper in phosphate buffer (pH 6.8) by using deluxe electrophoresis chamber (Gelman) system. Whatman No. 1 paper of 30 cm was marked as L at left side of the strip and R at right side of the strip. A drop of $^{99m}\text{Tc}$-vibramycin was poured at the middle of the strip and put in midpoint of electrophoresis chamber having buffer in such a way that left side was dipped at anode and right side at cathode. The electrophoresis was run for 60 to 90 min at a voltage of 300V. After completion of electrophoresis, the strip was scanned by using 2$\pi$ scanner to identify the charge on vibramycin.

3.10.4 Stability of $^{99m}\text{Tc}$-vibramycin in human serum

The stability of the $^{99m}\text{Tc}$-vibramycin was checked in human serum at 37 °C. Normal human serum (1.8 mL) was mixed with 0.2 mL of $^{99m}\text{Tc}$-vibramycin and incubated for 30 min. Aliquots of 0.2 mL were withdrawn during the incubation at different time intervals up to 24 hours and subjected to ITLC analysis for the determination of $^{99m}\text{Tc}$-vibramycin,
reduced/hydrolyzed $^{99m}$Tc and free $^{99m}$TcO$_4^-$; The increase in amount of free pertechnetate determined the degree of degradation.

### 3.10.5. *Staphylococcus aureus*

Bacterial strain of *Staphylococcus aureus* is frequently used in different microbiology labs. It was obtained from the American Type Culture Collection. Overnight cultures of the strain were prepared in brain heart infusion broth (BHI, Oxoid) at 37 ºC in a shaking water bath. Aliquots of suspensions comprising of the viable stationary phase bacteria were rapidly frozen in liquid nitrogen and stored at -70 ºC. Before using, immediately an aliquot of this suspension was thawed in a water bath at 37 ºC and diluted in sodium phosphate buffer.

### 3.10.6. Bacterial binding assay

Bacterial binding of Tc-99m-vibramycin was assessed using *S. aureus*. Sodium phosphate buffer (0.1 mL) containing ~5MBq of $^{99m}$Tc-vibramycin was shifted to a test tube. A known volume (0.8 mL) of acetic acid (0.01M) in Na-PB containing around 1× 10$^8$ viable bacteria was added to above tube. The mixture was incubated at 4 ºC for 1 h and centrifuged for 10 min. The supernatant was removed and the bacterial pellet was gently resuspended in 1 mL of ice cooled Na-PB and recentrifuged. The supernatant was removed and the radioactivity in bacterial pellet and supernatant was measured by gamma-counter. The radioactivity related to bacteria was expressed in percent of the added $^{99m}$Tc activity to viable bacteria with respect to total $^{99m}$Tc activity. For comparison purposes binding of $^{99m}$Tc-ascorbic acid to bacteria were also performed. Ascorbic acid (1mg) was dissolved in 1 mL pure water, the pH adjusted to 5 with 0.1 M NH$_4$OH solution, and then 0.2 mL of SnCl$_2$·H$_2$O (1 mg/1 mL 0.1 M HCl) and 0.5 mL of Na$^{99m}$TcO$_4$ (100 MBq) in saline were added to the vial. The reaction vial was
left at 25°C for 20 min. The radiochemical purity of the labeled compound was checked with TLC.

3.10.7. Induction of infection with live *S.aureus*

A single clinical isolation of *S.aureus* from biological samples was used to produce focal infection. Individual colonies were further diluted to obtain turbid suspension containing 2×10⁸ colony-forming units (cfu) of *S. aureus* in 0.2 mL of saline were intramuscularly injected into the left thigh of rats (Laverman *et al*., 2008; Oyen *et al*., 2001). Then, the rats were left for 24 h to get a visible swelling in the infected thigh. Three rats were used for one set of experiment.

3.10.8. Induction of non-infected inflammation with heat killed *S.aureus*

*Staphylococcus aureus* suspension was heated at 100°C for 2 h to obtain killed *S. aureus*. Sterile inflammation was induced by injecting 0.2 mL of heat killed *S.aureus*, intramuscularly in the left thigh muscle of the rats. Two days later, swelling appeared.

3.10.9. Induction of non-infected inflammation with irradiated *S. aureus*

*Staphylococcus aureus* (1mL suspension) containing about 2×10⁸ colony-forming units (cfu) was gamma irradiated with a 3 K Gy dose to get irradiated *S. aureus*. The non-viability of bacteria was tested by cultivating them in different media and 0.2 mL suspension were injected intramuscularly in the left thigh of rats.
3.10.10. Induction of inflammation with turpentine oil

Sterile inflammation was induced intramuscularly by injecting 0.2 mL of turpentine oil (Asikoglu et al., 2000) in the left thigh muscle of the rats. After two days the swelling appeared.

3.10.11. Biodistribution studies in animal models

The animals were intravenously injected with 0.2 mL of $^{99m}$Tc-vibramycin (~38 MBq) via the tail vein. After a definite time, the rats were sacrificed at 1, 4 and 24-hour post-injection after ether anesthesia and biodistribution study was done. Blood (1 mL) was taken by cardiac puncture and activity in total blood was calculated by assuming blood volume equal to 6.34% of body weight. Samples of weighed infected muscle, normal muscle, liver, spleen, kidney, stomach, intestine, heart, brain, bladder and lungs were taken and activity was measured by the use of a gamma counter. The results were expressed as the percent uptake of injected dose per organ. The results of the bacterial uptake of $^{99m}$Tc-vibramycin and other compounds were analyzed by an analysis of variance by setting the level of significance at 0.05.

3.10.12. Induction of experimental infection in rabbit

Saline (0.6 mL) containing viable S. aureus ($4\times10^8$ cfu) was injected into the left thigh muscle of each rabbit. After 72 hours when significant swelling appeared at the site of injection, scintigraphy was done.

3.10.13. $^{99m}$Tc-vibramycin Scintigraphy

The model animal in triplicate was placed on a flat hard surface with both hind legs spread out and was fixed with the help of a surgical tape. Diazepam (5 mg) was injected into the
right thigh muscle. Saline (0.2 mL) containing 15 MBq of $^{99m}$Tc-vibramycin was then injected intravenously into the marginal ear vein. A single headed Siemens Integrated ORBITER Gamma Camera System interfaced with high-resolution parallel hole collimator and an on-line dedicated computer was used for imaging. Immediately after injection, dynamic acquisition with both thighs in focus was done for 120 min. For the biodistribution study of the radiotracer, whole body acquisition was done at 1, 4 and 24 h after injection.

### 3.11. Labeling and Biological characterization of Tc-99m labeled Cercopin A for infection foci imaging

Cecropin A (Catalog number C6830; HPLC ≥97%) in the form of white powder was obtained from Sigma Aldrich, USA. All reagents used were of analytical grade and purchased from E. Merck, Germany. Na$^{99m}$TcO$_4$ eluted with 0.9% saline from a locally produced fission based PAKGEN $^{99}$Mo/$^{99m}$Tc generator. *E. coli* (ATCC 25922), was acquired from Quaid-e-Azam University Islamabad. The radioactivity of tissue and organs was measured with a gamma counter (Ludlum model 261). Scintigraphic imaging of rabbits was performed by using Gamma scintillation camera.

### 3.11.1. Animals and ethics

Rabbits and Swiss albino mice were obtained from National Institute of Health (NIH), Islamabad. Swiss Albino mice weighing 20-25g were used for biodistribution study and were given free access to food and water. All animal experiments were followed by the principles of laboratory animal care and approval for animal’s experiments was taken from the Animal Ethics Committee of the Pakistan Institute of Nuclear Science and Technology (Document no. IPDs-H-SOP-04-003).
3.11.2. Labeling of $^{99m}$Tc-Cecropin A

The experiments were performed to determine the optimum conditions for the synthesis of $^{99m}$Tc-Cecropin A. The different amounts of Cecropin A and reducing agent (SnCl$_2$.2H$_2$O) + tartaric acid were used at room temperature. The pH of the mixture was adjusted by using 0.5 M NaOH. After adding all reagents, freshly eluted ~296 MBq Na$^{99m}$TcO$_4^-$ in physiological saline (0.9% NaCl) was injected into the vial. Finally the mixture was kept in boiling water bath for about 30 minutes. The volume of reaction mixture was ~1.5 mL. All experiments were carried out under sterile conditions at room temperature.

3.11.3. Quality assurance

To determine the Radiochemical purity of $^{99m}$Tc-Cecropin A the sample of 1-µL was spotted on ITLC silica gel strips (1×6 cm strips, Gelman Laboratories) and developed using 0.5 M NaOH as the mobile phase. By using this system, $^{99m}$Tc-Cecropin A and free pertechnetate migrated with the solvent front of the mobile phase of the strip and the colloid was found at the origin. To determine the $^{99m}$TcO$_4^-$ content of the preparations, a strip of Whatman Paper No. 3 was developed using acetone as the mobile phase. In this system, $^{99m}$TcO$_4^-$ migrated with the solvent front of the mobile phase ($R_f = 1.0$) while labeled/reduced hydrolysed $^{99m}$Tc remained at origin. The distribution of labeled, free and hydrolyzed on chromatographic strips was measured by a $2\pi$ Scanner (Berthold, Germany).

Radiolabelled cecropin A was tested for stability in human serum, cysteine challenge test and bacterial binding studies.
3.11.4. Stability in serum

The stability of $^{99m}$Tc-Cecropin A was studied *in vitro* by mixing of 1.8 mL of normal human serum with 0.2 mL of $^{99m}$Tc-Cecropin A and incubated at 37°C for 24 hours. 0.2 mL aliquots were withdrawn at different time intervals and analyzed by Instant Thin-Layer Chromatography for determination of $^{99m}$Tc-Cecropin A, reduced/hydrolyzed $^{99m}$Tc and free $^{99m}$TcO$_4^-$.

3.11.5. Cysteine challenge test

The cysteine solutions were prepared in Phosphate buffered saline. The pH was adjusted to 7.4 with 1.0M NaOH. Aliquots of 100µL were diluted into separate vials to provide serial dilutions. One vial contained only saline. Aliquots of 100 µL of $^{99m}$Tc-cecropin A were allowed to react with different concentrations of cysteine ranging between 1:1 to 500:1 of cysteine compound and allowed to stand at 37 °C for 1 h. After incubation each sample was spotted on an ITLC-SG strip and chromatographed in PBS of pH 7. The radioactivity was determined by gamma counting. The amount of displacement was expressed as the percentage of total radioactivity associated with the solvent front. The $^{99m}$Tc displaced by cysteine migrated near the solvent front.

3.11.6. Bacterial Strains

At first took *E.coli* (10mL), Centrifuge at 5000rpm for 8 seconds. Pour the supernatant away. Add 5mL of PBS in pellet and mixed well with the pipette, centrifuge again. Repeated the process two times and checked the absorbance with the help of spectrophotometer. $1 \times 10^8$ viable bacteria per mL were used for each set of experiment.
3.11.7. Binding to bacteria

*In vitro* binding of $^{99m}$Tc-Cecropin A can be used for accessing infection caused by *E.coli* using the procedure reported (Britton et al., 1997). Tc-99m labeled Cecropin A (~6MBq) in 0.1mL of sodium phosphate buffer (Na-PB) was transferred in a sterilized test tube. After this 0.8 mL of 50% (v/v) of 0.01M acetic acid in sodium phosphate buffer containing almost $1 \times 10^8$ viable bacteria were added. The mixture was incubated at 4ºC for 1 h and centrifuged for 10 minutes. The supernatant was removed and the bacterial pellet was suspended in 1mL of ice cooled Na-PB. The supernatant was removed and the radioactivity in the bacterial pellet was determined using a γ-counter. The supernatant were also counted. The radioactivity bound to bacteria was expressed in percent of the added $^{99m}$Tc activity bound to viable bacteria compared to total $^{99m}$Tc activity.

3.11.8. *In vivo* assays

*E.coli* was chosen as microorganism for muscle infection induction. A single clinical isolation of *E.coli* from biological samples was used to produce focal infection. Individual colonies were diluted in order to obtain turbid suspension of *E.coli*. $2 \times 10^8$ colony-forming units (cfu) of *E.coli* in 0.2 mL of saline were intramuscularly injected into the left thigh muscle of mice (Laken *et al.*, 2000). Mice were than left for 24 h to get a visible swelling in the infected thigh. Sterile inflammation was induced by injecting 0.2 mL of turpentine oil intramuscularly in the left thigh muscle of the mice (Asikoglu *et al.*, 2000). After two days the swelling appeared. Sterile inflammation with the help of heat killed bacteria was induced by injecting 0.2 mL of heat killed *E.coli* intramuscularly in the left thigh muscle of the mice. The swelling appeared after 2 days. The *E.coli* suspension was provided the temperature of
100°C for 2 h for heat killing and turpentine oil was sterilized by autoclaving at 121°C for 20 min. Groups of three mice were used for each set of experiment.

3.11.9. Biodistribution of $^{99m}$Tc-Cecropin A in Pathological animal models

The Swiss Albino mice (infected, heat killed inflamed and turpentine oil inflamed) were injected intravenously with 0.2 mL of $^{99m}$Tc-Cecropin A (~38 MBq) via the tail vein. Then, the mice were sacrificed at 1h, 4h and 24h post-injection after ether anesthesia. Samples of the different organs were removed and counted for activity distribution in well type gamma counter. The average percent values of the administrated dose/organ were calculated. By cardiac puncture 1mL blood was taken and weighed. Activity in total blood was calculated by assuming blood volume 6.34% of the body weight.

3.11.10. SPECT Imaging of $^{99m}$Tc-Cecropin A in Infected Rabbits

The evaluation of $^{99m}$Tc-Cecropin A as potential infection imaging agent was further confirmed by scintigraphic study in rabbit models by single headed Siemens Integrated Orbiter Gamma Camera System interfaced with high-resolution parallel hole collimator. The infection was induced intramuscularly by injecting 0.4mL of $E$.coli containing $2 \times 10^8$ colony-forming units (cfu) in saline in left thigh muscle of rabbit. After ethical approval, animal was placed on a flat hard surface with both hind legs spread out and all legs fixed with surgical tape. After injecting Diazepam (5 mg) into the right thigh muscle, Saline (0.2 mL) containing 74 MBq of $^{99m}$Tc-Cecropin A was injected intravenously into the marginal ear vein. Immediately after injection, dynamic acquisition was done with both thighs in focus.
3.12. Preparation, Quality control and Bio-evaluation of $^{99m}$Tc-epirubicin for Tumor Imaging

3.12.1 Reagents

All the chemical reagents of AR grade were used in experiment without further purification. Epirubicin hydrochloride for intravenous injection was the product of Pfizer, Australia was purchased from Lahore, Pakistan. Medical tracer Tc-99m was eluted as $^{99m}$TcO$_4^-$ from locally produced PAKGEN $^{99m}$Mo/$^{99m}$Tc generator. A HPLC (C-18 column, waters, $\mu$-Bondapak$^\text{TM}$C18, 3.9×300mm) was used to examine the purity of the prepared $^{99m}$Tc-epirubicin. The NaI(Tl) $\gamma$-ray scintillation counter (Ludlum model 261) was used for the measuring tissue and organ radioactivity.

3.12.2 Experimental animals

Swiss Webster mice (25-28g), with naturally developed tumors were obtained from the Animal House at School of Biological Sciences (SBS), University of the Punjab, Lahore and Swiss Albino mice weighing approximately 28 g were purchased from National Institute of Health (NIH), Islamabad, Pakistan. The experiments with animals were performed according to the principles approved by the Animals Ethics committee of institute (Document no. IPDs-H-SOP-04-003). For SPECT imaging studies of mice Gamma scintillation camera was used.

3.12.3. Labeling methodology of $^{99m}$Tc-epirubicin

The $^{99m}$Tc-epirubicin labeling was optimized by varying the amounts of ligand 100–350 µg, stannous chloride dihydride 20–50 µg and pH range 2–10. The pH of solution under reaction was adjusted by using NaOH or HCl. The vial was incubated at room temperature for
different time intervals to determine the stability and highest labeling efficiency of $^{99m}$Tc-epirubicin. Finally $^{99m}$Tc-epirubicin was prepared by adding 200 µg of ligand and stannous chloride dihydride 35 µg at pH 6. After addition of all reagents ~ 296 MBq $^{99m}$Tc in isotonic saline was added into vial and incubated at room temperature for 30 minutes. In all experiments reaction mixture volume was 1.0 ± 0.2 mL.

3.12.4. Quality control of $^{99m}$Tc-epirubicin

The radiochemical purity of $^{99m}$Tc-epirubicin was checked by ascending chromatographic methods, Free Pertechnetate ($^{99m}$TcO$_4^-$) in the reaction mixture was analyzed with Whatman No. 3 paper as the stationary phase and acetone as mobile phase. The amount of reduced and hydrolyzed was checked by using ITLC-SG strips (instant thin layer paper chromatography) as the stationary phase and THF (tetrahydrofuran) as a mobile phase. The stability of $^{99m}$Tc-epirubicin was analysed at room temperature for 5 h. The distribution of labeled, free and hydrolyzed on chromatographic strips was traced and quantitated using 2π Scanner (Berthold, Germany).

3.12.5. HPLC analysis of $^{99m}$Tc-epirubicin

HPLC of $^{99m}$Tc-epirubicin was performed by using D-200 Elite HPLC system. The C-18 column was used as stationary phase and mixture containing acetonitrile and disodium hydrogen phosphate buffer (pH was set to 5-6 by using 0.5 M NaOH) ratio 45:55 (v/v %) was used as mobile phase. Sodium Iodide (NAI) crystal detector was used for measuring radioactivity. The flow rate was adjusted at 1 mL/min at 254 nm wavelength. UV detector was used for detection of pure epirubicin compound.
3.12.6. Stability studies of $^{99m}$Tc-epirubicin

The stability of $^{99m}$Tc-epirubicin was determined by ITLC-SG and paper chromatography at room temperature and human blood serum. The $^{99m}$Tc-epirubicin complex was spotted on chromatographic strips after 30m, 1h, 2h, 4h, 5h and 24h, developed and scanned by virtue of which in vitro stability of the labeled preparation was ascertained. The stability of the labeled product was investigated in human serum sample by adding 0.2 mL of $^{99m}$Tc-epirubicin and 1.8mL of human serum at 35ºC. Then, the samples were analyzed at different time intervals upto 24 hours.

3.12.7. Electrophoresis procedure

Electrophoresis of $^{99m}$Tc-epirubicin was done with Deluxe electrophoresis chamber of Gelman system. Strip of Whatman No. 1 paper having 30cm length was marked as R at right side of strip and L at left side of strip. Then, the strips were soaked with 0.05 M phosphate buffer, put a drop of $^{99m}$Tc-epirubicin and introduced into the chamber. The strip was retained for 45-60 minutes under applied voltage (300V), dried and scanned with the help of $2\pi$ scanner.

3.12.8. Bioassay in normal and tumor bearing mice

Biological distribution studies of $^{99m}$Tc-epirubicin were carried out in naturally developed mammary tumor bearing Swiss Webster mice and normal Swiss Albino mice having weight ranges 25-28g. The mice were anestesized in a jar having cotton swab dipped in chloroform. The prepared $^{99m}$Tc-epirubicin injection containing ~ 74 MBq (2 mCi) was injected intravenously into the tail. The mice were weighed, sacrificed and biological distribution
$^{99m}$Tc-epirubicin in various organs was determined. Blood samples (0.2mL) were taken by cardiac puncture, weighed and activity in total blood was calculated by assuming blood volume=6.5% of body weight. The activity was measured using a gamma counter. The results were expressed as the percentage of injected dose per gram of tissue (% ID/g).

3.12.9. Scintigraphic imaging of $^{99m}$Tc-epirubicin

For the evaluation of potential of prepared radiopharmaceutical as a feasible imaging agent, the scintigraphic study was carried out in mice bearing tumor. The animal was anesthetized and was put on a hard flat board with both hind legs spread out and fixed with surgical tape. After preparing the labeled kit under sterilized conditions, 200µL of $^{99m}$Tc-epirubicin was intravenously injected in tail. The animal was placed under the head of gamma camera projecting the dorsal view of the animal. The energy window of 20% was set on 140 keV. Images were developed on 256×256 matrix size for five minutes each. Whole body static images were taken after $^{99m}$Tc-epirubicin injection at various time intervals (30 min-4 h). Single headed Siemens Integrated Orbiter Gamma Camera System fitted with high-resolution parallel hole collimator connected to an on-line dedicated computer was used for study.
3.13 Labeling, Quality control and Biological evaluation of $^{99m}$Tc-DOTA-Lanreotide for Diagnostic purposes

Lanreotide (8-mer, Cys 2–7, cyclo) was a product of piCHEM Austria and was supplied by International Atomic Energy Agency (IAEA), Vienna. The $^{99m}$Tc-pertechnetate was obtained from a $^{99m}$Mo-$^{99m}$Tc radionuclide generator Pakgen, PINSTECH, Pakistan. The generator contains fission produced molybdenum-99 adsorbed on alumina. $^{99m}$Tc may be eluted using saline as an eluent. Other chemicals and reagents were obtained from commercial suppliers. Rabbits and normal Swiss webster mice were obtained from the animal house of the National Institute of Health (NIH), Islamabad, Pakistan. Swiss Webster mice, weighing approximately 28 g with naturally developed tumors, were obtained from the Animal House at Microbiology and Molecular genetics (MMG), University of the Punjab, Lahore, Pakistan.


A known amount of stannous chloride dihydrate was dissolved in 0.1 mL of concentrated HCl and diluted with distilled water for the required amount of reducing agent. To the varied amount of ligand (DOTA-lanreotide); specific amounts of SnCl$_2$.2H$_2$O and 220-250 MBq of $^{99m}$TcO$_4^-$ were added. The pH of the solution was adjusted with a NaOH solution. The mixture was then incubated for different time periods at room temperature (23±2 °C) for labeling purposes. At least five sets of experiments were performed for each point.

3.13.2. Quality control

The radiochemical purity of labeled biomolecules was determined by the standard chromatography techniques like paper chromatography, ITLC and by HPLC. Radiochemical yield of $^{99m}$Tc- DOTA-lanreotide was checked by chromatographic method using Whatman
No. 3 paper and ITLC-SG strips (Gelman Science). Free $^{99m}$TcO$_4^-$ in the preparation was determined by using Whatman No. 3 paper as the stationary phase and acetone as mobile phase. Reduced and hydrolyzed activity was determined by using instant thin layer paper chromatography (ITLC-SG strips) as the stationary phase and 0.5 M NaOH as a mobile phase. The distribution of labeled, free and hydrolyzed compounds on chromatographic strips was measured by a $2\pi$ Scanner (Berthold, Germany). Alternatively, the strips were cut into 1 cm segments and counted by a gamma-counter.

3.13.3. Electrophoresis of $^{99m}$Tc-DOTA-lanreotide

Electrophoresis of radiotracer $^{99m}$Tc-DOTA-lanreotide was studied by using Deluxe electrophoresis chamber [Gelman] system. The Na-phosphate buffer (0.2M) of pH 6.8 was used and Whatman No.1 as support in this experiment. The strip was placed in the electrophoresis chamber containing buffer in such a way that left side dip at anode and right side at cathode; one drop of $^{99m}$Tc-DOTA-Lanreotide was poured at the middle of the strip and electrophoresis was run for 45 to 60 minutes at a voltage of 300 V. After completion of electrophoresis, the strip was scanned by using $2\pi$ scanner to know the charge on $^{99m}$Tc-DOTA-lanreotide.

3.13.4. High performance liquid chromatography of $^{99m}$Tc-DOTA-lanreotide

HPLC of $^{99m}$Tc-DOTA-lanreotide was studied by using D-200 Elite HPLC system. A sodium iodide crystal detector was used for radioactivity measurement. The column of C-18 (waters, $\mu$-Bondapak$^{TM}$C18, 3.9×300mm) was used as stationary phase and a mixture of acetonitrile and water were used as mobile phase in the ratio 80:20 [v/v %]. The flow rate of the mobile phase was adjusted up to 1 mL per minute. UV detector was used for detection purpose and
work was done at wavelength of 230 nm, while gamma detector (NaI) was used for monitoring of $^{99m}$Tc activity.

3.13.5. *In vitro* stability study of $^{99m}$Tc-DOTA-lanreotide

After choosing suitable amount of ligand/$^{99m}$Tc ratio and pH, the complex was incubated for 24 hours at room temperature. To observe the stability of the complex $^{99m}$Tc-DOTA-lanreotide after 30 min, 1 h, 2 h, 4 h, 6 h and 24 h, the complex was spotted on paper strips, developed and scanned.

3.13.6. *In vitro* stability in normal human serum

Human serum (1.8mL) was mixed with 0.2 mL (~74 MBq) of $^{99m}$Tc-DOTA-lanreotide and incubated at 35 °C. 0.2 mL aliquots were withdrawn during the incubation at 1h, 4h and 24 hours and subjected to chromatography for determination of $^{99m}$Tc-DOTA-lanreotide, reduced/hydrolyzed $^{99m}$Tc and free $^{99m}$TcO$_4^-$.

3.13.7. Cysteine Challenge

About 100µL aliquots of labelled DOTA-lanreotide were allowed to react with serially diluted aliquots of solutions of cysteine with prepared in phosphate buffer solution (0.2M) resulting the molar ratios of cysteine compound from 1:1 to 500:1. The reaction mixtures were allowed to stand for 45 min at 37°C and after this time the mixtures were controlled by chromatography.
3.13.8. Biodistribution of $^{99m}$Tc-DOTA-lanreotide in normal mice

The biological distribution study was done using 12 Swiss webster mice divided into four groups (3 animals in each group) weighing 25–30 g. Each anesthetized animal was injected in tail vein with 0.3 mL containing ~74 MBq of $^{99m}$Tc-DOTA-lanreotide. The mice were sacrificed after anesthesia and biodistribution was determined. The sample of blood (1 mL) was taken by cardiac puncture, weighted and activity in total blood was calculated by assuming blood volume = 6.5% of body weight. The whole animals were then weighed and dissected after 1h, 4h and 24 h. Samples of muscle, liver, spleen, lungs, kidney, stomach, femur, heart and brain were removed, weighed and content of radioactivity was measured using a gamma counter. Corrections were made for background radiation during the experiment. The results were expressed as the percentage uptake of injected dose per gram organ tissue.

3.13.9. In vivo biodistribution studies in tumor bearing mice

_In vivo_ biological distribution study was performed in Swiss webster mice bearing the breast tumors. A 0.3mL (74MBq) solution of $^{99m}$Tc-DOTA-lanreotide was injected intravenously in the tail vein. The mice were dissected at 1h, 2h, 4h and 24h after drug administration. The organs of interest were collected and after weighing radioactivity were measured with a well type gamma counter. The results were expressed as the percent uptake of injected dose per gram organ (%ID/g).

3.13.10. Scintigraphic Studies in tumor induced mice and normal rabbits

The imaging study was performed in healthy rabbits with weight range of 2.0–2.5 Kg respectively. $^{99m}$Tc-DOTA-lanreotide 111 MBq was administered in the marginal ear vein of
rabbit. Similarly 74 MBq of activity was injected through the tail of Swiss Webster mice having breast tumors. The animal was given one mL of intramuscular diazepam injection and was immobilized on gantry table under the head of gamma camera projecting the dorsal view of the animal. The energy window of 20% was set on 140 keV. Images were acquired on 256×256 matrix size for five minutes each. Whole body static images were taken at 5min, 10 min, 15min, 20min, 30 min, 4h, and 24 h after $^{99m}$Tc- DOTA-lanreotide injection in normal rabbits while the images after 30min, 1h, 2h and 4h were obtained in case of breast tumor bearing mice. Single headed Siemens Integrated Orbiter Gamma Camera System interfaced with high-resolution parallel-hole collimator was used to acquire digital images.

3.14 Preparation, Quality control and Biological characterization of $^{99m}$Tc-vincristine

Vincristine sulphate injection was obtained from Lahore Pakistan. Technetium-99m was obtained from a locally produced fission based PAKGEN $^{99}$Mo/$^{99m}$Tc generator. All chemicals used were AR grade. The approval for animal’s experiments was taken from the Animal Ethics Committee of the Pakistan Institute of Nuclear Science and Technology.


Known amount of stannous chloride dihydrate was dissolved in 0.1 mL of concentrated HCl and diluted with distilled water to get required amount of reducing gent. To the varying amount of ligand (Vincristine sulphate); certain amount of SnCl$_2$·2H$_2$O and 1 mL of $^{99m}$TeO$_4^-$ was added. The pH of the solution was adjusted with the dilute NaOH solution. The mixture
was then incubated for different time periods at room temperature (25 ± 2°C) for labeling purposes. At least five set of experiments were performed for each point.

### 3.14.2. Quality control of $^{99m}$Tc-vinc

Radiochemical yield of $^{99m}$Tc-vinc was checked by chromatographic method using Whatman No. 3 paper and ITLC-SG strips (Gelman Science). Free $^{99m}$TcO$_4^-$ in the preparation was determined by using Whatman No. 3 paper as the stationary phase and acetone as mobile phase. Reduced and hydrolyzed activity was determined by using instant thin layer paper chromatography (ITLC-SG strips) as the stationary phase and 0.5 M NaOH as a mobile phase. The stability of $^{99m}$Tc-vinc was checked for 5 hours at room temperature. The distribution of labeled, free and hydrolyzed on chromatographic strips was measured by a 2π Scanner (Berthold, Germany). Alternatively, the strips were cut into 1 cm segments and counted by a gamma-counter.

### 3.14.3. In vitro stability study of $^{99m}$Tc-vinc

After choosing suitable Vincristine sulphate/$^{99m}$Tc ratio and pH, the complex was incubated for 24 hours at room temperature. To observe the stability of the complex $^{99m}$Tc-vinc after 30 min, 1 h, 2 h, 4 h, 6 h and 24 h was spotted on paper strips, developed and scanned likewise by virtue of which in vitro stability of the labeled preparation was ascertained.

### 3.14.4. Electrophoresis of $^{99m}$Tc-vinc

Electrophoresis of radiotracer $^{99m}$Tc-vinc was studied by using Deluxe electrophoresis chamber (Gelman) system. The phosphate buffer of pH 6.8 was used in this experiment. Whatman No. 1 paper of 30 cm was used marked with L at left side of the strip and R at right
side of the strip. The strip was placed in the electrophoresis chamber containing buffer in such a way that left side dip at anode and right side at cathode; one drop of $^{99m}$Tc-vinc was poured at the middle of the strip and electrophoresis was run for 45 to 60 minutes at a voltage of 300 V. After completion of electrophoresis, the strip was scanned by using 2π scanner to know the charge on $^{99m}$Tc-vinc.

3.14.5. HPLC of $^{99m}$Tc-vinc

HPLC of radiotracer $^{99m}$Tc-vinc was studied by using D-200 Elite HPLC system. Sodium Iodide crystal detector was used for radioactivity. The column of C-18 was used as stationary phase and a mixture of acetonitrile and water were used as mobile phase in the ratio 80:20 (v/v %). The flow rate of the mobile phase was adjusted up to 1 ml per minute. UV detector was used for detection purpose and work was done at wavelength of 230 nm, while gamma detector was used for monitoring of $^{99m}$Tc activity.


Stability of the radiotracer $^{99m}$Tc-vinc was studied in vitro. 1.8 mL of normal human serum was mixed with 0.2 mL of $^{99m}$Tc-vinc and incubated at 35°C. 0.2 mL aliquots were withdrawn during the incubation at different time intervals up to 24 hours and subjected to chromatography for determination of $^{99m}$Tc-vinc, reduced/hydrolyzed $^{99m}$Tc and for free $^{99m}$TcO$_4^-$.

3.14.7. Biological distribution of $^{99m}$Tc-vinc in mice

The biological distribution study was done using 9 Swiss Albino mice bearing tumor divided into three groups (3 animals in each group) weighing 30–35 g. Each anesthetized animal was
injected in tail vein with 0.4 mL containing ~74 MBq (2mCi) of $^{99m}$Tc-vinc. The mice were sacrificed after ether anesthesia and biodistribution was determined. The sample of blood (1mL) was taken by cardiac puncture, weighted and activity in total blood was calculated by assuming blood volume = 6.5% of body weight. The whole animal were then weighted and dissected after 1h, 4h and 24 h. Samples of muscle, liver, spleen, lungs, kidney, stomach, femur, heart, brain and tumor were removed, weighed and content of radioactivity was measured using a gamma counter. Corrections were made for background radiation and physical decay while performing the experiment. The results were expressed as the % uptake of injected dose per gram organ tissue.

3.14.8. SPECT-Imaging

The imaging study was performed using tumor induced mice, normal Swiss Albino mice and normal rabbits with weight range of 30-35 g and 2.0–2.5 Kg respectively. Each animal was placed on a flat hard surface with both hind legs spread out, fixed with surgical tape and injection of Diazepam was given. $^{99m}$Tc-vinc of activity 111 MBq (3 mCi) was injected intravenously into the tail of mice and marginal ear vein of rabbit. A single headed Siemens Integrated ORBITER Gamma Camera System interfaced with high-resolution parallel hole collimator was used which was connected to an on-line dedicated computer (Macintosh® Operating System 7.5 Software used on the ICON™ Workstation). For studying the biodistribution whole body static images were taken at 1 h, 4h and 6 h after $^{99m}$Tc-vinc injection.
CHAPTER 4

RESULTS AND DISCUSSION

4.1. $^{99m}$Tc-clarithromycin

Clarithromycin is a potent macrolide antibiotic employed for the treatment of different bacterial diseases. The chemical structure of clarithromycin is shown in Fig 4.1. The therapeutic property of the clarithromycin has been exploited for diagnostic purposes.

4.1.1. Quality control

The $R_f$ value of labeled Reduced or hydrolysed and free $^{99m}$Tc is represented in Fig 4.2 and Fig. 4.3. The effect of amount of SnCl$_2$.2H$_2$O as reducing agent on the labeling efficiency of clarithromycin with Tc-99m is shown in Fig. 4.4. The maximum labeling yield of $^{99m}$Tc-clarithromycin was observed at 25 µg and as the amount of reducing agent was increased the labeling efficiency reduced (Fig. 4.4). In order to determine the most appropriate pH on the labeling efficiency, the labeling of $^{99m}$Tc-clarithromycin was carried out at different pH in range from 3-11. The complexation rate of clarithromycin with Tc-99m was maximum (>99%) at pH 7. Labeling efficiency of $^{99m}$Tc-clarithromycin was decreased to ~42% in acidic conditions (pH 3). In basic media at pH 11 the radiochemical yield decreased to 78% (Fig. 4.5). At room temperature, after 30 min the maximum labeling of clarithromycin with $^{99m}$Tc is achieved and maintained for up to 12 hours (Fig. 4.6). The influence of amount of ligand having the highest labeling efficiency was checked by labeling of varying amounts of clarithromycin with $^{99m}$Tc and the amount of 500µg ligand was observed to show high yield of labeling as shown in (Fig.4.7).
The labeling efficiency of $^{99m}$Tc-clarithromycin was evaluated by instant thin-layer chromatography and ascending paper chromatography on silica gel. In ITLC-SG chromatography 0.5M NaOH was used as the solvent for $^{99m}$Tc-clarithromycin and reduced/hydrolyzed $^{99m}$Tc, while paper chromatography was performed using acetone as the solvent for free $^{99m}$TcO$_4^-$ In current study, clarithromycin was labeled with $^{99m}$Tc with high radiochemical yields.

4.1.2. Paper Electrophoresis and HPLC analysis

The electrophoresis results demonstrate the neutral nature of ligand and the free pertechnetate movement was observed towards the anode after 1 hour (Fig. 4.8). The HPLC analysis of $^{99m}$Tc-clarithromycin is given in Fig. 4.9 which shows a very sharp peak at 1.7 min of retention. This signal at 1.7 min represents the $^{99m}$Tc-clarithromycin labeling efficiency greater than 99%. The HPLC analysis by UV detector (inactive ligand) show percent purity of clarithromycin (Fig. 4.10).

4.1.3. In vitro binding with Staphylococcus aureus

In vitro binding of $^{99m}$Tc-clarithromycin to Staphylococcus aureus was in the range of 70-76% as represented in Table 4.1. The amounts of $^{99m}$Tc-clarithromycin in the range of 10-50 µg showed similar binding efficiency with bacteria while as the amount was increased to 100µg binding efficiency was decreased. In vitro studies and animal experiments have shown that $^{99m}$Tc-clarithromycin localizes in bacteria infected sites significantly. The uptake of $^{99m}$Tc-clarithromycin was found to be greater than $^{99m}$Tc-azithromycin (47-65%) (Sanad, 2013). Similarly due to the ease of $^{99m}$Tc-clarithromycin preparation and rather greater infection uptake, it may be an excellent substitute to $^{99m}$Tc-ciprofloxacain for infection
diagnosis. Clarithromycin has the same macrolide, 14-membered lactone ring as erythromycin with difference of a methoxy group which replaces the hydroxyl group at the 6-position. Clarithromycin demonstrates greater activity against gram-positive organisms compared with erythromycin and azithromycin (Retsema et al., 1987).

4.1.4. Stability of $^{99m}$Tc-clarithromycin

$^{99m}$Tc-clarithromycin was completely stable in human serum during incubation as determined by Instant thin layer chromatography (ITLC). It was found that 97% labeling was found at 24 hours of incubation with very little increase in free $^{99m}$TcO$_4^-$ and reduced/hydrolyzed $^{99m}$Tc at 37ºC. The total impurities found were less than 3% (Table 4.2). The results of the stability of $^{99m}$Tc- clarithromycin at room temperature and human blood serum showed that after starting the reaction, the stability remains 99% upto 12 hours at room temperature and 97% upto 24 hours in human blood serum, respectively. Therefore, it was confirmed that the radiolabeled product has a good stability. Actually, $^{99m}$Tc labeling often requires the introduction of chemical modifications on the studied biologically active molecule. Results showed that labeling of $^{99m}$Tc-clarithromycin is rapid and effective with a high yield (99%) for up to 12 hours stability. Based on the findings of, these kits can be easily formed without any requirement for boiling and post-labeling purification. The results of human plasma binding study indicated that the blood serum enzymes were unable to degrade the $^{99m}$Tc-clarithromycin within 24 hours and $^{99m}$Tc-clarithromycin can bind with bacterial DNA gyrase before blood protease degradation.
4.1.5. Biodistribution and Scintigraphy

The in vivo uptake of $^{99m}$Tc-clarithromycin (%injected activity/g) in different organs of the animals infected with living *S. aureus*, heat killed *S. aureus* and turpentine oil induced at 30min, 1h, 4h and 24h after intravenous administration is shown in Table 4.3. Mice with infectious lesions (live *S. aureus*) injected with $^{99m}$Tc-clarithromycin showed a mean target-to-non target (T/NT) ratio equal to 4.61±0.09 at 30 min post injection, 6.92±0.5 at 1h post injection, 4.84±0.01 at 2h post injection and 1.90±0.1 at 24h post injection. $^{99m}$Tc-clarithromycin shows higher T/NT in the infected muscle (live *S. aureus*) at all-time intervals than that of sterile inflamed muscle (heat killed *S. aureus* and turpentine oil). The $^{99m}$Tc-clarithromycin showed slightly lower uptake in infected muscle than $^{99m}$Tc-rufloxacin (T/NT = 8.5 ± 0.1) (Motaleb & Ayoub, 2013) and rifampicin (7.3 ± 0.7) (Shah et al., 2010). The mean abscess-to-muscle (T/NT) ratio for $^{99m}$Tc-clarithromycin was higher than that of other recently reported $^{99m}$Tc-labeled antibiotics such as $^{99m}$Tc-azithromycin (T/NT = 6.20 ± 0.12) (Sanad, 2013), $^{99m}$Tc erythromycin (T/NT = 5 ± 0.6) (Ercan et al., 1992), difloxacin (5.5 ± 0.5) (Motaleb, 2010), sparafloxacin (5.9 ± 0.7) (Motaleb, 2009), ceftraixone (5.6 ± 0.6) (Mostafa et al., 2010), N-sulfanilamide (2.9 ± 0.1) (Imen et al., 2010) and streptomycin (2.4 ± 0.1) (Meral et al., 1992). It was found that $^{99m}$Tc-clarithromycin is approximately two times more effective than $^{99m}$Tc-ciprofloxacin (T/NT = 3.8 ± 0.8) (Kleisner et al., 2002). Mice having infected sites injected with $^{99m}$Tc-clarithromycin showed a good target-to-non target ratio, which are in accordance with the results obtained from the in vitro binding assay. The greater activity of $^{99m}$Tc-clarithromycin in bacterial infection sites than inflammation sites can be attributed to specific binding to living bacteria in mice. The $^{99m}$Tc-clarithromycin was removed from the circulation mainly through the kidneys. The liver uptake was
decreased prominently with time from (11.9±0.9% ID at 1 h 9.66±0.19% ID at 4 h and 4.35±0.5 at 24 h). Whole body gamma camera images of infection induced rabbits at 1 hour, 4 hours, and 24 hours after administration of $^{99m}$Tc-clarithromycin are presented in Fig.4.11 A, B, and C respectively. The rapid uptake seen at 1 hour, 4 hours and 24 hours most likely is due to the physiologic changes at the site of infection. In rabbits S. aureus infection was induced in left thigh and the area was seen with increased tracer accumulation after injection of technetium-99m labeled clarithromycin proving it a potential infection diagnostic agent.
Fig. 4.1: Structure of Clarithromycin.

Fig. 4.2: Instant thin layer chromatography (ITLC) pattern of the $^{99m}$Tc-clarithromycin, free, reduced/hydrolyzed $^{99m}$Tc. Thus, the migration from the point of spotting is as follows:

- 1 cm = Reduced/Hydrolyzed Tc-99m
- 10 cm = Tc-99m-clarithromycin and Free Tc-99m
**Fig. 4.3:** $^{99m}$Tc-clarithromycin, Paper chromatography pattern of the free, reduced/hydrolyzed $^{99m}$Tc.

**Fig. 4.4:** The effect of amount of SnCl$_2$.2H$_2$O on radiochemical yield of $^{99m}$Tc-clarithromycin (500µg clarithromycin, pH=7, 30 minutes)
**Fig. 4.5:** Effect of pH on radiochemical yield of $^{99m}$Tc-clarithromycin (500µg clarithromycin, 50µg SnCl$_2$.2H$_2$O, 30 minutes)

**Fig. 4.6:** Effect of reaction time and stability of $^{99m}$Tc-clarithromycin at room temperature (500µg clarithromycin, 50µg SnCl$_2$.2H$_2$O, pH=7)
Fig. 4.7: Effect of clarithromycin amount on radiochemical yield of $^{99m}Tc$-clarithromycin (50µg SnCl$_2$.2H$_2$O, pH=7, 30 minutes)

Fig. 4.8: Electrophoresis of $^{99m}Tc$-clarithromycin representing its neutral nature
**Fig. 4.9:** HPLC profile representing the percentage labeling of clarithromycin with Tc-99m

**Fig. 4.10:** HPLC analysis (UV detector) showing purity of clarithromycin
**Fig. 4.11:** Whole body gamma camera image of rabbit injected with $^{99m}$Tc-clarithromycin at 30 min, 1-hour, 2 hours and 4-hours post administration.
Table 4.1: *In vitro* binding of the $^{99m}$Tc-clarithromycin to live *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>$^{99m}$Tc-clarithromycin</th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hour</td>
</tr>
<tr>
<td>10 µg</td>
<td>75.5</td>
</tr>
<tr>
<td>50 µg</td>
<td>74.7</td>
</tr>
<tr>
<td>100 µg</td>
<td>69.9</td>
</tr>
</tbody>
</table>

Table 4.2: *In vitro* stability of $^{99m}$Tc-clarithromycin in normal human serum

<table>
<thead>
<tr>
<th>Incubation Tie, h</th>
<th>$^{99m}$Tc-clarithromycin</th>
<th>Free pertechnetate</th>
<th>Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98.9± 1.2</td>
<td>1.1 ± 0.5</td>
<td>0.0± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>98.7 ± 1.1</td>
<td>1.3 ± 0.8</td>
<td>0.0 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>97.1 ± 2.0</td>
<td>0.9 ± 1.1</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>24</td>
<td>97.0 ± 1.9</td>
<td>2.2 ± 1.4</td>
<td>0.8 ± 0.9</td>
</tr>
</tbody>
</table>
Table 4.3: Biodistribution of $^{99m}$Tc-clarithromycin (%ID/g) in live *S. aureus*, heat killed *S. aureus* and turpentine oil inflamed mice at different time intervals (means ± SD)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Percentage of injected dose per gram of tissue weight</th>
<th>Live <em>Staphylococcus aureus</em></th>
<th>Heat Killed <em>Staphylococcus aureus</em></th>
<th>Turpentine oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 3/time, interval, iv)$^1$</td>
<td>30 min</td>
<td>1h</td>
<td>4h</td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
<td>3.74±0.6</td>
<td>3.66±0.8</td>
<td>2.99±0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>26.85±1.09</td>
<td>29.4±0.2</td>
<td>16.4±1.0</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>5.41±0.05</td>
<td>11.9±0.9</td>
<td>9.66±0.19</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td>2.73±0.9</td>
<td>3.28±0.6</td>
<td>3.84±0.12</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td>2.54±1.01</td>
<td>4.53±1.2</td>
<td>6.99±2.3</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>3.93±0.5</td>
<td>3.32±0.1</td>
<td>1.57±0.9</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td>1.76±0.1</td>
<td>3.88±0.6</td>
<td>5.72±0.25</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>0.1±0.01</td>
<td>0.02±0.1</td>
<td>0.03±0.9</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td>5.3±0.5</td>
<td>8.07±1.0</td>
<td>4.7±0.5</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td>1.35±0.8</td>
<td>5.08±1.9</td>
<td>6.91±1.09</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.54±0.2</td>
<td>0.17±0.3</td>
<td>0.11±0.5</td>
</tr>
<tr>
<td>Control muscle</td>
<td></td>
<td>0.21±0.05</td>
<td>0.27±0.2</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>Target muscle</td>
<td></td>
<td>1.04±0.1</td>
<td>1.7±0.05</td>
<td>1.6±0.04</td>
</tr>
<tr>
<td>T/NT</td>
<td></td>
<td>4.61±0.09</td>
<td>6.92±0.5</td>
<td>4.84±0.01</td>
</tr>
</tbody>
</table>
4.2. $^{99m}$Tc-clindamycin

4.2.1. Labeling and radiochemical purity

The direct labeling of clindamycin (Fig.4.12) with $^{99m}$Tc was evaluated. Fig. 4.13 and Fig. 4.14 depict the $R_f$ of labeled Reduced/hydrolysed and free $^{99m}$Tc. At low pH (3-5) the labeling efficiency was minimum (80%), while at pH 6-7 labeling efficiency of $^{99m}$Tc-clindamycin was $>95\%$ (Fig. 4.15). In basic media at pH 9 the labeling efficiency decreased to 68%. The amount of reducing agent, SnCl$_2$.2H$_2$O with the highest labeling efficiency, was 2.5-3 µg and a value of 2.8 µg of SnCl$_2$.2H$_2$O was chosen (Fig. 4.16). The amount of ligand having the highest labeling efficiency was 100 µg as shown in (Fig. 4.17). The maximum complexation of $^{99m}$Tc with clindamycin is achieved after 30 minutes and maintained for up to 12 hours (Fig. 4.18). The radiochemical purity was assessed by ascending paper chromatography and instant thin-layer chromatography on silica gel. In paper chromatography using acetone as the solvent for free $^{99m}$TcO$_4^-$ while ITLC-SG chromatography using 0.5M NaOH as the solvent for $^{99m}$Tc-clindamycin and reduced/hydrolyzed $^{99m}$Tc. In current study, clindamycin was labeled with $^{99m}$Tc with high radiochemical yields. During the labeling of clindamycin < 2% colloid and < 2% free pertechnetate were observed. Results obtained by this method were in excellent agreement with $^{99m}$Tc-cefuroxime in which 95% stability was seen in labeled kit (Chattopadhyay et al., 2012).
4.2.2. Stability test

$^{99m}$Tc-clindamycin was fully stable in human serum during incubation as determined by ITLC. At 37°C upto 92% labeling was found at 24 hours of incubation and there was almost no increase in reduced/hydrolyzed $^{99m}$Tc while very little increase was observed in free $^{99m}$TcO$_4^-$ . The total impurities were <5% (Table 4.4).

4.2.3. Electrophoresis and HPLC

The electrophoresis results illustrate the neutral nature of ligand and the movement of free pertechnetate towards anode after 1 hour (Fig. 4.19). The lipophilicity test indicates that $^{99m}$Tc-clindamycin is >99% in organic layer and <1% in aqueous layer hence proving the lipophilic nature of $^{99m}$Tc-clindamycin. Clindamycin contains a basic pyrollidene ring attached to a sugar group through an amide bond. The replacement of the hydroxyl group in lincomycin to a chloride atom increases the lipophilicity, and therefore clindamycin show better absorption and penetration into bacterial cells. The radiochromatogram of $^{99m}$Tc-clindamycin is given in Fig. 4.20 which shows a very sharp peak at 8.5 min of retention. This signal at 8.5 min represents the $^{99m}$Tc-clindamycin labeling efficiency greater than 95%. The HPLC analysis by UV detector (inactive ligand) show percent purity of clarithromycin (Fig. 4.21).

4.2.4. In Vitro Binding Studies

In vitro binding of $^{99m}$Tc-clindamycin to bacteria’s was compared to $^{99m}$Tc-ascorbic acid. Binding of $^{99m}$Tc-clindamycin was in the range of 95-98% (Table 4.5), while binding of $^{99m}$Tc-ascorbic acid was <5% (Table 4.6). Different amounts of $^{99m}$Tc-clindamycin (10-100 µg) showed similar binding efficiency with bacteria. In vitro studies and animal experiments have shown that $^{99m}$Tc-clindamycin localizes in bacteria infected sites significantly. Due to the ease of $^{99m}$Tc-
clindamycin preparation and infection uptake, it may provide an alternative to $^{99m}$Tc-ciprofloxacin in a variety of patients referred for infection evaluation (Ugur et al., 2006; Laken et al., 2000).

4.2.5. $^{99m}$Tc-clindamycin Biodistribution and Scintigraphic Images

The biodistribution results (%injected activity/g) of $^{99m}$Tc-clindamycin in different organs of the animals infected with living, heat killed S.aureus and turpentine oil induced, 1, 4 and 24 hours after intravenous administration are shown in Table 4.7. The results show that the $^{99m}$Tc-clindamycin accumulates significantly high at the infected thigh muscle as compared to heat killed S.aureus and turpentine oil infected group of animals. Our studies in rats with intramuscular infection indicated that the uptake in the infected tissue is attributed to specific binding to living bacteria. Thus in this study we can establish the basis for the potential of $^{99m}$Tc-clindamycin to distinguish bacterial from non-bacterial infection. As shown in Table 4.7, rats with infectious lesions injected with $^{99m}$Tc-clindamycin showed a mean target-to-non target (T/NT) ratio equal to 3.1 ± 0.3, after 1h post injection, 2.56 ± 0.2 at 2h post injection and 2.37 ± 0.5 at 24h post injection. $^{99m}$Tc-clindamycin shows higher T/NT in the infected muscle (live S.aureus) at all-time intervals than that of sterile inflamed muscle (heat killed S.aureus and turpentine). This $^{99m}$Tc-clindamycin showed slightly lower uptake in infected tissue than $^{99m}$Tc-ciprofloxacin (T/NT = 3.8 ± 0.8) (Chattopadhyay et al., 2012; Kleisner et al., 2002). The mean abscess-to-muscle (T/NT) ratio for $^{99m}$Tc-clindamycin was higher than that of other recently published $^{99m}$Tc-labeled antibiotics such N-sulfanilamide (T/NT = 2.9 ± 0.1) (Rien et al., 2004), and streptomycin (T/NT = 2.4 ± 0.1) (Imen et al., 2010). Our studies in rats with intramuscular infection indicated that the uptake in the infected tissue is attributed to specific binding to living bacteria. Thus in this study we can establish the basis for the potential of $^{99m}$Tc-clindamycin to
distinguish bacterial from non-bacterial infection. The infection is clearly visible at 3 hours post administration. The uptake of radioactivity in infected thigh muscle (target) at intervals of 1, 4, and 24 hours post injection were $1.94 \pm 0.3$, $2.38 \pm 0.2$, and $1.99 \pm 1.2$ respectively, and those of normal (non-target) were $0.61 \pm 0.3$, $0.93 \pm 0.2$, and $0.84 \pm 0.2$ respectively indicating higher binding affinity to the \textit{S. aureus} induced infection (Table. 4.7). Rats with infectious lesions injected with $^{99m}$Tc-clindamycin showed a good target-to-non target ratio, which are in accordance with the results obtained from the \textit{in vitro} binding assay. Whole body images of infected rabbit at 1 hour, 4 hours, and 24 hours after $^{99m}$Tc-clindamycin administration are presented in Fig. 4.22 A, B, and C respectively. \textit{S. aureus} infection was induced in rabbit left thigh and the area was seen with increased tracer accumulation after injection of labeled clindamycin in as shown in Fig. 4.23. The rapid uptake seen on images at 1 hour, 3 hours and 4 hours most likely is due to the physiologic changes at the site of infection.
Fig. 4.12: Structure of clindamycin.

Fig. 4.13: Paper chromatography pattern of the $^{99m}$Tc-clindamycin, free, reduced/hydrolyzed $^{99m}$Tc.
Fig. 4.14: ITLC-SG chromatography pattern of the $^{99m}$Tc-clindamycin, free, reduced/hydrolyzed$^{99m}$Tc.

Fig. 4.15: The effect of pH on labeling efficiency of $^{99m}$Tc-clindamycin
**Fig. 4.16**: The effect of amount of stannous chloride on labeling efficiency of $^{99m}$Tc-clindamycin

**Fig. 4.17**: Effect of amount of clindamycin on labeling efficiency of $^{99m}$Tc-clindamycin
Fig. 4.18: Stability study of the $^{99m}$Tc-clindamycin at room temperature.

Fig. 4.19: Electrophoresis result of $^{99m}$Tc-clindamycin
Fig. 4.20: HPLC analysis illustrates the percentage labeling of clindamycin with Tc-99m

Fig. 4.21: HPLC analysis (UV detector) showing the purity of Clindamycin
Fig. 4.22: Whole body gamma camera image of rabbit injection with $^{99m}$Tc-clindamycin at 1-hour post administration A, at 4-hours post administration B, and 24-hours post administration C.

Fig. 4.23: *S. aureus* infection in rabbit left thigh and right thigh was visualized as area of increased tracer accumulation 3 hours after post injection of $^{99m}$Tc-clindamycin.
Table 4.4: *In vitro* stability of $^{99m}$Tc-clindamycin in normal human serum

<table>
<thead>
<tr>
<th>Incubation time, h</th>
<th>$^{99m}$Tc-Clindamycin</th>
<th>Free pertechnetate</th>
<th>Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>97.3 ± 1.8</td>
<td>0.0</td>
<td>2.66 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>97.8 ± 2.2</td>
<td>0.8 ± 0.6</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>97.7 ± 2.1</td>
<td>0.6 ± 0.8</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>97.6 ± 2.0</td>
<td>0.4 ± 1.1</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>24</td>
<td>97.0 ± 1.9</td>
<td>0.8 ± 1.4</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
</table>

Table 4.5: *In vitro* binding of the $^{99m}$Tc-clindamycin to viable *S. aureus*

<table>
<thead>
<tr>
<th>$^{99m}$Tc-Clindamycin</th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hour</td>
</tr>
<tr>
<td>10 µg</td>
<td>96.23</td>
</tr>
<tr>
<td>50 µg</td>
<td>95.29</td>
</tr>
<tr>
<td>100 µg</td>
<td>89.84</td>
</tr>
</tbody>
</table>

Table 4.6: *In vitro* binding of the $^{99m}$Tc-ascorbic Acid to viable *S. aureus*

<table>
<thead>
<tr>
<th>$^{99m}$Tc-Ascorbic acid</th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hour</td>
</tr>
<tr>
<td>10 µg</td>
<td>0.13</td>
</tr>
<tr>
<td>50 µg</td>
<td>0.32</td>
</tr>
<tr>
<td>100 µg</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Table 4.7: Biodistribution of $^{99m}$Tc-clindamycin in live *S. aureus*, heat killed *S. aureus* and turpentine oil inflamed rats at different time intervals (means ± SD), (%ID/g).

<table>
<thead>
<tr>
<th>Organs</th>
<th>Percentage of injected dose per gram of tissue weight (n=3/time interval, iv) $^1$</th>
<th>Live <em>S. aureus</em></th>
<th>Heat killed <em>S. aureus</em></th>
<th>Turpentine oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
<td>24h</td>
<td>1h</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.87±0.8</td>
<td>2.41±0.2</td>
<td>1.12±0.6</td>
<td>5.76±0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.43±0.4</td>
<td>0.53±0.6</td>
<td>0.39±0.1</td>
<td>3.40±0.2</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.05±0.6</td>
<td>2.23±0.4</td>
<td>0.32±0.4</td>
<td>4.15±0.3</td>
</tr>
<tr>
<td>Intestine</td>
<td>3.62±0.9</td>
<td>1.05±0.8</td>
<td>0.95±0.8</td>
<td>3.59±0.12</td>
</tr>
<tr>
<td>Lungs</td>
<td>7.10±1.1</td>
<td>4.54±0.7</td>
<td>1.68±0.9</td>
<td>7.05±1.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.26±2.4</td>
<td>8.71±0.9</td>
<td>2.70±0.5</td>
<td>12.40±2.3</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.76±0.2</td>
<td>10.32±3.5</td>
<td>15.55±4.6</td>
<td>0.61±1.2</td>
</tr>
<tr>
<td>Heart</td>
<td>6.88±0.7</td>
<td>3.68±0.5</td>
<td>0.93±0.3</td>
<td>6.16±0.1</td>
</tr>
<tr>
<td>Brain</td>
<td>0.51±0.4</td>
<td>0.35±0.2</td>
<td>0.19±0.1</td>
<td>0.46±0.1</td>
</tr>
<tr>
<td>Blood</td>
<td>6.34±1.6</td>
<td>3.66±1.1</td>
<td>1.35±0.9</td>
<td>6.46±1.0</td>
</tr>
<tr>
<td>Body</td>
<td>1.75±0.5</td>
<td>1.13±0.8</td>
<td>0.89±0.2</td>
<td>1.79±0.2</td>
</tr>
<tr>
<td>Inflamed muscle</td>
<td>1.94±0.3</td>
<td>2.38±0.2</td>
<td>1.99±1.2</td>
<td>1.15±0.4</td>
</tr>
<tr>
<td>Control muscle</td>
<td>0.61±0.3</td>
<td>0.95±0.2</td>
<td>0.84±0.2</td>
<td>0.69±0.5</td>
</tr>
</tbody>
</table>

$^1$values represent the mean± standard deviation of data from 3 animals.
4.3. $^{99m}$Tc-vibramycin

The vibramycin (Fig. 4.24) was labeled with Technetium-99m and labeling efficiency of 95% was achieved (Fig. 4.25, 4.26). The effect of pH is shown in (Fig. 4.27). At pH 2 the minimum labeling efficiency was achieved (80%), while at pH 3-4 the labeling efficiency of $^{99m}$Tc-vibramycin was increased to > 95%. In basic media (pH 8) the labeling efficiency was decreased to 68%. A known quantity of 2-3 µg of reducing agent, SnCl$_2$.2H$_2$O gave the highest labeling efficiency, and thus the value of 2.5 µg of SnCl$_2$.2H$_2$O was chosen for further procedures (Fig. 4.28). The highest labeling efficiency at 200 µg of ligand was achieved (Fig. 4.29). The complexation of $^{99m}$Tc with vibramycin is achieved after about 30 min and retained for up to 12 h (Fig. 4.30). The radiochemical purity was assessed by a combination of ascending paper chromatography and instant thin-layer chromatography on a silica gel. In paper chromatography acetone was used as solvent for free $^{99m}$TcO$_4^-$ while ITLC-SG chromatography 0.5M NaOH was the solvent used for $^{99m}$Tc-vibramycin and reduced/hydrolyzed $^{99m}$Tc. After following above mentioned procedures results were in excellent agreement. In this study, vibramycin was labeled with $^{99m}$Tc with high radiochemical yields. During the labeling of vibramycin <2% colloid and <2% free pertechnetate were observed. The stability of radiopharmaceutical was checked by its incubation in human serum. $^{99m}$Tc-vibramycin was found to be fully stable as determined by ITLC. Up to 98% labeling was found at 24 hours of incubation at 37°C and there was almost no increase in reduced/hydrolyzed $^{99m}$Tc, while very little increase was observed in free pertechnetate. The total impurities were <2% (Table 4.8). The results of electrophoresis illustrated neutral behavior of the ligand (Fig. 4.31). In vitro binding of $^{99m}$Tc-vibramycin to bacteria was compared to $^{99m}$Tc-Ascorbic Acid. Binding of varying amounts of $^{99m}$Tc-vibramycin with S. aureus was in the range of 97-99% (Fig. 4.32), while binding of $^{99m}$Tc-
ascorbic acid was <5% (Table 4.9). Significant uptake of $^{99m}$Tc-vibramycin was observed in liver, stomach, lungs and heart during biodistribution studies. *In vivo* stability of $^{99m}$Tc-vibramycin was noticed in body since stomach, lungs and intestine showed significant activity. The biodistribution results (%injected activity/g) of $^{99m}$Tc-vibramycin in different organs of the animals infected with living, heat killed *S.aureus* and turpentine oil induced, 1, 4 and 24 hours after intravenous administration are shown in Table 4.10. The results show that the $^{99m}$Tc-vibramycin accumulates significantly high at the infected thigh muscle as compared to heat killed *S.aureus* and turpentine oil infected group of animals. Our studies in rats with intramuscular infection indicated that the uptake in the infected tissue is attributed to specific binding to living bacteria. Whole body images of infected rabbits at 1, 4, and 24 h post $^{99m}$Tc-vibramycin administration are presented in Fig. 4.33a, 4.33b, and 4.33c respectively. *S. aureus* infection in rabbit left thigh was visualized as the area of increased tracer accumulation just after injecting labeled vibramycin. The infection is clearly visible at 3 hours post administration. The rats with infectious lesions injected with $^{99m}$Tc-vibramycin showed a mean target-to-non target (T/NT) ratio equal to 2.6 ± 0.3, after 1 h post injection (Table 4.10). $^{99m}$Tc-vibramycin shows higher T/NT in the infected muscle (live *S.aureus*) at all-time intervals than that of sterile inflamed muscle (heat killed *S.aureus* and turpentine oil). This $^{99m}$Tc-vibramycin showed higher uptake in infected tissue than $^{99m}$Tc-streptomycin (T/NT = 2.4 ± 0.1). The high T/NT ratio for the live *S. aureus* model as compared to turpentine, Irradiated and heat killed *S. aureus* models provides evidence that $^{99m}$Tc-vibramycin accumulated at the infectious site due to its specific binding to bacterial cells. Thus in this study we can establish the basis for the potential of $^{99m}$Tc-vibramycin to distinguish bacterial from non-bacterial infection.
Fig. 4.24: Structure of vibramycin

Fig. 4.25: Paper chromatography pattern of the $^{99m}\text{Tc}$-vibramycin, free, reduced/hydrolyzed $^{99m}\text{Tc}$. 
Fig. 4.26: ITLC-SG chromatography pattern of the $^{99m}$Tc-vibramycin, free, reduced/hydrolyzed $^{99m}$Tc.

Fig. 4.27: Effect of pH on labeling Efficiency of $^{99m}$Tc-vibramycin
Fig. 4.28: Effect of amount of stannous chloride dihydrate on labeling efficiency of $^{99m}$Tc-vibramycin

Fig. 4.29: Effect of amount of ligand on labeling efficiency of $^{99m}$Tc-vibramycin
Fig. 4.30: The Rate of complexation and stability of $^{99m}\text{Tc}$-vibramycin at room temperature.

Fig. 4.31: Electrophoresis of radiolabeled compound ($^{99m}\text{Tc}$-vibramycin at 0cm; $^{99m}\text{TcO}_4^-$ at 10cm).
Fig. 4.32: *In vitro* binding of the $^{99m}$Tc-vibramycin to viable *Staphylococcus aureus*

Fig. 4.33: Whole body gamma camera image of rabbit injection with $^{99m}$Tc-vibramycin at 1-h post administration (a), at 4-h post administration (b), and 24-h post administration (c).
Table 4.8: *In vitro* stability of $^{99m}$Tc-vibramycin in normal human serum

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>$^{99m}$Tc-vibramycin</th>
<th>Free pertechnetate</th>
<th>Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>99.9 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>99.8 ± 0.2</td>
<td>0.2 ± 0.01</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>99.7 ± 0.3</td>
<td>0.3 ± 0.03</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>99.6 ± 0.4</td>
<td>0.4 ± 0.04</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>99.5 ± 0.5</td>
<td>0.5 ± 0.06</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 4.9: *In vitro* binding of the $^{99m}$Tc-ascorbic acid to viable *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>$^{99m}$Tc-ascorbic acid</th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>10 µg</td>
<td>0.25</td>
</tr>
<tr>
<td>50 µg</td>
<td>0.39</td>
</tr>
<tr>
<td>100 µg</td>
<td>4.7</td>
</tr>
</tbody>
</table>
Table 4.10: Biodistribution of $^{99m}$Tc-vibramycin in live *S.aureus*, heat killed *S.aureus*, irradiated *S.aureus* and turpentine oil inflamed rats at at different time intervals (means ± SD), (%ID/g).

<table>
<thead>
<tr>
<th>Organs</th>
<th>Percentage of injected dose per gram of tissue weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live <em>S.aureus</em></td>
</tr>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Liver</td>
<td>4.60 ± 0.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.20 ± 0.6</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.19 ± 0.2</td>
</tr>
<tr>
<td>Intestine</td>
<td>4.28 ± 1.1</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.29 ± 0.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.64 ± 0.1</td>
</tr>
<tr>
<td>Bladder</td>
<td>7.28 ± 2.3</td>
</tr>
<tr>
<td>Heart</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>Brain</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Blood</td>
<td>5.46 ± 1.5</td>
</tr>
<tr>
<td>Body</td>
<td>0.91 ± 0.3</td>
</tr>
<tr>
<td>Inflamed</td>
<td>0.66 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>0.25 ± 0.1</td>
</tr>
</tbody>
</table>

1 values represent the mean± standard deviation of data from 3 animals
4.4. $^{99m}$Tc-cercopin A

4.4.1. Preparation and Quality control

We have developed a simple, rapid and efficient method of labeling cecropin A with Technetium-99m using special antibacterial property of cecropin A and the marking property of $^{99m}$Tc to produce a complex for treatment as well as imaging purpose. Cecropin A is a potent macrolide antibiotic employed for the treatment of different bacterial diseases. The amino acid sequence of cecropin A is (KWKLFKKIEKVGQNIIRDGIKAGPAVAVGQATQIAK). The radiolabeling of cecropin A with $^{99m}$Tc resulted 95% radiolabeling yield. To determine the optimal conditions to gain the highest labeling yield with the maximum stability, the reaction was repeated at different ranges of pH and various amounts of cecropin A and SnCl$_2$.2H$_2$O. The effect of amount of SnCl$_2$.2H$_2$O as reducing agent with tartaric acid on the labeling efficiency of cecropin A with Tc-99m is shown in Fig. 4.34. The maximum labeling yield of $^{99m}$Tc-cecropin A was observed at 20 µg and as the amount of reducing agent was increased the labeling efficiency reduced. In order to determine the most appropriate pH on the labeling efficiency, the labeling of $^{99m}$Tc-cecropin A was carried out at different pH in range from 3-11. The complexation rate of cecropin A with Tc-99m was maximum (~95%) at pH 7 (Fig. 4.35). After boiling for 30min the maximum labeling of cecropin A with $^{99m}$Tc is achieved and maintained for up to 12 hours (Fig. 4.36). The influence of amount of ligand having the highest labeling efficiency was checked by labeling of varying amounts of cecropin A with $^{99m}$Tc and the amount of 200 µg ligand was observed to show high yield of labeling as shown in (Fig. 4.37). The results of optimization of affecting factors on radiolabeling showed that at reaction condition of 200 µg cecropin A, 20 µL SnCl$_2$.2H$_2$O+tartaric acid at pH=7, the labeling yield of cecropin A reached 95%. Results showed that labeling of $^{99m}$Tc-cecropin A is rapid and effective with a high yield (95%) with
good stability. The labeling efficiency of $^{99m}$Tc-cecropin A was evaluated by instant thin-layer chromatography and ascending paper chromatography on silica gel. In ITLC-SG chromatography 0.5M NaOH was used as the solvent for $^{99m}$Tc-cecropin A and reduced/hydrolyzed $^{99m}$Tc, while paper chromatography was performed using acetone as the solvent for free $^{99m}$TcO$_4^-$.

4.4.2. In vitro binding with E.coli

*In vitro* binding of $^{99m}$Tc-cecropin A was checked with *E.coli* which was in the range of 65-70% as represented in Table 4.11. The maximum binding of $^{99m}$Tc-cecropin A with *E.coli* at 10µg showed while as the amount was increased to 100µg binding efficiency was decreased. *In vitro* studies and animal experiments have shown that $^{99m}$Tc-cecropin A localizes in bacteria infected sites significantly. The results of human plasma binding study indicated that the blood serum enzymes were unable to degrade the $^{99m}$Tc-cecropin A within 24 hours and $^{99m}$Tc-cecropin A can bind with bacterial DNA gyrase before blood protease degradation. From the present results pertains to our method of labeling peptides with technetium-99m for imaging of infections. The results showed that labeling of cecropin A is rapid and effective, i.e. a high (95%) yield already at 10 mins after reaction, and safe, as assessed with an in vitro killing assay. In addition, the radiochemical purity (little free radionuclide or colloid formation) and stability of the radiolabeled peptides in diluted human serum are excellent. *In vitro* Incubation of $^{99m}$Tc-cecropin A with the bacterial strain *E.coli* has demonstrated a high bacterial retention of tracer. About >70% of the total activity was found in the bacterial pellet of *E.coli* after incubation with $^{99m}$Tc-cecropin A in a fresh bacterial culture.
4.4.3. Stability in human serum and cysteine challenge

$^{99m}$Tc-cecropin A was completely stable in human serum during incubation as determined by Instant thin layer chromatography (ITLC). It was found that 85% labeling was found at 24 hours of incubation (Table 4.12). The stability of the $^{99m}$Tc-cecropin A bond was analyzed by cysteine challenge test which showed that dissociation of 47% took place at cysteine ratio of 500:1 (Fig. 4.38).

4.4.4. Biodistribution and Scintigraphy

The in vivo uptake of $^{99m}$Tc-cecropin A (%injected activity/g) in different organs of the mice infected with living E. coli, heat killed E. coli and turpentine oil induced at 1h, 4h and 24h after intravenous administration is shown in Table 4.13. Mice with infectious lesions (live E. coli) injected with $^{99m}$Tc-cecropin A showed a mean target-to-non target (T/NT) ratio equal to 2.98±0.5 at 1h post injection, 1.92±0.01 at 2h post injection, 0.71±0.1 at 24h post injection. $^{99m}$Tc-cecropin A shows higher T/NT in the infected muscle (live E. coli) at all-time intervals than that of sterile inflamed muscle (heat killed E. coli and turpentine oil) (Table 4.13). Mice having infected sites injected with $^{99m}$Tc-cecropin A showed a good target-to-non target ratio, which are in accordance with the results obtained from the in vitro binding assay. The greater activity of $^{99m}$Tc-cecropin A in bacterial infection sites than inflammation sites can be attributed to specific binding to living bacteria in mice. The $^{99m}$Tc-cecropin A was removed from the circulation mainly through the kidneys. Whole body gamma camera images of infection induced rabbits in left thigh at 1 hour post- administration of $^{99m}$Tc-cecropin A are presented in Figure 4.39. The rapid uptake is most likely due to the physiologic changes at the site of infection. The infected area was seen with increased tracer accumulation after injection of technetium-99m labeled cecropin A proving it a potential infection diagnostic agent.
Fig. 4.34: The effect of amount of SnCl$_2$.2H$_2$O on radiochemical yield of $^{99m}$Tc-cecropin A (200µg Cecropin A, pH=7, 20 minutes)

Fig. 4.35: Effect of pH on radiochemical yield of $^{99m}$Tc-cecropin A (200µg cecropin A, 20µg SnCl$_2$.2H$_2$O, 20 minutes)
Fig. 4.36: Effect of reaction time and stability of $^{99m}$Tc-cecropin A at room temperature

(200µg Cecropin A, 20µg SnCl$_2$.2H$_2$O, pH=7)

Fig. 4.37: Effect of Cecropin A amount on radiochemical yield of $^{99m}$Tc-cecropin A (20µg SnCl$_2$.2H$_2$O, pH=7, 20 minutes)
**Fig. 4.38:** Cysteine challenge of $^{99m}$Tc-ecropin A

**Fig 4.39:** Whole body gamma camera image of infected rabbit with $^{99m}$Tc-ecropin A at 1h post administration.
**Table 4.11:** *In vitro* binding of the $^{99m}$Tc-ecropin A to live *E.coli*

<table>
<thead>
<tr>
<th>$^{99m}$Tc-ecropin A</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hour</td>
</tr>
<tr>
<td>10 µg</td>
<td>65.5</td>
</tr>
<tr>
<td>50 µg</td>
<td>70.7</td>
</tr>
<tr>
<td>100 µg</td>
<td>62.2</td>
</tr>
</tbody>
</table>

**Table 4.12:** *In vitro* stability of $^{99m}$Tc-ecropin A in normal human serum

<table>
<thead>
<tr>
<th>Incubation time, h</th>
<th>$^{99m}$Tc-ecropin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.1 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>90.7 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>90.1 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>85.0 ± 0.8</td>
</tr>
</tbody>
</table>
Table 4.13: Biodistribution of $^{99m}$Tc-cecropin A (%ID/g) in live E.coli, heat killed E.coli and turpentine oil inflammed mice at different time intervals (means ± SD)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Percentage of injected dose per gram of tissue weight</th>
<th>(n = 3/time, interval, iv)$^1$</th>
<th>Live E.coli</th>
<th>Heat Killed E.coli</th>
<th>Turpentine oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1h</td>
<td>4h</td>
<td>24h</td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
<td></td>
<td>2.66±0.8</td>
<td>2.99±0.1</td>
<td>1.57±0.2</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td>35.4±0.2</td>
<td>20.4±1.0</td>
<td>6.16±0.1</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td>3.9±0.9</td>
<td>4.66±0.19</td>
<td>3.35±0.4</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td>4.28±0.6</td>
<td>3.84±0.10</td>
<td>0.19±0.7</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
<td>7.53±1.2</td>
<td>7.99±2.3</td>
<td>4.57±0.9</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td>2.32±0.1</td>
<td>1.57±0.9</td>
<td>1.59±0.2</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td>3.88±0.6</td>
<td>4.72±0.25</td>
<td>0.32±0.7</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td>0.02±0.1</td>
<td>0.03±0.9</td>
<td>0.05±0.4</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td></td>
<td>6.07±1.0</td>
<td>4.0±0.5</td>
<td>3.55±0.8</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
<td>5.08±1.8</td>
<td>6.51±1.06</td>
<td>2.07±0.8</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td>0.17±0.3</td>
<td>0.11±0.5</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>Control muscle</td>
<td></td>
<td></td>
<td>0.57±0.2</td>
<td>0.52±0.02</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Target muscle</td>
<td></td>
<td></td>
<td>1.7±0.05</td>
<td>1.0±0.04</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>T/NT</td>
<td></td>
<td></td>
<td>2.98±0.5</td>
<td>1.92±0.01</td>
<td>0.71±0.1</td>
</tr>
</tbody>
</table>

$^1$values represent the mean± standard deviation of data from 3 animals
4.3. $^{99m}$Tc-epirubicin

4.5.1. Structure and labeling of epirubicin with Tc-99m

The design of radiopharmaceuticals to trace biochemical and physiological processes in small animal models are very important for preclinical radiobiological studies. The central emphasis of this work was to design and develop new $^{99m}$Tc-labeled chemotherapeutic drug epirubicin which can specifically accumulate in cancer cells performing the function of imaging as well as chemotherapy. Radiochemical stability of radiopharmaceuticals is crucial for their effective and safe use in the diagnosis and therapy of disease. The current study was done to assess the potential of chemotherapeutic drug epirubicin with Tc-99m for diagnostic purpose in normal and tumor-bearing animal models. The structure of Epirubicin is given in Fig. 4.40.

The factors affecting the labeling yield were accessed. The effect of amount of reducing agent is summarized in Fig. 4.41. The highest labeling efficiency was achieved at 35 µg of SnCl$_2$.2H$_2$O. The labeling was sufficiently increased by the increase in amount of SnCl$_2$.2H$_2$O from 10 to 35 µg and then decreased as the amount of reducing agent was further increased.

The influence of pH on labeling yield of $^{99m}$Tc-epirubicin is displayed in Fig. 4.42. At low pH (2-5) the labeling efficiency was to 70-73%, while at pH 6 the labeling efficiency of $^{99m}$Tc-epirubicin is 99%. In basic media 9 the labeling efficiency was decreased to a small extent. Hence further experiments were performed at pH 6. The complexation of $^{99m}$Tc with epirubicin is quite rapid and maximum labeling efficiency is achieved within minutes. The amount of ligand has no effect on labeling efficiency for upto 300µg but as the amount is increased significantly beyond this limit the labeling efficiency was decreased (Fig. 4.43) thus 200µg of $^{99m}$Tc-epirubicin was fixed to obtain the maximum labeling yield. As far as incubation time is
concerned, ~95% labeling took place in first 10 minutes which became maximum at 30 minutes (Fig 4.44). So, by optimizing all the factors affecting the labeling yield of $^{99m}$Tc-epirubicin beginning from amount of epirubicin, pH, amount of SnCl$_2$.2H$_2$O, and incubation time, it was found that the 99% labeling efficiency was acheived with 200µg epirubicin and 35µg SnCl$_2$.2H$_2$O at pH 6 after 30 minutes retention time.

Labeling efficiency was assessed by paper chromatography using acetone as the solvent, free technetium pertechnetate ($^{99m}$TcO$_4^-$) moved towards the solvent front (Rf= 1), while $^{99m}$Tc-epirubicin and reduced/hydrolyzed technetium-99m remained at the origin. In ITLC-SG chromatography using THF as solvent, reduced/hydrolyzed technetium-99m remained at the origin, whereas $^{99m}$Tc-epirubicin and free technetium pertechnetate moved towards the solvent front. The electrophoresis of $^{99m}$Tc-epirubicin shows the neutral nature of compound (Fig. 4.45). High performance liquid chromatographic results of inactive ligands demonstrate that purity of ligand was 90%. The HPLC analysis of $^{99m}$Tc-epirubicin demonstrates that about 99% of the compound binds with technetium-99m as represented in Fig.4.46 and 4.47.

4.5.2. Stability of $^{99m}$Tc-epirubicin at room temperature

*In vitro* stability of $^{99m}$Tc-epirubicin at room temperature at 10m, 30m, 1h, 2h, 4h and 5h is represented in Fig. 4.44. These results indicated that the labeled complex stayed stable for about 5 hours after labeling at room temperature. As far as stability is concerned, epirubicin remained intact with the radiometal in complex. The $^{99m}$Tc-epirubicin retained 99% labeling efficiency after 6 hours and hence could be considered quite stable and suitable for *in vivo* use for at least 6 h following the preparation.
4.5.3. Stability of $^{99m}$Tc-epirubicin in normal human serum

When $^{99m}$Tc-epirubicin was incubated at 25ºC with normal human serum, it remained sufficiently stable up to 4 hours. After incubation of 4 hours, the total impurities were about 12% indicating that enzymes present in the blood serum were not able to degrade the $^{99m}$Tc-epirubicin up to 4 hours of incubation period (Fig. 4.48).

4.5.4. Biodistribution of $^{99m}$Tc-epirubicin in normal mice

The *in vivo* behavior of $^{99m}$Tc-epirubicin in normal mice illustrate that the large amount of drug accumulated in liver at all-time points after injection. The amount of radioactivity observed in spleen, liver, lungs and stomach. The $^{99m}$Tc-epirubicin revealed quick blood clearance, with only 0.96% ID/g at 30 min, followed by more decrease at 4 h. The activity found in spleen was 12.93±0.05, 3.57±0.05, 2.32±1.01 and 1.59±0.8 at 30 min, 1h, 4h and 24 h respectively (Table 4.11). $^{99m}$Tc-epirubicin was washed out from kidney after 1 hour but in liver significant amount remained even after 24 hours of post-administration in normal mice. $^{99m}$Tc-epirubicin was delivered to the liver and kidneys because of high levels of blood flow through these organs. The retention of $^{99m}$Tc-epirubicin in liver might be due to its metabolism in liver. The high amount of radioactivity observed in liver may also be contributed to its hepatobiliary route of excretion. Stomach and spleen showed a high uptake of 3.54±0.2 and 3.57±0.05 ID%/g at 4 h of post administration respectively. Some organs showed significant decrease of $^{99m}$Tc-epirubicin at 1 h post injection like lung, kidneys, heart and stomach. The dissimilarities of the physiological processes between the tumor and normal cells allow understanding the differentiation of both types of tissues (Schottelius & Wester. 2009; Barros *et al.*, 2010)
4.5.5. Biodistribution of $^{99m}$Tc-epirubicin in tumor bearing mice

The biodistribution analysis of $^{99m}$Tc-epirubicin in mammary tumor bearing mice showed significant localization in tumor sites. The activity observed at tumor sites was $19.03\pm0.7$, $18.12\pm0.1$, $18.99\pm0.2$, $7.06\pm0.05$ at 30 min, 1 h, 4 h and 24 h post administration which was higher than that in the brain, heart, spleen, stomach and lung (Table 4.15). In tumor bearing mice, $^{99m}$Tc-epirubicin showed very high uptake in tumor sites and can be compared to $^{99m}$Tc-labelled 5-fluorouracil (Dar et al., 2013) and 5,10,15,20-tetrakis[4-(carboxymethyleneoxy)phenyl] porphyrin (T4CPP) which accumulated selectively in tumors of mammary bearing rats (Chatterjee et al., 1997) and 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)phenyl] porphyrin (T3,4BCPP) complexes in mice bearing abdominal sarcoma (Shetty et al., 1996). The chemotherapy dose of epirubicin resulting myocardial toxicity causing heart failure is typically 550 mg/m$^2$ and the risk increases quickly with increasing doses of epirubicin to 900 mg/m$^2$ (Waters et al., 1999; Cunningham et al., 1999). The therapeutic efficacy of epirubicin is similar to doxorubicin at equimolar doses, but epirubicin has a more favorable hematologic and non-hematologic toxicity profile, especially about cardiotoxicity (Findlay et al., 2007). The amount of $^{99m}$Tc-epirubicin administered for imaging purposes is 200 μg and is very low as compared to chemotherapy doses. The amounts of $^{99m}$Tc-epirubicin can be reduced further by the use of higher activities of $^{99m}$Tc during labeling process.
4.5.6. Imaging of $^{99m}$Tc-epirubicin in tumor bearing mice

Scintigraphic imaging have played an important role in cancer diagnosis and determining response to treatment. The results of imaging showed that $^{99m}$Tc-epirubicin accumulated significantly in tumor sites of Swiss Webster mice bearing naturally developed tumors (Fig. 4.49) which is in accordance to results obtained by biodistribution study. In tumor bearing mice the Scintigraphic imaging performed with $^{99m}$Tc-epirubicin indicated good visualization of the tumors from 30 min to 4 h after administration showing good stability \textit{in vivo}. Thus biodistribution and scintigraphy of $^{99m}$Tc-epirubicin in mice bearing naturally developed tumors shows high efficacy of the prepared compound for diagnostic purpose.
Fig. 4.40: Epirubicin chemical structure

Fig. 4.41: Effect of amount of stannous chloride on percent labeling of $^{99m}$Tc-epirubicin
Fig. 4.42: Effect of pH of reaction medium on labeling yield of $^{99m}$Tc-epirubicin

Fig. 4.43: Percent labeling yield of $^{99m}$Tc-epirubicin as a function of amount of epirubicin
Fig. 4.44: $^{99m}$Tc-epirubicin percent labeling yield according to reaction time at room temperature

Fig. 4.45: Electrophoresis result of $^{99m}$Tc-epirubicin
Fig. 4.46: HPLC analysis illustrates the percentage purity of epirubicin.

Fig. 4.47: HPLC study showing the percentage labeling of epirubicin with $^{99m}$Tc.
**Fig.4.48:** Stability of $^{99m}$Tc-epirubicin in normal human serum at different time intervals.

**Fig.4.49:** Scintigraphic images of tumor bearing mice injected with $^{99m}$Tc-epirubicin at 30 min, 1 hour, 2 hour and 4 hours of Post administration.
Table 4.14: Biodistribution of $^{99m}$Tc-epirubicin in normal Swiss albino mice at 30 min, 1h, 4h and 24h after intravenous post administration (%ID/g*).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percent Injected Dose per gram organ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.74±1.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.41±1.25</td>
</tr>
<tr>
<td>Liver</td>
<td>23.85±0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>5.73±0.9</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.54±0.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>12.93±0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>0.63±0.5</td>
</tr>
<tr>
<td>Blood</td>
<td>0.94±1.0</td>
</tr>
<tr>
<td>Brain</td>
<td>0.18±0.6</td>
</tr>
<tr>
<td>Carcass</td>
<td>9.38±0.2</td>
</tr>
<tr>
<td>Urine</td>
<td>0.05±0.9</td>
</tr>
<tr>
<td>Bladder</td>
<td>1.35±0.2</td>
</tr>
</tbody>
</table>

*All data presented in mean percentage (n = 3) of the injected dose of $^{99m}$Tc-epirubicin per gram tissue ± Standard deviation of the mean
Table 4.15: Biodistribution of $^{99m}$Tc-epirubicin at 30 min, 1h, 4h and 24h after intravenous post administration in Swiss Webster mice with mammary carcinoma (%ID/g$^*$).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percent Injected Dose per gram organ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.55±1.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.21±0.15</td>
</tr>
<tr>
<td>Liver</td>
<td>21.45±0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.9±0.9</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.44±0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>9.33±0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>0.66±0.3</td>
</tr>
<tr>
<td>Blood</td>
<td>2.64±1.0</td>
</tr>
<tr>
<td>Brain</td>
<td>0.28±0.5</td>
</tr>
<tr>
<td>Carcass</td>
<td>7.38±0.2</td>
</tr>
<tr>
<td>Urine</td>
<td>0.15±0.5</td>
</tr>
<tr>
<td>Bladder</td>
<td>1.35±0.2</td>
</tr>
<tr>
<td>Tumor</td>
<td>19.03±0.7</td>
</tr>
</tbody>
</table>

*All data presented in mean percentage (n = 3) of the injected dose of $^{99m}$Tc-epirubicin per gram tissue ± Standard deviation of the mean
4.6. $^{99m}$Tc-DOTA-lanreotide

Over the past decades, interest in the development of radio probes for detection of tumors has increased dramatically. The introduction of small peptides as carrier molecules for radionuclides has opened up new possibilities for development of diagnostic agents. Somatostatin analogues such as lanreotide were synthesized because the somatostatin peptide is susceptible to very rapid enzymatic degradation. The $^{99m}$Tc-DOTA-lanreotide is a cyclic peptide having the structure (DOTA-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$) as shown in Fig.4.50. The synthesis of bifunctional chelating agents and radiolabelling of the DOTA-lanreotide with Tc-99m was carried out to determine the best conditions ensuring high radiochemical yield, high purity, and stability of $^{99m}$Tc- DOTA-lanreotide. The radiolabeled peptide with technetium-99m must have specific receptor binding affinity and resistance against degradative enzymes. The examination of the effect of the different parameters on the labeling yield such as amount of DOTA-lanreotide, pH of the reaction mixture, amount of SnCl$_2$·2H$_2$O, and reaction time was studied.

The effects of pH are shown in Fig. 4.51. At low pH (3-5) the labeling efficiency was about 80%, while at pH 7 the labeling efficiency of $^{99m}$Tc- DOTA-Lanreotide was 96%. In basic media 7-8 the labeling efficiency was decreased significantly. Hence further experiments were performed at pH 7. The amount of reducing agent, SnCl$_2$.2H$_2$O, which gave the highest labeling efficiency, was 4µg and thus this amount of SnCl$_2$.2H$_2$O was chosen (Fig. 4.52) to avoid colloid formation.

The preparation of the variety of $^{99m}$Tc radiopharmaceuticals involves reduction of $^{99m}$Tc from 7+ to lower-valence state enabling its chelation by other compounds of diagnostic importance. The reduction of disulfide bridges is done by incubation of the ligand with stannous ions. The complexation of $^{99m}$Tc with 5 µg DOTA-Lanreotide at pH 7 with 4 µg of SnCl$_2$.2H$_2$O gave a maximum labeling efficiency of 96% within few minutes (Fig. 4.53). Labeling yield at different
time intervals 10 min, 30 min, 1 h, 2 h, 3 h and 4 h after the initiation of reaction came out to be 90% at initial 10 minutes and labeling of 96% was achieved at 30 minutes. Graphical representation of these results is depicted in Fig. 4.54. The final formulation for the radiotracer $^{99m}$Tc-DOTA-Lanreotide was: DOTA-Lanreotide 5 µg; SnCl$_2$.2H$_2$O 4µg; pH ~7; $^{99m}$Tc 222 MBq and reaction mixture volume 1.5 mL. The molar ratios of DOTA-Lanreotide to reducing agent were found to be 1:2.

Labeling efficiency, radiochemical purity and stability were assessed by a combination of ascending chromatography and instant thin layer chromatography impregnated with silica gel. In paper chromatography using acetone as the solvent, free $^{99m}$TcO$_4^-$ moved towards the solvent front (Rf = 1), while $^{99m}$Tc-DOTA-lanreotide and reduced/hydrolyzed $^{99m}$Tc remained at the point of spotting. In ITLC-SG chromatography using 0.5M NaOH as solvent, reduced/hydrolyzed $^{99m}$Tc remained at the point of spotting, whereas $^{99m}$Tc-DOTA-lanreotide and free $^{99m}$TcO$_4^-$ moved towards the solvent front. The electrophoresis illustrate that complex is neutral in nature (Fig. 4.55). HPLC results of inactive ligand illustrate that ligand is > 90% pure (Fig. 4.56). HPLC analysis of $^{99m}$Tc-DOTA-Lanreotide illustrate that ~95% $^{99m}$Tc binds with available ligand (Fig. 4.57).

When the preparation (labeled radiopharmaceutical) was incubated with normal human serum at 35ºC, little increase in free $^{99m}$TcO$_4^-$ and reduced/hydrolyzed $^{99m}$Tc was seen up to 24 hours. The total impurities were found to be < 5%. $^{99m}$Tc-DOTA-lanreotide showed good stability during incubation in serum (Fig. 4.58). The results of cysteine challenge indicate that labelled peptide is sufficiently stable toward cysteine (Table 4.16).

Biodistribution of $^{99m}$Tc-DOTA-lanreotide in various organs of the normal mice at 15 min, 1 h, 4 h and 24 h after intravenous administration is presented in Table 4.17. The in vivo behavior of
the $^{99m}$Tc-DOTA-lanreotide was expressed as percentage of injected dose per gram organ tissue (%ID/gram organ tissue). Results obtained from in vitro studies suggested that $^{99m}$Tc-DOTA-lanreotide can be used as a radiotracer. In normal mice $^{99m}$Tc-DOTA-lanreotide was taken up by stomach and intestine as the receptors of the lanreotide are normally present in the gastrointestinal tract. $^{99m}$Tc-DOTA-lanreotide was up taken by liver, from where it was cleared to the small intestine. The activity uptake of stomach was 1.69% and 2.04% at 60 min and 120 min post injection respectively and this is in accordance to $^{188}$Re-Lanreotide (De Castiglia et al., 1998).

The $^{99m}$Tc- DOTA-lanreotide was rapidly distributed after intravenous injection as shown by the renal elimination. Biodistribution of $^{99m}$Tc-DOTA-lanreotide in various organs of the tumor induced mice at 1 h, 2 h, 4 h and 24 h after intravenous administration is presented in Table 4.18.

In the study of biodistribution of $^{99m}$Tc-DOTA-lanreotide in Swiss Webster mice bearing naturally developed tumors significant localization in tumors are observed as compared to the minor uptake in the normal organs. The radiopharmaceuticals should have high and long lasting uptake in the targeted tumour tissue and low uptake and fast excretion from the normal organs/tissues. The radioactivity in the tumor was higher than that in the brain, heart, spleen, and lung. The tumor activity of the $^{99m}$Tc-DOTA-lanreotide was 5.44±0.10, 8.96±0.05, 9.14±0.02 and 1.1±0.02 respectively at 1h, 2h, 4h and 24 h post injection. Tumor-to-organ ratios are shown in Table 4.18. $^{99m}$Tc-DOTA-lanreotide showed ratios as tumor to liver (2.18); tumour to muscle (45.7); tumour to blood (24.05) and tumour to kidney(2.33) which were comparable with $^{99m}$TC-HYNIC-TOC with EDDA ( 9.11, 31.29, 33.97, 2.05), $^{99m}$TC-HYNIC-TOC with tricine-nicotinic acid (7.77, 51.14, 22.67, 1.59), $^{99m}$TC-HYNIC-TOC with tricine (4.61, 10.46, 8.38, 0.66) and $^{111}$In-DTPA-octreotide with tumor to liver (9.14); tumour to muscle (52.28); tumour to blood.
(62.54) and tumor to kidney (0.19) ratios respectively after 4h post injection (Decristoforo et al., 2000). High uptake of $^{99m}$Tc-DOTA-lanreotide in tumor presents its significant application in diagnosis of cancer.

The whole body SPECT imaging results agree well with *in vivo* biodistribution studies of $^{99m}$Tc-DOTA-lanreotide. The scan showed a high activity in liver, spleen and thorax 2 min after administration of the $^{99m}$Tc-DOTA-lanreotide in rabbits (Fig.4.59) as in rats (Faintuch et al., 2004). The activity gradually accumulated in urinary bladder and decreased in liver and spleen with the passage of time. The scintigraphic images thus suggest that $^{99m}$Tc-DOTA-lanreotide possesses excellent characteristics for promising application as a novel imaging agent in rabbits. In planar imaging study of breast tumor bearing mice, tumors could be visualized very clearly (Fig. 4.60). Prominent uptake was observed in the liver and the tumor specimens. The whole body SPECT imaging results agree well with *in vivo* biodistribution studies of $^{99m}$Tc-DOTA-lanreotide. $^{99m}$Tc-DOTA-lanreotide was excreted through kidneys.

When compared with direct labeling of lanreotide (Pervez et al., 2001) with $^{99m}$Tc, the labeling of DOTA-lanreotide was simple. The pH of labeled compound (pH 7) was most appropriate for injection purposes. The incubation period of lanreotide with reducing agent is more than 15 h while DOTA-lanreotide can be labeled with $^{99m}$Tc within half an hour. Hence instant kit formulation of DOTA-lanreotide is quite appropriate. Boiling step is also not required in DOTA-lanreotide labeling with $^{99m}$Tc. Thakur et al. (1997) demonstrated that direct labeling of octreotide with Tc-99m via disulfide reduction, reduced receptor affinity by four orders of magnitude, confirming that alterations of the cyclic part of octreotide can dramatically reduce biological activity. HYNIC was also used to label somatostatin analogues with Tc-99m. $^{99m}$Tc-DOTA-lanreotide showed comparable tumor uptake 9.14 ±0.02 with $^{99m}$Tc-labeled HYNIC-
octreotide conjugates such as $^{99m}$Tc-HYNIC-TOC with EDDA 9.65±2.16, $^{99m}$Tc-HYNIC-TOC with tricine-nicotinic acid 5.80±2.31, $^{99m}$Tc-HYNIC-TOC with tricine 9.58±0.90 and $^{111}$In-DTPA-octreotide 4.26± 1.00 respectively (Decristoforo et al., 2000). Since very small amount of DOTA-lanreotide was used for $^{99m}$Tc labeling, hence multiple doses can be used for imaging purposes.
$^{99m}$Tc-DOTA-D-$\beta$-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$

**Fig 4.50:** Chemical structure of $^{99m}$Tc-DOTA-lanreotide

**Fig. 4.51:** Effect of pH on labelling efficiency of $^{99m}$Tc-DOTA-lanreotide

**Fig. 4.52:** Effect of amount of stannous chloride on labelling efficiency of $^{99m}$Tc-DOTA-lanreotide
**Fig. 4.53:** Effect of amount of ligand on labelling efficiency of $^{99m}$Tc-DOTA-lanreotide

**Fig. 4.54:** Stability of the $^{99m}$Tc-DOTA-lanreotide at room temperature
Fig. 4.55: Electrophoresis result of $^{99m}$Tc-DOTA-lanreotide

Fig. 4.56: HPLC analysis (UV detector) showing the purity of $^{99m}$Tc-DOTA-lanreotide
Fig. 4.57: HPLC analysis illustrate the percentage labeling of $^{99m}$Tc-DOTA-lanreotide

Fig. 4.58: *In vitro* stability of $^{99m}$Tc-DOTA-lanreotide in normal human serum
Table 4.16: Cysteine challenge test of $^{99m}$Tc-DOTA-lanreotide

<table>
<thead>
<tr>
<th>Molar ratio (cysteine to peptide)</th>
<th>Percent of $^{99m}$Tc-DOTA-lanreotide transchelated to cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td>50</td>
<td>83</td>
</tr>
<tr>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>500</td>
<td>66</td>
</tr>
</tbody>
</table>

Table 4.17: Biodistribution data in percent injected dose per gram organ/tissue for $^{99m}$Tc-DOTA-lanreotide at 15min, 1h, 4h and 24h after intravenous administration in normal mice (means±SD)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percentage of Injected Dose per Gram organ (n=3/time, interval, iv)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15min</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.89±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.4±0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>1.5±0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.69±0.09</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.97±0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>Femur</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Blood</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>Brain</td>
<td>0.12±0.04</td>
</tr>
<tr>
<td>Body</td>
<td>2.09±0.02</td>
</tr>
<tr>
<td>Urine</td>
<td>1.03±0.01</td>
</tr>
<tr>
<td>Bladder</td>
<td>2.8±0.02</td>
</tr>
<tr>
<td>Heart</td>
<td>0.94±0.05</td>
</tr>
</tbody>
</table>

$^a$Values represent the mean±standard deviation of data from three animals
Table 4.18: Biodistribution data for \(^{99m}\text{Tc}\)-DOTA-lanreotide at 1h, 2h, 4h and 24h after intravenous administration in tumor bearing mice in % injected dose/gram organ or tissue (mean±SD)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percentage of Injected Dose per Gram organ (n=3/time, interval, iv)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>liver</td>
<td>12.12±0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.82±0.05</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.46±0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.24±0.10</td>
</tr>
<tr>
<td>Brain</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Body</td>
<td>0.17±0.1</td>
</tr>
<tr>
<td>Blood</td>
<td>0.40±0.05</td>
</tr>
<tr>
<td>femur</td>
<td>0.086±0.01</td>
</tr>
<tr>
<td>Urine</td>
<td>1.20±0.1</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.50±0.1</td>
</tr>
<tr>
<td>Tumor</td>
<td>5.44±0.10</td>
</tr>
</tbody>
</table>

Ratios

<table>
<thead>
<tr>
<th></th>
<th>Tumor to blood</th>
<th>Tumor to muscle</th>
<th>Tumor to liver</th>
<th>Tumor to stomach</th>
<th>Tumor to kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor to blood</td>
<td>13.6</td>
<td>56</td>
<td>0.44</td>
<td>49.45</td>
<td>1.20</td>
</tr>
<tr>
<td>Tumor to muscle</td>
<td>11.06</td>
<td>45.7</td>
<td>2.18</td>
<td>6.44</td>
<td>2.01</td>
</tr>
<tr>
<td>Tumor to liver</td>
<td>24.05</td>
<td>27.5</td>
<td>1.32</td>
<td>3.63</td>
<td>2.33</td>
</tr>
<tr>
<td>Tumor to stomach</td>
<td>6.47</td>
<td>0.36</td>
<td>1.11</td>
<td>1.11</td>
<td>0.55</td>
</tr>
</tbody>
</table>

\(^a\) Values represent the mean±standard deviation of data from three animals
**Fig. 4.59:** Whole body gamma camera image of rabbits injected with $^{99m}$Tc-DOTA-lanreotide at 1-hour post administration (a), at 4-hours post administration (b), and 6-hours post administration (c).
Fig. 4.60: Whole body gamma camera image of tumor induced mice injected intravenously with $^{99m}$Tc-DOTA-lanreotide after post administration at 30min, 1h, 2h and 4 hours post-administration.
4.7. $^{99m}$Tc-vincristine

The Vincristine contains the sulphate salt of vinca alkaloid from the *Catharanthus roseus* formerly Vincarosea. It has molecular formula $C_{46}H_{56}N_{4}O_{10}$ as shown in (Fig. 4.61). The synthesis of $^{99m}$Tc-vinc was performed to determine the best conditions ensuring high radiochemical yield, high purity, and stability of $^{99m}$Tc-vinc. The examination of the effect of the different parameters on the labeling yield such as amount of vincristine, pH of the reaction mixture, amount of SnCl$_2$·2H$_2$O, and reaction time was studied. The effects of pH are shown in Fig. 4.62. At low pH (2-3) the labeling efficiency was 60 to 80%, while at pH 4 the labeling efficiency of $^{99m}$Tc-vinc was ~100%. In basic media 7-8 the labeling efficiency was decreased (~50%). Hence further experiments were performed at pH 4. The amount of reducing agent, SnCl$_2$·2H$_2$O, which gave the highest labeling efficiency, was 4-8 µg and 6 µg of SnCl$_2$·2H$_2$O was chosen (Fig. 4.63) to avoid colloid formation. The complexation of $^{99m}$Tc with 5 µg (Fig. 4.64) vincristine at pH 4 with 6 µg of SnCl$_2$·2H$_2$O gave a maximum labeling efficiency of ~100% within few minutes. Labeling yield at different time intervals 5 min, 30 min 1 h, 2 h, 4 h and 6 h after the initiation of reaction came out to be 97.458 at initial 5 minutes and 100% was maintained till 5 hours (Fig. 4.65). The final formulation for the radiotracer $^{99m}$Tc-vinc was: Vincristine sulphate 5 µg; SnCl$_2$·2H$_2$O 6 µg; pH ~4; $^{99m}$Tc 370 MBq (10 mCi) and reaction mixture volume 1.5 mL.

4.7.1. Radiochemical purity and stability

Labeling efficiency, radiochemical purity and stability were assessed by a combination of ascending chromatography and instant thin layer chromatography impregnated with silica gel. In paper chromatography using acetone as the solvent, free $^{99m}$TcO$_4^-$ moved towards the solvent
front (Rf = 1), while $^{99m}$Tc-vinc and reduced/hydrolyzed $^{99m}$Tc remained at the point of spotting. In ITLC-SG chromatography using 0.5M NaOH as solvent, reduced/hydrolyzed $^{99m}$Tc remained at the point of spotting, whereas $^{99m}$Tc-vinc and free $^{99m}$TcO$_4^-$ moved towards the solvent front. The electrophoresis illustrate that complex is neutral in nature (Fig. 4.66). HPLC results of inactive ligand illustrate that ligand is > 90% pure (Fig. 4.67) HPLC analysis of $^{99m}$Tc-vinc illustrate that ~100% $^{99m}$Tc binds with available ligand (Fig. 4.68). When the preparation (labeled radiopharmaceutical) was incubated with normal human serum at 35ºC, no significant increase in free $^{99m}$TcO$_4^-$ or reduced/hydrolyzed $^{99m}$Tc was seen up to 24 hours. The total impurities were found to be < 5% (Fig. 4.69).

4.7.2. Biodistribution and Scintigraphic Studies in animals

Biodistribution of $^{99m}$Tc-vinc in various organs of the mice at 1, 4 and 24 hours after intravenous administration is presented in Table 4.18. The in vivo behavior of the $^{99m}$Tc-vinc was expressed as percentage of injected dose per gram organ tissue (%ID/gram organ tissue). $^{99m}$Tc-vinc is accumulated in spleen (~8%), liver (>18%), and kidney (>19%) after 1h post administration. Spleen showed significant uptake of $^{99m}$Tc-vinc being the organ system with high cell turns over. The activity uptake of stomach was low, 0.8% (60 min post injection), indicating that $^{99m}$Tc-vinc stable inside the body. The $^{99m}$Tc-vinc was rapidly distributed after intravenous injection as shown by the renal elimination, although liver and spleen uptake was significant. The retention of $^{99m}$Tc–vinc in liver may be attributed to its metabolism in liver. The study shows that uptake of a radiotracer is dependent on several factors, such as the nature of the radiotracer, blood flow, pH and plasma concentration indicating a slow transfer of charged metabolites formed across the cell membrane. The whole body SPECT imaging results agree well with in vivo biodistribution studies of $^{99m}$Tc-vinc. The scan showed a high activity in liver, spleen and normal distribution in
the whole body after 1h administration of the $^{99m}$Tc-vinc in mice and rabbits as depicted in Fig. 4.70(a) and 4.71(a) respectively. The activity was gradually increased in liver and slightly decreased in spleen with the passage of time. The scintigraphic images thus suggest that $^{99m}$Tc-vinc possesses excellent characteristics for promising application as a novel splenic imaging agent in mice and rabbits. The high hydrophilic character of $^{99m}$Tc-vinc is in accordance with its predominant renal clearance. When the distribution of activity in tumor induced mice was studied with the help of gamma camera, the tumor uptake was found to be very significant (Fig. 4.72). Biodistribution of $^{99m}$Tc-vinc in solid tumor bearing mice was found to be greatest in liver and intestine at different times after post injection. The great tumor count density indicated the tumor targeting potential of $^{99m}$Tc-vinc. When the biodistribution was carried out in mice bearing naturally developed breast tumor, the major activity was observed in tumors (Fig. 4.72). Thus it was concluded that $^{99m}$Tc-vinc can be a very important diagnostic agent for tumors.
Fig. 4.61: Structure of vincristine sulphate

Fig. 4.62: Effect of pH on labelling efficiency of $^{99m}$Tc-vinc
**Fig. 4.63:** Effect of amount of stannous chloride on labelling efficiency of $^{99m}$Tc-vinc.

**Fig. 4.64:** Effect of amount of ligand on labelling efficiency of $^{99m}$Tc-vinc.
Fig. 4.65: Stability of the $^{99m}$Tc-vinc at room temperature

Fig. 4.66: Electrophoresis result of $^{99m}$Tc-vinc
**Fig. 4.67:** HPLC analysis (UV detector) showing the purity of vincristine

**Fig. 4.68:** HPLC analysis illustrate the percentage labeling of vincristine with $^{99m}$Tc
**Fig. 4.69:** *In vitro* stability of $^{99m}$Tc-vinc in normal human serum

**Fig. 4.70:** Whole body gamma camera image of mice injection with $^{99m}$Tc-vinc at 1-hour post administration (a), at 4-hours post administration (b), and 6 hours post administration (c).
Fig. 4.71: Whole body gamma camera image of rabbits injected with $^{99m}$Tc-vine at 1-hour post administration (a), at 4-hours post administration (b), and 6-hours post administration (c).
**Fig. 4.72:** Whole body gamma camera image of tumor bearing mice injected with $^{99m}$Tc-vinc at 30 min, 1-hour, 2-hours, 3-hours and 4-hours post administration.
Table 4.19: Biodistribution data of $^{99m}$Tc-vinc in percent injected dose per gram organ at 1, 4, and 24 hours after intravenous administration in tumor bearing mice.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percentage of Injected Dose per Gram organ</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
<td>4 hours</td>
<td>24 hours</td>
</tr>
<tr>
<td>Liver</td>
<td>18.51±0.5</td>
<td>19.06±0.3</td>
<td>20.67±0.2</td>
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<td>Spleen</td>
<td>8.02±0.08</td>
<td>4.54±0.1</td>
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<tr>
<td>Stomach</td>
<td>0.18±0.5</td>
<td>0.28±0.04</td>
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<tr>
<td>Intestine</td>
<td>3.32±0.09</td>
<td>4.48±0.5</td>
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<tr>
<td>Lungs</td>
<td>0.21±0.02</td>
<td>0.22±0.1</td>
<td>0.24±0.06</td>
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<tr>
<td>Kidney</td>
<td>19.97±0.1</td>
<td>15.8±0.05</td>
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<td>Urine</td>
<td>13.43±0.2</td>
<td>27.18±0.05</td>
<td>48.29±0.6</td>
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<tr>
<td>Heart</td>
<td>0.98±0.1</td>
<td>0.75±0.9</td>
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<tr>
<td>Blood</td>
<td>0.96±0.05</td>
<td>0.82±0.02</td>
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<tr>
<td>Brain</td>
<td>0.63±0.7</td>
<td>0.41±0.4</td>
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<tr>
<td>Body</td>
<td>24.77±0.4</td>
<td>22.09±0.02</td>
<td>21.08±0.5</td>
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<tr>
<td>Bladder</td>
<td>11.53±0.09</td>
<td>6.26±0.02</td>
<td>4.69±0.1</td>
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<tr>
<td>Muscle</td>
<td>1.42±0.08</td>
<td>1.1±0.06</td>
<td>0.9±0.05</td>
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<tr>
<td>Tumor</td>
<td>5.87±0.9</td>
<td>6.27±0.4</td>
<td>4.98±0.11</td>
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</table>
CHAPTER 5

SUMMARY

$^{99m}$Tc-labeled antibiotics, antitumor agents and peptides have opened a new, exciting field of research in infection and tumor diagnosis. Small animals are frequently used in clinical research as models of infection, inflammation and tumor due to rapid production rate and thus provide suitable models for different human disorders. Labeling of clarithromycin with $^{99m}$Tc was performed for targeting detection of \textit{S. aureus} infection in animal models. We discovered that $^{99m}$Tc-clarithromycin can be labeled at pH 7 using 500 µg of clarithromycin and 25µg SnCl$_2$·2H$_2$O as reducing agent in 30 minutes incubation time which can be used for treatment as well identification of infection sites in the body. Quality control analysis was done by ITLC, and HPLC which was as high as ~99 %. The stability of $^{99m}$Tc-clarithromycin in human serum was fairly high and \textit{in vitro} bacterial binding assay showed higher bacterial cellular uptake of $^{99m}$Tc-clarithromycin. The biodistribution and scintigraphic results confirmed that mean Target to non-target ratio in the infected muscle (live \textit{S. aureus}) was higher at different time intervals than in sterile-inflamed muscle (heat-killed \textit{S. aureus} & turpentine oil), which indicates that $^{99m}$Tc-clarithromycin may be used as a possible bacterial infection imaging agent for diagnosis of infection.

Due to high labeling efficiency and high uptake in infected muscle, $^{99m}$Tc-clindamycin may be an important radiotracer for the diagnosis of deep-seated infections. Radiochemical purity was monitored by ITLC and paper chromatography which was greater than 95%. The $^{99m}$Tc-clindamycin complex is quite stable and labeling of $\geq 92\%$ was maintained for up to 24 hours. No post-labeling purification was required. HPLC, lipophilicity test, \textit{in vitro} binding in human
serum and in vivo study by checking biodistribution and scintigraphy of $^{99m}$Tc-clindamycin may be used as a bacterial infection imaging agent as well as for the purpose of chemotherapy.

The $^{99m}$Tc-vibramycin prepared by direct method possessed high radiochemical purity of 95%. $^{99m}$Tc-vibramycin was stable and $\geq 95\%$ labeling was maintained for up to 12 h and there was no requirement of post-labeling. The biological activity of $^{99m}$Tc-vibramycin and $^{99m}$Tc-ascorbic acid was compared. The $^{99m}$Tc-vibramycin is found to have greater ability to localize in bacterial infection sites induced by S. aureus in animal models. Thus data obtained from bio-evaluation studies showed that the prepared $^{99m}$Tc-vibramycin is accumulated at the infectious site and may be a good bacterial infection imaging agent due to its specific binding to bacterial cells.

$^{99m}$Tc-cecropin A can be labeled at pH 7 using 500 µg of Cecropin A and 25µg SnCl$_2$·2H$_2$O as reducing agent in the presence of tartaric acid in 30 minutes. It can be used for treatment as well as identification of infection sites in the body. Quality control analysis was done by ITLC, and HPLC which was as high as $\sim 99\%$. The high stability of $^{99m}$Tc-cecropin A in human serum and was observed. This is the first time that radiolabeling of cecropin A with $^{99m}$Tc is performed for targeting detection of S. aureus infection in animal models. The biodistribution and scintigraphic results confirmed that mean target to non-target ratio in the infected muscle (live E.coli) was higher at different time intervals than in sterile-inflamed muscle (heat-killed E.coli & turpentine oil).

Epirubicin was labeled with technetium-99m as a source to carry technetium-99m to tumor cells. Chemotherapeutic agent epirubicin (200 µg) can be efficiently labeled with $^{99m}$Tc using 35 µg of SnCl$_2$·2H$_2$O as a reducing agent at pH 6 in 30 min reaction time at room temperature with a high radiolabeling efficiency ($\sim 100\%$) monitored by paper and ITLC-SG chromatography. HPLC
indicates the presence of a single species of $^{99m}$Tc-epirubicin. Electrophoresis shows neutral nature of resulting complex. $^{99m}$Tc-epirubicin has shown good stability in human serum. $^{99m}$Tc-epirubicin enters the circulation and reaches different organs systems especially stomach, spleen and liver. *In vitro* and *in vivo* studies showed significantly selective uptake of $^{99m}$Tc-epirubicin in the tumor, indicating use of $^{99m}$Tc-epirubicin both as a tumor diagnostic as well as a chemotherapeutic agent for cancer.

The radiopharmaceutical $^{99m}$Tc-DOTA-lanreotide was designed and evaluated. Radiolabeling efficiency of $^{99m}$Tc-DOTA-lanreotide monitored by thin layer chromatography was 96%. Neutral charge on complex was determined by electrophoresis while HPLC results showed a single species. Biological distribution and scintigraphic studies in normal mice and rabbit indicated higher accumulation of $^{99m}$Tc-DOTA-lanreotide in thorax, liver and spleen. Due to the attractive biological properties, $^{99m}$Tc-DOTA-lanreotide may serve as functional agent for diagnostic purposes. Radiolabeling of lanreotide by direct method is more complex, while DOTA-lanreotide labeling was quite simple.

The novel $^{99m}$Tc-vinc complex was designed, synthesized and evaluated biologically. Radiolabeling efficiency of $^{99m}$Tc-vinc monitored by paper and ITLC-SG was ~100%. Neutral charge on complex was determined by electrophoresis while HPLC results showed single species. Biological distribution and scintigraphic studies in normal rats indicated higher accumulation of $^{99m}$Tc-vinc in liver and spleen. *In vivo* biodistribution in tumor bearing mice indicated large amount of activity in tumors. During scintigraphic imaging in mice, tumor was clearly visible which is consistent with the results from *in-vivo* biodistribution studies proving its potential as a tumor imaging agent. Vinc labeled with inexpensive and commonly available medically interesting Tc-99m can be used in all medical centres for planar or SPECT studies.
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