

**FRACTIONATION METHOD ADAPTATION FOR METHYLENE
DIPHOSPHONATE (MDP) RADIOPHARMACEUTICAL KIT USED IN THE
DIAGNOSIS OF INFECTIOUS DISEASES LUSAKA, ZAMBIA**

BY

ZULU JENIPHER

**A THESIS SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE**

OF

DOCTOR OF PHILOSOPHY IN INFECTIOUS DISEASES

UNIVERSITY OF ZAMBIA

LUSAKA

©2024

COPYRIGHT DECLARATION

No part of this work may be replicated or conveyed in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the author or the University of Zambia. I, Zulu Jenipher as the writer of this thesis have checked with sources understood to be reliable in my efforts to provide information that is accurate or complete and generally in accordance with the standards accepted at the time of this Thesis. I disprove all accountability for any inaccuracies or inadvertences that may perhaps be made. Permission is however granted to the University of Zambia to reproduce this thesis in part or whole in any format which may deem fit for purposes of research and study as long as it is published in a manner satisfactory to the University. Copyright © Zulu Jenipher, University of Zambia

DECLARATION

I, Zulu Jenipher hereby declare that this thesis herein presented for the degree of Doctor of Philosophy in Infectious Diseases has not been formerly submitted in every detail or in part at this or any other university.

Signed: Date:

Student: Jenipher Zulu (VET 2100011)

CERTIFICATE OF APPROVAL

This thesis by Zulu Jenipher, has been approved as fulfilling the requirements for the award of the Doctor of Philosophy in Infectious diseases by the University of Zambia

Supervisor

Name: Date.....

Signature.....

Internal Examiner 1

Name: Date.....

Signature.....

Internal Examiner 2

Name: Date

Signature

External Examiner

Name: Date

Signature

Chairperson, Board of examiners

Name: Date

Signature

ABSTRACT

The global emergence of antimicrobial resistance (AMR) highlights the urgent need for accurate diagnostic tools to combat infectious diseases effectively. Radiopharmaceuticals, such as the metastable state of Technetium-99m Methylene Diphosphonate ($^{99m}\text{Tc-MDP}$), are recognized as reliable diagnostic methods; however, challenges related to their storage persist especially in developing countries like Zambia because storage facilities are quite costly and maintenance is challenging particularly during power outages. While the literature has revealed that MDP aliquots kept at frozen and refrigerated conditions remain of good quality, this research explored the viability of MDP aliquots made from four

different kinds of normal saline. The research enquired into the potential of ambient-stored MDP radiopharmaceuticals to overcome the hurdles associated with cold-chain maintenance and storage infrastructure limitations. By addressing these challenges, the study aimed to contribute valuable insights that could pave way to a more sustainable and accessible deployment of nuclear medicine technologies, thereby improving diagnostic capabilities and patient outcomes in resource-challenged environments. The research was conducted at the University Teaching Adult Hospital (Nuclear Hot Laboratory) in Lusaka, Zambia from October 2022 to February 2023. A mixed methodology design was applied and data was collected from both the laboratory and biodistribution tests. In all, 80 MDP aliquots made with different types of normal saline were tested and biodistribution studies were done on *staphylococcus aureus* infected rat models. Data analysis using Stata 14 revealed that the Radiochemical Purity (RCP) for ambiently-stored MDP aliquots ranged from 98 to 99%, exceeding the World Health Organization's expected required minimum of 90%. Furthermore, the efficacy of the radiolabeled MDP fractions using biodistribution animal models demonstrated accurate localization. The study concluded that MDP aliquots stored at ambient temperature demonstrated exceptional RCP and favorable biodistribution patterns, effectively localizing in regions of interest in infected rat models. This suggests potential application for ambient-stored MDP in low-resource countries facing challenges in maintaining cold chain storage, particularly during power outages.

ACKNOWLEDGEMENT

First of all, I would like to acknowledge the Zambian Government Ministry of higher Education and the Africa centre for Infectious Diseases at the School of Veterinary Medicine, University of Zambia for co-funding this study.

My Supervisors; Professor Bernard Hang'ombe (School of Veterinary Medicine, University of Zambia), Dr Reuben Katebe (Zambian National Institute of Scientific Research/Ministry of higher education), and Dr Martalena Ramli (Indonesian Research Center for Radioisotope and Radiopharmaceutical Technology) for their untiring guidance and support during my research, from the concept note to writing this dissertation and ensuring that I successfully complete. Further, thanks go to Dr. apt N. Elly Rosilawati, M.Kes, M.Si, SpFN (Department of Nuclear Medicine and Molecular Theranostics, Hassan Sadikin Hospital, Bandung, Indonesia) for the research idea, which developed into the research concept. Special thanks also go to the research assistants who helped with data collection at the University Teaching Hospital in Lusaka, Zambia.

In a special way, I would also like to thank the Radiation Protection Authority for issuing the licence to handle radioactive substances for research, and the University Teaching Hospital Management for granting permission to conduct research at the Institution. Colleagues in the Nuclear Medicine Department (especially members of staff under Radiopharmacy and Radiology units) are also appreciated for their support.

This Research would have not been possible without genuine support from KANSMA Laboratory Distributors, relatives, friends, colleagues, and members of staff under ACEIDHA and the School of Veterinary Medicine. Thank you all for your encouragements. I would like to thank the ERES CONVERGE for granting clearance to conduct the research, in a similar manner the National Health Research Authority.

I would like to express my sincere gratitude to my son-in-law, Mr. Bwalya Mwenechanya, for his invaluable assistance in refining this document. His efforts in correcting figures, aligning tables, and ensuring content clarity and coherence were greatly appreciated.

Above all, I would like to thank God for His grace in my life which is so abundant and sees me through every rough passages. I fully give honour to Him for His never-ending support and faithfulness in my life.

DEDICATION

I dedicate this piece of work to my Lord Jesus Christ through whom I find strength every day and also to my husband Dr Ernest Chipasha and our five sons; Ernest Junior, Clement, Dalitso, Mwila, Theophilus, my Niece Violet Zulu for standing in for me as a mother when the times became so rough. Many thanks indeed for their encouragement given physically, emotionally and spiritually. I cannot forget the love that they have always shown me during and outside this research period despite the less time I spent with them.

TABLE OF CONTENTS

.....	
Page	
COPYRIGHT DECLARATION	i
DECLARATION	ii
CERTIFICATE OF APPROVAL	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
DEDICATION	vii
LIST OF APPENDICES	xi
LIST OF TABLES	x
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS AND ACRONYMS	xvi
CHAPTER ONE	
1	
INTRODUCTION	1
1.1. General Overview	1
1.2. Diagnosis of Infectious diseases	1
1.3. Background to Research Study	4
1.4. Statement of the problem	5
1.5. Research Questions	6
1.6. Research Objectives	6
1.7. Significance of the study	7

CHAPTER TWO	9
LITERATURE REVIEW	9
2.1. General Overview	9
2.2. Trends in general use of Radiopharmaceuticals for diagnosis and treatment	9
2.3. Current Trends in Radiopharmaceuticals use in Medicine for diagnosis and treatment	10
2.4. Fractionation of Radiopharmaceuticals.....	16
2.5. Biodistribution and mechanism of localization of Radiopharmaceuticals	18
2.6. Possible challenges in the use of Radiopharmaceuticals	23
2.7. Future Trends in Radiopharmaceuticals use in Medicine for diagnosis and treatment	24
CHAPTER THREE	25
MATERIALS	25
AND	25
METHODS	25
3.1. Study Design	25
3.2. Study Site	27
3.3. Study Sample Size Calculation and Sampling method	29
3.4. Research Variable	33
3.5. Sampling Method and Techniques	37
3.6. Viability Procedures	38

3.7. Animal Studies	49
3.8. Data Analysis and Interpretation	55
3.9. Ethical considerations	
55	
CHAPTER	FOUR
.....	56
RESULTS	
56	
4.1. Introduction	
.....	56
4.2. Compliance of Laboratory products to standards	56
4.3. Clarity of ^{99m} Tc	59
4.4. pH of all ^{99m} Tc-MDP aliquots.....	60
4.5. Chemical purity (CP) of ^{99m} Tc	61
4.6. Sterility Testing of ^{99m} Tc-MDP Aliquots	63
4.7. Radionuclidic purity (RNP) of ^{99m} Tc	65
4.8. Radiochemical Purity (RCP) Findings	66
4.9. Observations made on infected animals Models	89
4.10. Biodistribution Studies of ^{99m} Tc-MDP radiopharmaceutical aliquots in uninfected and infected rat models	89
CHAPTER FIVE	
95	
DISCUSSION	
.....	95
5.1. Brief Introduction	
95	
5.2. Radiochemical Purity (RCP) of Ambiently Stored MDP Fractions versus Conventional Storage (Frozen and Refrigerated Conditions)	95
5.3. RCP of MDP Fractions Prepared Using Argon-Purged Normal Saline (NSA) Versus Plain Normal Saline (NS).....	96

5.4. RCP of MDP Fractions Reconstituted with Tin Chloride Augmented NS (NSSnCl ₂) Versus Tin Chloride Augmented NS Purged with Argon (NSSnCl ₂ A)	96
5.5. Efficacy of Radiolabeled MDP Fractions through Biodistribution in Animal Models	97
CHAPTER SIX	98
CONCLUSIONS AND RECOMMENDATIONS	98
6.1. Conclusions	98
6.2. Limitations.....	98
6.3. Future Work and Recommendations	99
REFERENCES	101
APPENDICES.....	125

LIST OF APPENDICES

.	
Page	
APPENDIX A: Permission Letters	125
APPENDIX A1: Letter of approval of the Research Proposal	125
APPENDIX A2: Permission to conduct research from research Site	126
APPENDIX A3: Ethical Approval Letter	127
APPENDIX A4: National Health Research Authority Approval letter	129
APPENDIX B: Data Collection Forms	131
APPENDIX B1: Laboratory form: Data collection form for NSSCl ₂ A Stream	131
APPENDIX B2: Laboratory form: Data collection form for NSSCl ₂ Stream	132
APPENDIX B3: Laboratory form: Data collection form for NSA Stream	133
APPENDIX B4: Laboratory form - Data collection form for NS Stream	134
APPENDIX B5: Laboratory Form - To Show compliance of products (+ = Complies, - = Does not comply)	135
APPENDIX C: Radioactivity	136
APPENDIX D: Publication s	137
APPENDIX D1: Published Article (World Journal of Nuclear Medicine)	137
APPENDIX D2: Articles presented at 4th IPET IAEA Conference on 10th October, 2024.	143
APPENDIX E: Elution Process ^{99m} Tc Eluate	144
APPENDIX F: Labelling Process of ^{99m} Tc-MDP	146

LIST OF TABLES

...	
Page	
Table 3.1: Sample size distribution.....	30
Table 3.2: Conceptual Framework	34
Table 3.3: Research Experimental Setup	34
Table 3.4: Acceptable Radiochemical Purity from the standard references	42
Table 3.5: Primer Set used in Identifying and confirming <i>S. aureus</i>	50
Table 4.1: Compliance of laboratory products to show compliance of products (+ = Complies, - = Does not comply)	57
Table 4.2: Comparisons of MDP between Global standards and study results	57
Table 4.3: Number of aliquots analysed	58
Table 4.4: Clarity of MDP aliquots from all streams (NS, NSA, NSSnCl ₂ , and NSSnCl ₂ A) kept at ambient conditions	59
Table 4.5: Clarity of ^{99m} Tc used to label MDP aliquots kept at recommended conditions	60
Table 4.6: pH of ^{99m} Tc-MDP aliquots stored at recommended conditions on day 22	60

Table 4.7: pH of ^{99m}Tc -MDP aliquots kept at ambient conditions from all streams	
61 Table 4.8: Chemical Purity for ^{99m}Tc used to label aliquots kept at recommended conditions by day 22	62
Table 4.9: Chemical purity (CP) of ^{99m}Tc used to label MDP aliquots kept at ambient conditions from all streams	
63 Table 4.10: Sterility Testing of Methylene diphosphonate (MDP) aliquots	64
Table 4.11: RNP of ^{99m}Tc used to label all ambiently stored MDP aliquots	65
Table 4.12: RNP of ^{99m}Tc for MDP aliquots stored at recommended conditions on day 22	66
Table 4.13: Results for control Aliquots kept at recommended conditions (Frozen and refrigerated)	67
Table 4.14: RCP of ^{99m}Tc -MDP aliquots reconstituted with Argon purged Normal Saline (NSA) and with non-Argon purged (NS).....	
68 Table 4.15: Pearson Chi-square results for (MDP) aliquots reconstituted with Argon purged Normal Saline (NSA) versus those reconstituted with non-Argon purged NS	
70 Table 4.16: RCP of MDP aliquots reconstituted with Argon purged Normal Saline and Stannous Chloride and with non-Argon purged and Stannous Chloride	71
Table 4.17: Pearson Chi-square results for MDP aliquots reconstituted with NSSnCl_2 and NSSnCl_2A	73
Table 4.18: Pearson Chi-square results for MDP aliquots reconstituted with plain NSA and versus those reconstituted with Argon purged NS with Stannous Chloride	
74 Table 4.19: Pearson Chi-square results for MDP aliquots reconstituted with non-Argon purged NS versus those reconstituted with non-Argon purged NS and Stannous Chloride	76
Table 4.20: Pearson Chi-square results for MDP aliquots reconstituted with non-	

Argon purged NS versus those reconstituted with Argon purged NS and Stannous Chloride	78
Table 4.21: Pearson Chi-square results for MDP aliquots reconstituted with non-Ar NS and Stannous Chloride (NSSnCl ₂ A)	80
Table 4.22: Comparisons of the average RCP for different MDP aliquots kept at ambient conditions to those kept at recommended conditions (Frozen and refrigerated once-off)	82
Table 4.23: Imaging Information Requirements for ^{99m} Tc-MDP Based Images for Experimental Rats	90

LIST OF FIGURES

...	
Page	
Figure 2.1: Cost of Radiopharmaceuticals globally	13
Figure 2.2: Global Market for Radiopharmaceuticals	14
Figure 2.3: Cost of Radiopharmaceuticals in Zambia over 4 years	14
Figure 2.4: An example of a gamma camera in nuclear medicine Department	19
Figure 2.5: Whole body anterior bone image	20
Figure 2.6: Gamma camera image showing biodistribution of ^{99m} Tc-MDP	
Radiopharmaceutical liquots	21
Figure 2.7: Localization of ^{99m} Tc-MDP radiopharmaceutical in cancer.....	22
Figure 2.8: Image produced from a gamma camera, of a rat model injected with radionuclide shows better localization than images from a planar	23
Figure 3.1: MDP kit Formula labelled with Tc	25
Figure 3.2: The map of Zambia highlighting research site	28
Figure 3.3: Flow of research methodology activity	34
Figure 3.4: Laboratory Work flow	37

Figure 3.5: Student purging the NS with argon gas	38
Figure 3.6: Various types of normal saline; plain normal saline (NS), normal saline purged with argon gas and augmented with stannous chloride (NSSnCl ₂ A) and normal saline with stannous chloride (NSSnCl ₂)	39
Figure 3.7: Aliquots arranged according to different streams of NS and agar plates for sterility test	40
Figure 3.8: Paper chromatography procedure	42
Figure 3.9: Student performing the Radiochemical Purity	43
Figure 3.10: Strips of whatman paper	43
Figure 3.11: An example of a background for particle size inspection	44
Figure 3.12: Student verifying clarity of MDP aliquots	44
Figure 3.13: pH paper strips	45
Figure 3.14: Student conducting pH reading of pH paper strip	45
Figure 3.15: Calorimeter paper strip	46
Figure 3.16: Sterility Testing in Laminar flow	47
Figure 3.17: Sterility Test conditions in Laminar flow.....	48
Figure 3.18: Student conducting Radionuclidic Purity	48
Figure 3.19: Retrieving <i>Staphylococcus aureus</i> culture plates from the refrigerator	51
Figure 3.20: Preparation of inoculum	52
Figure 3.21: <i>Staphylococcus aureus</i> blood agar cultured disk with haemolysed blood... ..	52
Figure 3.22: Separated well fed caged rats	53
Figure 3.23: Injection of ^{99m} Tc-MDP aliquots into rat tail vein prior to imaging.	54
Figure 3.24: Nuclear Radiographer imaging the infected rat models	55

Figure 4.1: RCP of $^{99m}\text{TcMDP}$ aliquots reconstituted with NSA and with NS	69
Figure 4.2: RCP of MDP aliquots reconstituted with NSSnCl_2A and with NSSnCl_2	72
Figure 4.3: RCP of MDP aliquots reconstituted NSA) and NSSnCl_2	75
Figure 4.4: RCP of MDP aliquots reconstituted with NS and with NSSncl_2	77
Figure 4.5: RCP of MDP aliquots reconstituted with NS and NSSnCl_2A	78
Figure 4.6: RCP of MDP aliquots reconstituted with NSSnCl_2A and NSSnCl_2	81
Figure 4.7: RCP comparison for aliquots made from NS	83
Figure 4.8: Average RCP of aliquots made from NSA	84
Figure 4.9: Average RCP of aliquots prepared from NSSnCL_2	86
Figure 4.10: Average RCP of aliquots made from NSSCL_2A	90
Figure 4.11: Radiographer imaging one of the infected rat model	91
Figure 4.12: Biodistribution images of control and infected rats on NSA Stream	93

LIST OF ABBREVIATIONS AND ACRONYMS

^{99m}Tc	Technetium-99m
AIDS	Acquired Immune Deficiency Syndrome
BP	British Pharmacopeia
CFUs	Colony Forming Units
COVID 19	CoronaVirus Disease of 2019
CP	Chemical Purity
CT	Computed Tomography
DMSA	DiMercaptoSuccinic acid
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration of the United States of America
FDG	Fluorodeoxyglucose

FTM	Fluid Thioglycolate Medium
FTG	Fluid Thioglycolate
GMP	Good Manufacturing Processes
% HR	Percentage hydrolysed reduced of ^{99m} Tc (Technetium-99m)
HR	Hydrolysed reduced of ^{99m} Tc (Technetium-99m)
HIDA	Hepatobiliary Iminodiacetic Acid
HIV	Human immunodeficiency virus
HMPAO	Hexamethylpropylene amine oxime
IAEA	International Atomic Energy Agency
ITLC	Instant Thin-layer chromatography
KM	Kilometer
LAL	Limulus Amebocyte Lysate
MAA	Macro aggregated albumin
MAG3	Mercaptoacetylglycylglycylglycine
MBT	Molybdenum Breakthrough
mCi	millicurie
MDP	Methylene Diphosphonate
MEK	Methyl Ethyl Ketone
MRI	Magnetic Resonance Imaging
NaCl	Sodium Chloride
NHREB	National Health Research Ethics Board
NISIR	National Institute of Scientific and Industrial Research
NM	Nuclear Medicine
NS	Normal Saline
NSA	Normal Saline purged with Argon gas
NSSnCl ₂	Normal Saline augmented with Stannous Chloride

NSSnCl ₂ A	Normal Saline augmented with Stannous Chloride and purged with argon gas
NSTS	Normal Saline with Stannous Chloride
°C	Degrees Celsius
P	Probability value
	Polymerase Chain Reaction
PCR	Potential of Hydrogen
pH	Quality Control
QC	Radiochemical Purity
RCP	Reference front value
RF	Radionuclidic Purity
RNP	Sonography
S	<i>Staphylococcus aureus</i>
<i>S. aureus</i>	Standard Error
SE	Single-photon Emission Computed Tomography
SPECT	Thin Layer Chromatography
TLC	Trypticase Soy Broth
TSB	United States Dollars
US\$	United States Pharmacopeia
USP	University Teaching Hospital
UTH	World Health Organisation
WHO	Zambia Radiation Regulation Authority
ZRPA	
>	Greater than
<	Less than
+	Clear and correct under labelling
-	Not clear and correct under labelling

CHAPTER ONE

INTRODUCTION

1.1. General Overview

Infectious diseases, caused by different microorganisms such as *Staphylococcus aureus*, present a global health challenge with substantial morbidity and mortality rates, especially in underdeveloped countries (Hamid et al., 2023; Boora et al., 2023; Huang and Chen, 2023; Lessa and Sievert, 2023). A significant proportion of the 13 million annual deaths from infectious diseases occurs in underdeveloped regions, highlighting the urgent need for accurate diagnostic approaches (IAEA, 2021). Infectious diseases, including HIV, AIDS, and tuberculosis, particularly impact underdeveloped nations, necessitating effective diagnostic strategies (Bates et al., 2004; Peer, 2015; Shirwaiker et al., 2015; Willyard, 2017; Makam et al., 2021). Certain infections, especially those that are deep-seated like bone and joint infections are widespread diseases that pose difficult diagnostic and therapeutic challenges for both the patient and orthopaedic Doctor (Garg, 2012; Hakkarainen et al., 2014; Lipsky et al., 2020).

Among infectious diseases, bone and joint infections pose intricate diagnostic and therapeutic challenges, and potentially lead to permanent disability if left untreated (Nade, 2003; Shah et al., 2014). The prevalence of deep-seated infections, including osteomyelitis, has tremendously gone up, thereby necessitating advanced diagnostic tools, especially in the background of increased antimicrobial resistance (Pigrau and Rodrigue, 2013; Charani et al., 2023; Magnano San Lio et al., 2023; Rogers Van Katwyk et al., 2023; Salam et al., 2023; Walsh et al., 2023). Therefore, it is necessary to find and make every effort that can help in the correct diagnosis of infectious diseases.

1.2. Diagnosis of Infectious diseases

Understanding the nature of the infection is critical for clinicians in choosing optimal diagnostic and therapeutic strategies. While clinical assessments, laboratory tests, and imaging modalities have traditionally been employed, technological advancements have facilitated more rapid and accurate diagnostic procedures (Ng, 2023; Fuchsova, 2023;

Augustine et al., 2023). Common methods for detecting infections include Clinical Evaluation which is a common practice for clinicians, where they begin with a thorough patient history and physical check-up. Fever, soreness, a rash, or swelling are just few of the symptoms that might indicate the presence and severity of an infection (Crump et al., 2015). For example, a clinical prediction algorithm for the diagnosis of septic arthritis (pyogenic bacterial joint infections) was developed (Kocher et al., 1999; Gutierrez, 2005) based on four clinical variables which are history of fever of above 38°C, a Red Blood Cell sedimentation rate of 40 mm/hour and a serum white blood cell count of greater than 12,000/mm³. If all of the above conditions are met, there is a 99% chance that the disease is septic arthritis. The probability of septic arthritis is 93% if only 75% of the above conditions are met, 40% if only 50% of the above criteria are met, and 3% if only 25% of the above criteria are met (Kocher et al., 1999).

Laboratory Blood tests with infection signs like; high white blood cell counts or certain antibodies may be tested for in blood samples. Bacteria and fungi found in the blood may also be identified with the use of a blood culture. Urine Analysis tests are used to detect presence of bacteria and to diagnose urinary tract infections. Analysis of Cerebrospinal Fluid obtained by a lumbar puncture may help in the diagnosis of meningitis and other illnesses of the central nervous system (Mahon and Lehman, 2022). Fluids exhaled by the Lungs maybe used to detect the presence of viruses and bacteria in the respiratory system by analysing swabs taken from the throat, nose, or lungs. Other methods include Microbiological Cultures where it becomes possible to isolate and identify the offending microorganism (bacteria, fungi, or viruses) by cultivating samples from infected locations (such as wound swabs, throat swabs, or sputum) on specialized growth medium.

Antibiotic susceptibility testing relies heavily on this technique (Mahon and Lehman, 2022). Molecular Diagnostics of Pathogen genetic material may be detected by polymerase chain reaction (PCR) and other nucleic acid-based diagnostics. These tests can detect even the smallest amounts of viruses, germs, and parasites. They are often used in the diagnosis of viruses like HIV and COVID-19 among others. Serology is another method where antibodies created by the body in response to an infection may be detected by a serological test. Hepatitis, HIV, and certain bacterial illnesses like syphilis may all be diagnosed with the use of these tests. Antigen testing is used to identify infections

based on the presence of a certain protein or molecule on their surface. Virus infections, such as COVID-19, are often diagnosed with rapid antigen assays.

For immunological tests, antibodies and antigens related to infections may be detected using a variety of immunological tests like the enzyme-linked immunosorbent assay (Brockwell et al., 2013). Biopsy is applied when diagnosing an infection in a hard-to-reach area, such as the bone or an internal organ, a tissue biopsy may be required. Pathogens may be detected by microscopic examination of biopsied tissue. Photography from X-rays, Computed Tomography scans, Magnetic Resonance Images, and ultrasounds are only few of the imaging modalities that may aid in the diagnosis of infections by detecting anomalies or alterations in the structure of organs or tissues. Pneumonia and other lung infections are often diagnosed with the use of chest X-rays (Jaiswal et al., 2019). When diagnosing chronic osteomyelitis, x-ray radiographs appear quite normal at first, since approximately 40 to 50% of focal bone loss is required to produce visible bone damage on plain images (Shah et al., 2014). Therefore, when it comes to infectious diseases of the bones and joints, other precise diagnostic options must be considered like the use of radiopharmaceuticals that are able to trace exactly where the infections is sitting (van der Bruggen et al., 2010).

When it comes to infections of the bones and joints, where accurate diagnoses are crucial, conventional approaches might not be enough (Chianelli et al., 1997; Silverman and Phelps, 1999; Verburg et al., 2007; Gulyas and Halldin, 2012). Ionizing radiation-emitting radiopharmaceuticals improve specificity, sensitivity, and accuracy, which aids in diagnosing these difficult illnesses. Microorganisms can travel through the circulation and infect bones and joints from far away due to trauma, infection at a local location, or postoperative sepsis (Shirtliff and Mader, 2002; Olsen and van, 2014). According to Dartnell et al. (2012), Pigrau-Serrallach and Rodriguez-Pardo (2013), Saavedra-Lozano et al. (2017), Li and Webster (2018), and McNally et al. (2021), most of these infections affecting bones and joints are often rather difficult to diagnose or treat. Direct trauma or illness is the usual route of contamination for microorganisms that enter the circulatory system and can go to any part of the body (Gristina, 1987; Blanchette et al., 2017). Since ionizing radiation-emitting radiopharmaceuticals are more targeted, sensitive, and

accurate, their usage boosts diagnostic trust (Chianelli et al., 1997; Silverman and Phelps, 1999; Verburg et al., 2007; Gulyas and Halldin, 2012).

1.3. Background to Research Study

1.3.1. Current Trends in optimising use of radiopharmaceuticals - Central

Radiopharmacy

A centralized radiopharmaceutical laboratory manufactures radiopharmaceuticals for a large group of patients from multiple nearby hospitals at the same time. Given the generally high cost of nuclear medicine services, several publications describing cost reduction strategies have appeared in the literature. As early as 1973, Gnau and others began exploring ways to centralize radiopharmaceutical facilities to lower the price of nuclear medicine services. Nuclear medicine services were reduced by 50 to 70% due to the plan to centralize radiopharmacy services. Other notable benefits included improved radiopharmaceutical quality control, reduced labour, and an improvement in both community-based nuclear medicine practice and patient care (Dhingra et al., 2015). The University Teaching Hospital is currently the only facility in Zambia providing nuclear medicine services, therefore the central pharmacy model cannot be used there.

1.3.2. Current Trends in optimising use of radiopharmaceuticals -Fractionation of Radiopharmaceuticals

In radiopharmaceutical fractionation, a reconstituted radiopharmaceutical kit is divided with normal saline to save part or parts for later use (Fleming, 1912). Fractionation is another strategy to reduce costs associated with the use of radiopharmaceuticals in nuclear medicine. This has been demonstrated in the literature when aliquots are stored under refrigerated and frozen conditions. Fractionation has been shown to be beneficial in the literature (Srivastava et al., 1985; Huigen et al., 1990; Bhatia et al., 2013; Thokchom et al., 2022). Fractionation of several radiopharmaceuticals is performed to facilitate the diagnosis and treatment of diseases, make them more affordable, available, and reduce their waste. No study has been conducted to demonstrate the impact of ambient conditions on aliquots or possibly places with unreliable appropriate backup mechanisms to maintain cold chain.

The feasibility of ambient-stored MDP radiopharmaceuticals in resolving the issues of cold-chain maintenance and storage infrastructure constraints was therefore investigated in this

study. Improving diagnostic capabilities and patient outcomes in resource- challenged locations might be achieved by a more sustainable and accessible deployment of nuclear medicine technology. The study aimed to provide significant insights by addressing these obstacles. In this study, therefore, the different aliquots prepared from different normal saline solutions were evaluated with the aim of adapting the fractionation method for the Methylene Diphosphonate radiopharmaceutical (MDP), one of the most used radiopharmaceuticals. MDP is used to diagnose various diseases, including infectious diseases.

1.4. Statement of the problem

Despite the widespread practice of fractionating radiopharmaceuticals to enhance accessibility and minimize waste, the impact of ambient storage conditions on aliquots remains unexplored, particularly in regions lacking reliable cold chain infrastructure. Given the common use of MDP in diagnostic nuclear medicine, this study aimed at assessing and comparing the stability of MDP aliquots stored at ambient conditions with those stored at conventional temperatures, to refine and adapt the fractionation method.

While fractionation method is not currently employed in Zambia, it has potential to reduce the running cost of radiopharmaceuticals, enhance their affordability and accessibility. Fractionated radiopharmaceutical kits, stored under controlled conditions, can maintain viability for several months (Penglis and Tsopelas, 1998; Bayomy et al., 2009; Bhatia et al., 2013; Dhingra et al., 2019; Thokchom et al., 2022). However, the absence of local evidence on the quality of fractionated radiopharmaceuticals and real-time biodistribution studies contributes to the lack of utilization of the fractionation method in Zambia.

1.5. Research Questions

1.5.1. Main Research question

How can the fractionation method of methylene diphosphonate radiopharmaceutical kits be adapted for diagnosing infectious diseases at the University Teaching Hospital in Zambia at a low cost?

1.5.2. Specific Research Questions

1. How does the radiochemical purity (RCP) of radiolabelled methylene diphosphonate (^{99m}Tc -MDP) fractions stored under ambient conditions compare with those stored conventionally (frozen and refrigerated)?

2. What differences in RCP are observed between radiolabelled MDP fractions prepared from argon-purged normal saline (NSA) versus those prepared from plain normal saline (NS)?
3. How does the RCP of radiolabelled MDP fractions reconstituted with tin chloride augmented NS (NSSnCl₂) compare with those reconstituted with tin chloride augmented NS purged with argon (NSSnCl₂A)?
4. What is the efficacy of radiolabelled MDP fractions determined through biodistribution studies in animal models?

1.6. Research Objectives

1.6.1. General Objective

The aim of the study was to adapt the MDP radiopharmaceutical kit fractionation method in the diagnosis of infectious diseases at the University Teaching Hospital in Zambia.

1.6.2. Specific Objectives

1. To compare the Radiochemical purity (RCP) of ambiently stored radiolabelled methylene diphosphonate MDP fractions with those stored at conventional (frozen and refrigerated) conditions.
2. To compare the RCP of radiolabelled MDP fractions prepared from argon-purged normal saline (NSA) with those prepared from plain normal saline (NS).
3. To compare the RCP of radiolabelled MDP fractions reconstituted with tin chloride augmented NS (NSSnCl₂) with those reconstituted with tin chloride augmented NS and purged with argon (NSSCl₂A).
4. To determine the efficacy of the radiolabelled MDP fractions through biodistribution in animal models.

1.7. Significance of the study

This study was justified by the lack of information regarding the exploration on the impact of ambient storage conditions on fractionated radiopharmaceutical aliquots, especially in areas where maintaining a reliable cold chain infrastructure is challenging. Considering the widespread usage of MDP in diagnostic nuclear medicine, it was essential to assess and compare the stability of MDP aliquots stored under ambient conditions versus conventional temperatures. The aim was to refine and adapt the fractionation method to ensure the accessibility and efficacy of radiopharmaceuticals while minimizing waste.

The paramount goal of utilizing appropriate radiopharmaceuticals in medical diagnostics is to enhance disease diagnosis and subsequently alleviate the mortality and morbidity rates associated with various medical conditions (IAEA, 2006). However, the accessibility of nuclear medicine services for all patients hinges on the availability of affordable radiopharmaceuticals. To address this challenge, the fractionation of radiopharmaceuticals has emerged as a potential solution. Studies have demonstrated that fractionated radiopharmaceutical kits, when stored under controlled conditions, maintain their quality for extended periods, offering a prospect to reduce costs and minimize wastage (Bhatia et al., 2013; Dhingra et al., 2019; Thokchom et al., 2022). Despite the proven benefits, the absence of local evidence and guidelines has hindered the adoption of this cost-effective strategy in Zambia, where adaptation to local conditions, such as power disruptions affecting the cold chain system, is crucial.

This research was imperative to assess the impact of ambient conditions on the stability of MDP fractions. The study also recognized the challenge where a single vial of MDP, adequate for multiple patients, may be wasted due to a low patient volume or a limited radionuclide dose. In the context of Zambia, a resource-constrained country where nuclear medicine services are predominantly subsidized by the government, any initiative to enhance resource availability and affordability is of paramount importance. The research not only contributes valuable insights into the radiochemical purity of radiolabelled MDP aliquots stored at ambient temperatures but also sheds light on the implications of varied normal saline preparation methods on radiochemical purity. Notably, this study addresses crucial considerations in the diagnostic realm, specifically for infectious diseases. Therefore, this study contributes significantly to this field by investigating methods to optimize the utilization of radiopharmaceutical resources, addressing both economic and diagnostic efficacy aspects, and potentially making nuclear medicine services more sustainable and accessible in Zambia.

CHAPTER TWO

LITERATURE REVIEW

2.1. General Overview

The literature review gives a thorough introduction to radiopharmaceuticals, highlighting their use in medical diagnosis and treatment. It delves into the past, present, and potential future of radiopharmaceutical applications in healthcare. Challenges in radiopharmaceutical use are also discussed, with the high cost of these medications being a major one. As a result, strategies like fractionation are being investigated to see if they may reduce costs. According to Saha (2010) and Payolla et al. (2019), radiopharmaceuticals are novel drug formulations made up of radioisotopes and pharmaceutical ligands. While the latter serve as carriers of the preparation to the targeted portion of the body, the former generates the necessary radiation (Dash et al., 2013). A sterile and pyrogen-free reaction vial is used for the preparation of radiopharmaceuticals. It contains nonradioactive chemicals like complexing agents (ligand/pharmaceuticals), reducing agents, and stabilizers that are necessary to produce a particular radiopharmaceutical after reaction with a radioactive component (Saha, 2010). Once administered and distributed, radiopharmaceuticals concentrate in the target organ and produce radiation that can be used for diagnosis or treatment of various ailments including infectious diseases (Catarino, 2017).

2.2. Trends in general use of Radiopharmaceuticals for diagnosis and treatment

The rapid expansion of the usage of radioisotopes in medicine following Roentgen's discovery of x-rays in 1895 led to Becquerel and Marie Curie's discovery of radioactivity (Anderson, 2019). Irène and Frédéric Joliot-Curie's invention of artificial radioactivity was one of many in the first few decades of the 20th century (Welch et al., 2003). Iodine-131 was effectively utilized to treat Graves' illness and thyroid cancer in 1946 thanks to nuclear scientific research that had previously been used for devastation during World War II (IAEA, 2006). Radiographic imaging was carried out essentially in the same way since Wilhelm Conrad Roentgen first discovered the X-ray until the early 1970s, but then radiography underwent a revolution. One of the most significant developments in diagnostic imaging since the invention of the X-ray itself occurred in the 1970s with the integration of computers into the image creation process.

The evolution of novel imaging techniques, including Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Sonography (S), and Nuclear Medicine (NM), which employs radiopharmaceuticals has been facilitated by computers. Over the past 50 years, a large number of radiopharmaceuticals have been developed for a variety of industrial and medicinal uses (Brugarolas et al., 2020). Some of them are utilized for infectious disease diagnostic imaging (Walter et al., 2014; Dadgar et al., 2022). Other are employed in the management and detection of non-infectious illnesses. Particle-emitting radioisotopes connected to tissue-targeting radiopharmaceutical agents are used as unsealed therapeutic sources in radiopharmaceuticals for therapy (Knapp et al., 2016). For instance, bone-seeking radiopharmaceuticals have been used in cancer pain palliation therapy for bone metastases (D'Angelo et al., 2012).

2.3. Current Trends in Radiopharmaceuticals use in Medicine for diagnosis and treatment

The use of radiopharmaceuticals is increasing vividly worldwide (Duncan, 1998; Giovacchini et al., 2018; Filippi et al., 2021). Effective application of radiopharmaceuticals demands that we use more clinically validated radiopharmaceuticals (Stephenson et al., 2015). To increase the availability of more radiopharmaceuticals on one hand, new and better radiochemical preparation methods are being developed (Weissleder et al., 2016; Herrero et al., 2021). Recent developments in the production of ^{18}F -radiopharmaceuticals used to diagnose different types of cancers are focused on synthesis methods that are simple to perform and therefore enabling easy distribution (Schirmacher and Schirmacher, 2007; Elsinga, 2012; Gower et al., 2021).

2.3.1. Use of radiopharmaceuticals in diagnosing infectious diseases

Radiopharmaceuticals being used in diagnosing infectious diseases include but not limited to Gallium Citrate (^{67}Ga) and Indium (^{111}In) which are used in abscess and infection, while Technetium Methylene diphosphonate ($^{99\text{m}}\text{Tc-MDP}$) is being used in bone and joint infections (Welch and Redvanly, 2003; Ballinger, 2021). White cell labelling for imaging infections is being done in advanced Nuclear Centers (Britton et al., 1997; McCartney, 2006). The use of radiolabeling with white blood cells is the gold standard techniques for detecting infections. It is based on the property of leukocytes which are able to migrate

into infected areas (Vinjamuri et al., 1996). The imaging of the areas to which the cells spread in the body then allows the infected regions to be identified through a gamma camera.

Many other Radiopharmaceuticals are currently being used to diagnose different types of infectious diseases (Signore et al., 2023; Lau and Mostafa, 2023; Palestro, 2023; Calabria et al., 2023; Benfante et al., 2023; Kleynhans et al., 2023). This is usually done in the nuclear medicine department of a hospital. One of the procedures frequently used in nuclear medicine is bone scintigraphy, which is used, among other things, to detect infectious diseases. The optimal agent for bone scintigraphy is technetium labelled with methyl diphosphate ($^{99m}\text{Tc-MDP}$), which has high bone-soft tissue absorption, fast blood clearance, and good in vivo stability (Leslie et al., 2011; Chen et al., 2016; Looker, 2017). The high bone-tissue absorption is based on the increased osteoblastic activity of bone remodelling and blood flow in the affected bone tissue. $^{99m}\text{Tc-MDP}$ exhibits high chemisorption and therefore localizes to the region of interest easily (Zuckier and Freeman, 2010; Chakraborty et al., 2013; Rowe et al., 2016; Zhang et al., 2019), while the rest is excreted via the kidneys (Leide-Svegborn et al., 2016; Mandiwana et al., 2018; Khawar et al., 2019; Jiang et al., 2021). Diagnosing bone diseases like osteomyelitis can best be done by using low-dose ionizing radiation studies with $^{99m}\text{Tc-MDP}$ bone scan (Kaul et al., 2013; Dammann et al., 2014; Shah et al., 2014; Versari and Casali, 2021; Woods et al., 2021).

In vivo methods using ionizing radiation, for example, provide images of infectious diseases of the bones and joints, which can be caused by *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), among others (Agaja and Ayorinde, 2008; Wu et al., 2019; Van Dijk et al., 2020). In addition, different in vivo radioisotope techniques are used to identify different physiological and pathological conditions in humans to detect early pathophysiological changes before structural changes take place (Signore and Glaudemans, 2011). For example, $^{99m}\text{Tc-Sestamibi}$ used for heart disease, hepatobiliary radioisotope iminodiacetic acid ($^{99m}\text{Tc-HIDA}$) scans for liver and gallbladder disease, thyroid scans using radioactive iodine-131 to detect thyroid disease,

and ^{99m}Tc -MDP scans for bone and joint disease are just a few of these procedures (Landau et al., 2013; Cook et al., 2013; Radiation Studies, 2014; Cherry et al., 2018; Orlhac et al., 2018; Mayerhoefer et al., 2020). Rapid test kits are used to diagnose diseases such as HIV/AIDS, Ebola and malaria using *in vitro* methods using ionizing radiation (IAEA, 2021).

2.3.2. Use of radiopharmaceuticals in diagnosing non-infectious diseases

As noted by Ballinger (2018), certain radiopharmaceuticals play a dual role in both diagnosing and treating benign and malignant diseases. Diagnostic radiopharmaceuticals find application in various medical conditions, spanning cerebral vascular disorders like Alzheimer's disease, schizophrenia, epilepsy, and studies related to neurotransmitters (Tian, 2016). Techniques such as scintimamography, sentinel node identification, myocardial perfusion studies, neuroreceptor imaging, and renography serve as effective diagnostic methods for tumors, encompassing both benign and cancerous varieties (Censullo and Vijayan, 2017). These diagnostic tools contribute significantly to identifying the precise locations of distant metastases, assessing injuries to bones and joints, and evaluating metabolic bone disorders. In essence, the versatile nature of radiopharmaceuticals facilitates a comprehensive approach to diagnosis, enabling healthcare professionals to address a wide spectrum of medical conditions with precision and efficacy.

Only very low doses of radiopharmaceuticals are administered to the patient to diagnose medical issues (Saha, 2010; O'Malley and Ziessman, 2020). Depending on the radiopharmaceutical employed and how it is administered, the radiopharmaceutical either passes through or is absorbed by an organ of the body where the imaging is supposed to be done (Saha, 2010). The nuclear physician can then examine how the organ is functioning by using specialized imaging equipment to detect radioactivity and create photographs of the organ, and determine the different ailments like infections, tumors, or any other pathologies that may be present in the organ. The concept of localization remains the same whether it's a therapeutic dose which is usually higher than diagnostic doses.

2.3.3. Cost of radiopharmaceuticals on the global market

Radiopharmaceuticals used to diagnose and treat a variety of diseases, including infectious diseases, are quite expensive (Saha, 2011). The global market for radiopharmaceuticals is expected to be worth US\$11 billion by 2030, growing at 10.5%. Nuclear medicine services are vital and important in the medical field but are not come cheap, this applies to Zambia as well (figures 2.1 to 2.3).

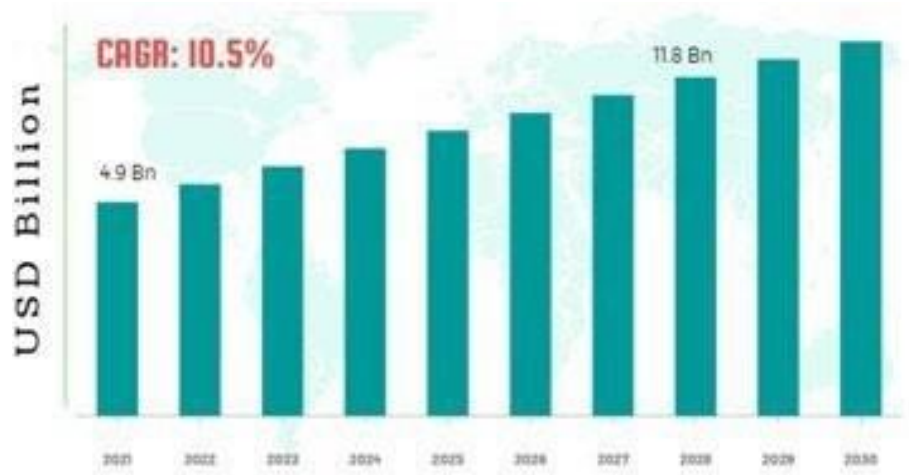


Figure 2.1 Cost of radiopharmaceuticals globally

(www.futuremarketinsights.com/reports/radiopharmaceuticals)

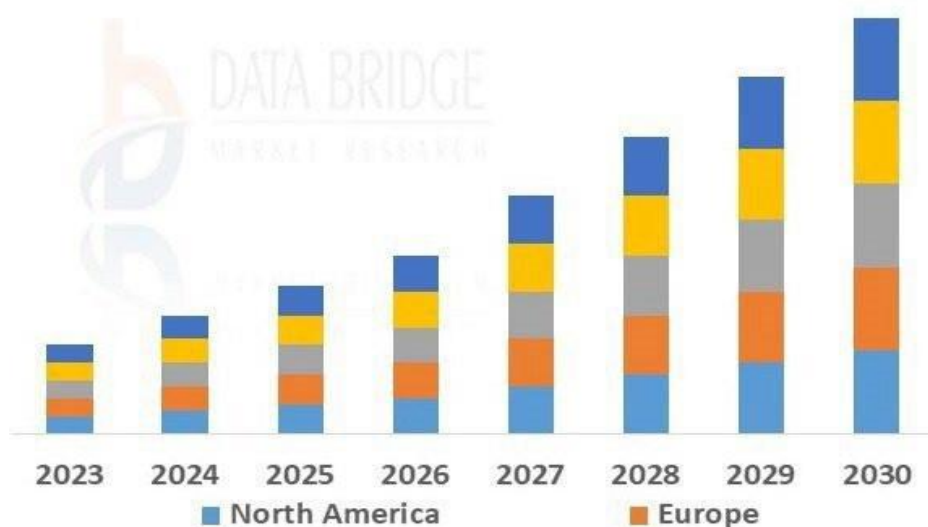


Figure 2.2: Radiopharmaceutical Global Market growth
(www.futuremarketinsights.com/reports/radiopharmaceuticals-market)

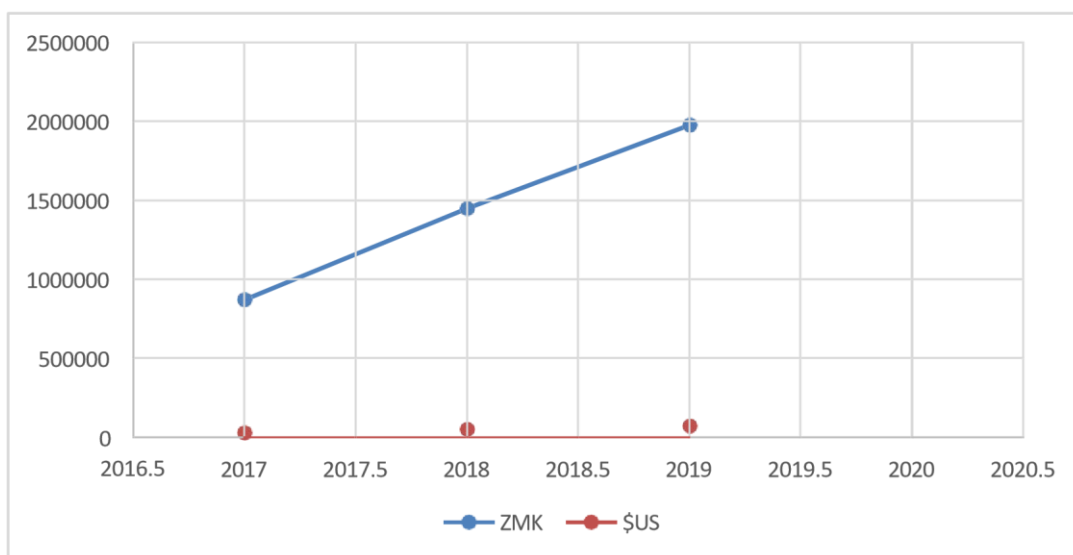


Figure 2.3: Showing cost of Radiopharmaceuticals in Zambia over 4 years (Annual reports, 2020) UTH Nuclear medicine)

2.3.3.1. Cost of Methylene diphosphonate Radiopharmaceutical

Methylene diphosphonate (MDP) is one of the most commonly used radiopharmaceuticals and like most other radiopharmaceuticals, it is manufactured by as a lyophilized agent, which increases the cost. The United States Food and Drug Administration (FDA) has approved the use of MDP radiopharmaceutical. It is one of the most used radiopharmaceuticals worldwide (Jurisson et al., 1993; Evbuomwan, 2018; Nur et al., 2018; Anderson, 2020) and in Zambia. Typically, radiopharmaceutical kits containing multiple doses of methylene diphosphonate are supplied in vial containers. If patients are pooled at the same time, one vial can treat up to ten patients. However, a whole vial of MDP is consumed, resulting in the waste of these expensive items, regardless of whether the number of patients is pooled at one time or the radioactive dose has dropped.

Patients in Zambia currently pay between US\$15 and US\$35 for a single dose of the methylene diphosphate radiopharmaceutical bone scan, as it is subsidized by the Zambian government. In addition, most of the patients who need these treatments fall into the financially underclass category and do not pay the affordable fee; As a result, they receive a full exemption from social security. Despite the seemingly untenable reality mentioned above, the government still provides subsidies for the continued operation of these very expensive services. In Zambia, the average cost of a vial of MDP is about US\$90, excluding the handling cost of delivering radiopharmaceutical kits. It is therefore necessary to ensure that these services do not burden the state budget at the expense of other equally important services, even if the government may prefer to offer them at a greatly reduced price. Therefore, any strategy that can optimise the use of these expense products and make them cost effective is worthy exploring. This is where this research became relevant and necessary.

At Hassan Sadikin Central Hospital in Indonesia, an MDP diagnosis set costs US\$320 for a low-cost patient and US\$450 for a high-cost patient (Personal communication). The minimum cost of therapy is US\$900 for a low-cost patient and \$1200 for a high-cost patient. Diagnostic MDP dosing costs US\$350 in South Africa (Personal communication). In the Indonesian hospital mentioned above and in South Africa therapeutic doses are far more expensive (average greater than US\$1000), but in Zambia they are only \$38. Patients currently pay between US\$15 and US\$35 for a single dose of MDP for diagnostic purposes at the University Teaching Hospital (UTH) Nuclear Medicine Unit. While these fees may seem small, money is being spent by the government of Zambia to subsidize these services, hence the need to find mechanisms to try and curb this.

2.4. Fractionation of Radiopharmaceuticals

Radiopharmaceutical fractionation involves dividing a reconstituted radiopharmaceutical kit using ordinary saline, allowing portions to be saved for later use (Saha, 2010). The literature reports that fractionation can be a cost-saving method, with radiopharmaceutical kits reconstituted, split into portions, and frozen for future use. When the vials are thawed (a process that could take hours or days), ^{99m}Tc pertechnetate is added. Studies have shown that the radiochemical purity of these fractionated kits typically meets the minimum

requirement of 90%, making them suitable for clinical and research use (USP, 2005; Saha, 2010; BP, 2016; WHO, 2019).

Thokchom et al. (2022) evaluated how fractionation affects the radiochemical purity of an MDP radiopharmaceutical kit. Using either the vial or syringe method, the MDP kit was fractionated and stored at -20°C. The effectiveness of the fractionated ^{99m}Tc-MDP was assessed and compared with ^{99m}Tc-MDP prepared conventionally. The study involved 100 patients, and both the vial and syringe methods resulted in >95% radiochemical purity. However, by the eighth day, radiochemical purity declined to 83.6% and 88%, respectively. No microbial growth was detected during the storage period, confirming the safety of the techniques.

Dhingra et al. (2019) tested multiple samples of MDP, dimercaptosuccinic acid (DMSA), and diethylene pentaacetate (DTPA) kits for radiochemical purity. The average purity was 95.1%, 91.4%, and 95.7%, respectively. These samples were divided into aliquots, stored at -20°C to -30°C, and showed stable purity for up to 15 days. Bhatya et al., (2013) similarly found that DTPA and MDP aliquots stored at -10°C for up to 10 days maintained over 90% radiochemical purity.

Ballinger et al., (1995) demonstrated the stability of fractionated ^{99m}Tc-Exametazime (^{99m}Tc-HMPAO) kits. These kits were divided into aliquots, frozen, and later reconstituted with ^{99m}Tc pertechnetate. Even after five days of freezing, the radiochemical purity ranged from 90% to 96%. Notably, when stored in vials purged with argon, the radiochemical purity of aliquots remained above 97%, even after 21 days.

Penglis et al., (1998) fractionated and stored Mercaptoacetyl triglycine (MAG3) at -70°C for 28 days, achieving an average radiochemical purity of 98.4% upon reconstitution. Similarly, ^{99m}Tc-Tetrofosmin, stored at -80°C for 3 months, retained purity of 96.5 ± 1.6% (Penglis et al., 2000). Sestamibi kits, fractionated and stored at -20°C, showed labeling efficiency above 90% (Mansur et al., 2006). Cooper et al. (2006) stored sestamibi kits in nitrogen-filled vials for 8 hours, maintaining purity above 90%. Fractionation

studies have also demonstrated cost savings. Prabakaran et al. (1997) showed an average radiochemical purity of $92.1 \pm 2.7\%$ for fractionated sestamibi and exametazime kits. Malzac et al. (1997) successfully fractionated several kits, including HMDP, MAG3, and HMPAO, with significant cost savings and radiochemical purity within acceptable limits.

However, Decristoforo et al. (1996) found that MAG3 kits stored at -10°C to -70°C had low radiochemical purity (63%) due to tin (II) oxidation caused by oxygen exposure in the eluate. This highlights the need for careful handling of kits containing stannous chloride, as an inert gas atmosphere (e.g. argon) is necessary to prevent oxidation and maintain purity. Bayomy et al. (2009) investigated different methods to reduce MDP kit costs. They found that fractions stored in non-oxygenated vials at -20°C to -28°C maintained 98.6% radiochemical purity. However, fractions stored in oxygenated vials at the same temperature had lower purity (88.9%), underscoring the importance of using inert gas or antioxidants during storage to preserve ligand stability. In conclusion, fractionation of radiopharmaceutical kits has been shown to be a viable method for cost savings, with most studies reporting radiochemical purity levels that meet clinical and research standards. However, certain kits, like MAG3, require additional precautions to maintain stability, particularly when stannous chloride is involved.

2.5. Biodistribution and mechanism of localization of Radiopharmaceuticals

By combining a biologically active molecule that mimics the physiological properties of the imaging site of interest with a radioactive molecule (radionuclide), which allows external detection by emitting radiation (Palm et al., 2003; Ziessman et al., 2013; Ranjbar et al., 2021; Jang et al., 2022), radiopharmaceuticals are created. This process is known as labelling. This ensures that the compounded radiopharmaceutical will be localized or distribute in the patient's region of interest. The labelling efficiency is determined by performing a radiochemical purity test, which must be above 90% (Saha, 2010; Sadeghpour et al., 2015; Maioli et al., 2017; Rathmann et al., 2019). This accurate localization makes it easier to obtain a high-quality diagnostic image and a precise diagnosis. Factors related to radiopharmaceutical preparation and formulation are significant variables influencing the biodistribution of radiopharmaceuticals (Ripoll-Hamer et al., 1998; Saha., 2010; Kurniawan et al., 2020; Elgebaly et al., 2023). To ensure successful biodistribution, some quality control tests, such as those for radiochemical

purity, must be carried out before to injection (Vallabhajosula et al., 2010; Nagai, et al., 2011; Swanepoel., 2014; Doot et al., 2020). Numerous mechanisms, including active transport, capillary blockade, sequestration, antigen-antibody reactions, receptor binding, and metabolic trapping, enable the biodistribution and localization of radiopharmaceuticals (Palm et al., 2003; Newberg et al., 2004; Saha, 2010; Taylor, 2013; Kunos et al., 2021).

In many instances, the determination of the diagnosis of infectious diseases, including those involving deep-sited illnesses like osteomyelitis depends on the enhanced absorption, localization, and biodistribution of radiolabelled radionuclides like ^{99m}Tc -MDP, which are often higher in damaged bone or tissue where there are infections (Horst et al., 2012; Lin et al., 2021), when the bone heals. The interaction with specific medications is one of the factors that can interfere with the efficient biodistribution of radiopharmaceuticals (Lentle et al., 2007; Ji-xin and Yan-hua, 2010). Gamma rays released by the ^{99m}Tc -MDP are captured by a Single Photon Emission Computed Tomography (SPECT) gamma camera connected to a computer, creating images that indicate the location and biodistribution of the ^{99m}Tc -MDP. Figure 2.4 below shows an example of a SPECT gamma camera. A strong foundation for the idea that this method can be used has been provided by biodistribution studies on fractionated MDP that have shown positive results in the literature (Negi et al., 2019; Thokchom et al., 2022). According to a study by Thokchom and colleagues, the fractionation of cold kits using standardized technology, which is a cost- and time-effective process, had a very good RCP hence had shown excellent localization in hundred human patients. Similar to this, a study on various radiopharmaceuticals was conducted in 2019 by Negi and others. Imaging in human patients with numerous samples showed that the biodistribution of the fractions was precise in the region of interest (Negi et al., 2019).



Figure 2.4: An example of a gamma camera in nuclear medicine Department (<https://www.bing.com/images/search>).

Figure 2.5 below shows images of a SPECT gamma camera used on a human patient (Thokchom et.al., 2022).

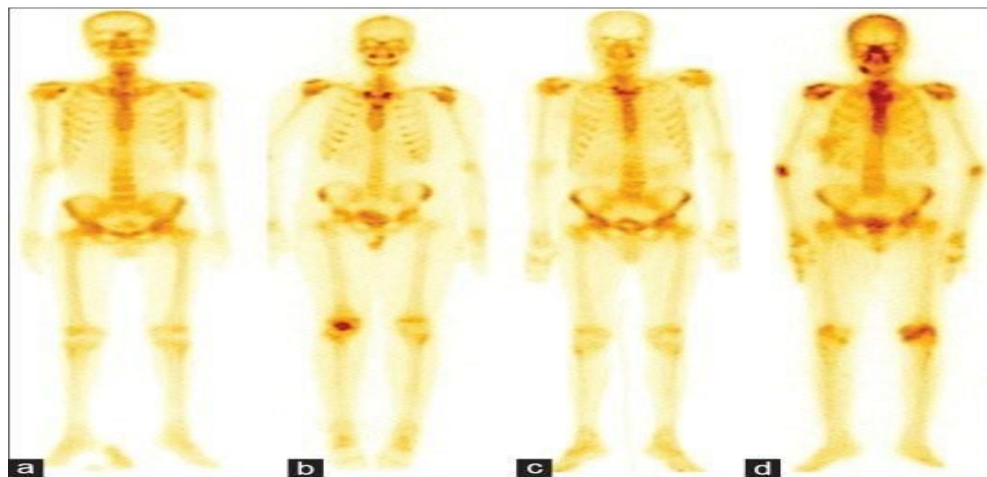


Figure 2.5: Whole body anterior bone image. (a) Day 1 (vial method). (b) Day 2 (vial method). (c) Day 1 (syringe method). (d) Day 2 (Syringe method)

Key to figure 2.5: Whole Body Anterior Bone Image using SPECT gamma camera:

1. **High Uptake Areas (Bright/Intense Regions):** Represent areas of increased radiopharmaceutical accumulation, indicating high metabolic or osteoblastic activity. This is commonly seen in joints, spine, pelvis, and healing fractures. Possible indications include metastatic bone disease, fractures, infections (osteomyelitis), or arthritis.

2. Moderate Uptake Areas (Intermediate Intensity): Normal physiological distribution of the radiopharmaceutical in bones. Symmetrical uptake in ribs, long bones, and skull suggests a normal scan.
3. Low Uptake Areas (Dark/Cold Spots): Indicate reduced or absent radiopharmaceutical uptake. May be due to ischemia, bone necrosis, or certain bone lesions.
4. Differences Between Day 1 and Day 2: Show changes in radiopharmaceutical distribution may indicate clearance patterns of radiopharmaceutical, disease progression, or treatment response.
5. Vial Method vs. Syringe Method: Indicates differences in image quality and uptake patterns as the radiopharmaceutical wears out due following biodistribution efficiency. The parts showing more uptake (darker than others) of MDP indicate presence of disease, hence aids in disease localisation in region of interest. Similarly, figure 2.7 a Google image also clearly shows the distribution of the concentration of the ^{99m}Tc -MDP radiopharmaceutical can be seen as hot spots showing dark brown, this significantly indicates needy areas where the bone is highly taking up the methyl diphosphonate in a bid to repair and heal. This can be seen also in Figure 2.6 below extracted from the study by Dhingra et al. (2019). These images were generated by a SPECT gamma camera done on a human patient. The distribution of the concentration of the ^{99m}Tc -MDP radiopharmaceutical can also be seen in Figure 2.8, this one was also generated by a gamma camera but the imaging was done in a rat. The parts showing more uptake of MDP indicates location of pathological condition.



Figure 2.6: Gamma camera image showing biodistribution of ^{99m}Tc -MDP radiopharmaceutical aliquots (Negi et al., 2019)

Key to figure 2.6: Gamma Camera Image Interpretation

1. High Uptake Areas (Bright/Intense Regions): Bones (Skeleton): Normal physiological uptake occurs in bones due to the affinity of ^{99m}Tc -MDP for hydroxyapatite, reflecting bone metabolism. Fractures, Bone Metastases, Osteomyelitis: Increased radiotracer concentration in these regions indicates active bone remodelling or pathology.
2. Moderate Uptake Areas (Intermediate Intensity): Joints: Slightly increased uptake may be seen in arthritic or inflammatory conditions. Soft Tissues: Some soft tissue uptake can occur due to inflammation or calcification.
3. Low Uptake Areas (Dark/Cold Regions): Normal Soft Tissues & Background Activity: Minimal radiotracer accumulation in muscles, fat, or blood, except in cases of abnormal calcium deposition. Photon Deficient Lesions: Areas of decreased uptake may indicate avascular necrosis or certain bone tumors.

The image below shows pictures of a SPECT gamma camera done on a human patient. The parts showing more uptake of MDP indicate disease. SPECT Gamma camera has better imaging than planar hence highly recommended.

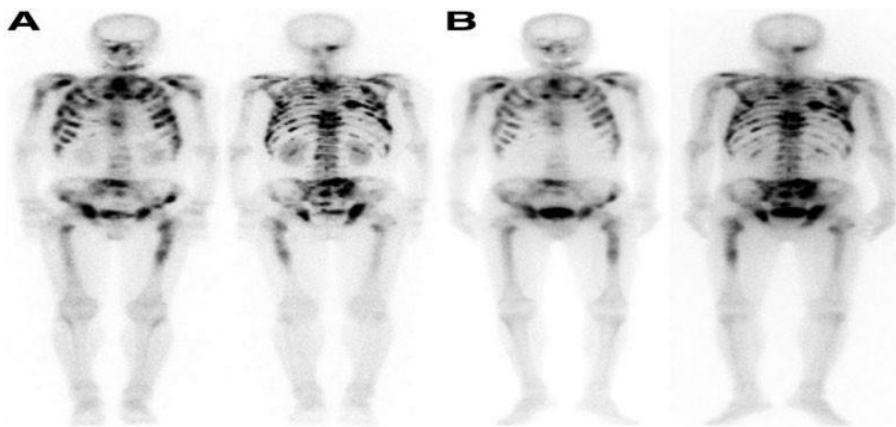


Figure 2.7: Localization of ^{99m}Tc -MDP radiopharmaceutical in cancer metastases (<https://www.jnm.snmjournals.org>)

Key to Figure 2.7: Localization of ^{99m}Tc -MDP Radiopharmaceutical in Cancer Metastases

1. High Uptake Regions (Bright/Hot Spots): Indicates areas of increased osteoblastic activity. Common sites: Spine, ribs, pelvis, long bones, and skull. Possible interpretation: Bone metastases, fractures, or degenerative bone disease.
2. Normal Physiological Uptake: Symmetrical uptake in major joints (e.g., knees, shoulders) and along vertebral bodies.
3. Low or Absent Uptake (Cold Spots): Indicates areas of reduced perfusion or bone destruction.

The image of a SPECT gamma camera done on a rat with peritonitis. The parts showing more uptake of MDP indicate disease.

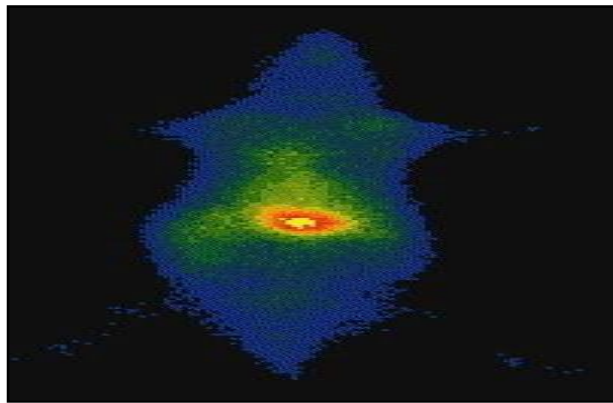


Figure 2.8: Image produced from a gamma camera, of a rat model injected with radionuclide shows better localization than images from a planar (Sulaiman et al., 2018)

Key for Figure 2.8: SPECT Gamma Camera Image of a Rat with Peritonitis

1. High Uptake Areas (Bright/Hot Spots): regions with increased MDP accumulation, indicating active infection or inflammation.
2. Low Uptake Areas (Dark/Cold Spots): Regions with minimal radionuclide localization, representing normal or less affected tissue.
3. Bone Structures: Areas naturally accumulating MDP due to bone metabolism, serving as anatomical landmarks.
4. Soft Tissue Background: Areas with general radionuclide distribution but without specific targeting, providing contrast for lesion identification.

2.6. Possible challenges in the use of Radiopharmaceuticals

2.6.1. Cost of Radiopharmaceuticals

Worldwide, the expense of using radiopharmaceuticals is still considerable (Zimmermann, 2013; Sgouros et al., 2020; Cutler, 2019). Additionally, papers indicating fractionation as a method of optimizing their use have been published in the literature (Prabhakaran et al., 1997; Penglis et al., 2000; Mansur et al., 2006; Bhatia et al., 2013; Dhingra et al., 2019). The method of fractionating radiopharmaceuticals involves dividing up radiopharmaceutical kits that have been reconstituted with normal saline into different portions for usage in the future (Fleming and Ryo, 1992).

2.6.2. Design and delivery of Radiopharmaceuticals to point of focus

To understand the specific difficulties faced during the design, radiosynthesis, in vitro and in vivo evaluation, and clinical translation of novel radiopharmaceuticals, this review discusses general concepts about radiopharmaceuticals for diagnostic or therapeutic applications (Vermeulen et al., 2019). The type and location of the molecular target, the desired application, and the time restrictions imposed by the relatively short half-life of radionuclides must all be considered when choosing a suitable vector molecule and an appropriate radionuclide (Ballinger, 2018).

2.7. Future Trends in Radiopharmaceuticals use in Medicine for diagnosis and treatment

In an effort to better human health, innovative research is still being done to create radiopharmaceuticals (Anderson, 2019; Kaushik et al., 2021; Holik et al., 2022; Vallabhajosula., 2023). The development of even more specialized agents for bacterial infection imaging has been made possible by the careful design of new radiopharmaceutical kits (Auletta et al., 2019). For instance, a dried ciprofloxacin radiopharmaceutical kit (Nurlaila et al., 2009) and an Ethambutol radiopharmaceutical kit (Kartamihardja et al., 2018) are being tested for clinical usage in Indonesia to diagnose extra pulmonary tuberculosis and bacterial infections, respectively. This study will examine potential applications for MDP aliquots stored at ambient temperatures with or without addition of stannous chloride. If they work, these aliquots can be employed in low resource settings with low volume Nuclear Medicine facilities.

CHAPTER THREE

MATERIALS AND METHODS

This chapter outlines and discusses the research methodology in terms of the research design, research approach, sample size calculation determination, data collection tools, data collection methods, data analysis and ethical clearance respectively.

3.1. Study Design

The research implored a mixed (quantitative and qualitative) method research design which conducted laboratory experiments and observational studies. The purpose of this research was to modify a previously established method for fractionating Methylene diphosphonate (MDP) radiopharmaceuticals for use in diagnosing deep seated infectious diseases once labelled with Technetium (^{99m}Tc). Figure 3.1 below shows the formula for ^{99m}Tc -MDP.

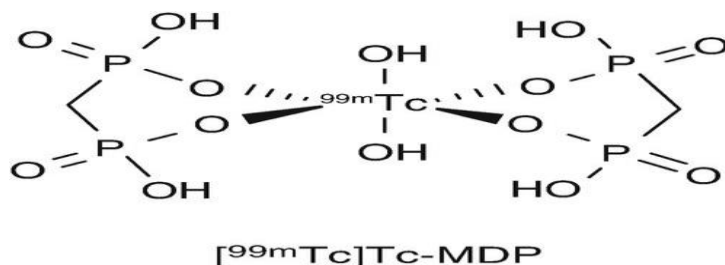


Figure 3.1: MDP kit Formula labelled with Tc

3.1.1. Quantitative Component: Experimental (quantitative) Design The experimental group design involved:

- a. Intervention which involved the aliquoting process. The MDP radiopharmaceutical kit was subjected to aliquoting with the addition of stannous chloride and Argon gas. This process aimed to evaluate the impact of these specific interventions on the stability and characteristics of the quality of MDP fractions.
- b. Control Group included MDP Aliquots without addition of Stannous Chloride and Argon gas. The control group consisted of MDP aliquots prepared without the inclusion of stannous chloride and argon gas. This control setup allowed for a comparative analysis to discern the specific effects of stannous chloride on the stability of the

radiopharmaceutical. Also, MDP aliquots kept at recommended (frozen and refrigerated) conditions to compare the difference in quality with those MDP aliquots kept at ambient conditions.

c. Conditions involved ambient storage. Both the experimental and control groups were stored under ambient conditions. This choice of storage conditions reflects real-world scenarios where radiopharmaceuticals may be stored before use during power outage and lack of appropriate back up plans.

d. A duration of eighteen days stability assessment was considered. The study spanned a defined period of eighteen days to comprehensively assess the stability of the MDP fractions over time. This duration was selected to capture potential changes in radiochemical purity and other quality indicators during an extended storage period.

e. Measurements involved quantitative assessments. The stability of MDP fractions was measured through periodic quantitative assessments. These quantitative assessments included Radiochemical Purity which measures the extent to which the radiopharmaceutical retained its intended chemical composition, pH Measurements which monitors changes in pH levels as an indicator of potential alterations in the chemical characteristics of the MDP fractions and other additional Quality Indicators were conducted to evaluate various quality indicators, providing a comprehensive understanding of the stability of the radiopharmaceutical under different conditions as detailed under 3.6.3.

3.1.2. Real-Time Biodistribution Study: Observational (qualitative) Design

a. Research objects were infected and uninfected rat models. The uninfected rat models were used as controls. The study involved the participation of a cohort of infected rats and uninfected rat models undergoing nuclear medicine procedures using MDP aliquots. These rat models were selected to mimic real-world scenarios where the radiopharmaceutical is used for diagnostic purposes in the presence of various bone infections.

b. The objective was to assess the diagnostic potential assessment. The primary aim of this observational study was to evaluate the diagnostic potential of MDP aliquots stored under ambient conditions. This involved investigating the effectiveness of the MDP radiopharmaceutical aliquots in accurately localising in regions of interest (those with infections) within the rat models.

- c. Data Collection involved real-time monitoring and imaging, upon which the biodistribution patterns were observed in real-time during a nuclear medicine procedure. The nuclear planar was employed to capture the dynamic images showcasing the distribution of MDP within the rat models over time. This real-time approach allowed for a qualitative understanding of how the radiopharmaceutical interacted with infected tissues.
- d. Data Analysis involved qualitative analysis of biodistribution patterns. The observed biodistribution patterns were qualitatively analyzed to identify areas of increased uptake or concentration of the radiopharmaceutical within the infected rat models. This qualitative assessment provided insights into the performance of MDP in diagnosing and highlighting infection sites.
- e. Correlation of Qualitative and Quantitative Findings: A holistic approach involved correlating qualitative observations with quantitative data to enhance the overall understanding of how MDP aliquots, stored under ambient conditions, contributed to the diagnostic process in the context of infection in the rat models.

3.2. Study Site

The modified fractionation procedure was tested in a controlled setting at the University Teaching Adult Hospital Nuclear Hot Laboratory in Lusaka, Zambia. The location of the site is as shown in Figure 3.2 below. The University Teaching Hospital is the only tertiary referral hospital in Zambia that offers services in Nuclear Medicine and is certified by the Zambia Radiation Protection Authority (ZRPA), hence the research was conducted there.

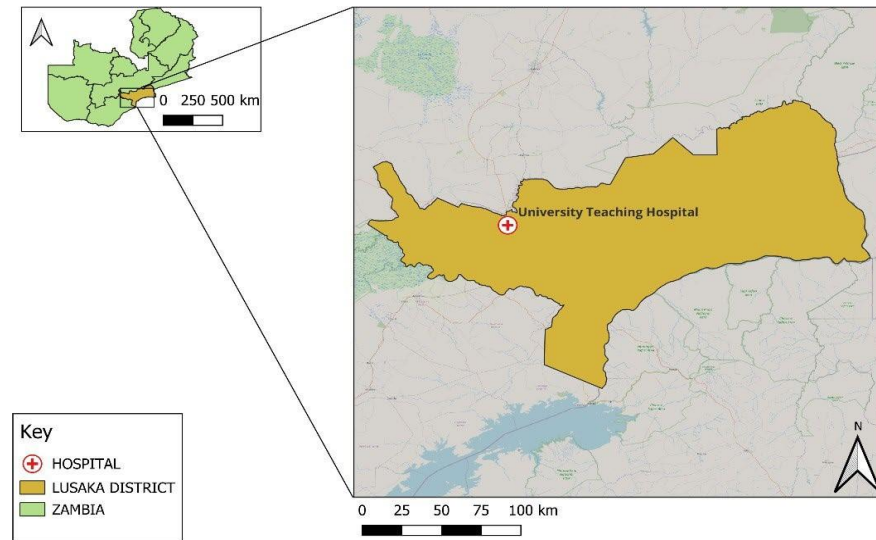


Figure 3.2: The map of Zambia highlighting Lusaka Province the location for the research site. Map of study sites constructed in Quantum Geographic Information System (QGIS) software, version 3.40.3.

Campus and Distance Scale for Figure 3.2

Campus Scale: Hospital Complex Size covers approximately ~30-40 hectares (300,000 to 400,000) square meters (<http://yourfreetemplates.com> google maps). Comprises multiple buildings, specialized wards, and research facilities with bed Capacity of over 1,800 beds, making it the largest main tertiary referral hospital offering nuclear medicine services hospital in Zambia. University Teaching Adult Hospital Nuclear Hot Laboratory marked as the specific research site within the University Teaching Hospital (UTH) campus.

Distance Scale: from Lusaka City Center to UTH, it is approximately 3–5 km, while from Kenneth Kaunda International Airport to UTH it is approximately 20–25 km (Maps from <http://yourfreetemplates.com>, google maps).

3.3. Study Sample Size Calculation and Sampling method

3.3.1. Sample Size Calculation for Number of MDP aliquots

The sample size was determined by using Cochran's formula, which uses a 95% confidence interval (so $Z=1.96$) and a mean from a comparable study, in this case a study conducted by Dhingra et al. (2019) with SD of 5.43 and sample size of 30. Because the

outcome variable is a continuous (RCP: >90% to 100%; Saha, 2010), the formula used a 95% confidence interval (so $Z=1.96$) and a mean from the similar study done by Dhingra et al. (2019).

Therefore, Sample size calculation for the research was;

$$N = (Z^2) \times SD^2 / \text{Precision}^2$$

Precision = $Z \times \text{Standard Error (SE) of mean from similar study}$

$SE = SD / \sqrt{n}$ (from similar study)

$SE = 5.43 / \sqrt{30} = 0.9914$ while

Precision = $Z \times \text{Standard Error (SE)}$ Precision = 1.96

$\times 0.9914 = 1.194$.

Sample size (n) therefore was calculated as $= 1.962 \times 5.432 / 1.1942 = 75.19 \approx 76$ tests $\pm 5\%$ fallout = 80 tests of 1 drop (0.05mL) each from a 1mL mixture of MDP aliquots with TC-99m liquid. Therefore, in this research, 80 was our study population and were subjected to various analysis tests and were used to calculate the RCP of aliquots made from various types of NS. The 80 was distributed across all the NS stream and control as can be seen in Table 3.1 below.

Table 3.1: Sample size distribution

Specific Objectives	Type of Aliquots	Storage conditions	QC time for aliquots	Total No of aliquots
1. To establish whether the radiochemical purity (RP) of	MDP kit + NS + Ar	Ambient	1 day to 18 days	18

Methylene diphosphonate (MDP) aliquots reconstituted with Argon purged Normal Saline (NS) is different from those reconstituted with non-Argon purged NS.	MDP kit + NS	Ambient	1 day to 18 days	18
2. To determine whether the RP of MDP aliquots reconstituted with non-Argon	MDP kit + NS + SnCl ₂ + Ar	Ambient	1 day to 18 days	18
	MDP kit + NS+ SnCl ₂	Ambient	1 day to 18 days	18
purged NS and Stannous Chloride is different from those reconstituted Argon purged NS with Stannous Chloride and without stannous.				

3. To compare the RCP for different MDP aliquots kept at ambient conditions to those kept at recommended conditions (Frozen and refrigerated-Once off).	Four from each NS stream	Refrigerated And frozen conditions	Day 28	8
---	--------------------------	------------------------------------	--------	---

4. To determine the bio distribution of MDP aliquots using animal models (rats/mice) kept at ambient. (Once off)	2 aliquots reconstituted with Tc to be purposively sampled from those kept at ambient conditions with	Could not be done due to malfunctioning gamma camera	Could not be done due to malfunctioning gamma camera	Could not be done due to malfunctioning gamma camera
	acceptable RCP			

TOTAL SAMPLE SIZE				80 aliquots
----------------------	--	--	--	-------------

3.3.2. Sample Size Calculation for Number of Rat Models

The sample size for comparing groups of animals was determined employing the one-way resource equation method. Within this approach, a parameter denoted as E is calculated based on the predetermined sample size range, within the optimal range of 10 to 20 as suggested by Arifin and Zahiruddin (2017). $E = \text{total number of animals} - \text{total number of groups for each of the groups}$. $n = DF/k + 1$, where Degree of freedom (DF) lies between 10 as minimum and 20 as maximum, k = number of groups, and n = number of subjects per group. This research had four groups, though biodistribution studies were done in two groups that recorded lowest and highest RCP. Therefore; Sample size = degrees of freedom (Df)/number of treatment group+1. Minimum sample = Df 10 = Df/2 groups +1 = $10/2+1 = 6$. And maximum sample size = Df 20 = Df/2 groups +1 = $20/2+1 = 11$ animals.

Therefore, total minimum sample size = minimum min number of animals x number of groups of animals; $6 \times 2 = 12$ or maximum sample size = maximum number of animals x number of groups of animal groups = $11 \times 2 = 22$. In this research, the minimum number of sample size was used, therefore the experiment was done in twelve rats. The twelve rats were to be distributed 3 per stream (one control and two infected models), however, due to records of high activity in the hot lab only the stream that recorded the lowest and highest RCP were considered.

3.3.3. Inclusion criteria

- All clear uncolored MDP aliquot mixtures with visibly particle-free during the research period.
- Rats between 2 to 3 months old weighing between 30 to 65g.

3.3.4. Exclusion criteria

- All unclear colored MDP aliquot mixtures with discernable particles.
- Rats that became sickly or distressed.

3.4. Research Variable

The research variables included the following;

- a. Independent Variables: Stannous Chloride and Argon gas Addition and this was at four levels; which are; level 1: MDP aliquots without stannous chloride or Argon gas addition (Control Group- NS), level 2: MDP aliquots with stannous chloride addition (Experimental Group- NSA), level 3: MDP aliquots with stannous chloride and Argon gas addition (Control group- NSSnCl₂A) and level 4: MDP aliquots with stannous chloride addition (Experimental Group - NSSnCl₂)
- b. Dependent Variables: Stability of MDP Fractions which measured; radiochemical purity, pH and other quality control parameters and biodistribution Patterns which measured the real-time monitoring of MDP during diagnostic procedures (qualitative and quantitative analysis of biodistribution.
- c. Moderator Variables: Ambient Conditions which are controlled conditions representing typical storage in Zambia and their impacts stability of MDP aliquots
- d. Control Variables: Duration of Storage entailing controlled Variable of uniform storage duration for both experimental and control groups and characteristics of infected rat models undergoing real-time biodistribution studies with consistency in patient profiles during diagnostic procedures. Figure 3.3 below shows a summary of flow of research methodology activity, Table 3.2 shows the conceptual framework and 3.4 below shows a summary of research variables.

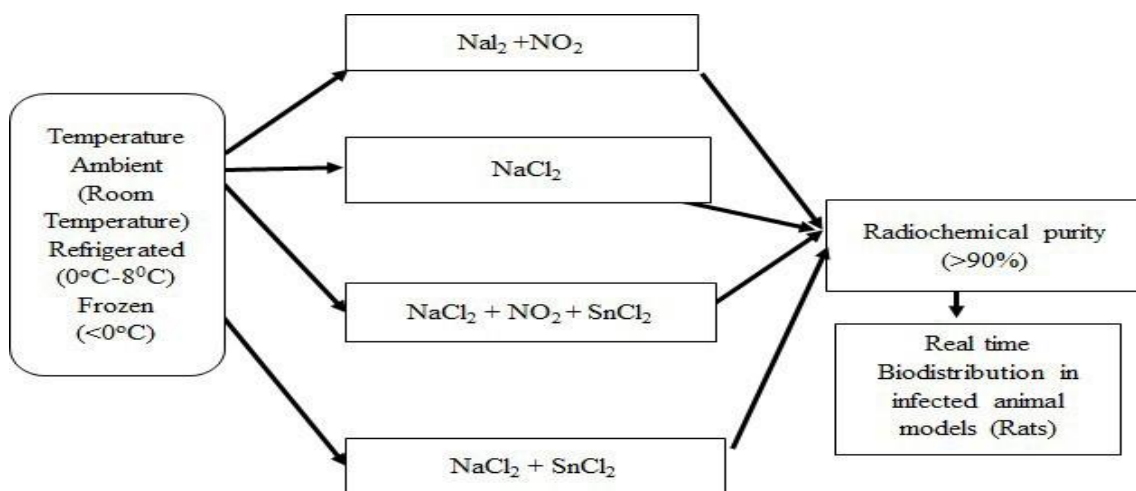


Figure 3.3: Summary of flow of research methodology activity

The conceptual framework for this study illustrates the relationships between the independent, dependent, moderator, and control variables, providing a structured understanding of the research process. It interprets the relationship between the independent variables (Stannous Chloride and Argon Gas Addition), the moderator variable (Ambient Conditions), and the dependent variables (Stability of MDP Fractions and Biodistribution Patterns). The control variables (Duration of Storage and Characteristics of Infected Rat Models) ensure methodological rigor and control for extraneous factors, enhancing the validity and reliability of the study's findings.

Table 3.2: Conceptual Framework

	Variable	Measure
Dependent variables	Stability of MDP aliquots (RCP) Biodistribution Patterns	pH, RCP Monitor MDP distribution <i>in vivo</i>
Moderator variables	Ambient temperature	Impact on stability and biodistribution of MDP aliquots
Controlled variables	Duration of storage	Uniformity and consistency of QCs
Independent variables	Non-NO ₂ purged NS (NS stream A)	
	NO ₂ purged NS (NSA/stream B)	
	Non-NO ₂ purged NS with SnCl ₂ (NSSnCl ₂ /stream C)	

	NO ₂ purged NS with SnCl ₂ (NSSnCl ₂ A, stream D)	
--	---	--

Table 3.3: Showing Research Experimental Setup

Study Question	Does ambient conditions affect RCP of aliquots from MDP Kit	
Independent variables (Ambient condition)	Controlled setup	Experimental setup
	Aliquots kept at recommended [frozen (below 0°C) and refrigerated (2 to 8°C)]	Aliquots kept at ambient (room)
Dependent variables (Radiochemical purity)		
Controlled variables	Controlled variables	Controlled variables
1. Plain Normal Saline (NS)	NS	NS
2. Argon purged NS (NSA)	NSA	NSA
3. plain NS with Stannous Chloride (NSSnCl ₂)	NSSnCl ₂	NSSnCl ₂
4. N ₂ purged Argon and with SnCl ₂ (NSSnCl ₂ A)	NSSnCl ₂ A	NSSnCl ₂ A

Figure 3.4 below shows a comparison of the laboratory workflow of preparing MDP radiopharmaceutical with and without fractionation

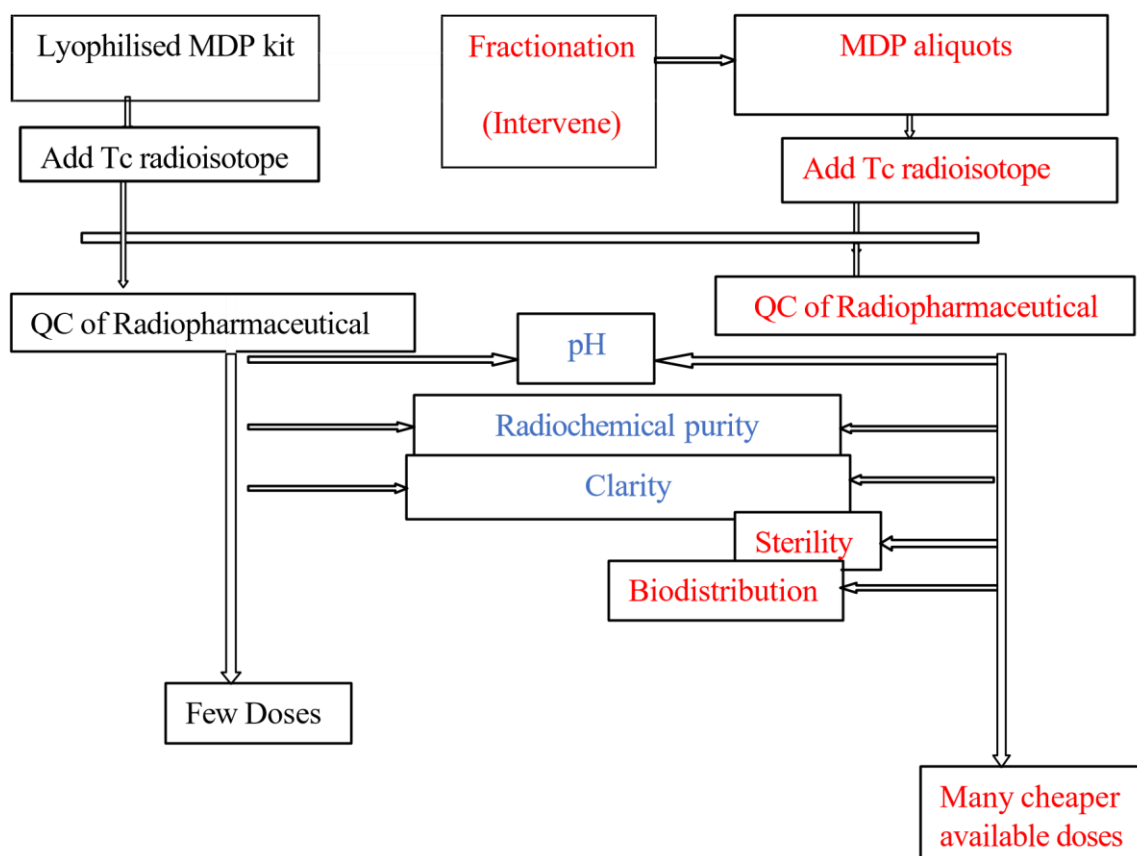


Figure 3.4: Laboratory Work flow

3.5. Sampling Method and Techniques

Knowing that we were dealing with very rare expensive substances that requires making the most of it, purposive sampling method was applied where the selection of the micro-sample was intentionally made to capture specific characteristic components of interest from within the larger sample. The emphasis was on obtaining a sample that was representative of the overall quality. Similarly, purposive sampling was applied on selecting the rat that were used for the biodistribution test of MDP aliquots.

3.6. Viability Procedures

Using laboratory reports in the form of tables, data was collected by observing the results from the quality control of MDP aliquots prepared from several types of normal saline. The Technetium radiolabelled MDP aliquots were made to go through different tests in this laboratory to establish their viability. These tests were; radiochemical purity,

radionuclidic purity, chemical purity, sterility test, pyrogen test, bio distribution, and physical properties. The data collection procedure was done as follows:

3.6.1. Preparation of Various Types of Normal Saline

Figures 3.5 and 3.6 below are showing the student preparing the different streams of Normal Saline. Four streams of various types of normal saline were prepared using four 500 mL packets of NS. The first NS packet (NS Stream) was left unchanged, while the second was purged with argon (A) gas and was labelled NSA. The third NS packet was purged with argon gas and 0.5g of Stannous Chloride was added and was labelled NSSnCl₂A while the fourth NS packet received an addition of 0.5g of Stannous Chloride and was labelled NSSnCl₂. These were marked as such and kept in an ambient environment.



Figure 3.5: Student purging the NS with argon gas (NSA)



Figure 3.6: Various types of normal saline; plain normal saline (NS), normal saline purged with argon gas and augmented with stannous chloride (NSSnCl₂A) and normal saline with stannous chloride (NSSnCl₂)

3.6.2. Preparation of MDP Aliquots

The MDP (Methylene diphosphonate) kit and Molybdenum - Technetium generator (⁹⁹Mo/^{99m}Tc Generator) were imported from South Africa. Each MDP Radiopharmaceutical (RP) vial was reconstituted with 4 mL of each type of the four normal saline (NS) mentioned above that is NS, NSA, NSSnCl₂A, and NSSnCl₂. Then, four aliquots by 1 mL of MDP mixture was extracted and placed in 4 sterile vials. Each stream of NS contained 20 aliquots), making a total of 80 aliquots in 80 sterile vials. 1 aliquot from each stream making a total of 4, were stored under refrigeration (2°C to 8°C), while 1 aliquot from each stream making a total of 4 were stored at frozen (below 0°C). The remaining 72 aliquots from all streams were stored at ambient conditions. Figure 3.7 below is showing one set of the four streams of MDP aliquots. Thereafter, these aliquots were made to through a radiochemical test and other quality checks, as described below.



Figure 3.7: Aliquots arranged according to different streams of NS and agar plates for sterility test

3.6.3. Quality Control of labelled aliquots

3.6.3.1. Radiochemical Purity Test (RCP)

RCP is usually done after compounding a radiopharmaceutical like MDP with a radioisotope like Technetium-99m (^{99m}Tc) eluate (Appendix E) to determine the quantity of the desired amount. Therefore, the RCP test was done by getting a drop of the labelled (Appendix F) Technetium-99m-labelled MDP (^{99m}Tc -MDP) radiopharmaceutical kit aliquots was applied during the operation to two Whatman chromatographic paper strips, they were then submerged in two distinct mobile phases (Normal Saline and Methyl ethyl Ketone). Finding the component concentrations (radiochemical impurities and intended form of radiochemical compound) in relation to the interaction of the combination with the mobile phase came next. Free Technetium pertechnetate ($^{99m}\text{TcO}_4^-$), as well as the colloid also known as hydrolyzed reduced pertechnetate ($^{99m}\text{TcO}_2$) in ^{99m}Tc labeled

complexes, were among the radiochemical impurities analyzed (Zolle, 2017). Poor-quality pictures and an extra dosage of radiation for the patient come from the presence of an unanticipated level of radiochemical contaminants in a radiopharmaceutical.

Whatman paper was employed as the immobile phase to detect free $^{99m}\text{TcO}_4^-$, while methyl ethyl ketone (MEK) and normal saline were utilized as the mobile phase. Free $^{99m}\text{TcO}_4^-$ is soluble in both NS and MEK; as a result, it will migrate with the mobile phase as the paper is wetted until the expected mark and the reference (rf) value are noted. Whatman paper number 1 served as an immobile phase and MEK served as a mobile phase to determine the colloid, $^{99m}\text{TcO}_2$. Sodium chloride is insoluble in the colloid ($^{99m}\text{TcO}_2$) and required radiopharmaceutical. To do this, a strip of 0.9 cm by 8.5 cm Whatman paper was cut, marked with a pencil to indicate the origin at 1 cm from the bottom, and marked with a pencil to indicate the solvent front line at 6.5 cm from the origin.

The two chromatography paper strips were immediately put in the developing chamber for chromatography with a lid containing MEK and normal saline and allowed to develop once a drop (0.05 mL) of the mixture was detected on the origin line as illustrated in Figure 3.8 below. The strip was removed and left to dry when the solvent got to the solvent front line. The amount of ^{99m}Tc -MDP, free $^{99m}\text{TcO}_4^-$ (pertechnetate), and $^{99m}\text{TcO}_2$ colloid were typically measured on chromatograms made from radiochemical purity using the dosage calibrator.

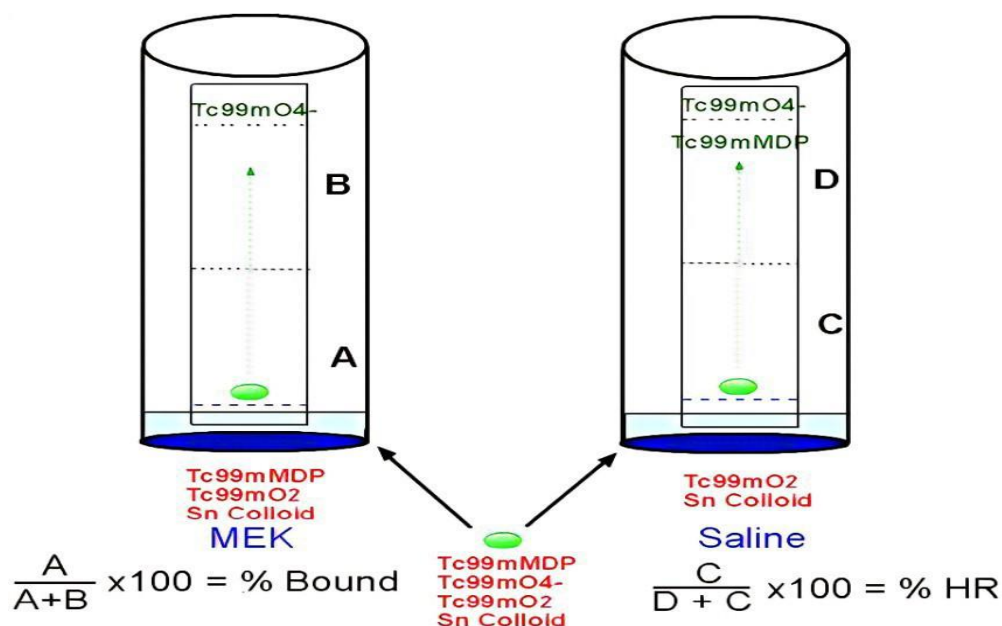


Figure 3.8: Showing a detailed illustration of paper chromatography procedure (<https://www.bing.com/images/search?>)

The RP was done on all aliquots sequentially according to the age of aliquots starting from 1 day old up to the oldest aliquot generated of about 18 days according to our sample size. According to the WHO (2021) and USP (2011), ^{99m}Tc -MDP should only be used if the RCP is at least 90%. Table 3.4 below indicates the minimum levels of acceptable standards of usable ^{99m}Tc -MDP while Figures 3.9 and 3.10 are showing a student carrying out the RCP test.

Table 3.4: Showing acceptable Radiochemical Purity from the standard references

Radiopharmaceutical	US Pharmacopoeia	WHO Pharmacopoeia
Methylene diphosphonate (MDP)	% RCP > Or = to 90%	% RCP > Or = to 95%



Figure 3.9: Student performing the Radiochemical Purity

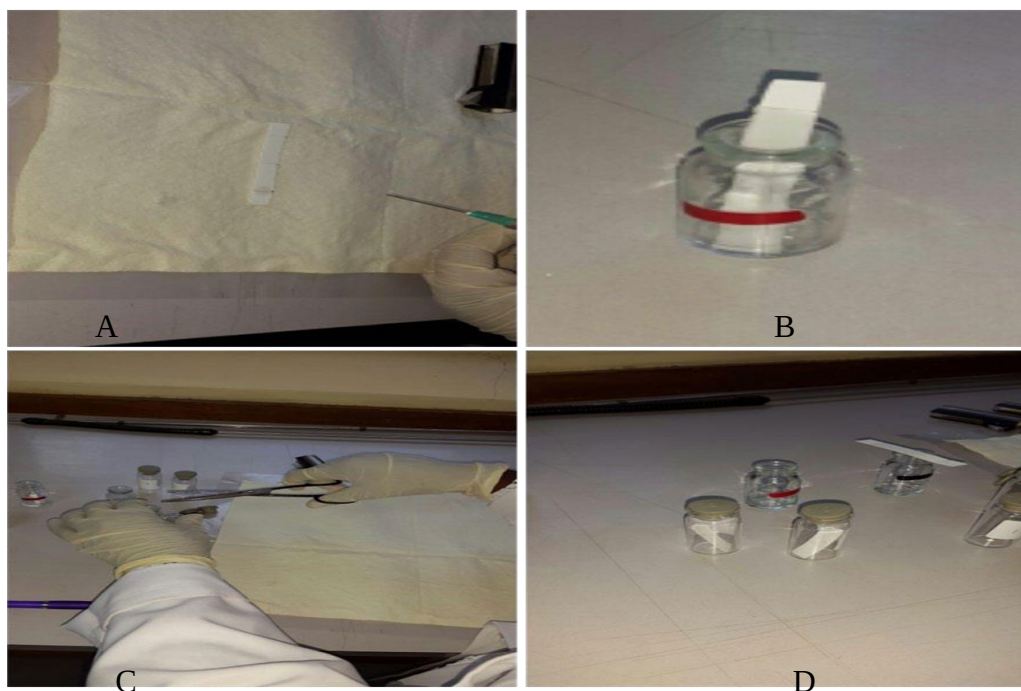


Figure 3.10: Strip of whatman paper with a drop of chemical to be analysed (A), Whatman paper in mobile phase (B), Student cutting the whatman papers (C), Cut whatman papers prepared for RCP test as explained above (D)

3.6.3.2. Clarity of ^{99m}Tc

A radiopharmaceutical's physical appearance is important. A true solution of a ^{99m}Tc eluate should be clear, colourless with no particulate matter. The materials involved were

^{99m}Tc eluate and a clear vial. The physical check involved visual inspection of the colour

of the ^{99m}Tc eluate using the naked eyes against a white and black background as shown in Figure 3.11 and 3.12 below.



Figure 3.11: An example of a background for particle size inspection (Amal, 2018)



Figure 3.12: Student verifying clarity of MDP aliquots

3.6.3.3. Potential for Hydrogen (pH) Test for Technetium Methylene

Diphosphonate

For stability and integrity, all radiopharmaceuticals should have a pH that is acceptable. By using the universal pH papers, the pH was determined by dipping the pH in the compounded ^{99m}Tc -MDP as illustrated by Figures 3.13 and 3.14. A parenteral radiopharmaceutical's pH should ideally match that of blood (7.2 to 7.4).



Figure 3.13: pH paper strips



Figure 3.14: Student conducting pH reading of pH paper strip

3.6.3.4. Chemical Purity

According to Simon et al. (2012), chemical purity is the percentage of the total mass that is in the specified chemical form. This is the quantity of unwanted, non-radioactive chemical species that are present in the radiopharmaceutical. Because the parent radionuclide, ^{99}Mo , is embedded onto an aluminium column and may occasionally be milked into the elate in more than the specified quantities, aluminium is always a potential chemical contaminant in the elution of $^{99\text{m}}\text{Tc}$. The needed components were the test, the ammonium salt of the auring tricarboxylic acid indicator, and the $^{99\text{m}}\text{Tc}$ eluate from the sampled $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators. Applying a drop of $^{99\text{m}}\text{Tc}$ eluate and a drop of a standard solution containing roughly 10 mcg/mL of aluminium to a calorimeter test paper impregnated with ammonium salt of auring tricarboxylic acid indicator was the approach used to detect the presence of aluminium. Aluminium combines with the aforementioned acid indicator to produce a faint pink colour, which is used to compare with the standard as shown in Figure 3.15 below. The amount of aluminium present determines how intense the pink colour is.

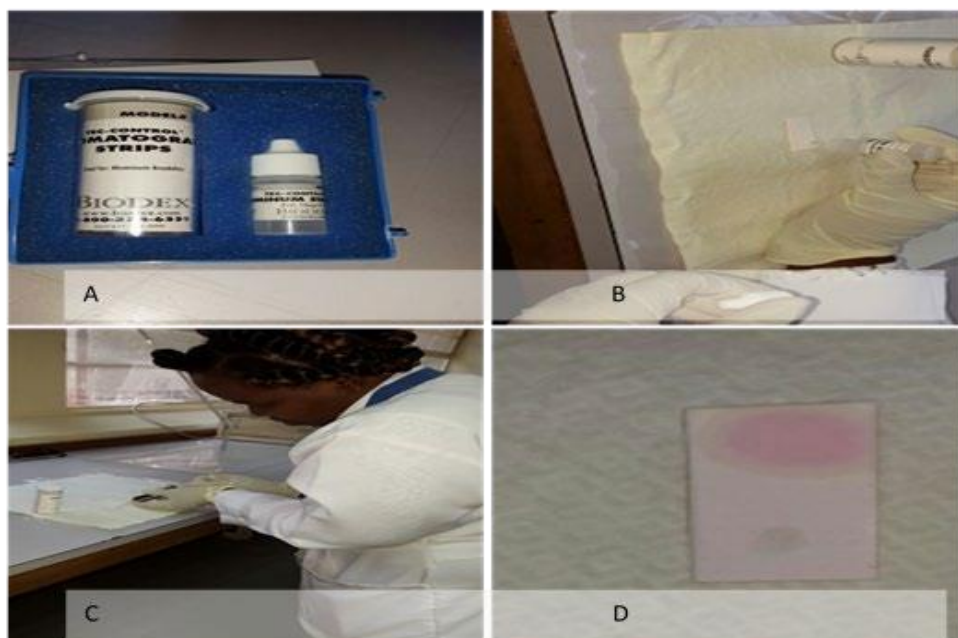


Figure 3.15: Calorimeter paper strip (A), Dropping standard liquid on Calorimeter paper strip (B), Student dropping Eluate on Calorimeter paper strip (C), Comparison of test liquid with standard (D)

3.6.3.5. Sterility of fractionated radiopharmaceuticals

For parenteral delivery, all radiopharmaceuticals must be sterile. Based on the protocols outlined in the reference books, sterility tests were conducted (USP, 2005). To make it easier to find contamination whose cause might be people, workspaces, materials, gear, a lack of engineering know-how or expertise, or the environment. Due to the requirement that GMP be followed, the labelled aliquots were put through a sterility test using two different media, Trypticase Soy Broth (TSB) and Fluid Thioglycolate Medium (FTM) as shown in Figure 3.16 and 3.17. While FTM is used to culture anaerobic bacteria, TSB is suited for the culture of both fungal and aerobic bacteria. The sample of labeled aliquots was then poured on top of the media in the appropriate agar plates. Without shaking, this combination was kept at room temperature for 14 days. If there were any indications of microbial development, the test was considered positive; otherwise, it was negative.



Figure 3.16: Sterility Testing in Laminar flow



Figure 3.17: Sterility Test conditions in Laminar flow

3.6.3.6. Radionuclidic Purity



Figure 3.18: Student conducting Radionuclidic Purity

According to Welch and Redvanly (2003), radionuclidic purity is the ratio of the indicated radionuclide activity to the total radioactivity, which is typically expressed as a percentage. A dosage calibrator was used to determine radionuclidic purity. The operation, which is part of an internal mechanism in the dose calibrator, includes measuring Molybdenum (^{99}Mo) impurity step by step as can be seen in Figure 3.18 above, which shows the student performing one of the steps of radionuclidic purity test.

3.6.3.7. Real time Determination of Bio distribution Test of radiolabelled MDP aliquots

The localization of radiopharmaceuticals of interest within an experimental animal or human subject can be followed via biodistribution research. Using MDP Radiopharmaceutical in conjunction with a radioactive isotope like Technetium-99m is one example. To detect deep-seated illnesses or malignancies, these are chemically combined and injected into an animal or a person. The real-time bio distribution of radiolabelled MDP aliquots was done in animals (rats) using *Staphylococcus aureus* (*S. aureus*) isolates (Ripoll-Hamer et al., 1998; Monzon et al., 2001; Nair et al., 2011; Reizner et al., 2014; Colston et al., 2018; Roukoz et al., 2020).

3.7. Animal studies

These studies involved culture of *Staphylococcus aureus* bacteria and infecting the animals with the confirmed bacteria to induce bone inflammation. After the research, the animals were given non-steroidal anti-inflammatory drug (Brustan syrup) and ampiclox syrup (ampicillin cloxacillin) to cure the induced inflammatory reactions and infection. The procedures were carried as described below.

3.7.1. Culture and confirmation of *Staphylococcus aureus* (*S. aureus*)

A *S. aureus* isolate from a stored clinical sample was cultured on Blood agar and incubated overnight at 37°C (Figures 3.19 and 3.20). Briefly a wire loop was used to inoculate stored isolates onto nutrient agar and incubated at 37°C for 24 hours. From the aspect of morphology, the gram-positive cocci bacteria in pairs or clusters were observed and morphologically confirmed using catalase, coagulase, mannitol fermentation and DNase tests. Following morphological confirmation, the PCR was done to confirm the identity of *S. aureus*. To conduct PCR, the DNA was extracted by the boiling method technique described by Dashti et al. (2009) from 24 hours old colonies.

Thereafter, 200 µL of sterilized distilled water was used to emulsify a loop full of pure colonies from nutrient agar. The mixture was then vortexed and heated at 95°C for 15 minutes on a heating block. This was followed by centrifugation at 5,000 *xg* for 5 minutes. The supernatant containing the DNA was then transferred to another tube and stored at -20°C until further analysis. PCR was done through the amplification using species-specific (*nuc*) gene (Table 3.4). Thereafter, the PCR was performed in a final volume of 25µl and the reaction compositions included 0.5µl (5ng/µl) of each Nuc1 and Nuc2

primers (10 Pmol), 10µl of deionized water, 12.5µl of reaction buffer of OneTaq Quick-load Master Mix (BioLabs, New England) and 1.5µl of the template DNA extracted. The amplification was performed on a Veriti thermal cycler (Thermo Scientific, Hanover, MD, USA), using the following PCR conditions: an initial denaturation step at 94°C for 5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, and a final extension step at 72 °C for 10 min. The PCR products were electrophoresed in 1.5% agarose gels with 1X Tris-acetate-EDTA (Baba et al., 2008) buffer at 100V for 30 min. The gels were then pre-stained with ethidium bromide to visualise the amplified DNA fragments (279 bp) under UV light box by comparison with a molecular size marker (100 bp ladders, eurobio, UK). Positive and negative controls were *S. aureus* ATCC29213 and molecular grade water, respectively.

Table 3.5: Primer Set used in Identifying and confirming *S. aureus*

Primer Name	Primer Sequence (5'-3')	Amplicon size (bp)	Reference
Nuc1	GCG ATT GAT GGT GAT ACG GTT	279	(Zhang et al., 2004)
Nuc2	AGC CAA GCC TTG ACG AAC TAA AGC		

3.7.2. Preparation of Inoculum for animal infection

McFarland Standards are a series of turbidity standards used to visually match the density of a bacterial suspension (McFarland, 2000; Christoflogiannis, 2001; Wiegand and Hancock, 2008; Koeth et al., 2015; Nazarian et al., 2008 World Health Organization, 2018). The most common standard used is the 0.5 McFarland standard, which is equivalent to approximately 1.5×10^8 colony-forming units (CFU) per milliliter. In this research, a 0.5 McFarland standard was used. The bacteria culture was suspended into sterile Phosphate buffered saline (PBS), using a sterilized wire loop and test tube. The dilutions to 0.5 McFarland were made using sterile Phosphate buffered saline (Figure 3.21) to obtain an equal turbidity. Following equalization of turbidity, the colony was suspended in saline in a test tube and combined with additional *S. aureus* bacterium colonies or saline as necessary. The mixer was vortexed and the density was then checked

visually by comparing against a 0.5 Mcfarland standard. The rats were placed in a glass container with a lid and diethyl ether wetted cotton wool. The prepared suspension was administered via injection into the rats' right thigh bone within 15 to 30 minutes. The injection site was shaven and cleaned with methylated spirit. A 25G needle and 2mL syringe were filled with the suspension consisting of *S. aureus* was injected into the left thigh bone. The intraosseous method involved going through the bone by feeling the hardness of the bone, bypassing that layer until the soft feeling was sensed; that was when the mixture was released.



A

B

Figure 3.19: Retrieving *Staphylococcus aureus* culture plates from the refrigerator (A)
Placing *Staphylococcus aureus* culture plates in an incubator at 37°C for 24 hours to allow growth (B)

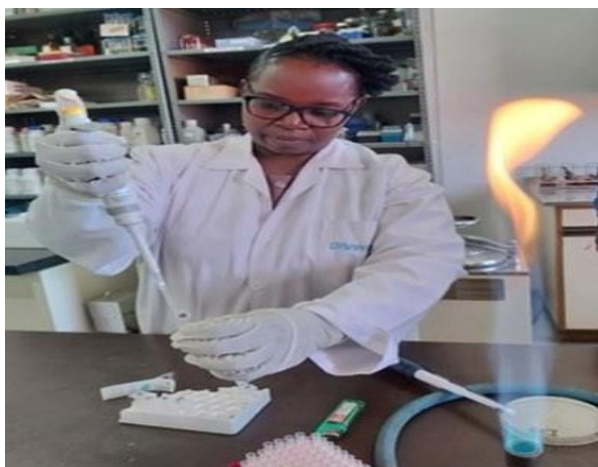


Figure 3.20: Preparation of inoculum



A

B

Figure 3.21: *Staphylococcus aureus* blood agar cultured disk with haemolysed blood illustrating how virulent the bacteria was (A). Inoculum prepared by comparing with McFarland standard solution (McFarland, 2000), placed in a test tube rack (B)

3.7.3. Animal study model

Twelve rats between the weights of 30 and 65g that were healthy and had never been the subject of any tests were separated and housed in clean cages (Figure 3.22) with adequate hydration and food (Shah and Khan, 2013). The rats were placed in a glass container and exposed to cotton wool soaked in diethyl ether, which served as an anaesthetic. Once the rats were sedated, they were injected with a suspension of *Staphylococcus aureus* using aseptic techniques. (Tatara et al., 2015; Anastasio et al., 2020; Vantucci et al., 2021).

Direct trauma or illness is the usual route of contamination for microorganisms that enter the circulatory system and can go to any part of the body (Gristina, 1987; Blanchette et al., 2017). Therefore, the rats were inoculated with *S. aureus* bacterium colonies into the right thigh bone prior to the planar imaging to induce osteoarthritis (Shah and Khan., 2011; Shah et al., 2011; Siaens et al., 2004). The infected rats were then kept for up to fourteen days (Lucke et al., 2003; Horst et al., 2012). The rats were observed twice a day, fresh food and water were supplied and changed daily while the surroundings (Figure

3.21) were also cleaned once a day (National Research Council, 2010; Couto. and Cates, 2019).



Figure 3.22: Separated well fed caged rats in preparation for developing rat models. Four rats kept cage A, were painted blue and not inoculated with *S. aureus*. Eight rats kept in cages (four per cage) B and C, were painted red and inoculated with *S. aureus*.



Figure 3.23: Injection of ^{99m}Tc -MDP aliquots into rat tail vein prior to imaging

3.7.3.1. Imaging of Infected and Uninfected Rats to Assess Biodistribution of ^{99m}Tc -MDP Radiopharmaceutical Aliquots

In this experimental study, the biodistribution of ^{99m}Tc -MDP radiopharmaceutical aliquots was assessed in both infected and uninfected rats over a fourteen-day post- inoculation period. The infected rats were carefully monitored during this time to allow the infection to fully establish. On the fourteenth day, the rats were restrained, and the ^{99m}Tc -MDP aliquots were administered via tail vein injection to evaluate the biodistribution of the radiopharmaceutical, particularly its accumulation in infected regions (Volk et al., 1994; Motaleb, 2007; Shah et al., 2015).

A dosage of 80 to 100 mCi of ^{99m}Tc -MDP was injected into each rat's tail vein using a 25-gauge needle, as shown in Figure 3.22. The tail was held under slight tension, and the needle was inserted 3-5 mm into the vein with the bevel facing upward. Proper placement of the needle was confirmed by gently pulling back the plunger and observing a flash of blood in the hub. After injection, a two-hour interval was observed to allow the radiopharmaceutical to localize within the targeted regions.

Following this, diethyl ether anaesthesia was administered to minimize the animals' movement, allowing for accurate imaging. The rats were placed in a sternal recumbence position on the imaging bed (Figure 3.24), ensuring consistent imaging conditions. Planar imaging was then performed, capturing the biodistribution of the ^{99m}Tc -MDP in both infected and uninfected rats.



A

B

Figure 3.24: Nuclear Radiographer imaging the infected rat models using a planar (A, B)

3.8. Data Analysis and Interpretation

Stata version 14 was used to analyse the study for both qualitative and quantitative data. The Stata software has the capacity to verify, validate and check the reliability of the obtained data and can be customized with the research scale, in which case a nominal was used scale. A p - value of less than 0.05 was regarded as statistically significant for testing.

3.9. Ethical considerations

The National Health Research Authority, management of the University Teaching Adult Hospital and ERES Converge were consulted regarding permission to conduct the study. Authority to conduct research was given from all the relevant authorities. To further safeguard the security of researchers, the general public, and the environment, Standard Operating Procedures for the use of radiopharmaceuticals were adhered to.

CHAPTER FOUR

RESULTS

4.1. Introduction

The study was meant to modify an existing (Methylene Diphosphonate) MDP fractionation method that would optimise the University Teaching Hospital of Zambia's use of MDP radiopharmaceuticals for infectious disease detection. In this investigation, a total of 80 fractions were examined. MDP radiopharmaceutical, which is sold as a multidose preparation in a 5 mL vial, was used to create the fractions. The MDP radiopharmaceutical powder was initially reconstituted with 4 mL of various types of normal saline before being separated into 1mL fractions that were stored at ambient, frozen, and chilled settings, respectively. MDP aliquots were reconstituted with Normal Saline in four different ways as; NS, NSA, NSSnCl₂, and NSSnCl₂A. From each NS stream 1mL of the mixture was taken out and placed into separate sterile vials and stored at ambient conditions, refrigerator and in the freezer.

After the set period of storage time, these aliquots were subsequently subjected to a number of analytical procedures. Several QC and QA procedures were subsequently performed on the fractions kept frozen and cooled. The frozen aliquots were tested on day 28, while those kept at ambient conditions were tested daily from days 1 to day 18. The findings revealed that fractions kept at room temperature were viable up to the eighteenth day. Additional research demonstrated that the biodistribution was quite successful because they were able to localize in the area of interest.

4.2. Compliance of Laboratory products to standards

MDP RP and the Molybdenum - Technetium generator (⁹⁹Mo/^{99m}Tc Generator) were examined upon arrival to ensure that they complied with global standards (Morgan-Warren, 2006). The ⁹⁹Mo/^{99m}Tc Generator with MDP RP kit demonstrated complete compliance with all requirements. The Table 4.1 below reveals complete conformity to all anticipated parameters.

Table 4.1: To Show compliance of laboratory products to show compliance of products (+ = Complies, - = Does not comply)

⁹⁹ Mo/ ^{99m} Tc Generator/ MDP RP kit	Labelling Compliance (Clear and correct = +, Not clear and correct = -)	Packaging (Intact = +, Not intact = -)	Shelf life (Not expired = +, Expired = -)	Batch No (Available =+, not available = -)
Month 01	+	+	+	+
Month 02	+	+	+	+
Month 03	+	-	+	+
% Compliance	100%	100%	100%	100%

Radiolabelled MDP RP must have a RCP of 90% or above (USP, 2005; BP, 2016; WHO, 2019). This is in accordance with the internationally agreed-upon norms, which are displayed in the Table 4.2 below. Thus, all ^{99m}Tc-MDP radiopharmaceuticals, for example, must have an RCP of 90% or higher in accordance with the guidelines set forth by the suggested reference materials, as shown in Table 4.2 below;

Table 4.2: Comparisons of MDP between Global standards and study results

Radiopharmaceutical	US Pharmacopoeia % RCP	WHO Pharmacopoeia % RCP	Study Research lab Results % RCP
MDP	X > Or = 90%	X > Or = 95%	X > Or = 98%

In this study, 80 aliquots in total were analysed. The radio chemical purity data was recorded as the Quality Control standards were being monitored. The aliquots were made using MDP

radiopharmaceutical, which is offered as a multidose preparation in a 5 mL vial. The MDP radiopharmaceutical powder was first reconstituted with 4 mL of several types of NS, and then divided into 1 mL fractions that were kept at ambient, frozen and refrigerated conditions respectively as can be seen in Table 4.3 below.

Table 4.3: Number of aliquots analysed

Specific Objectives	Type of Aliquots	Storage conditions	QC time for aliquots	Total No. of aliquots
1. To establish whether the RP MDP) aliquots reconstituted with NSA is different from those reconstituted NS	MDP kit + NS + Ar	Ambient	1 day to 18 days	18
	MDP kit + NS	Ambient	1day to18 days	18
2. To determine whether the RP of MDP aliquots reconstituted with NSSnCl ₂ and NSSnCl	MDP kit + NS + SnCl ₂ + Ar	Ambient	1 day to 18 days	18
	MDP kit + NS+ SnCl ₂	Ambient	1 day to 18 days	18
3. To compare the RCP for different MDP aliquots kept at ambient conditions to those kept at recommended conditions	Four from each NS stream	Refrigerated/frozen conditions	Day 28	8

Total Number of aliquots				80
--------------------------	--	--	--	----

4.3. Clarity of ^{99m}Tc

Clarity refers to a test where ^{99m}Tc eluate is visually inspected to determine presence of particles using a naked eye against a white and black background (Saha, 2010). A genuine solution of a ^{99m}Tc eluate must be transparent, devoid of any colour or particles. Clarity validates the absence of particles in the ^{99m}Tc eluate as seen in Tables 4.4 and 4.5 below.

Table 4.4: Clarity of MDP aliquots from all streams kept at ambient conditions

Aliquot Age in Days	Type of NS Stream				Clarity	Average Temperature
1	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	28
2	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	27
3	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	26
4	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29
5	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	28
6	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	25
7	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29
8	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	27
9	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	33
10	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	35
11	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	27
12	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	28
13	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29

14	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29
15	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	30
16	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29
17	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	27
18	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Clear	29

Table 4.5: Clarity of ^{99m}Tc used to label MDP aliquots kept at recommended conditions

Average Temperature	Stream	Clarity
3	NS	Clear
-23	NS	Clear
3	NSA	Clear
-23	NSA	Clear
3	NSSnCl ₂	Clear
-23	NSSCl ₂	Clear
2	NSSCl ₂ A	Clear
-23	NSSCl ₂ A	Clear

4.4. pH of all ^{99m}Tc-MDP aliquots

Radiopharmaceuticals must be produced at the proper pH to maintain stability and integrity. A parenteral radiopharmaceutical's pH should ideally be the same as blood's pH (7.4), a pH range of 4 to 8 is acceptable (BP 2016). The pH paper strips were used to during this research investigation. All the MDP aliquots kept at recommended conditions with a pH value of 7 are shown in the Tables 4.6 and 4.7 below.

Table 4.6: pH of ^{99m}Tc-MDP aliquots stored at recommended conditions on day 22

Average pH	Temperature AM (°C)	Stream
7	3	NS

7	-23	NS
7	2	NSA
7	-23	NSA
7	2	NSSnCl ₂
7	-23	NSSCl ₂
7	2	NSSCl ₂
7	-23	NSSCl ₂ A

Table 4.7: pH of ^{99m}Tc-MDP aliquots kept at ambient conditions from all streams

Aliquot Age in Days	Type of NS Stream				Average pH	Average Temperature
1	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	28
2	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	27
3	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	26
4	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29
5	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	28
6	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	25
7	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29
8	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	27
9	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	33
10	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	35
11	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	27
12	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	28
13	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29

14	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29
15	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	30
16	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29
17	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	27
18	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	7	29

4.5. Chemical purity (CP) of ^{99m}Tc

CP is the proportion of total mass present in the stated chemical form (Simon et al., 2012 and Thomson, 2002). This refers to the amount of undesirable aluminium chemical species (which are non-radioactive) present in the radiopharmaceutical. In solution of ^{99m}Tc, aluminium is always the possible chemical contaminant since the parent radionuclide ⁹⁹Mo is embedded onto aluminium column, hence may at times be milked into the eluate in more than the limited quantities. The materials that were required included ^{99m}Tc eluate from the sampled ⁹⁹Mo/^{99m}Tc generators, ammonium salt of aurin tricarboxylic acid indicator and the test. The procedure to detect the presence of aluminium was done by applying a drop of ^{99m}Tc eluate and a drop of a standard solution containing approximately 10mcg/ml of aluminium to a calorimeter test paper impregnated with ammonium salt of aurin tricarboxylic acid indicator. The two spots were compared in pink colour formation (aluminium forms a light pink colour when it reacts with the mentioned acid indicator). The intensity of the pink colour is proportionate to the amount of the aluminium present.

This procedure was done according to the leaflet instruction by the manufacturer (Biodex Medical Systems found at 20 Ramsey Road, Shirley, New York, USA) of the aluminium kit. This method is validated alternative, cheaper method of checking for aluminium content. Other, more expensive, sophisticated methods exist. Results for all aliquots kept at ambient and recommended conditions are as are shown in the Tables 4.8 and 4.9 below.

Table 4.8: Chemical Purity for ^{99m}Tc used to label aliquots kept at recommended conditions by day 22

Chemical Purity	Morning Temperature	Stream
Colourless	3	NS
Colourless	-23	NS
Colourless	2	NSA
Colourless	-23	NSA
Colourless	2	NSSnCl_2
Colourless	-23	NSSCl_2
Colourless	2	NSSCl_2A
Colourless	-23	NSSCl_2A

NB: Colourless mean that Al breakthrough less than 10 ppm

Table 4.9: Chemical purity (CP) of ^{99m}Tc used to label MDP aliquots kept at ambient conditions from all streams

Aliquot Age in Days	Type of NS Stream				Chemical Purity	Average Temperature
1	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	28
2	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	27
3	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	26
4	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	29
5	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	28
6	NS	NSA	NSSnCl_2	NSSnCl_2A	Colourless	25

7	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	29
8	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	27
9	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	33
10	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	35
11	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	27
12	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	28
13	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	29
14	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	29
15	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	30
16	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	29
17	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	27
18	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	Colourless	29

4.6. Sterility Testing of ^{99m}Tc-MDP Aliquots

Radiopharmaceuticals must be sterile to be administered parenterally. The radiolabelled aliquots were subjected to a sterility test using two different media, Trypticase Soy Broth (TSB) and Fluid Thioglycolate Medium (FTG), in accordance with the requirements of standard practice (Cooper, 2010). TSB is suitable for the cultivation of both fungal and aerobic bacteria, whereas FTM is utilized for anaerobic bacteria. The sample of the labelled aliquots was added to the media in the relevant agar plates. This mixture was left at room temperature for 14 days without being shaken. The test was positive if there were any signs of microbial development; otherwise, it was negative. The bulk of the studied aliquots, as can be seen in Table 4.10 below, unfortunately exhibited a significant positive result, meaning there was microbial growth in most aliquots. This is despite efforts carried out in applying the aseptic techniques like sterilising materials and tools, and having

carried out the process in a sterilised laminar flow. However, the hot lab was not sterilised with ultraviolet or alcohol.

Table 4.10: Sterility Testing of Methylene diphosphonate (MDP) aliquots

Aliquot age (days)	Average Temp (C)	Incubation period (days)	Stream	Sterility (FTM)	Sterility (TSB)
1	28	14	NS	Negative	Positive
3 6	26		NSA	Negative	Positive
9	25		NSSnCL ₂	Negative	Positive
	33		NSSCL ₂ A	Negative	Positive
12	28	14	NS	Positive	Positive
15	30		NSA	Positive	Positive
17	27		NSSCL ₂	Positive	Negative
18	29		NSSCL ₂ A	Positive	Positive
28	2 2	14	NS	Positive	Positive
28	-23		NSA	Positive	Positive
28	-23		NSSCL ₂	Positive	Positive
28			NSSCL ₂ A	Positive	Positive

4.7. Radionuclidic purity (RNP) of ^{99m}Tc

Radionuclidic purity test tries to check for Molybdenum an impurity through conducting a Molybdenum breakthrough. The acceptable amount of molybdenum is 0.15. As can be seen in the Table 4.11 and 4.12 below, all the aliquots kept at ambient and recommended conditions passed this test.

Table 4.11: RNP of ^{99m}Tc used to label all ambiently stored MDP aliquots

Aliquot Age (Days)	Type of NS Stream				Radionuclidic Purity (>0.015%)	Average Temperature
1	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.948	28
2	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.993	27
3	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.985	26
4	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.982	29
5	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.997	28
6	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.997	25
7	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.986	29
8	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.998	27
9	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.987	33
10	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.997	35
11	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.939	27
12	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.970	28
13	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.941	29
14	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.991	29

15	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.993	30
16	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.991	29
17	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.784	27
18	NS	NSA	NSSnCl ₂	NSSnCl ₂ A	99.983	29

Table 4.12: RNP of ^{99m}Tc for MDP aliquots stored at recommended conditions on day 22

Radionuclidic Purity	Afternoon Temperature	Stream
99.97%	3	NS
99.967%	-23	NS
99.964%	2	NSA
99.958%	-23	NSA
99.965%	2	NSSnCl ₂
99.968%	-23	NSSCl ₂
99.968%	2	NSSCl ₂ A
99.972%	-23	NSSCl ₂ A

4.8. Radiochemical Purity (RCP) Findings

RCP is the percentage of the desired form of a substance (Saha, 2016). In this case, it is a very important indicator in the purity of MDP to be used in diseases diagnosis.

4.8.1. RCP of aliquots kept at recommended conditions

Eight MDP fractions were regarded as control fractions, therefore two from each stream of the NS type were maintained in the suggested conditions as per the literature (Dhingra, 2013; Bayomy, 2019). These are frozen (<0°C) and refrigerated conditions (2°C to 8°C). The Table 4.13 below shows the RCP results for the control MDP fractions. Eight MDP fractions were regarded as control fractions, therefore two from each stream of the normal

saline type were maintained in the suggested conditions as per the literature. These are frozen and refrigerated conditions. The Table below shows the RCP results for the control MDP fractions.

Table 4.13: Results for control Aliquots kept at recommended conditions (Frozen and refrigerated)

Aliquot Age in Days	RCP (%)	Temperature (°C)	Stream
28	99	3	NS
28	100	-23	NS
28	99	2	NSA
28	100	-23	NSA
28	100	2	NSSnCl ₂
28	100	-23	NSSnCl ₂
28	100	2	NSSnCl ₂ A
28	100	-23	NSSnCl ₂ A

4.8.2. Radiochemical Purity of aliquots kept at ambient conditions and made from NS and NSA

Fractions were prepared and split into two groups to respond to the aforementioned objective. When preparing one group, plain normal saline was used, and when preparing the second group, argon-purged normal saline was used. Fractions from both streams were kept in ambient storage. From day one to day eighteen, RCP was evaluated on aliquots from both streams, one fraction per day. The results for RCP for the MDP fractions from both streams are shown in the Table 4.14 below.

Table 4.14: RCP of ^{99m}Tc-MDP aliquots reconstituted with Argon purged Normal Saline (NSA) and with non-Argon purged (NS).

Aliquot Age (Days)	Radiochemical purity % (%)	Radiochemical purity % (%)
	NS	NSA
1	99	99
2	99	98
3	99	99
4	99	99
5	99	99
6	99	99
7	99	99
8	99	99
9	99	99
10	99	98
11	99	98
12	99	100
13	99	99
14	99	99
15	99	98
16	99	100
17	99	99
18	99	99

The RCP findings comparison between the NSA and NS stream is shown in Figure 4.1 below. The NSA stream is represented by the colour grey, whereas the NS stream is represented by the colour black

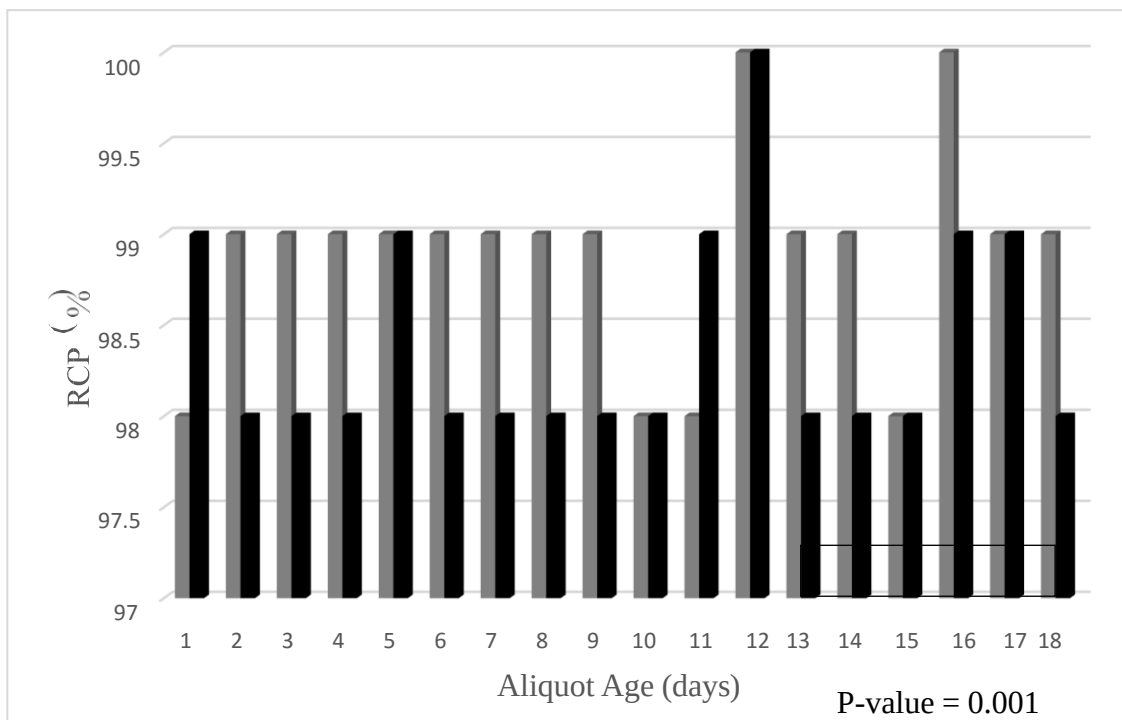


Figure 4.1: RCP of $^{99m}\text{TcMDP}$ aliquots reconstituted with Argon purged Normal Saline (NSA) and with non-Argon purged NS

Key for Figure 4.1

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon- purged Normal Saline (NSA).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with non Argon-purged Normal Saline (NS).

The comparison of the findings of the NS and NSA streams using Pearson statistical analysis is shown in the Table 4.15 below.

Table 4.15: Pearson Chi-square results for (MDP) aliquots reconstituted with Argon purged Normal Saline (NSA) versus those reconstituted with non-Argon purged NS.

		Argon purged Normal Saline Aliquot (NSA)				
Normal Saline NS RCP %						Total
	0.98	3 0.1	9 0.3	0 1.3	0 0.6	12 2.3
	0.99	1 0.0	3 0.0	1 0.4	0 0.3	5 0.7
	1.00	0 0.2	0 0.6	1 7.6	0 0.1	1 8.5
	NS RCP	0 0.2	0 0.6	0 0.1	1 17.1	1 18.0
	Total	4 0.5	12 1.5	2 9.4	1 18.0	19 29.4
		Pearson χ^2 (9) = Pr = 0.001 29.4500				

The P-value in Table 4.6 was 0.001 which indicated that there is a significant difference between aliquots of NSA and aliquots reconstituted with NS, and that the observed difference in the RCP results from the two aliquots was not by chance

4.8.3. Radiochemical Purity of aliquots kept at ambient conditions and made from NSSnCl₂ and NSSnCl₂A

Fractions were prepared and split into two groups to respond to the aforementioned objective. When preparing one group, normal saline augmented with stannous Chloride was used, and when preparing the second group, argon-purged normal saline augmented with stannous Chloride was used. Fractions from both streams were kept in ambient storage. From day one to day eighteen, RCP was evaluated on aliquots from both streams.

The results for RCM for both streams are shown in Table 4.16 below.

Table 4.16: RCP of MDP aliquots reconstituted with Argon purged Normal Saline and Stannous Chloride and with non-Argon purged and Stannous Chloride.

Aliquot Age (Days)	Radiochemical purity (%)	Radiochemical purity (%)
	NSSnCl ₂	NSSnCl ₂ A
1	99	99
2	99	99
3	100	100
4	99	99
5	100	100
6	100	100
7	100	100
8	100	100
9	100	100
10	100	100
11	100	100
12	100	100
13	98	98
14	99	99
15	100	99
16	99	99
17	99	99
18	98	99

The RCP findings comparison between the NSSnCl₂ and NSSnCl₂A stream is shown in Figure 4.2 below. The NSSnCl₂ stream is represented by the colour grey, whereas the NSSnCl₂A stream is represented by colour black.

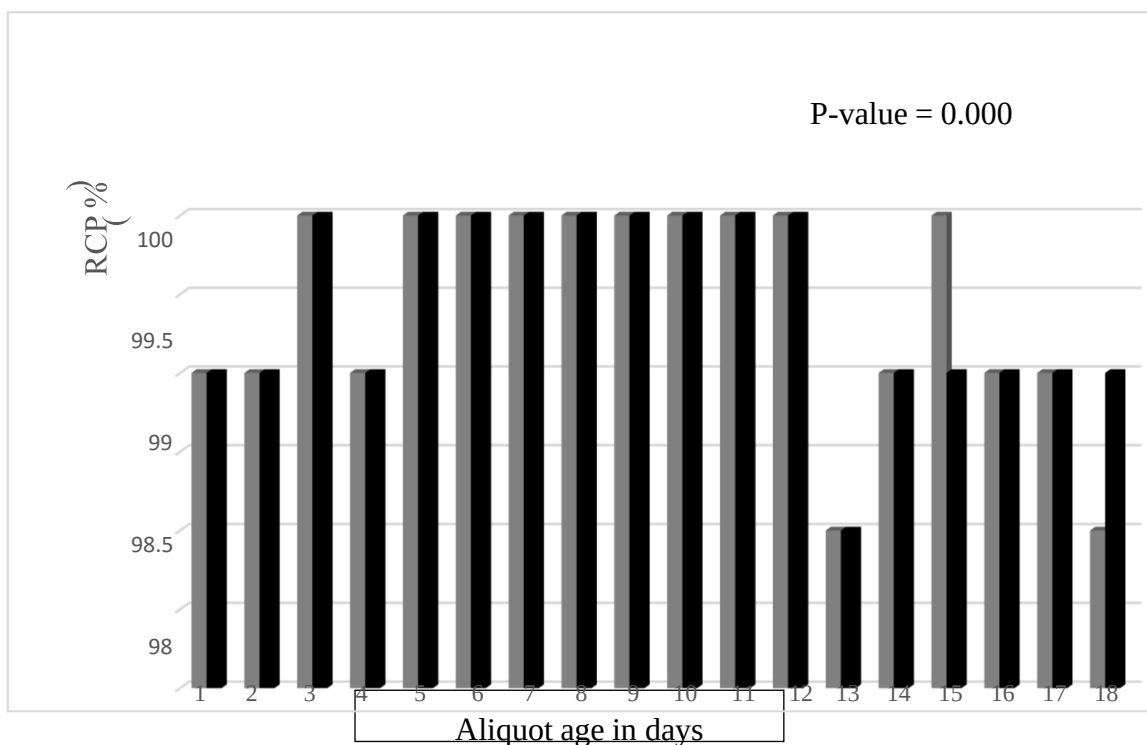


Figure 4.2: RCP of MDP aliquots reconstituted with NSSnCl₂A and with NSSnCl₂

The comparison of the findings of the NSSnCl₂ and NSSnCl₂A streams using Pearson statistical analysis is shown in Table 4.17.

Key for Figure 4.2

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with non- Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂A).

The comparison of the findings of the NSSnCl₂ and NSSnCl₂A streams using Pearson statistical analysis is shown in Table 4.17.

Table 4.17: Pearson Chi-square results for MDP aliquots reconstituted with NSSnCl₂ and NSSnCl₂A

	NSSnCl ₂ A	
--	-----------------------	--

NSSnCl ₂						Total
	0.98	1 7.6	1 0.0	0 0.9	0 0.1	2 8.7
	0.99	0 0.3	6 4.8	0 2.8	0 0.3	6 8.3
	1.00	0 0.5	1 2.4	9 3.8	0 0.5	10 7.3
	NSSnCl ₂	0 0.1	0 0.4	0 0.5	1 17.1	1 18.0
	Total	1 8.5	8 7.7	9 8.1	1 18.0	19 42.3
		Pearson χ^2 (9) = Pr = 0.000 42.2750				

The P-value in Table 17 was 0.000 which indicated that there is a significant difference between MDP aliquots reconstituted with non-Argon purged NS and Stannous Chloride versus those reconstituted with Argon purged NS and Stannous Chloride, and that the observed difference in the Radiochemical Purity results from the two aliquots were not by chance.

4.8.4. Comparison of Radiochemical Purity of aliquots kept at ambient conditions and made from NSA and NSSnCl₂

The comparison of the findings of the NSA and NSSnCl₂ streams using Pearson statistical analysis is shown in the Table 4.18 below.

Table 4.18: Pearson Chi-square results for MDP aliquots reconstituted with plain NSA and versus those reconstituted with Argon purged NS with Stannous Chloride

	Non-Argon purged Normal Saline and Stannous Chloride Aliquot (NSSnCl ₂)	
--	---	--

Argon purged Normal Saline without Stannous Chloride NSA						Total
	0.98	0 0.2	2 0.1	2 0.0	0 0.2	4 0.5
	0.99	1 0.2	5 0.0	6 0.0	0 0.6	12 0.9
	1.00	0 0.1	1 0.0	1 0.0	0 0.1	2 0.2
	NSA	0 0.1	0 0.4	0 0.5	1 17.1	1 18.0
	Total	1 0.6	8 0.5	9 0.5	1 18.0	19 19.6
		Pearson χ^2 (9) = 19.5938			Pr = 0.021	

The P-value in Table 4.18 was 0.021 which indicated that there is a significant difference between MDP aliquots reconstituted with non-Argon purged NS and Stannous Chloride versus those reconstituted with Argon purged NS without Stannous Chloride, and that the observed difference in the RCP results from the two aliquots were not by chance.

The RCP findings comparison between the NSA and NSSnCl₂ stream is shown in Figure 4.3 below. The NSA stream is represented by the colour grey, whereas the NSSnCl₂ stream is represented by the colour black.

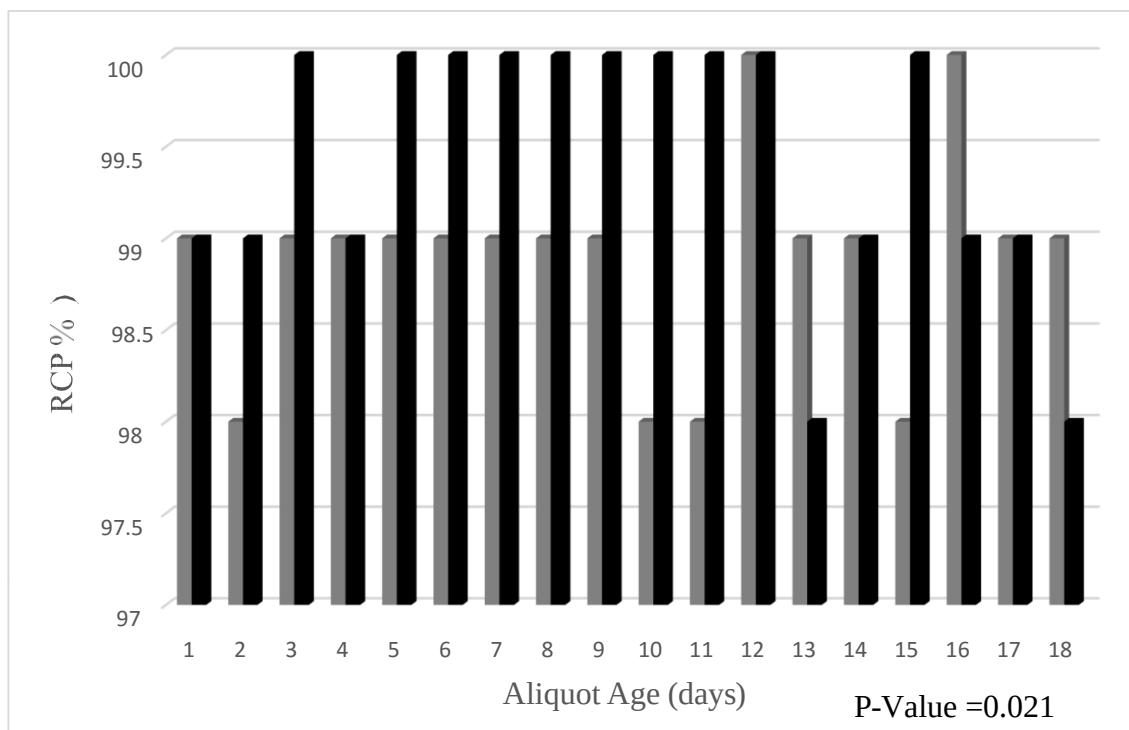


Figure 4.3: RCP of Methylene diphosphonate (MDP) aliquots reconstituted with Argon purged Normal Saline and Stannous Chloride (NSA) and with non-Argon purged and Stannous Chloride (NSSnCl₂)

Key for Figure 4.3

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with non-Argon-purged Normal Saline and Stannous Chloride (NSA).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂A).

4.8.5. Comparison of RCP of aliquots kept at ambient conditions and made from NS and NSSnCl₂

The comparison of the findings of the NSA and NSSnCl₂ streams using Pearson statistical analysis is shown in Table 4.19 below.

Table 4.19: Pearson Chi-square results for MDP aliquots reconstituted with non-Argon purged NS versus those reconstituted with non-Argon purged NS and Stannous Chloride

	Non-Argon purged Normal Saline and Stannous Chloride Aliquot (NSSnCl ₂)					
Non Argon purged Normal Saline (NS)		0.98	0.99	1.00	NSSnCl ₂ (%)	Total
	0.98	2 0.4	3 0.2	7 0.1	0 0.6	12 1.3
	0.99	0 0.5	3 1.3	2 0.2	0 0.3	5 2.2
	1.00	0 0.1	0 0.3	1 0.4	0 0.1	1 0.9
	NS RCP (%)	0 0.1	0 0.3	0 0.5	1 17.1	1 18.0
	Total	2 1.2	6 2.1	10 1.2	1 18.0	19 22.4
		Pearson χ^2 (9) = 22.4200			Pr = 0.008	

The P-value in Table 19 was 0.008 which indicated that there is a significant difference between MDP aliquots reconstituted with non-Argon purged NS with Stannous Chloride versus those reconstituted with Argon purged NS without Stannous Chloride, and that the observed difference in the Radiochemical Purity results from the two aliquots were not by chance.

The RCP findings comparison between the NS and NSSnCl₂ stream is shown in Figure 4.4 below. The NS stream is represented by the colour grey, whereas the NSSnCl₂A stream is represented by the colour black.

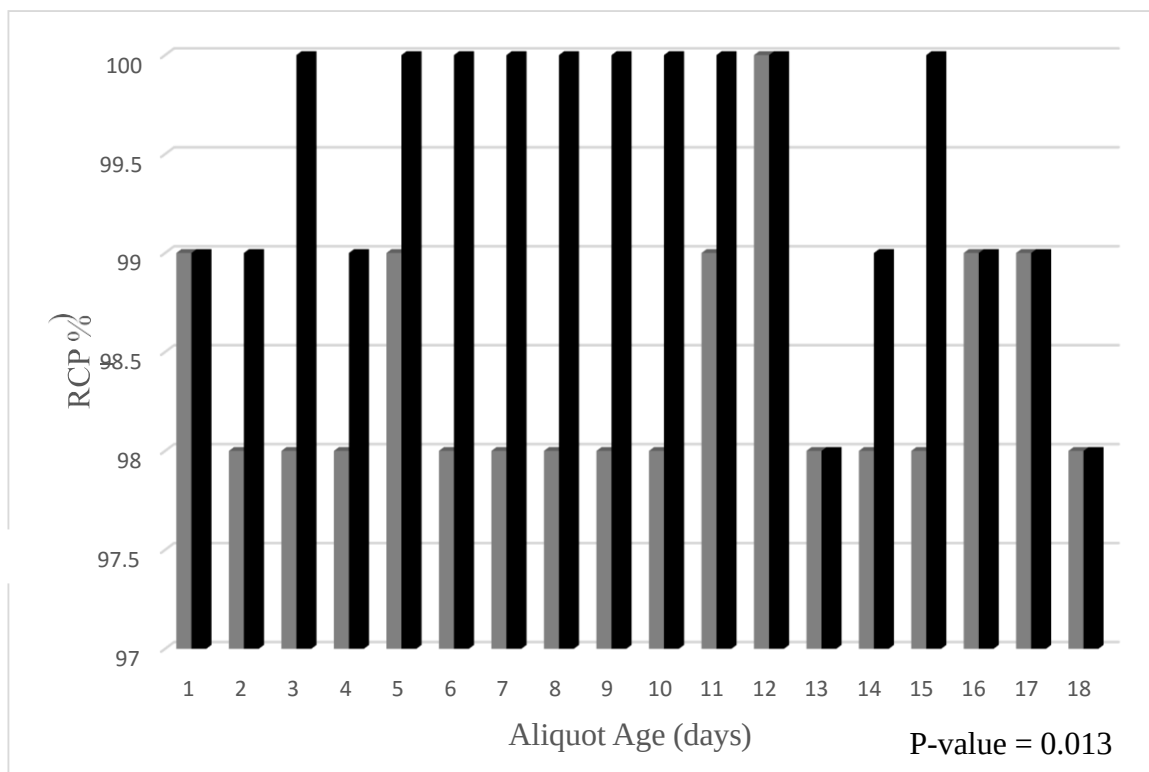


Figure 4.4: Radiochemical purity (RCP) of Methylene diphosphonate (MDP) aliquots reconstituted with Argon purged Normal Saline and Stannous Chloride (NS) and with non-Argon purged and Stannous Chloride (NSSnCl₂).

Key for Figure 4.5

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with non-Argon-purged Normal Saline and Stannous Chloride (NS).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon- purged Normal Saline and Stannous Chloride (NSSnCl₂A).

4.8.6. Comparison of Radiochemical Purity and aliquots kept at ambient conditions and made from NS and NSSnCl₂A

The comparison of the findings of the NS and NSSnCl₂A streams using Pearson statistical analysis is shown in Table 4.20 below.

Table 4.20: Pearson Chi-square results for MDP aliquots reconstituted with non-Argon purged NS versus those reconstituted with Argon purged NS and Stannous Chloride.

	Argon purged Normal Saline and Stannous Chloride Aliquot (NSSnCl ₂ A)					
Non Argon purged Normal Saline NS		0.98	0.99	1.00	NSSnCl ₂ A (%)	Total
	0.98	1 0.2	5 0.0	6 0.0	0 0.6	12 0.9
	0.99	0 0.3	3 0.4	2 0.1	0 0.3	5 1.0
	1.00	0 0.1	0 0.4	1 0.6	0 0.1	1 1.1
	NS RCP (%)	0 0.1	0 0.4	0 0.5	1 17.1	1 18.0
	Total	1 0.6	8 1.2	9 1.1	1 18.0	19 20.9
		Pearson χ^2 (9) = 20.9396				Pr = 0.013

The P-value in Table 4.20 was 0.013 which indicated that there is a significant difference between MDP aliquots reconstituted with non-Argon purged NS with Stannous Chloride versus those reconstituted with Argon purged NS without Stannous Chloride, and that the observed difference in the Radiochemical Purity results from the two aliquots were not by chance.

The RCP findings comparison between the NS and NSSnCl₂A stream is shown in 4.5 below. The NS stream is represented by the colour grey, whereas the NSSnCl₂A stream is represented by the colour black.

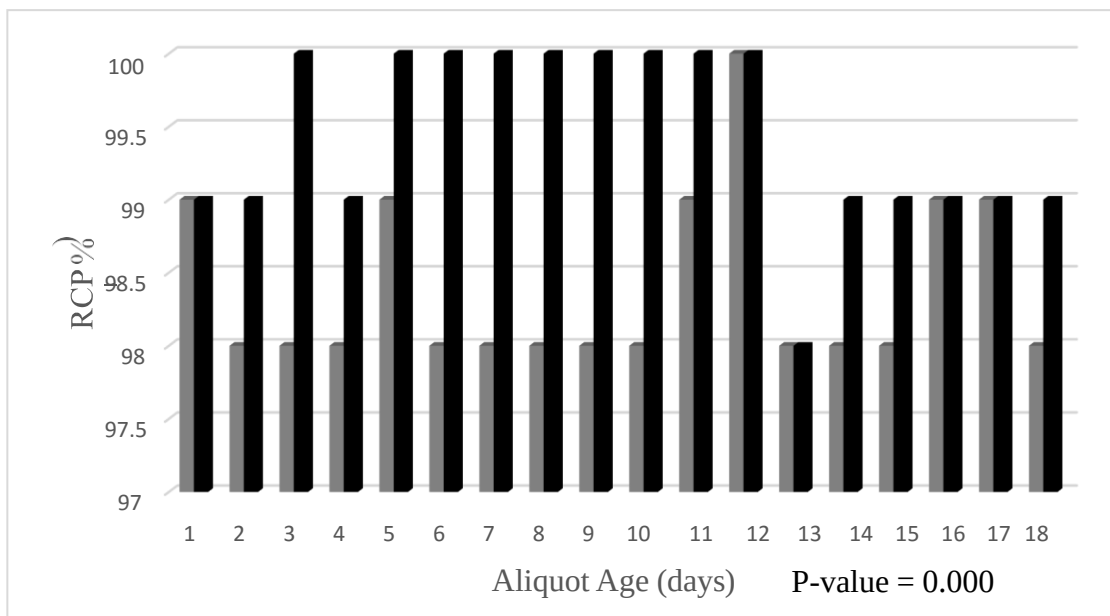


Figure 4.5: RCP of MDP aliquots reconstituted Normal Saline and with Argon purged NS augmented with Stannous Chloride (NSSnCl₂A).

Key for Figure 4.5

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with Normal Saline (NS).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂A).

4.8.7. Comparison of Radiochemical Purity of aliquots kept at ambient conditions and made from NSSnCl₂ and NSSnCl₂A

The comparison of the findings of the NSSnCl₂ and NSSnCl₂A streams using Pearson statistical analysis is shown in Table 4.21 below.

Table 4.21: Pearson Chi-square results for MDP aliquots reconstituted with non-Argon purged (NSSnCl₂) and Stannous Chloride versus those reconstituted with Argon purged NS and Stannous Chloride (NSSnCl₂A)

		Argon purged Normal Saline and Stannous Chloride Aliquot (NSSnCl ₂ A)	
--	--	--	--

Non-Argon purged Normal Saline and Stannous Chloride NSSnCl ₂			0.98	0.99	1.00	NSSnCl ₂ A (%)	Total
		0.98	1 7.6	1 0.0	0 0.9	0 0.1	2 8.7
		0.99	0 0.3	6 4.8	0 2.8	0 0.3	12 0.9
		1.00	0 0.5	1 2.4	9 3.8	0 0.5	10 7.3
		NSSnCl ₂ RCP (%)	0 0.1	0 0.4	0 0.5	1 17.1	1 18.0
		Total	1 8.5	8 7.7	9 8.1	1 18.0	19 42.3
		Pearson χ^2 (9) = 42.2750				Pr = 0.000	

The P-value in Table 4.21 was 0.000 which indicated that there is a significant difference between MDP aliquots reconstituted with non-Argon purged NS with Stannous Chloride versus those reconstituted with Argon purged NS without Stannous Chloride, and that the observed difference in the Radiochemical Purity results from the two aliquots were not by chance.

The RCP findings comparison between the NSSnCl₂ and NSSnCl₂A stream is shown in Figure 4.6 below. The NSSnCl₂ stream is represented by the colour grey, whereas the NSSnCl₂A stream is represented by the colour black.

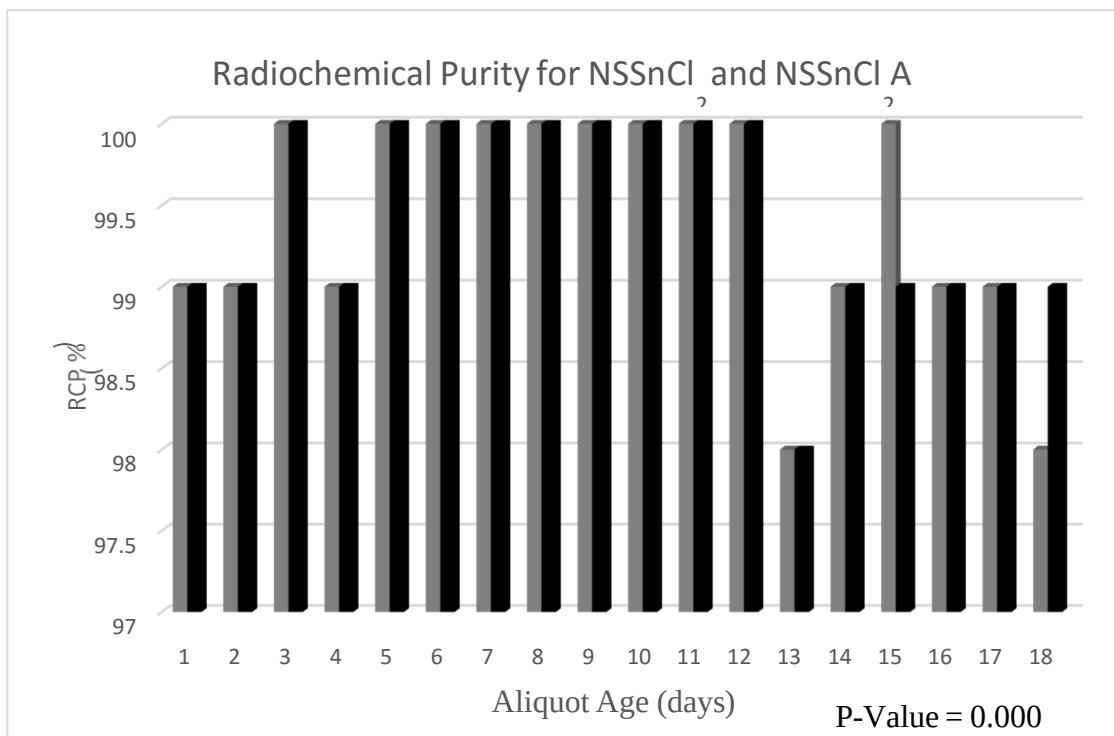


Figure 4.6: RCP of MDP aliquots reconstituted with Argon purged Normal Saline and Stannous Chloride (NSSnCl₂A) and with non-Argon purged and Stannous Chloride (NSSnCl₂).

Key for Figure 4.6

- Grey Bars: Represent the RCP findings of MDP aliquots reconstituted with non-Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂).
- Black Bars: Represent the RCP findings of MDP aliquots reconstituted with Argon-purged Normal Saline and Stannous Chloride (NSSnCl₂A).

4.8.8. Comparison of Radiochemical Purity of aliquots kept at ambient conditions and those made from NSSnCl₂ and NSSnCl₂A

The comparison of the findings of the fractions from all kept at recommended conditions shown in Table 4.22 below.

Table 4.22: Comparisons of the average RCP for different MDP aliquots kept at ambient conditions to those kept at recommended conditions (Frozen and refrigerated once-off)

Aliquot stream	Aliquot age (days)	Storage Temp. (°C)	Average RCP (%)
NS	28	-23	100
	28	3	99
	18	27	98
NSA	28	-23	100
	28	2	99
	18	27	99
NSSnCl ₂	28	-23	100
	28	2	100
	18	27	98
NSSnCl ₂ A	28	-23	100
	28	2	100
	18	27	99

The RCP findings comparison between the NS stream fractions kept at and the fractions kept at recommended (frozen and refrigerated) conditions shown in Figure 4.7 below. The NS stream is represented by the colour light grey, whereas the frozen stream is represented by dark grey and the refrigerated stream is represented by the colour black.

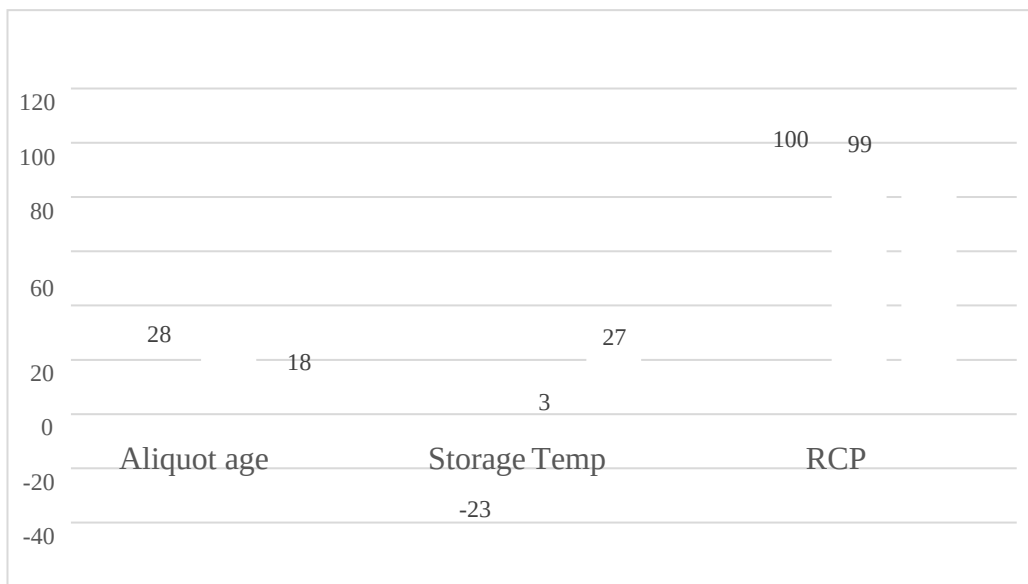


Figure 4.7: RCP comparison for aliquots made from Normal Saline (NS) kept at ambient and those kept at recommended conditions (frozen and refrigerated, once-off).

Key to Figure 4.7: RCP Comparison for Aliquots Made from Normal Saline (NS) Kept at Ambient vs. Recommended Conditions

Color Representation

- Light Grey: Ambiently Stored NS Stream
- Dark Grey: Frozen NS Stream
- Black: Refrigerated NS Stream Key Interpretations

1. Radiochemical Purity (RCP) Variations: Higher RCP in frozen and refrigerated conditions (black and dark grey) indicates better preservation of radiopharmaceutical integrity. Lower RCP in ambient storage (light grey) suggests degradation or oxidation over time, reducing radiolabeling efficiency.
2. Impact of Storage Conditions: Frozen and Refrigerated NS Streams (Black and Dark Grey) maintain higher RCP, implying reduced hydrolysis or oxidation of tin and ^{99m}Tc complexes. Ambient NS Stream (light grey) shows a decline in RCP, likely due to exposure to oxygen and temperature fluctuations.
3. Stability and Usability: Aliquots with RCP above the acceptance threshold (typically >90%) are considered suitable for clinical and research use. Decreased RCP in ambient conditions may lead to suboptimal imaging quality and inaccurate diagnostics.

4. Practical Implications: Storing NS aliquots under recommended conditions (frozen or refrigerated) preserves RCP and ensures high-quality radiopharmaceutical preparation. Ambient storage may not be ideal for long-term stability, requiring optimization or modification in fractionation methods.

The RCP findings comparison between the NSA stream fractions kept at and the fractions kept at recommended (frozen and refrigerated) conditions shown in Figure 4.8 below. The NSA stream is represented by the colour light grey, whereas the frozen stream is represented by dark grey and the refrigerated stream is represented by the black.

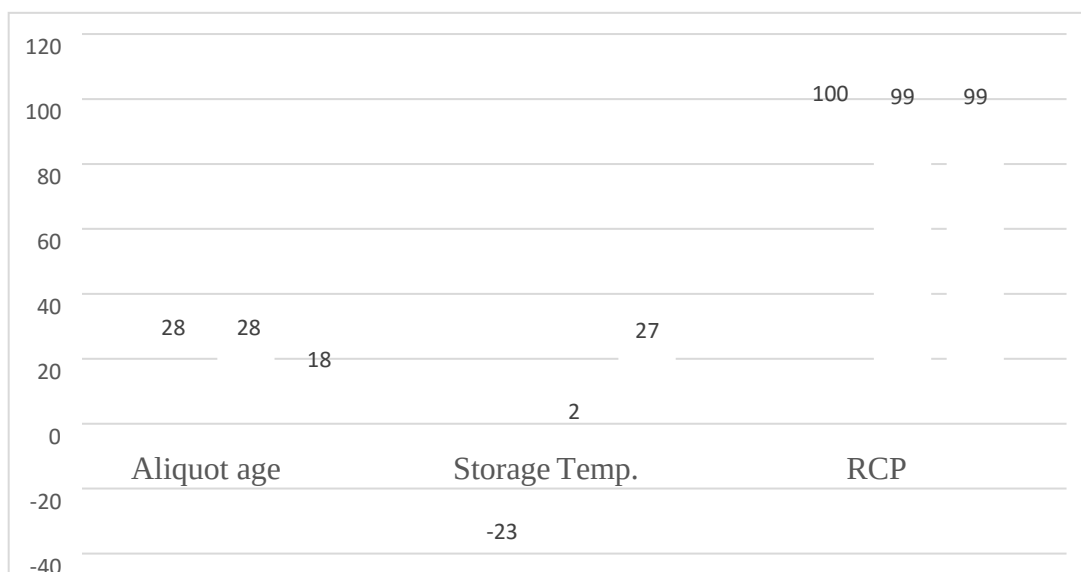


Figure 4.8: Average Radio-Chemical Purity (RCP) of aliquots made from Argon purged Normal Saline (NSA) and kept at ambient conditions compared to those kept at recommended conditions (frozen and refrigerated once-off).

Key to Figure 4.8: RCP Comparison for Aliquots Made from Argon augmented Normal Saline (NSA) Kept at Ambient vs. Recommended Conditions

Color Representation

Light Grey: Ambiently Stored NSA Stream

Dark Grey: Frozen NSA Stream

Black: Refrigerated NSA Stream

Key Interpretations

1. Radiochemical Purity (RCP) Variations: Higher RCP in frozen and refrigerated conditions (black and dark grey) indicates better preservation of radiopharmaceutical integrity. Lower RCP in ambient storage (light grey) suggests degradation or oxidation over time, reducing radiolabeling efficiency.
2. Impact of Storage Conditions: Frozen & Refrigerated NSA Streams (black and dark grey) maintain higher RCP, implying reduced hydrolysis or oxidation of tin and ^{99m}Tc complexes. Ambient NSA Stream (light grey) shows a decline in RCP, likely due to exposure to oxygen and temperature fluctuations.
3. Stability and Usability: Aliquots with RCP above the acceptance threshold (typically >90%) are considered suitable for clinical and research use. Decreased RCP in ambient conditions may lead to suboptimal imaging quality and inaccurate diagnostics.
4. Practical Implications: Storing NSA aliquots under recommended conditions (frozen or refrigerated) preserves RCP and ensures high-quality radiopharmaceutical preparation. Ambient storage may not be ideal for long-term stability, requiring optimization or modification in fractionation methods.

The RCP findings comparison between the NSSnCl_2 stream fractions kept at and the fractions kept at recommended (frozen and refrigerated) conditions shown in Figure 4.9 below. The NSSnCl_2 stream is represented by the colour light grey, whereas the frozen stream is represented by colour dark grey and the refrigerated stream is represented by the colour black.

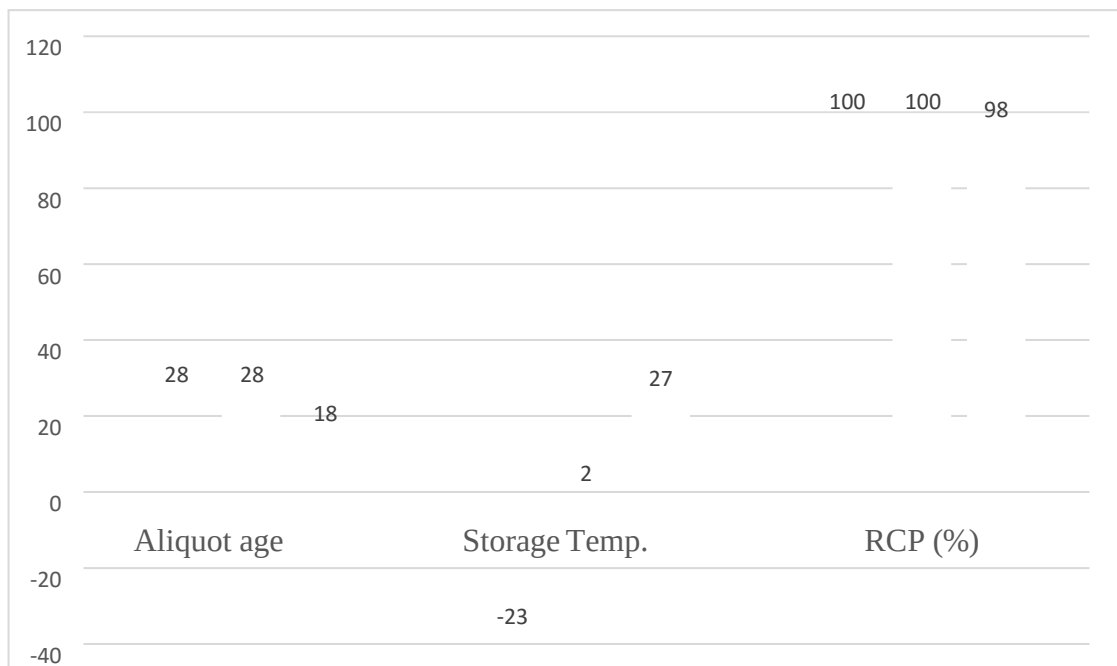


Figure 4.9: Average RCP of aliquots prepared from non-Argon purged Normal Saline and Stannous Chloride (NSSnCL₂) kept at ambient compared to those kept recommended conditions (frozen and refrigerated once-off).

Key to Figure 4.9: RCP Comparison for Aliquots Made from Normal Saline (NSSnCL₂) Kept at Ambient vs. Recommended Conditions

Color Representation

Light Grey: Ambiently Stored NSSnCL₂ Stream

Dark Grey: Frozen NSSnCL₂ Stream

Black: Refrigerated NSSnCL₂ Stream

Key Interpretations

1. Radiochemical Purity (RCP) Variations: Higher RCP in frozen and refrigerated conditions (black and dark grey) indicates better preservation of radiopharmaceutical integrity. Lower RCP in ambient storage (light grey) suggests degradation or oxidation over time, reducing radiolabeling efficiency.
2. Impact of storage conditions: Frozen and Refrigerated NSSnCL₂ Streams (black

and dark grey) maintain higher RCP, implying reduced hydrolysis or oxidation of tin and ^{99m}Tc complexes. Ambient NSSnCl_2 Stream (light grey) shows a decline in RCP, likely due to exposure to oxygen and temperature fluctuations.

3. Stability and usability: Aliquots with RCP above the acceptance threshold (typically >90%) are considered suitable for clinical and research use. Decreased RCP in ambient conditions may lead to suboptimal imaging quality and inaccurate diagnostics.
4. Practical implications: Storing NSSnCl_2 aliquots under recommended conditions (frozen or refrigerated) preserves RCP and ensures high-quality radiopharmaceutical preparation. Ambient storage may not be ideal for long-term stability, requiring optimization or modification in fractionation methods.

The RCP findings comparison between the NSSnCl_2A stream fractions kept at and the fractions kept at recommended (frozen and refrigerated) conditions shown in Figure 4.10 below. The NSSnCl_2A stream is represented by the colour light grey, whereas the frozen stream is represented by colour dark grey and the refrigerated stream is represented by the colour black.

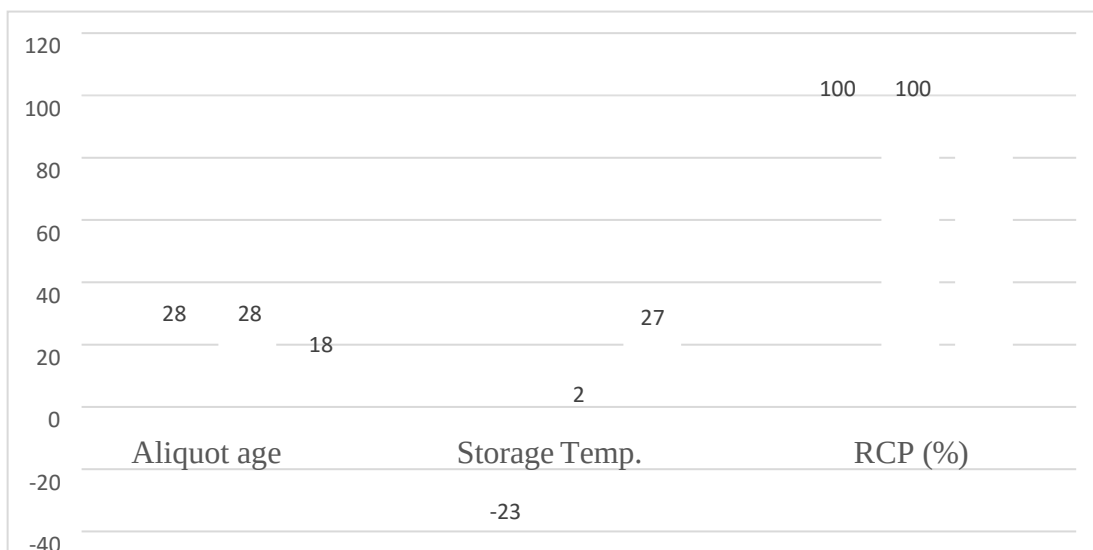


Figure 4.10: Average RCP of aliquots made from Argon purged Normal Saline and Stannous Chloride (NSSCL_2A) kept at ambient compared to those kept at recommended conditions (frozen and refrigerated once-off).

Key to Figure 4.9: RCP Comparison for Aliquots Made from Normal Saline (NSSnCL₂)
Kept at Ambient vs. Recommended Conditions

Color Representation

Light Grey: Ambiently Stored NSSnCL₂A Stream

Dark Grey: Frozen NSSnCL₂A Stream

Black: Refrigerated NSSnCL₂A Stream

Key Interpretations

1. Radiochemical Purity (RCP) Variations: Higher RCP in frozen and refrigerated conditions (black and dark grey) indicates better preservation of radiopharmaceutical integrity. Lower RCP in ambient storage (light grey) suggests degradation or oxidation over time, reducing radiolabeling efficiency.

2. Impact of storage conditions: Frozen and Refrigerated NSSnCL₂A Streams (black and dark grey) maintain higher RCP, implying reduced hydrolysis or oxidation of tin and

^{99m}Tc complexes. Ambient NSSnCL₂A Stream (light grey) shows a decline in RCP, likely due to exposure to oxygen and temperature fluctuations.

3. Stability and usability: Aliquots with RCP above the acceptance threshold (typically >90%) are considered suitable for clinical and research use. Decreased RCP in ambient conditions may lead to suboptimal imaging quality and inaccurate diagnostics.

4. Practical implications: Storing NSSnCL₂A aliquots under recommended conditions (frozen or refrigerated) preserves RCP and ensures high-quality radiopharmaceutical preparation. Ambient storage may not be ideal for long-term stability, requiring optimization or modification in fractionation methods.

4.9. Observations made on infected animals Models

On the second day after the inoculation, the signs and symptoms that indicated the efficacy of the inoculum were seen in our rat models. This experience was like what was seen in a study done by Reizner et al. (2014). It was realised that by day two, the rats were hobbling and clinging to each other, which was an indication of illness. By the fifth day, it was seen

that the rats were having difficulties standing or walking. The animals were also dragging the injected leg, showing a typical limp.

4.10. Biodistribution Studies of ^{99m}Tc -MDP radiopharmaceutical aliquots in uninfected and infected rat models

The biodistribution studies involved injecting two ^{99m}Tc -MDP aliquots into the left thigh bone of the rat models through the tail vein after which they were subjected to a nuclear imaging using a planar (Figure 4.11). The MDP aliquots used were those with the stannous chloride-which recorded the highest RCP and the one which was from the plain normal saline stream which recorded the lowest RCP. The biodistribution studies were contrasted with these. The imaging specifications as seen in Table 23 were those of a thyroid scanning which uses the smallest pin hole suitable for small animals like rats (Saha, 2006).

Table 4.23: Imaging Information Requirements for ^{99m}Tc -MDP Based Images for Experimental Rats

Width	20%
Energy	140 Kev
No Background subtraction	
Imaged in Thyroid Protocol	
Matrix	256 x 256 x 8
Total time	300 Seconds
Maximum Pixel	184
Sum of Counts	289421

The above table expresses the imaging information of the experimental rats. The planar was not working up to maximum capabilities in that it could not process the images effectively and could not get rid of the back ground noise.



Figure 4.10: Radiographer imaging one of the infected rat model

The biodistribution studies were compared using uninfected rats as the control and infected rat models. The ^{99m}Tc -MDP radiopharmaceutical aliquots was injected on the fourteenth day post-inoculation. One of the radiopharmaceutical aliquots was prepared from normal saline alone, while the other was prepared from normal saline augmented with stannous chloride.

4.10.1. Rat images comparing the biodistribution of ^{99m}Tc -MDP prepared from ambientally stored NS stream aliquots taken on day fourteen post inoculation

The use of ^{99m}Tc -MDP prepared from ambientally stored NS exhibited a good biodistribution in region of interest (Figure 4.12). The resulting images following biodistribution studies indicated a high concentration of ^{99m}Tc -MDP in the left thigh for infected rats one and two compared to the uninfected one. This is illustrated by darker spots in region of interest (left thigh) compared to the non-region of interest.

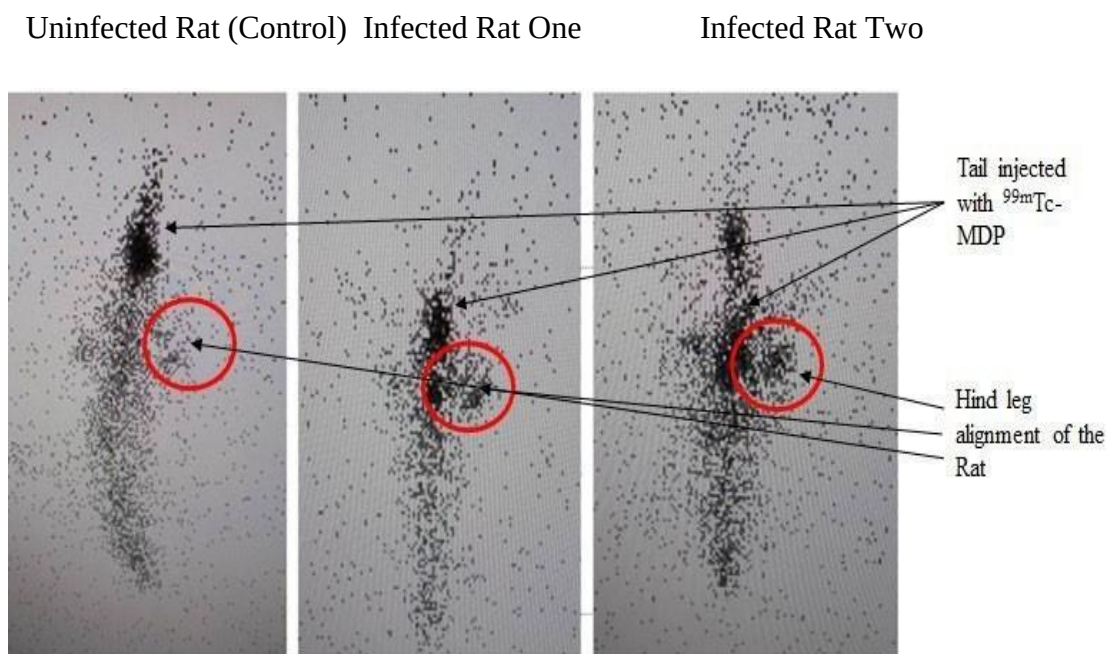


Figure 4.11: Biodistribution images of control and infected rats with *s. aureus* after being injected with ambientally stored ^{99m}Tc -MDP aliquots made from the NS stream in posterior position

Key to Figure 4.11: Biodistribution Images of Control and Infected Rats with *S. aureus* After Injection of Ambiently Stored ^{99m}Tc -MDP (Posterior View)

1. High Uptake Regions (Dark/Hot Spots): Indicates increased radiopharmaceutical accumulation. Control Rats had normal uptake expected in bones, kidneys, and bladder due to physiological distribution. Infected Rats had increased uptake at the site of *S. aureus* infection, suggesting localized osteoblastic activity or inflammatory response.
2. Moderate Uptake Regions (Intermediate Intensity): Represents normal physiological distribution of ^{99m}Tc -MDP. Common sites: bones, kidneys, and soft tissues involved in radiotracer clearance. Variations may occur based on infection severity and metabolic changes.
3. Low Uptake or Cold Areas (Brighter Regions): Indicates poor radiopharmaceutical accumulation or perfusion. May suggest tissue necrosis, vascular impairment, or reduced bone remodeling. Infected rats may show altered perfusion patterns due to inflammation-related vascular changes.
4. Differences Between Control and Infected Groups: Higher ^{99m}Tc -MDP uptake in infected sites suggests active infection-induced bone remodeling. Altered biodistribution patterns in infected rats may indicate systemic effects of bacterial infection, such as changes in renal or hepatic clearance. Control rats should show a more uniform, predictable uptake pattern.
5. Clinical and Research Implications: Increased radiopharmaceutical localization in infection sites could support ^{99m}Tc -MDP as a potential imaging agent for diagnosing infectious bone diseases. Comparison with other imaging modalities (e.g., histology, microbiological analysis) is necessary to confirm infection-related changes.

4.10.2. Rat images comparing the biodistribution of ^{99m}Tc -MDP prepared from ambiently stored NSSnCl_2 stream aliquots taken on day fourteen post inoculation.

The use of ^{99m}Tc -MDP prepared from ambiently stored NSSnCl_2 exhibited a good biodistribution in region of interest. The resultant images following biodistribution studies indicated a high concentration of ^{99m}Tc -MDP in the left thigh for infected rats one and

two compared to the uninfected one. This is illustrated by darker spots in region of interest (left thigh) compared to the non-region of interest.

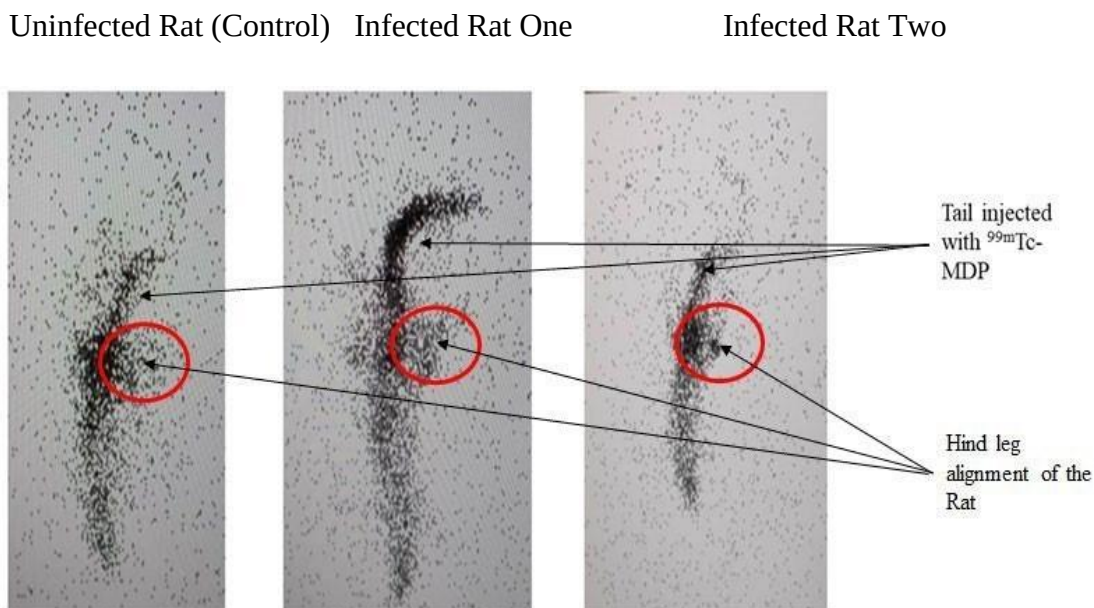


Figure 4.12: Biodistribution images of control and infected rats with *S. aureus* after being injected with ambiently stored ^{99m}Tc -MDP aliquots made from the NSSnCl_2 stream

Key to Figure 4.12: Biodistribution Images of Control and Infected Rats with *S. aureus* After Injection of NSSnCl_2 stream Ambiently Stored ^{99m}Tc -MDP aliquots (Posterior View)

1. High Uptake Regions (Dark/Hot Spots): Indicates increased radiopharmaceutical accumulation. Control Rats had normal uptake expected in bones, kidneys, and bladder due to physiological distribution. Infected Rats had increased uptake at the site of *S. aureus* infection, suggesting localized osteoblastic activity or inflammatory response.
2. Moderate Uptake Regions (Intermediate Intensity): Represents normal physiological distribution of ^{99m}Tc -MDP. Common sites: bones, kidneys, and soft tissues involved in radiotracer clearance. Variations may occur based on infection severity and metabolic changes.
3. Low Uptake or Cold Areas (Brighter Regions): Indicates poor radiopharmaceutical accumulation or perfusion. May suggest tissue necrosis, vascular impairment, or

- reduced bone remodeling. Infected rats may show altered perfusion patterns due to inflammation-related vascular changes.
4. Differences Between Control and Infected Groups: Higher ^{99m}Tc -MDP uptake in infected sites suggests active infection-induced bone remodeling. Altered biodistribution patterns in infected rats may indicate systemic effects of bacterial infection, such as changes in renal or hepatic clearance. Control rats should show a more uniform, predictable uptake pattern.
 5. Clinical and Research Implications: Increased radiopharmaceutical localization in infection sites could support ^{99m}Tc -MDP as a potential imaging agent for diagnosing infectious bone diseases. Comparison with other imaging modalities (e.g., histology, microbiological analysis) is necessary to confirm infection-related changes.

CHAPTER FIVE

DISCUSSION

5.1. Brief Introduction

This chapter provides in-depth discussion of the findings in relation to the specific research objectives. The discussion is organized according to the four specific objectives outlined for this study and interpreting the results in light of existing literature thereby providing insights into the implications especially concerning the formulation and efficacy of ambientally stored methylene diphosphonate (MDP) radiopharmaceutical kits in the diagnosis of infectious diseases.

In this research, a Methylene Di Phosphate (MDP) fractionation method was modified in an effort to optimise its use at the University Teaching Hospital in Zambia for infectious disease diagnosis. The research assessed the stability of MDP aliquots stored at room temperature made from various types of normal saline. The results demonstrated that fractions stored at room temperature remained useful for approximately a month, as they were able to demonstrate good RCP and localization in the region of interest, hence this research demonstrated that the biodistribution was quite successful.

5.2. Radiochemical Purity (RCP) of Ambiently Stored MDP Fractions versus Conventional Storage (Frozen and Refrigerated Conditions)

The first objective aimed to compare the RCP of radiolabeled methylene diphosphonate (MDP) fractions stored at ambient temperatures with those stored under conventional conditions (frozen and refrigerated). The study found that ambient storage resulted in a lower RCP (98% to 99%) compared to frozen and refrigerated storage conditions (100%) agreeing to earlier research done by Damasceno et al. (2022). These results align with this study's findings, which demonstrate that maintaining MDP fractions at lower temperatures preserves their radiochemical integrity better than higher temperatures, although in our case the RCP was far above the minimum threshold (90%) which qualifies the use of a radiopharmaceutical.

This degradation in MDP RCP below the minimum threshold of 90% may result in reduced diagnostic efficacy. This finding highlights the importance of appropriate storage conditions in maintaining the functionality and safety of MDP radiopharmaceutical kits, particularly in resource-limited settings where cold chain management is often a challenge.

5.3. RCP of MDP Fractions Prepared Using Argon-Purged Normal Saline (NSA) Versus Plain Normal Saline (NS)

The second objective focused on the comparison of the RCP of radiolabeled MDP fractions prepared using NSA (99%) with those prepared using NS (98%). The results demonstrated a significant improvement in RCP when argon-purged normal saline was used. This is consistent with previous studies suggesting that oxygen in normal saline can lead to the oxidation of stannous ions (Sn^{2+}) that are critical in the radiolabeling process (Decristoforo et al., 2016; Cucurachi et al., 2019), which supports the idea that presence of too much oxygen exposure can reduce the capacity of stannous chloride, leading to less efficient binding of technetium-99m ($^{99\text{m}}\text{Tc}$) to the MDP molecule. By purging the normal saline with argon, this study effectively reduced the oxidation of tin ions, ensuring a higher RCP and thus, better radiolabeling efficiency. This finding has significant implications for the preparation of radiopharmaceutical kits in settings where controlling oxygen exposure

is feasible. Argon purging represents a cost-effective intervention to improve RCP without the need for sophisticated equipment, enhancing the overall performance of MDP kits in clinical practice.

5.4. RCP of MDP Fractions Reconstituted with Tin Chloride Augmented NS (NSSnCl₂) Versus Tin Chloride Augmented NS Purged with Argon (NSSnCl₂A)

The third objective involved comparing the RCP of radiolabeled MDP fractions reconstituted with tin chloride-augmented normal saline (NSSnCl₂) and those reconstituted with tin chloride-augmented saline purged with argon (NSSnCl₂A). The results demonstrated that the fractions reconstituted with NSSnCl₂A achieved higher RCP (100%) levels compared to NSSnCl₂ alone (99%).

The key difference here is the combined effect of tin chloride augmentation and argon purging, which effectively reduced oxidation and further enhanced radiolabeling efficiency. The importance of both maintaining reducing agents and minimizing oxidative exposure for optimal radiolabeling has been emphasized in literature by Saha, (2011), which aligns with this research findings.

The synergy between tin chloride and argon purging suggests that this preparation method can serve as a superior alternative in optimizing the quality of radiopharmaceuticals, especially in settings where cold storage and handling might not be ideal. This could be particularly advantageous in developing regions, where limited resources and infrastructure often presents barriers to the optimal preparation and storage of radiopharmaceutical kits.

5.5. Efficacy of Radiolabeled MDP Fractions through Biodistribution in Animal Models

The fourth and final objective was to determine the efficacy of the radiolabeled MDP fractions through biodistribution studies in animal models. Since all streams of aliquots passed the minimum (90%) RCP threshold, the biodistribution studies were done by administering two different types of aliquots to rat models, one with highest RCP which was made argon and stannous chloride (100%) and one with normal saline (98%). The results revealed an increased uptake of labelled ^{99m}Tc-MDP in the left thigh bone, where induction of *Staphylococcus aureus* infection was performed for both streams (NS and NSSncl₂A). This contrasted with other anatomical regions, such as the right thigh and the

whole-body regions of the control rat model, where no infection was induced. This biodistribution pattern is similar to the findings made by Dhingra et al. (2019).

The results also showed that MDP fractions with higher RCP levels particularly those prepared using argon-purged saline and tin chloride augmentation produced biodistribution images with poor contrast though with enhanced uptake in target tissues and minimal non-target accumulation.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. MDP aliquots reconstituted with NSA and NS, stored at room temperature for eighteen days, showed RCP typically ranging from 98 to 99%, falling within the expected range (>90%) and were suitable for diagnosing infectious diseases.
2. MDP aliquots reconstituted with NSSnCl_2 and NSSnCl_2A , stored at room temperature for eighteen days, exhibited RCP ranging from 99% to 100% suitable for therapeutic and diagnostic purposes.
3. Ambiently stored MDP aliquots recorded RCP of 98 to 99% in comparison to MDP aliquots stored under recommended conditions (-2 to 8°C), that recorded 100% RCP with both aliquots being found suitable for clinical applications.
4. Biodistribution studies using infected rat models revealed excellent localization in the region of interest, indicating viability for use.

6.2. Limitations

6.2.1. Data Collection Method

When the research was conducted, the modern SPECT gamma camera that would have tested for real-time biodistribution had broken down. As a result, the older model of a planar was used, with technical difficulties like non-clearer images of the biodistribution, failure to get rid of background noise and collect uptake data in various rat organs. Uptake data would help to numerically determine the difference in target to non - target ratio of radiopharmaceuticals in various organs. There was also non-availability of any equipment like single channel analyser

with detector. The diethyl ether used as anesthesia wore out faster than expected which also affected quality of imaging.

6.2.2. Scope

The only Nuclear Pharmacy Hot Laboratory at University Teaching Hospital (UTH) was used for the investigation because it is the only location equipped to handle radiopharmaceuticals. As a result, only Lusaka's ambient conditions were evaluated. Other locations in Zambia where ambient circumstances are different from those seen during this investigation may record higher temperatures and hence provide a different story.

6.3. Future Work and Recommendations

This research has laid a very good foundation for the characteristics of Methylene Di Phosphonate (MDP) aliquots stored at ambient conditions. The results have shown that plain Normal Saline can be used to make these aliquots and still give good Radiochemical purity. However, there is need to conduct further research to confirm how good the biodistribution could be regarding these MDP aliquots by using modern equipment like SPECT or SPECT-CT. These better machines are able to give clearer images of biodistribution.

6.3.1. Ministry of Health

The UTH facility's gamma cameras are dated. Modern ones like SPECT or SPECT-CT are required since more study must be done to show how well the biodistribution works. These more advanced devices can produce images of biodistribution that are more precise. Therefore, it is anticipated that the Ministry of Health will facilitate the purchase of new cutting-edge machinery.

6.3.2. Nuclear Medicine Department- Future Research

1. There is need to conduct further research to confirm how good the biodistribution will be regarding these aliquots by using modern equipment like SPECT or SPECT-CT.

Research can be conducted in the following areas;

- a) Comparing of cost of fractionated MDP verses the unfractionated MDP.
- b) Study on MDP aliquots at ambient conditions for longer than eighteen days and in hotter arts of Zambia and in a clean environment following sterilization of the hot lab.
- c) Assessing the biodistribution of MDP aliquots using a SPECT gamma camera in actual human patients' other conditions other than infections.

2. Deliberate measures must be made to support improvements in the nuclear laboratory's cleanliness. The growth of microorganisms on agar plates for more than 70% of the collected MDP aliquots during the sterility test was notable even though all QCs were in compliance with standards, which is a sign of contamination in the lab setting.

REFERENCES

- Agaja, S. and Ayorinde, R., 2008. Chronic osteomyelitis in Ilorin, Nigeria. *South African Journal of Surgery*, 46(4), pp.456-67.
- Anderson, C.J., Ling, X., Schlyer, D.J. and Cutler, C.S., 2019. A short history of nuclear medicine. In *Radiopharmaceutical Chemistry*. Springer, Cham. pp.11-26.
- Anastasio, A.T., Van Eps, J.L. and Fernandez-Moure, J.S., 2020. Surgical technique for development of a clinically-representative ventral hernia repair infection rat model. *Methods X*, 7, pp.100887.
- Anderson, C.J., Ling, X., Schlyer, D.J. and Cutler, C.S., 2019. A short history of nuclear medicine. In *Radiopharmaceutical Chemistry*. Springer, Cham. pp.1-26.
- Anderson, P.M., 2020. Radiopharmaceuticals for treatment of osteosarcoma. *Current Advances in Osteosarcoma: Clinical Perspectives: Past, Present and Future*, pp.45-53.
- Annual Reports., 2020. University Teaching Hospital, Department of Nuclear medicine, Lusaka, Zambia. pp.15
- Arifin, W.N. and Zahiruddin, W.M., 2017. Sample size calculation in animal studies using resource equation approach. *The Malaysian Journal of Medical Sciences: MJMS*, 24(5), p.101.
- Augustine, D., Sandhya, K.V., James, J.J., Sowmya, S.V., Pushpalatha, C., Saraswathy, G.R., Shakir, A. and Dhodwad, R., 2023. Drug Delivery and Therapeutics for the Treatment of Infectious Diseases. In *Nanovaccinology: Clinical Application of Nanostructured Materials Research to Translational Medicine*. Cham: Springer International Publishing, pp.59-77
- Auletta, S., Varani, M., Horvat, R., Galli, F., Signore, A. and Hess, S., 2019. PET radiopharmaceuticals for specific bacteria imaging: Advances in Nuclear Medicine; a systematic review. *Journal of Clinical Medicine*, 8(2), pp.197.
- Baba, T., Bae, T., Schneewind, O., Takeuchi, F. and Hiramatsu, K., 2008. Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. *Journal of Bacteriology*, 190, pp.300-310.

- Arifin, W.N. and Zahiruddin, W.M., 2017. Sample size calculation in animal studies using resource equation approach. *The Malaysian Journal of Medical Sciences: MJMS*, 24(5), p.101.
- Ballinger, J.R., Golan, H. and Ichise, M., 1995. Cost effective preparation of Tc-99m ECD by freezing excess ligand. *Journal of Nuclear Medicine*, pp 36.
- Ballinger, J.R. and Blower, P.J., 2011. Radiochemical purity testing of ^{99m}Tc -labelled radiopharmaceuticals: how much is enough? *Nuclear Medicine Communications*, 32(9), pp.761-763.
- Ballinger, J.R., 2018. Theranostic radiopharmaceuticals: established agents in current use. *The British Journal of Radiology*, 91(1091), pp.20170969.
- Ballinger, J.R., 2021. Radiopharmaceuticals in clinical diagnosis and therapy. In *Basic Sciences of Nuclear Medicine* Cham: Springer International Publishing. pp.103-118.
- Bates, I., Fenton, C., Gruber, J., Laloo, D., Lara, A.M., Squire, S.B., Theobald, S., Thomson, R. and Tolhurst, R., 2004. Vulnerability to malaria, tuberculosis, and HIV/AIDS infection and disease. Part 1: determinants operating at individual and household level: *The Lancet of infectious diseases*. 4(5), pp.267-277.
- Bayomy, T., Abdulrazzak, M., Moustafa, H., Khalil, W.M. and Pant, G.S., 2009. Radiopharmaceutical, Original Article Different Models for Cost-Effective Fractionation of Bone Radiopharmaceuticals; Methylene Diphosphonate (MDP) and hydroxy methylene Diphosphonate (HDP). *Egyptian J. Nucl. Med*, 2(2), pp.74.
- Benfante, V., Stefano, A., Ali, M., Laudicella, R., Arancio, W., Cucchiara, A., Caruso, F., Cammarata, F.P., Coronello, C., Russo, G. and Miele, M., 2023. An Overview of In Vitro Assays of ^{64}Cu -, ^{68}Ga -, ^{125}I -, and ^{99m}Tc -Labelled Radiopharmaceuticals Using Radiometric Counters in the Era of Radiotheranostics. *Diagnostics*, 13(7), p.1210.
- Bhatia, N., Dhingra, V.K., Watts, A., Ranjan, R., Singh, B., Shukla, J. and Mittal, B.R., 2013. Fractionation of common cold kits as a cost-saving method in a low volume nuclear medicine department. *Indian Journal of Nuclear Medicine*,

(28), pp. S36.

- Bhatia, N., Vatsa, R., Dhingra, V.K., Dhawan, D.K. and Chadha, V.D., 2022. Evaluation of Tetrofosmin Cold Kit Fractionation Using Alternative New Method of Quality Control for Testing Radiochemical Purity. *World Journal of Nuclear Medicine*, 21(04), pp.314-319.
- Blanchette, K.A., Prabhakara, R., Shirtliff, M.E. and Wenke, J.C., 2017. Inhibition of fracture healing in the presence of contamination by *Staphylococcus aureus*: effects of growth state and immune response. *Journal of Orthopaedic Research*, 35(9), pp.1845-1854.
- Boora, S., Sharma, V., Kaushik, S., Bhupatiraju, A.V., Singh, S. and Kaushik, S., 2023. Hepatitis B virus-induced hepatocellular carcinoma: a persistent global problem. *Brazilian Journal of Microbiology*, pp.1-11.
- British Pharmacopeia, 2016: 8099-9005
- Britton, K.E., Vinjamuri, S., Hall, A.V., Solanki, K., Siraj, Q.H., Bomanji, J. and Das, S., 1997. Clinical evaluation of technetium-99m infecton for the localization of bacterial infection. *European Journal of Nuclear Medicine*, 24(5), pp.553-556.
- Brockwell, Y.M., Spithill, T.W., Anderson, G.R., Grillo, V. and Sangster, N.C., 2013. Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. *Veterinary parasitology*, 196(3-4), pp.417-426.
- Brugarolas, P., Comstock, J., Dick, D.W., Ellmer, T., Engle, J.W., Lapi, S.E., Liang, S.H., Parent, E.E., Pillarsetty, N.V.K., Selivanova, S. and Sun, X., 2020. Fifty years of radiopharmaceuticals. *Journal of Nuclear Medicine Technology*, 48(Supplement 1), pp.34S-39S.
- Calabria, F., Leporace, M., Tavolaro, R. and Bagnato, A., 2023. Radiopharmaceuticals. *Radiology-Nuclear Medicine Diagnostic Imaging: A Correlative Approach*, pp.133-162.
- Carretta, R.F. and Matin, P., 1986. Marketing Nuclear Medicine. *Journal of Nuclear Medicine Technology*, 14(4), pp.225-227.

- Censullo, A. and Vijayan, T., 2017. Using nuclear medicine imaging wisely in diagnosing infectious diseases. In *Open Forum Infectious Diseases* (Vol. 4, No. 1, p. ofx011). US: Oxford University Press, pp.67-75.
- Chakraborty, D., Bhattacharya, A., Mete, U.K. and Mittal, B.R., 2013. Comparison of 18F fluoride PET/CT and ^{99m}Tc -MDP bone scan in the detection of skeletal metastases in urinary bladder carcinoma. *Clinical Nuclear Medicine*, 38(8), pp.616-621.
- Chen P, Li Z, and Hu Y., 2016. Prevalence of osteoporosis in China : a meta-analysis and systematic review. *BMC Public Health*; 1–11.
- Cherry, S.R., Jones, T., Karp, J.S., Qi, J., Moses, W.W. and Badawi, R.D., 2018. Total-body PET: maximizing sensitivity to create new opportunities for clinical research and patient care. *Journal of Nuclear Medicine*, 59(1), pp.3-12.
- Chianelli, M., Mather, S.J., Martin-Comin, J. and Signore, A., 1997. Radiopharmaceuticals for the study of inflammatory processes: a review. *Nuclear Medicine Communications*, 18(5), pp.437-455.
- Christofilogiannis, P., 2001. Current inoculation methods in MIC determination. *Aquaculture*, 196(3-4), pp.297-302.
- Christofilogiannis, P., 2001. Current inoculation methods in MIC determination. *Aquaculture*, 196(3-4), pp.297-302.
- Colston, J. and Atkins, B., 2018. Bone and joint infection. *Clinical Medicine*, Royal College of Physicians 18(2), p.150.
- Cook, G.J., Yip, C., Siddique, M., Goh, V., Chicklore, S., Roy, A., Marsden, P., Ahmad, S. and Landau, D., 2013. Are pretreatment 18F-FDG PET tumor textural features in non-small cell lung cancer associated with response and survival after chemoradiotherapy. *Journal of Nuclear Medicine*, 54(1), pp.19-26.
- Cooper, M., Dustan, K. and Rotureau, L., 2006. The effect on radiochemical purity of modifications to the method of preparation and dilution of ^{99m}Tc -sestamibi. *Nuclear medicine communications*, 27(5), pp.455-460.
- Cooper, J.F., Petry, N.A. and Director, B.C.N.P., 2010. USP Chapter 797 Pharmaceutical

- compounding; sterile preparations potential impact on handling radiopharmaceuticals. University of New Mexico Continuing Education Program for Nuclear Pharmacists, 13, p.19.
- Couto, M. and Cates, C., 2019. Laboratory guidelines for animal care. *Vertebrate Embryogenesis: Embryological, Cellular, and Genetic Methods*, pp.407-430.
- Crémieux, A.C. and Carbon, C., 1997. Experimental models of bone and prosthetic joint infections. *Clinical Infectious Diseases*, 25(6), pp.1295-1302.
- Crump, J.A., Sjölund-Karlsson, M., Gordon, M.A. and Parry, C.M., 2015. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Reviews*, 28(4), pp.901-937.
- Cutler, C.S., 2019. Economics of new molecular targeted personalized radiopharmaceuticals. In *Seminars in nuclear medicine*. WB Saunders, 49(5) pp. 450-457.
- Dadgar, H., Norouzbeigi, N., Jokar, N., Zareizadeh, J., Gholamrezanezhad, A., Ahmadzadehfar, H., Abbaszadeh, M. and Assadi, M., 2022. Comparison of 18F-NaF imaging, ^{99m}Tc -MDP scintigraphy, and 18F-FDG for detecting bone metastases. *World Journal of Nuclear Medicine*, 21(01), pp.001-008.
- Dammann, F., Bootz, F., Cohnen, M., Haßfeld, S., Tatagiba, M. and Kösling, S., 2014. Diagnostic imaging modalities in head and neck disease. *Deutsches Ärzteblatt International*, 111(23-24), p.417.
- Damasceno, A., Pijeira, M.S.O., Ricci-Junior, E., Alencar, L.M.R., İlem-Özdemir, D. and Santos-Oliveira, R., 2022. Exploiting the extemporaneousness of radiopharmaceuticals: Radiolabeling stability under diverse conditions. *Journal of Pharmaceutical and Biomedical Analysis*, 221, p.115024.
- D'angelo, G., Sciuto, R., Salvatori, M., Sperduti, I., Mantini, G., Maini, C.L. and Mariani, G., 2012. Targeted "bone-seeking" radiopharmaceuticals for palliative treatment of bone metastases: a systematic review and meta-analysis. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging: Official*

- Publication of the Italian Association of Nuclear Medicine (AIMN)[and] the International Association of Radiopharmacology (IAR). Section of the Society 56(6), pp.538-543.
- Dartnell, J., Ramachandran, M. and Katchburian, M., 2012. Haematogenous acute and subacute paediatric osteomyelitis: a systematic review of the literature. *The Journal of Bone and Joint Surgery British Volume*, 94(5), pp.584-595.
- Dash, A., F. Russ Knapp, F. and Ra Pillai, M., 2013. Targeted radionuclide therapy-an overview. *Current radiopharmaceuticals*, 6(3), pp.152-180.
- Dashti, A.A., Jadaon, M.M., Abdulsamad, A.M. and Dashti, H.M., 2009. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. *Kuwait Med J*, 41, pp.117-122.
- Decristoforo, C., Siller, R., Chen, F. and Riccabona, G., 2000. Radiochemical purity of routinely prepared ^{99m}Tc radiopharmaceuticals: a retrospective study. *Nuclear Medicine Communications*, 21(4), pp.349-354.
- Dhingra VK, Saini S. and Basu S., 2015 How a tertiary medical nuclear medicine department at the Himalayan area in India can be established and function in an exemplary manner. Basic rules revisited. *Hell J. Nucl Med*;18, pp.252-6.
- Dhingra, M. and Dhingra, V.K., 2019. Quality of radiochemical purity in multiple samples of various fractionated cold kits: Testing a cost and time effective technique. *Hellenic Journal of Nuclear Medicine*, 22(3), pp.200-205.
- Doot, R.K., Young, A.J., Daube-Witherspoon, M.E., Alexoff, D., Labban, K.J., Lee, H., Wu, Z., Zha, Z., Choi, S.R., Ploessl, K.H. and Schubert, E.K., 2020. Biodistribution, dosimetry, and temporal signal-to-noise ratio analyses of normal and cancer uptake of $[^{68}\text{Ga}]$ Ga-P15-041, a gallium-68 labeled bisphosphonate, from first-in-human studies. *Nuclear Medicine and Biology*, 86, pp.1-8.
- Duncan, K., 1998. Radiopharmaceuticals in PET imaging. *Journal of Nuclear Medicine Technology*, 26(4), pp.228-234.
- Elgebaly, R.H., Rageh, M.M., Adel, M. and Kamal, I., 2023. Studying Various Parameters Affecting Labeling Efficiency of Radiopharmaceuticals In Nuclear Medicine. *Journal of Pharmaceutical Negative Results*, pp.3512-3520.

- Elsinga, P.H., 2012. Present and future of PET-radiopharmaceuticals. *Nuclear Medicine Review*, 15(C), pp.13-16.
- Filippi, L., Frantellizzi, V., Chiaravalloti, A., Pontico, M., De Feo, M.S., Corica, F., Montebello, M., Schillaci, O., De Vincentis, G. and Bagni, O., 2021. Prognostic and theranostic applications of positron emission tomography for a personalized approach to metastatic castration-resistant prostate cancer. *International Journal of Molecular Sciences*, 22(6), pp.3036.
- Fuchsova, R., 2023. A Blood Biomarker Panel Recommended for Personalized Prediction, Prognosis and Prevention of Rheumatoid Arthritis. *Biomedical Journal of Scientific and Technical Research*, 50(2), pp.41580-41583.
- Fleming, W.K., Jay, M. and Ryo, U.Y., 1992. Reconstitution and fractionation of radiopharmaceutical kits. *Journal of Nuclear Medicine*, 33(10), pp.1915- 1915.
- Garg, P., 2012. Fungal, Mycobacterial, and Nocardia infections and the eye: an update. *Eye*, 26(2), pp.245-251.
- Giovacchini, G., Giovannini, E., Riondato, M. and Ciarmiello, A., 2018. PET/CT with ⁶⁸Ga-PSMA in prostate cancer: radiopharmaceutical background and clinical implications. *Current Radiopharmaceuticals*, 11(1), pp.4-13.
- Gnau, T.R. and Maynard, C.D., 1973. Reducing the cost of nuclear medicine: sharing radiopharmaceuticals. *Radiology*, 108(3), pp.641-645.
- Gower-Fry, L., Kronemann, T., Dorian, A., Pu, Y., Jaworski, C., Wängler, C., Bartenstein, P., Beyer, L., Lindner, S., Jurkschat, K. and Wängler, B., 2021. Recent Advances in the Clinical Translation of Silicon Fluoride Acceptor (SiFA) ¹⁸F-Radiopharmaceuticals. *Pharmaceutics*, 14(7), pp.701.
- Grandin, T., 2010. Recommended animal handling guidelines and audit guide. A systematic approach to animal welfare. American Industry Foundation: USA, pp.56
- Gristina, A.G., 1987. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science*, 237(4822), pp.1588-1595.
- Gulyas, B. and Halldin, C., 2012. New PET radiopharmaceuticals beyond FDG for brain tumor imaging. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging: Official Publication of the Italian Association of Nuclear Medicine*

- (AIMN) [and] the International Association of Radiopharmacology (IAR), [and] Section of the Society of., 56(2), pp.173-190.
- Gutierrez, K., 2005. Bone and joint infections in children. *Paediatric Clinics*, 52(3), pp.779-794.
- Hakkarainen, T.W., Kopari, N.M., Pham, T.N. and Evans, H.L., 2014. Necrotizing soft tissue infections: review and current concepts in treatment, systems of care, and outcomes. *Current problems in surgery*, 51(8), pp.344-362.
- Hamid, L., Alsayari, A., Tak, H., Mir, S.A., Almoyad, M.A.A., Wahab, S. and Bader, G.N., 2023. An Insight into the Global Problem of Gastrointestinal Helminth Infections amongst Livestock: Does Nanotechnology Provide an Alternative? *Agriculture*, 13(7), p.1359.
- Herrero Álvarez, N., Bauer, D., Hernández-Gil, J. and Lewis, J.S., 2021. Recent advances in radiometals for combined imaging and therapy in cancer. *Chem. Med. Chem*, 16(19), pp.2909-2941.
- Holik, H.A., Ibrahim, F.M., Elaine, A.A., Putra, B.D., Achmad, A. and Kartamihardja, A.H.S., 2022. The chemical scaffold of theranostic radiopharmaceuticals: radionuclide, bifunctional chelator, and pharmacokinetics modifying linker. *Molecules*, 27(10), pp.3062.
- Horst, S.A., Hoerr, V., Beineke, A., Kreis, C., Tuschcherr, L., Kalinka, J., Lehne, S., Schleicher, I., Köhler, G., Fuchs, T. and Raschke, M.J., 2012. A novel mouse model of *Staphylococcus aureus* chronic osteomyelitis that closely mimics the human infection: an integrated view of disease pathogenesis. *The American Journal of Pathology*, 181(4), pp.1206-1214.
- <https://www.bing.com/search2014'RaditionStudies'examples+of+studies+done+in+nucl ear+medicine&form=ANSPH1&refig=d8a1389d1fb54b7f9a8a73411052daf c&pc=U531#>, (Accessed on 31/08/2021)
- <https://www.pdsa.org.uk/pet-help-and-advice/looking-after-your-pet/small-pets/the-ideal-homeforyourmice#:~:text=The%20right%20cage%20for%20your%20mice%20A%20large%2C,mice%20because%20they%20don%E2%80%99t%20have%20very%20good%20ventilation.> (Accessed on 29/10/21)

- <https://www.wikihow.com/Take-Care-of-Your-Pet#> (Accessed on 29/10/21)
- Huang, S., Qi, M. and Chen, Y., 2023. Photonics-based treatments: Mechanisms and applications in oral infectious diseases. *Frontiers in Microbiology*, 14, pp.236.
- Huigen, Y.M., Krips, H.J., Hulleman, S., Gelsema, W.J. and De Ligny, C.L., 1990. The adsorption of ^{99m}Tc (Sn)—diphosphonate complexes on tri-calcium phosphate: the influence of preparation conditions, ligand-type, incubation media and adsorption conditions. The reversibility of the adsorption. *International journal of radiation applications and instrumentation. Part A. Applied radiation and isotopes*, 41(2), pp.189-194.
- Hurst, J.L., Barnard, C.J., Tolladay, U., Nevison, C.M. and West, C.D., 1999. Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Animal Behaviour*, 58(3), pp.563-586.
- <https://www.bing.com/images/search?view=detailV2&ccid=hr1VFKjK&id=FA1AE5EF E07F79D71E0BA4B193122366E5FB1781&thid=OIP.hr1VFKjKV8sYFqc 9SBFIXAAAA&mediaurl=https%3a%2f%2fi.pinimg.com>
- IAEA., 2006. Advances in medical radiation imaging for cancer diagnosis and treatment. *Nuclear Technology Review*, pp. 110-127.
- IAEA., 2021. Diagnosis of infectious diseases with nuclear medicine. available at <https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjHgeDIx6vxAhVzAWMBHc02C3gQFjAJegQIGRAD&url=https%3A%2F%2Fwww.iaea.org%2Ftopics%2F=AOvVaw386Skk-pEFHfiX-MfdYBcJ>, (Accessed:22/06/2021).
- International Atomic Energy Agency Hospital Guidelines., 2008. pp.28-36.
- Jang, J., Kumakura, Y., Tatenuma, K., Ozeki, A.N., Wada, Y., Akimitsu, N., Tsuguchi, A., Kikunaga, H., Higaki, S. and Uesaka, M., 2022. A preliminary biodistribution study of [^{99m}Tc] sodium pertechnetate prepared from an electron linear accelerator and activated carbon-based ^{99m}Tc generator. *Nuclear Medicine and Biology*, 110, pp.1-9.
- Jaiswal, A.K., Tiwari, P., Kumar, S., Gupta, D., Khanna, A. and Rodrigues, J.J., 2019. Identifying pneumonia in chest X-rays: A deep learning approach.

- Measurement, 145, pp.511-518.
- Jiang, D., Younis, M.H., Lan, X. and Cai, W., 2021. High-performance renal imaging with a radiolabelled, non-excretable chimeric fusion protein. *Theranostics*, 11(18), pp.9177.
- Ji-xin, W.A.N.G. and Yan-hua, Z.H.A.N.G., 2010. Experimental Study on “One-strip” Thin-layer Chromatography for Determining the Radiochemical Purity of ^{99m}Tcm Radiopharmaceuticals. *Journal of Isotopes*, 23(3), pp.170.
- Jurisson, S., Berning, D., Jia, W. and Ma, D., 1993. Coordination compounds in nuclear medicine. *Chemical Reviews*, 93(3), pp.1137-1156.
- Kartamihardja, A.H.S., Kurniawati, Y. and Gunawan, R., 2018. Diagnostic value of ^{99m}Tc -ethambutol scintigraphy in tuberculosis: compared to microbiological and histopathological tests. *Annals of nuclear medicine*. 32(1), pp.60-68.
- Kaul, A., Hazari, P.P., Rawat, H., Singh, B., Kalawat, T.C., Sharma, S., Babbar, A.K. and Mishra, A.K., 2013. Preliminary evaluation of technetium- ^{99m}Tc -labeled ceftriaxone: infection imaging agent for the clinical diagnosis of orthopedic infection. *International Journal of Infectious Diseases*, 17(4), pp.e263-e270.
- Kaushik, D., Jangra, P., Verma, R., Purohit, D., Pandey, P., Sharma, S. and Sharma, R.K., 2021. Radiopharmaceuticals: An insight into the latest advances in medical uses and regulatory perspectives. *Journal of Biosciences*, 46, pp.1-25.
- Khawar, A., Eppard, E., Roesch, F., Ahmadzadehfar, H., Kürpig, S., Meisenheimer, M., Gaertner, F.C., Essler, M. and Bundschuh, R.A., 2019. Preliminary results of biodistribution and dosimetric analysis of $[^{68}\text{Ga}]\text{Ga-DOTA ZOL}$: a new zoledronate-based bisphosphonate for PET/CT diagnosis of bone diseases. *Annals of Nuclear Medicine*, 33, pp.404-413.
- Kleynhans, J., Sathekge, M.M. and Ebenhan, T., 2023. Preclinical Research Highlighting Contemporary Targeting Mechanisms of Radiolabelled Compounds for PET Based Infection Imaging. In *Seminars in Nuclear Medicine*. WB Saunders, pp.678
- Knapp, F.F. and Dash, A., 2016. Radiopharmaceuticals for Therapy. New Delhi, India: Springer, pp.3-23.
- Kocher, M.S., Zurakowski, D. and Kasser, J.R., 1999. Differentiating between septic

- arthritis and transient synovitis of the hip in children: an evidence-based clinical prediction algorithm. *JBJS*, 81(12), pp.1662-70.
- Koeth, L.M., DiFranco-Fisher, J.M. and McCurdy, S., 2015. A reference broth microdilution method for dalbavancin in vitro susceptibility testing of bacteria that grow aerobically. *JoVE (Journal of Visualized Experiments)*, (103), pp.e53028.
- Kunos, C.A., Mankoff, D.A., Schultz, M.K., Graves, S.A. and Pryma, D.A., 2021. Radiopharmaceutical Chemistry and Drug Development—What's Changed? In *Seminars in radiation oncology*. WB Saunders. Vol. 31, No. 1, pp.3-11.
- Landau, S.M., Breault, C., Joshi, A.D., Pontecorvo, M., Mathis, C.A., Jagust, W.J. and Mintun, M.A., 2013. Amyloid- β imaging with Pittsburgh compound B and florbetapir: comparing radiotracers and quantification methods. *Journal of Nuclear Medicine*, 54(1), pp.70-77.
- Lapi, S.E. and Welch, M.J., 2012. A historical perspective on the specific activity of radiopharmaceuticals: what have we learned in the 35 years of the ISRC? *Nuclear Medicine and Biology*, 39(5), pp.601-608.
- Lau, C.Y. and Mostafa, M.Y.A., 2023. Reviews in: Radiopharmaceuticals in nuclear medicine. *Frontiers in Medicine*, 10, pp.1178528.
- Leide-Svegborn, S., Ahlgren, L., Johansson, L. and Mattsson, S., 2016. Excretion of radionuclides in human breast milk after nuclear medicine examinations. Biokinetic and dosimetric data and recommendations on breastfeeding interruption. *European Journal of Nuclear Medicine and Molecular Imaging*, 43, pp.808-821.
- Lentle, B., Attariwila, R. and Lyster, D., 2007. Drug-induced changes in radiopharmaceutical biodistributions. Albuquerque: The University of New Mexico Health Sciences Center College of Pharmacy, Albuquerque, New Mexico, pp.1-62.
- Leslie, W.D. and Lix LM, Yogendran MS., 2011. Validation of a case definition for osteoporosis disease surveillance, pp.37–46.
- Lessa, F.C. and Sievert, D.M., 2023. Antibiotic Resistance: A Global Problem and the Need to Do More. *Clinical Infectious Diseases*, 77(Supplement_1), pp.S1-S3.

- Li, B. and Webster, T.J., 2018. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *Journal of Orthopaedic Research*, 36(1), pp.22-32.
- Li, H., McLaurin, K.A., Mactutus, C.F. and Booze, R.M., 2021. A rat model of EcoHIV brain infection. *JoVE (Journal of Visualized Experiments)*, (167), pp.e62137.
- Liepe, K., Murray, I. and Flux, G., 2022. March. Dosimetry of bone seeking beta emitters for bone pain palliation metastases. In *Seminars in Nuclear Medicine*, WB Saunders 52 (2), pp. 178-190).
- Lin, M., Paolillo, V., Le, D.B., Macapinlac, H. and Ravizzini, G.C., 2021. Monoclonal antibody based radiopharmaceuticals for imaging and therapy. *Current Problems in Cancer*, 45(5), pp.100796.
- Lin, M., Ta, R.T., Kairemo, K., Le, D.B. and Ravizzini, G.C., 2021. Prostate-specific membrane antigen-targeted radiopharmaceuticals in diagnosis and therapy of prostate cancer: Current status and future perspectives. *Cancer Biotherapy and Radiopharmaceuticals*, 36(3), pp.237-251.
- Lipsky, B.A., Senneville, É., Abbas, Z.G., Aragón-Sánchez, J., Diggle, M., Embil, J.M., Kono, S., Lavery, L.A., Malone, M., van Asten, S.A. and Urbančič-Rovan, V., 2020. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes/metabolism research and reviews*, 36, pp.e3280.
- Looker AC., 2017. Trends in Osteoporosis and Low Bone Mass in Older US adults, 2005-2006 through 2013-2014. *Osteoporosis Int.* 28(6), pp.2005–6.
- Lucke, M., Schmidmaier, G., Sadoni, S., Wildemann, B., Schiller, R., Stemberger, A., Haas, N.P. and Raschke, M., 2003. A new model of implant-related osteomyelitis in rats. *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, 67(1), pp.593-602.
- Magnano San Lio, R., Favara, G., Maugeri, A., Barchitta, M. and Agodi, A., 2023. How

- antimicrobial resistance is linked to climate change: an overview of two intertwined global challenges. *International Journal of Environmental Research and Public Health*, 20(3), pp.1681.
- Mahon, C.R. and Lehman, D.C., 2022. Textbook of diagnostic microbiology-e-book. Elsevier Health Sciences.
- Maioli, C., Lucignani, G., Strinchini, A., Tagliabue, L. and Del Sole A., 2017. Quality control on radiochemical purity in Technetium-99m radiopharmaceuticals labelling: Three years of experience on 2280 procedures. *Acta Biomed*, pp.88:49.
- Makam, P. and Matsa, R., 2021. Big Three infectious diseases: tuberculosis, malaria and HIV/AIDS. *Current Topics in Medicinal Chemistry*, 21(31), pp.2779-2799.
- Malzac, D., Couret, I., Suquet, J. and Rossi, M., 1997. Interests in splitting-up four kits of radiopharmaceuticals intended for sup 99m, pp.106
- Mandiwana, V., Kalombo, L., Grobler, A. and Zeevaart, J.R., 2018. ^{99m}Tc-MDP as an imaging tool to evaluate the in vivo biodistribution of solid lipid nanoparticles. *Applied Radiation and Isotopes*, 141, pp.51-56.
- Mayerhoefer, M.E., Materka, A., Langs, G., Häggström, I., Szczypiński, P., Gibbs, P. and Cook, G., 2020. Introduction to radiomics. *Journal of Nuclear Medicine*, 61(4), pp.488-495.
- McCartney, A., 2006. A12 White cell labelling: How do you do yours? *Nuclear Medicine Communications*, 27(7), pp.A3.
- McFarland, D.C., 2000. Preparation of pure cell cultures by cloning. *Methods in cell science*, 22, pp.63-66.
- McNally, M., Sousa, R., Wouthuyzen-Bakker, M., Chen, A.F., Soriano, A., Vogely, H.C., Clauss, M., Higuera, C.A. and Trebše, R., 2021. The EBJIS definition of periprosthetic joint infection: a practical guide for clinicians. *The Bone and Joint Journal*, 103(1), pp.18-25.
- Motaleb, M.A., 2007. Preparation and biodistribution of 99m Tc-lomefloxacin and 99m Tc-ofloxacin complexes. *Journal of Radioanalytical and Nuclear Chemistry*, 272, pp.95-99.

- Monzon, M., García-Álvarez, F., Lacleriga, A., Gracia, E., Leiva, J., Oteiza, C. and Amorena, B., 2001. A simple infection model using pre-colonized implants to reproduce rat chronic *Staphylococcus aureus* osteomyelitis and study antibiotic treatment. *Journal of Orthopaedic Research*, 19(5), pp.820-826.
- Morgan-Warren, E.J., 2006. Principles for safe transport of radioactive material. *Packaging, Transport, Storage and Security of Radioactive Material*, 17(2), pp.71-77.
- Mueller, D., Klette, I., Baum, R.P., Gottschaldt, M., Schultz, M.K. and Breeman, W.A., 2012. Simplified NaCl based ⁶⁸Ga concentration and labelling procedure for rapid synthesis of ⁶⁸Ga radiopharmaceuticals in high radiochemical purity. *Bioconjugate chemistry*, 23(8), pp.1712-1717.
- Museru, L.M. and Mcharo, C.N., 2001. Chronic osteomyelitis: a continuing orthopaedic challenge in developing countries. *International orthopaedics*, 25(2), pp.127- 131.
- Nade, S., 2003. Septic arthritis. *Best Practice and Research Clinical Rheumatology*, 17(2), pp.183-200.
- Nagai, Y., Hatsukawa, Y., Kin, T., Hashimoto, K., Motoishi, S., Konno, C., Ochiai, K., Takakura, K., Sato, Y., Kawauchi, Y. and Sato, N., 2011. Successful Labeling of ^{99m}Tc-MDP Using ^{99m}Tc Separated from ⁹⁹Mo Produced by ¹⁰⁰Mo (n, 2 n) ⁹⁹Mo. *Journal of the Physical Society of Japan*, 80(8), p.083201.
- Nair, M.B., Kretlow, J.D., Mikos, A.G. and Kasper, F.K., 2011. Infection and tissue engineering in segmental bone defects—a mini review. *Current Opinion in Biotechnology*, 22(5), pp.721-725.
- National Research Council, Division on Earth, Life Studies, Institute for Laboratory Animal Research, Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2010. *Guide for the care and use of laboratory animals*, pp.89
- Nazarian, E.J., Bopp, D.J., Saylor, A., Limberger, R.J. and Musser, K.A., 2008. Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples. *Diagnostic Microbiology and Infectious disease*, 62(2), pp.125-132

- Negi, M., Dhingra, V.K. and Narayan, M.L., 2018. Quality of radiochemical purity in multiple samples of various fractionated cold kits Testing a cost-and time-effective technique. *Indian Journal of Nuclear Medicine*, 33(5, suppl.), pp.S89.
- Newberg, A.B., Plössl, K., Mozley, P.D., Stubbs, J.B., Wintering, N., Udeshi, M., Alavi, A., Kauppinen, T. and Kung, H.F., 2004. Biodistribution and imaging with ^{123}I -ADAM: a serotonin transporter imaging agent. *Journal of Nuclear Medicine*, 45(5), pp.834-841.
- Ng, T.L.T., 2023. Development of amplicon sequencing-based protocols for direct detection of antimicrobial resistance in mycobacterium tuberculosis and human immunodeficiency virus from clinical samples. lib.polyu.edu.hk/handle/200, pp.12538
- Notni, J. and Wester, H.J., 2018. Re-thinking the role of radiometal isotopes: Towards a future concept for theranostic radiopharmaceuticals. *Journal of Labelled Compounds and Radiopharmaceuticals*, 61(3), pp.141-153.
- Nur, R.H., Teguh, H.A.W., Isti, D., Deka, A. and Isnaini, N., 2018. Determination of residence time Radiopharmaceutical $^{99\text{m}}\text{Tc}$ -MDP using MS-EXCEL, MATLAB and OLINDA/EXM for human dose estimation. *INIS, Indonesia* 50 (41), RN:5006 (2), pp.868
- Nurlaila, Z., Rukmini, I. and Basuki, H., 2009. Development and clinical application of the radiopharmaceutical dried-kit of ciprofloxacin. *Jurnal Sains dan Teknologi Nuklir Indonesia*, 10(1), pp.11-23.
- Olsen, I. and van Winkelhoff, A.J., 2014. Acute focal infections of dental origin. *Periodontology* 2000, 65(1), pp.178-189.
- O'Malley, J.P. and Ziessman, H.A., 2020. *Nuclear Medicine and Molecular Imaging: The Requisites E-Book*. Elsevier Health Sciences, Philadelphia, 5th Edn, pp. 255- 360.
- Orlhac, F., Boughdad, S., Philippe, C., Stalla-Bourdillon, H., Nioche, C., Champion, L., Soussan, M., Frouin, F., Frouin, V. and Buvat, I., 2018. A post reconstruction harmonization method for multicenter radiomic studies in PET. *Journal of Nuclear Medicine*, 59(8), pp.1321-1328.
- Owunwanne, A., 2012. *The handbook of radiopharmaceuticals*. Springer. Chapman and

Hall USA. ISBN 978-1-4757-0414-3(eBook)

- Palestro, C.J., 2023. PET Imaging of Infection. Pericarditis-Diagnosis and Management Challenges. EBOOK (PDF) ISBN 978-1-83768-365-910.5772/intechopen.104157
- Palm, S., Enmon, R.M., Matei, C., Kolbert, K.S., Xu, S., Zanzonico, P.B., Finn, R.L., Koutcher, J.A., Larson, S.M. and Sgouros, G., .2003. Pharmacokinetics and biodistribution of ^{86}Y -trastuzumab for ^{90}Y dosimetry in an ovarian carcinoma model: correlative MicroPET and MRI. *Journal of Nuclear Medicine*, 44(7), pp.1148-1155.
- Payolla, F.B., Massabni, A.C. and Orvig, C., 2019. Radiopharmaceuticals for diagnosis in nuclear medicine: a short review. *Eclética Química Journal*, 44(3), pp.11-19.
- Peer, N., 2015. The converging burdens of infectious and non-communicable diseases in rural-to-urban migrant Sub-Saharan African populations: a focus on HIV/AIDS, tuberculosis and cardio-metabolic diseases. *Tropical diseases, travel medicine and vaccines*, 1(1), pp.1-8.
- Penglis, S. and Tsopelas, C., 1998. Feasibility of fractionating MIBI cold kits for cost reduction. *ANZ Nuclear Medicine*, 29(2), pp.27.
- Penglis, S. and Tsopelas, C., 2000. $^{99\text{m}}\text{Tc}$ -tetrofosmin: Evaluation of fractionated cold kits and two new methods of quality control. *Nuclear Medicine Communications*, 21(5), pp.469-472.
- Pigrau-Serrallach, C. and Rodríguez-Pardo, D., 2013. Bone and joint tuberculosis. *European Spine Journal*, 22(4), pp.556-566.
- Ponto, J.A., 2012. Mechanisms of radiopharmaceutical localization. *UNM College of Pharmacy*, 16(4), pp.28.
- Prabakaran, K. and Freund, J., 1997. Optimised fractionation and most cost-effective use of expensive radiopharmaceuticals. *ANZ Nuclear Medicine*, 28(3), pp.37.
- Qadri, S.S. and Ramachandra, S.G., 2018. Laws, regulations, and guidelines governing research animal Care and use in India. In *Laboratory animals*. Academic Press. pp.237-261.

- Rathmann, S.M., Ahmad, Z., Slikboer, S., Bilton, H.A., Snider, D.P. and Valliant, J.F., 2019. The radiopharmaceutical chemistry of technetium-99m. *Radiopharmaceutical Chemistry*, pp.311-333.
- Reizner, W., Hunter, J., O'Malley, N., Southgate, R., Schwarz, E., Kates, S., 2014. A systematic review of animal models for *Staphylococcus aureus* osteomyelitis. 601 Elmwood Ave, Box 665, Rochester, NY 14642, USA CM 27, pp.196–212.
- Ripoll-Hamer, E., De-Paula, E.F., Freitas, L.C., Pereira, M.J., Fonseca, L.M., Gutfilen, B., Carvalho, J.J., Pôrto, L.C. and Bernardo-Filho, M., 1998. In vitro radioautographic studies of the biodistribution of radiopharmaceuticals on blood elements. *Braz J Med Biol Res* 31, 303–306.
<https://doi.org/10.1590/s0100-879x1998000200014>
- Rogers Van Katwyk, S., Giubilini, A., Kirchhelle, C., Weldon, I., Harrison, M., McLean, A., Savulescu, J. and Hoffman, S.J., 2023. Exploring models for an international legal agreement on the global antimicrobial commons: lessons from climate agreements. *Health Care Analysis*, 31(1), pp.25-46.
- Roukoz, S., El Khoury, G., Saghbini, E., Saliba, I., Khazzaka, A. and Rizkallah, M., 2020. Does the induced membrane have antibacterial properties? An experimental rat model of a chronic infected nonunion. *International orthopaedics*, 44, pp.391-398.
- Rowe, S.P., Mana-Ay, M., Javadi, M.S., Szabo, Z., Leal, J.P., Pomper, M.G., Pienta, K.J., Ross, A.E. and Gorin, M.A., 2016. PSMA-based detection of prostate cancer bone lesions with 18F-DCFPyL PET/CT: a sensitive alternative to ^{99m}Tc-MDP bone scan and Na18F PET/CT? *Clinical Genitourinary Cancer*, 14(1), pp.e115-e118.
- Saavedra-Lozano, J., Falup-Pecurariu, O., Faust, S.N., Girschick, H., Hartwig, N., Kaplan, S., Lorrot, M., Mantadakis, E., Peltola, H., Rojo, P. and Zaoutis, T., 2017. Bone and joint infections. *The Pediatric Infectious Disease Journal*, 36(8), pp.788-799.

- Sadeghpour, H., Alavi, M., Shahedi, M., Entezarmahdi, S.M. and Sakhteman, A., 2015. Evaluation of radiochemical purities of some radiopharmaceuticals in Shiraz Namazi teaching hospital. *Trends in Pharmaceutical Sciences*, 1(1), pp.15-19.
- Saha, G.B., 2010. *Fundamentals of nuclear pharmacy*. New York: Springer. 1(6), pp.96- 100.
- Saha, G.B., 2010. *Fundamentals of nuclear pharmacy*. Vol. 7. New York: Springer, pp.78-89.
- Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A. and Alqumber, M.A., 2023. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. In *Healthcare*, 11 (13), pp. 1946
- Satpati, D., 2021. Recent breakthrough in ^{68}Ga -radiopharmaceuticals cold kits for convenient PET radiopharmacy. *Bioconjugate Chemistry*, 32(3), pp.430-447.
- Schirmacher, R., Wangler, C. and Schirmacher, E., 2007. Recent developments and trends in ^{18}F -radiochemistry: syntheses and applications. *Mini-Reviews in Organic Chemistry*, 4(4), pp.317-329.
- Sgouros, G., Bodei, L., McDevitt, M.R. and Nedrow, J.R., 2020. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nature reviews Drug discovery*, 19 (9), pp.589-608.
- Shirliff, M.E. and Mader, J.T., 2002. Acute septic arthritis. *Clinical microbiology reviews*, 15(4), pp.527-544.
- Shah, S.Q. and Khan, M.R., 2013. Synthesis of $^{99\text{m}}\text{Tc}$ labelled Temafloxacin complex and biodistribution in male Wistar rats artificially infected with streptococci pneumonia. *Advances in clinical and experimental medicine*, 22(3), pp.319-325.
- Shah, S.Q., Khan, A.U. and Khan, M.R., 2011. $^{99\text{m}}\text{Tc}$ -prulifloxacin in artificially infected animals. *Nuklearmedizin-Nuclear Medicine*, 50(03), pp.134-140.
- Shirwaiker, R.A., Springer, B.D., Spangehl, M.J., Garrigues, G.E., Lowenberg, D.W., Garras, D.N., Yoo, J.U. and Pottinger, P.S. 2015. A clinical perspective on musculoskeletal infection treatment strategies and challenges. *JAAOS-Journal of the American Academy of Orthopaedic Surgeons*, 23(suppl), pp.S44-S54.

- Siaens, R.H., Rennen, H.J., Boerman, O.C., Dierckx, R. and Slegers, G., 2004. Synthesis and comparison of ^{99m}Tc -enrofloxacin and ^{99m}Tc -ciprofloxacin. *Journal of Nuclear Medicine*, 45(12), pp.2088-2094.
- Signore, A. and Glaudemans, A.W., 2011. The molecular imaging approach to image infections and inflammation by nuclear medicine techniques. *Annals of nuclear medicine*, 25(10), pp.681-700.
- Signore, A., Soroa, V.E. and De Vries, E.F., 2009. Radiolabelled white blood cells or FDG for imaging of inflammation and infection? *QJ Nucl Med Mol Imaging*, 53(1), pp.23-25.
- Signore, Alberto, Valeria Bentivoglio, Michela Varani, and Chiara Lauri., 2023. Current Status of SPECT Radiopharmaceuticals for Specific Bacteria Imaging. In *Seminars in Nuclear Medicine*. WB Saunders. 50(4), pp.12-15
- Silverman, D.H., Small, G.W. and Phelps, M.E., 1999. Clinical value of neuroimaging in the diagnosis of dementia: sensitivity and specificity of regional cerebral metabolic and other parameters for early identification of Alzheimer's disease. *Clinical Positron Imaging*, 2(3), pp.119-130.
- Simon, RC, James, AS and Micheal, EP., 2012. *Physics in the nuclear medicine* 4th edition, pp.546-552
- Srivastava, S.C., Meinken, G.E., Richards, P., Som, P., Oster, Z.H., Atkins, H.L., Brill, A.B., Knapp Jr, F.F. and Butler, T.A., 1985. The development and in-vivo behavior of tin containing radiopharmaceuticals—I. Chemistry, preparation, and biodistribution in small animals. *International Journal of Nuclear Medicine and Biology*, 12(3), pp.167-174.
- Stephenson, N.A., Holland, J.P., Kassenbrock, A., Yokell, D.L., Livni, E., Liang, S.H. and Vasdev, N., 2015. Iodonium ylide-mediated radiofluorination of 18F-FPEB and validation for human use. *Journal of Nuclear Medicine*, 56(3), pp.489-492.
- Sulaiman, S., Aguswarini, S., Karyadi, K., Chairuman, C., Setiawan, G., Adang, H.G. and Subur, M., 2018. Uji Klirens dan Uji Pirogenitas sebagai Bagian dari Penentuan Mutu Biologi Sediaan 90Y-EDTMP. *Jurnal Kefarmasian Indonesia*, pp.118-124.

- Swanepoel, A.J., 2014. Radio-labelling as a tool to investigate the absorption and bio-distribution of selected antimalarial drugs, North-West University, South Africa, Potchefstroom, (Doctoral dissertation).
- Tatara, A.M., Shah, S.R., Livingston, C.E. and Mikos, A.G., 2015. Infected animal models for tissue engineering. *Methods*, 84, pp.17-24.
- Taylor, D.M., 2013. Radiopharmaceuticals for Tumour Localization–Rapporteur Report’. In *Radioactive Isotopes in the Localization of Tumours: The Proceedings of the International Nuclear Medicine Symposium Arranged by the Institute of Cancer Research: Royal Cancer Hospital and Held at the Imperial College of Science and Technology, London, in September, 1967* (3), pp. 73
- Thokchom, A.K., Kumar, R. and Bharati, S., 2022. Quality assessment of Tc-99m methylene diphosphonate (MDP) radiopharmaceutical prepared using different cold kit fractionation methods. *Indian Journal of Nuclear Medicine*: IJNM: the Official Journal of the Society of Nuclear Medicine, India, 37(1), pp.7.
- Thorson, L.M., Hung, J.C. and Hollar, B.S., 1992. Feasibility of fractionating MAG3 cold kit for cost reduction. *Nucl Med Commun*; (13), pp.832-7.
- Tian, M., 2016. *The Pathophysiologic Basis of Nuclear Medicine*. Springer-Verlag Berlin Heidelberg New York, 5th Edn, pp.345-540.
- United States Pharmacopeial Conversion., 2005. *United States Pharmacopeia 28 official monographs. Technetium -99m Medroante injection*. Rockville, MD pp.281- 297.
- Vallabhajosula, S., 2023. Metal Radionuclides for Molecular Imaging Molecular imaging (MI). In *Molecular Imaging and Targeted Therapy: Radiopharmaceuticals and Clinical Applications*, Cham: Springer International Publishing. pp. 259-289.
- Vallabhajosula, S., 2023., *Theranostics in Prostate Cancer*. In *Molecular Imaging and Targeted Therapy: Radiopharmaceuticals and Clinical Applications*, Cham: Springer pp. 655-704.
- Vallabhajosula, S., Killeen, R.P. and Osborne, J.R., 2010. July. Altered biodistribution of radiopharmaceuticals: role of radiochemical/pharmaceutical purity, physiological, and pharmacologic factors. In *Seminars in nuclear medicine*, WB Saunders. 40(4) pp. 220-241.

- van der Bruggen, W., Bleeker-Rovers, C.P., Boerman, O.C., Gotthardt, M. and Oyen, W.J., 2010. PET and SPECT in osteomyelitis and prosthetic bone and joint infections: a systematic review. In *Seminars in nuclear medicine*, WB Saunders. 40(1) pp. 3-15.
- Van Deemter, J.J., Zuiderweg, F.J. and Klinkenberg, A.V., 1956. Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. *Chemical Engineering Science*, 5(6), pp.271-289.
- Van Dijk, B., Lemans, J.V.C. and Hoogendoorn, R.M., 2020. Treating infections with ionizing radiation: a historical perspective and emerging techniques. *Antimicrobial Resist Infect Control* 9, 121 <https://doi.org/10.1186/s13756-020-00775>.
- Vantucci, C.E., Ahn, H., Fulton, T., Schenker, M.L., Pradhan, P., Wood, L.B., Guldberg, R.E., Roy, K. and Willett, N.J., 2021. Development of systemic immune dysregulation in a rat trauma model of biomaterial-associated infection. *Biomaterials*, 264, p.120405.
- Verburg, F.A., de Keizer, B. and van Isselt, J.W., 2007. Use of radiopharmaceuticals for diagnosis, treatment, and follow-up of differentiated thyroid carcinoma. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry- Anti-Cancer Agents)*, 7(4), pp.399-409.
- Vermeulen, K., Vandamme, M., Bormans, G. and Cleeren, F., 2019. September. Design and challenges of radiopharmaceuticals. In *Seminars in nuclear medicine* WB Saunders, 49(5), pp. 339-356.
- Versari, A. and Casali, M., 2021. Normal findings with different radiopharmaceuticals, techniques, variants, and pitfalls. *Radionuclide imaging of infection and inflammation: a pictorial case-based atlas*, pp.1-27.
- Vinjamuri, S., Solanki, K.K., Bomanji, J., Siraj, Q., Britton, K.E., Hall, A.V., O'Shaughnessy, E. and Das, S.S., 1996. Comparison of ^{99m}Tc infecton imaging with radiolabelled white-cell imaging in the evaluation of bacterial infection. *The Lancet*, 347(8996), pp.233-235.

- Volk, A., Crémieux, A.C., Belmatoug, N., Vallois, J.M., Pocidalo, J.J. and Carbon, C., 1994. Evaluation of a rabbit model for osteomyelitis by high field, high resolution imaging using the chemical-shift-specific-slice-selection technique. *Magnetic resonance imaging*, 12(7), pp.1039-1046.
- Walsh, T.R., Gales, A.C., Laxminarayan, R. and Dodd, P.C., 2023. Antimicrobial Resistance: Addressing a Global Threat to Humanity. *Plos Medicine*, 20(7), pp.10042-10064.
- Walter, G., Vernier, M., Pinelli, P.O., Million, M., Coulange, M., Seng, P. and Stein, A., (2014). Bone and joint infections due to anaerobic bacteria: an analysis of 61 cases and review of the literature. *European Journal of Clinical Microbiology and Infectious Diseases*, 33(8), pp.1355-1364.
- Weissleder, R., Schwaiger, M.C., Gambhir, S.S. and Hricak, H., 2016. Imaging approaches to optimize molecular therapies. *Science translational medicine*, 8(355), pp.355ps16-355ps16.
- Welch, M.J. and Redvanly, C.S. eds., 2003. *Handbook of radiopharmaceuticals: radiochemistry and applications*. John Wiley and Sons, pp. 245-354
- Wiegand, I., Hilpert, K. and Hancock, R.E., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols*, 3(2), pp.163-175.
- Willyard, C., 2017. The drug-resistant bacteria that pose the greatest health threats. *Nature News*, 543(7643), pp.15.
- Woods, Charles R., John S. Bradley, Archana Chatterjee, Lawson A. Copley, Joan Robinson, Matthew P. Kronman and Antonio Arrieta. Clinical practice guideline by the Paediatric Infectious Diseases Society and the Infectious Diseases Society of America: 2021. guideline on diagnosis and management of acute hematogenous osteomyelitis in paediatrics. *Journal of the Paediatric Infectious Diseases Society* 10, no. 8 (2021), pp.801-844.
- World Health Organization, 2019. International Atomic Agency (IAEA)/WHO guidelines on good manufacturing practices for radiopharmaceutical products. *WHO Drug Information*, 33(3), pp.395-409.
- World Health Organization., 2018. Technical manual for drug susceptibility testing of

- medicines used in the treatment of tuberculosis. ISBN 978-92-4-151484-2
- Wu, Z., Zeng, D., Yao, J., Bian, Y., Gu, Y., Meng, Z., Fu, J. and Peng, L., 2019. Research progress on diagnosis and treatment of chronic osteomyelitis. *Chinese Medical Sciences Journal*, 34(3), pp.211-220.
- www.futuremarketinsights.com/reports/radiopharmaceuticals-market
- Zhang, L., He, Q., Zhou, T., Zhang, B., Li, W., Peng, H., Zhong, X., Ma, L. and Zhang, R., 2019. Accurate characterization of ^{99m}Tc -MDP uptake in extraosseous neoplasm mimicking bone metastasis on whole-body bone scan: contribution of SPECT/CT. *BMC Medical Imaging*, 19(1), pp.1-9.
- Zhang, K., Sparling, J., Chow, B. L., Elsayed, S., Hussain, Z., Church, D. L., Gregson, D. B., Louie, T. and Conly, J. M. 2004. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *Journal of Clinical Microbiology*, 42, pp.4947-4955.
- Ziessman, H.A. and O'Malley, J.P., 2013. Nuclear medicine: the requisites e-book. Elsevier Health Science, pp.234-256.
- Zimmermann, R.G., 2013. Why are investors not interested in my radiotracer? The industrial and regulatory constraints in the development of radiopharmaceuticals. *Nuclear medicine and Biology*, 40(2), pp.155-166.
- Zolle, I., 2017. Technetium-99m Pharmaceuticals: Preparation and Quality Control in Nuclear Medicine; Springer: Berlin, Germany; ISBN 978-3-540-33990-8.
- Zuckier, L.S. and Freeman, L.M., 2010. Nonosseous, nonurologic uptake on bone scintigraphy: atlas and analysis. In *Seminars in nuclear medicine*. WB Saunders, 40(4), pp.242-256.

APPENDICES APPENDIX A: Permission Letters

APPENDIX A1: Letter of approval of the Research Proposal



**THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
OFFICE OF THE ASSISTANT DEAN (POSTGRADUATE)**

Telephone: 293727
Telegrams: UNZA LUSAKA
Telex: UNZALU ZA 44370
Fax: 293727/253952
School Fax: 293727
Vet. Clinic Telephone: 291515

P.O. Box 32379
Lusaka, Zambia

Your Ref:

Our Ref:

24th September, 2021

Jenipher Zulu
Department of Paraclinical Studies
School of Veterinary Medicine
University of Zambia
P.O. Box 32379
LUSAKA

Dear Jenipher Zulu,

RE: APPROVAL OF RESEARCH PROPOSAL

At the meeting of the School Board of Graduate Studies held on 24th September 2021, your research proposal entitled '**Adaptation of the fractionation methodology for methylene diphosphate radiopharmaceutical in the diagnosis of infectious diseases**' was tabled and discussed. I am therefore pleased to inform you that the research proposal was subsequently approved by the Board.

On behalf of the Board, I wish you success as you apply for ethical approval and carry on with your research activities.

Yours sincerely

Dr Chisoni Mumba

ACTING ASSISTANT DEAN (PG), SCHOOL OF VETERINARY MEDICINE

Cc Director, DRGS
 Dean, School of Veterinary Medicine
 Head, Paraclinical Studies
 File

APPENDIX A2: Permission to conduct research from research Site



REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH
University Teaching Hospitals (Adult)

Fax: +260 211 250305
E-mail: mduth@yahoo.com

P/Bag Rw 1X
Lusaka - Zambia
Tel: +260 211 253947 (Switch Board)
+260 211 251451

SENIOR MEDICAL SUPERINTENDENT

Our Ref:

Your Ref:

4th October, 2021

Ms. Zulu Jenipher
University of Zambia
School of Veterinary of Medicine
P O Box 32379
LUSAKA

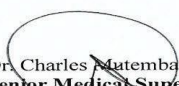
Dear Zulu Jenipher

RE: REQUEST FOR AUTHORITY TO CONDUCT RESEARCH

The University Teaching Hospital – Adult is in receipt of your letter date 19th September, 2021 in which you had requested to conduct a research titled **“Adaptation of the fractionation methodology for methylene diphosphate radiopharmaceutical in the diagnosis of infectious diseases at the University Teaching Hospital”**

I wish to inform you that permission has been granted and you are advised to liaise with the Head of Department.

Yours faithfully,


Dr. Charles Mutemba
Senior Medical Superintendent
UNIVERSITY TEACHING HOSPITALS - ADULT

APPENDIX A3: Ethical Approval Letter



Plot No. 277, Cnr Olive Tree Manswood Road,
Manswood Road
Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493 220
Email: eresconverge@yahoo.co.uk
I.R.B. No. 00005948
F.W.A. No. 00011697

08th November, 2021.

Ref. No. 2021-Oct- 085

The Principal Investigator
Ms. Jenipher Zulu
University of Zambia
School of Veterinary Medicine
LUSAKA, ZAMBIA

Dear Ms. Zulu

REF: ADAPTATION OF THE FRACTIONATION METHODOLOGY FOR METHYLENE DIPHOSPHATE RADIOPHARMACEUTICAL IN THE DIAGNOSIS OF INFECTIOUS DISEASE

Reference is made to your protocol resubmission. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Fasttrack	Approval No. 2021-Oct-085
Approval and Expiry Date	Approval Date: 08 th November, 2021	Expiry Date:
Protocol Version and Date	Version - Nil.	7 th November, 2022
Information Sheet, Consent Forms and Dates	• English.	7 th November, 2022
Consent form ID and Date	Version - Nil	7 th November, 2022
Recruitment Materials	Nil	7 th November, 2022
Other Study Documents	Questionnaire.	7 th November, 2022
Number of participants approved for study	-	7 th November, 2022

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB



Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Sc., PhD
CHAIRPERSON

PDF Scanned with
MOBILE SCANNER

APPENDIX A4: National Health Research Authority Approval letter



NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Chalala Office Lot No. 18961/M, Off Kasama Road, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@nhra.org.zm | www.nhra.org.zm

Ref No: NHRA0000015/01/06/2022

Date: 1st June, 2022

The Principal Investigator,
Zulu Jenipher,
UNZA-Vet,
Lusaka, Zambia

Dear Ms Zulu,

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for ethical clearance and authority to conduct research titled **"Adaptation of The Fractionation Methodology for Methylene Di Phosphate Radiopharmaceutical in The Diagnosis of Infectious Diseases."**

I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

Prof. Victor Chalwe,
Acting Director
National Health Research Authority

Completion of Online Research Ethics Review Submission

The Authority confirms receipt of your submission "Researcher New Registration - Ms. Jenipher Zulu". It is now undergoing the review process and we will provide feedback as soon as possible. For registration and accreditation, it takes 10 working days for you to receive feedback and issue of NHRA Certificate. Please note for study clearance for low-risk studies take about 10 working days: whereas the review of highrisk studies (i.e. MTAs, Clinical trials,

progress reports, request to disseminate and Special protocols) takes 20 working days. An email of your approval letter will be sent to you. For further details or follow up use: znhrasec@nhra.org.zm

Please use the reference number below for all follow ups.

Name of applicant: Ms Jenipher, Zulu

Certificate Reference number: NHRAR-R-2493/13/02/2025

Date and Time: 2025-02-13 07:09:54

CERTIFICATE OF REGISTRATION RESEARCHER

Your submission has been recommended for approval Review of registration takes 10 working day. An email will be sent to you to notify you that your certificate is ready for collection.

Email for further information znhrasec@nhra.org.zm Secretariat National Health Research Authority Lot No. 18961/M in Chalala, off Kasama Road Lusaka Email: znhrasec@nhra.org.zm www.nhra.org.zm

APPENDIX B: Data Collection Forms

APPENDIX B1: Laboratory form: Data collection form for NSSCl₂A Stream

QC Tests								
Aliquot Age in days	Amount of NSSCl ₂ A	Temp (AM/PM)	Clarity (Clear, not clear)	Chemical purity (pink or colorless)	Radio -nuclidic purity <, =, > 0.15MBq	RCP (> 90%)	pH (7.2 to 7.4)	Sterility (No growth=+, Growth=-)

1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								

APPENDIX B2: Laboratory form: Data collection form for NSSCl₂ Stream

QC Tests

Aliquot age in days	Amt of NSSCl ₂	Temp (AM/PM)	Clarity (Clear, not clear)	Chemical purity (pink or colorless)	Radio - nuclidic purity <, =, > 0.15MBq	RCP (> 90%)	pH (7.2 to 7.4)	Sterility (No growth=+, Growth=-)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								

23								
----	--	--	--	--	--	--	--	--

APPENDIX B3: Laboratory form: Data collection form for NSA Stream

QC Tests								
Aliquot age in days	Amt of NSAr	Temp (AM/PM)	Clarity (Clear, not clear)	Chemical purity (pink or colorless)	Radio - nuclidic purity <, =, > 0.15MBq	RCP (> 90%)	pH (7.2 to 7.4)	Sterility (No growth=+, Growth=-)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

21								
22								
23								

APPENDIX B4: Laboratory form - Data collection form for NS Stream

QC Tests								
Aliquot age in days	Amt of NS	Temp (AM/PM)	Clarity (Clear, not clear)	Chemical purity (pink or colorless)	Radio - nuclidic purity <, =, > 0.15MBq	RCP (> 90%)	pH (7.2 to 7.4)	Sterility (No growth=+, Growth=-)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								

18								
19								
20								
21								
22								

APPENDIX B5: Laboratory Form - To Show compliance of products

99-Mo/Tc-99m Generator/MDP RP kit	Labelling Compliance (Clear and correct = +, Not clear and correct = -)	Packaging (Intact = +, Not intact = -)	Shelf life (Not +, expired = -) Expired = -)	Batch No (Available =+, not available = -)
Month 01				
Month 02				
Month 03				
% Compliance				

APPENDIX C: Radioactivity

1 curie (Ci) = 3.7×10^{10} disintegrations per second (dps)

= 2.22×10^{12} disintegrations per minute (dpm)

1 millicurie (mCi) = 3.7×10^7 dps
= 2.22×10^9 dpm

1 microcurie (mCi) = 3.7×10^4 dps
= 2.22×10^6 dpm

1 becquerel (Bq) = 1 dps
= 2.703×10^{-11} Ci

1 kilobecquerel (kBq) = 10^3 dps
= 2.703×10^{-8} Ci

1 megabecquerel (MBq) = 10^6 dps
= 2.703×10^{-5} Ci

1 gigabecquerel (GBq) = 10^9 dps
= 2.703×10^{-2} Ci

1 terabecquerel (TBq) = 10^{12} dps
= 27.03 Ci

APPENDIX D: Publications

APPENDIX D1: Published Article (World Journal of Nuclear Medicine)

264 Original Article



Exploring the Use of Ambientally Stored Methylene Diphosphonate Radiopharmaceutical Aliquots in Solving Challenging Situations in Developing Countries

Jenipher None Zulu¹  Reuben None Katebe^{2,3} Martalena None Ramli⁴ Rita None Sakala⁵
Elias None Mwape⁶ Ernest None Chipasha⁷ Bernard Mudenda Hang'ombe⁸

¹ Pharmacy/School of Health Sciences, Levy Mwanawasa Medical University, Zambia

² Department of Technology and Science, Ministry of Higher Education, Lusaka, Zambia

³ Department of Nuclear Science, National Institute for Scientific Research, Lusaka, Zambia

⁴ Department of Radiopharmaceuticals, Centre for Radiopharmaceutical Production, Indonesia

⁵ Department of Nuclear Medicine/Radiopharmacy Unit, University Teaching Hospital, Zambia

Address for correspondence: Jenipher None Zulu, MSc, PhD Scholar, Pharmacy/School of Health Sciences, Levy Mwanawasa Medical University 10101, Zambia (e-mail: janzulu2003@yahoo.com).

⁶ Department of Nuclear Medicine, University Teaching Hospital, Zambia

⁷ Office of the Deputy Commandant, Northern Command Military Hospital, Zambia

⁸ School of Veterinary Medicine, University of Zambia, Zambia

World J Nuclear Med 2024;23:264–269.

Abstract

Objectives The primary aim was to evaluate the prolonged quality characteristics of methyl diphosphonate (MDP) aliquots during ambient storage over a specified duration. This study further investigated potential additives that could enhance the stability of MDP aliquots stored under such conditions.

Materials and Methods This was a laboratory-based experimental study conducted at the University Teaching Adult Hospital in Lusaka, Zambia. A total of 36 MDP aliquots stored at ambient conditions and 4 MDP aliquots stored at conventional refrigerated frozen conditions were labeled with technetium-99m (^{99m}Tc) and tested for radiochemical purity (RCP) and other quality characteristics. A comparative analysis of the stability and quality of MDP aliquots from the two cohorts was then conducted.

Statistical Analysis Stata 14 was used to analyze the data on the RCP of all MDP aliquots.

Results The RCP of ambient stored MDP aliquots was found to be ranging from 98 to 99%, while that for frozen and refrigerated ones ranged from 99 to 100%. There was also a 1% increase in RCP for both cohorts with argon gas purging (98 and 99%, respectively).

Conclusion The RCP of MDP aliquots from both cohorts was much higher than the required minimum of 90% implying that there was no significant association of their stability and quality with the mode of storage. However, purging with argon gas seemed to increase the stability further in both streams. The study findings show potential for application in resource-constrained environments and centers, especially in developing countries, where challenges to maintain the cold storage chain of these important radiopharmaceuticals are likely to be encountered due to power outages.

Keywords

- radiopharmaceutical
- technetium
- methylene diphosphate
- ambient conditions
- radiochemical purity

article published online
July 4, 2024

DOI: <https://doi.org/10.1055/s-0044-1788278>
ISSN: 1450-1147

© 2024. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (<https://creativecommons.org/licenses/by/4.0/>)
Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

Radiopharmaceuticals (RPs), consisting of a radioactive isotope combined with a pharmaceutical compound, offer exceptional precision when it comes to diagnosing and treating various medical conditions.¹ RPs have revolutionized the field of medicine, providing precise diagnostic capabilities and innovative treatments for a wide range of ailments.^{2,3} RPs are, therefore, widely used in diagnostic imaging procedures like single-photon emission computed tomography and positron emission tomography.⁴⁻⁷ The precision of these imaging techniques allows healthcare professionals to identify and evaluate various ailments with accuracy.⁸

However, while RPs offer remarkable accuracy in diagnosis and treatment, several usage challenges exist.⁹ These include the short half-lives of certain radioisotopes, limited availability, and cost considerations.⁶ Other challenges come in when there are low patient volumes and therefore a whole multidose vial of a RP kit that can cater for a good number of patients tends to go to waste.

Further, though the use of RPs is integral for diagnostic and therapeutic applications,¹⁰⁻¹³ logistical challenges associated with their storage and transportation, particularly in resource-constrained environments of developing countries, often hinder the seamless provision of nuclear medicine services. However, the compound methylene diphosphonate (MDP—supplied by AEC Armersham, 6 Indianapolis ST, Kyalami Park, Midland, 1684, South Africa), a RP commonly employed in bone imaging, holds promise for mitigating these challenges. While literature holds that MDP aliquots stored at refrigerated and frozen conditions remain viable for as long as 9 months,^{14,15} the effects of ambient conditions on aliquots in areas with unreliable cold chain backup strategies have not been studied and, therefore, the viability of MDP aliquots kept at ambient conditions has not been explored. This research, therefore, investigated the potential of ambient-stored MDP RPs to overcome the hurdles associated with cold-chain maintenance and storage infrastructure limitations.

It is hoped that the study findings would contribute valuable insights that could pave way to a more sustainable and accessible deployment of nuclear medicine technologies,¹⁶⁻¹⁹ thereby improving diagnostic capabilities and patient outcomes in resource-challenged regions.

Materials and Methods

Preparation of Various Types of Normal Saline

Two streams of different types of normal saline (NS) were prepared using two 500 mL packets of NS. The first packet (named as NS stream) was left unchanged, while the other 500 mL packet (named as NSA stream) was purged with argon gas.

Preparation of MDP Aliquots

MDP aliquots were prepared by diluting a vial of MDP RP kit with 4 mL of NS drawn from either stream (NS and NSA). Thereafter, 1 mL was withdrawn from each diluted MDP vial and dispensed into four separate sterile vials. This process

was done on ten vials of MDP RP kit making each stream of NS yield 20 MDP aliquots.

From each stream of NS, 18 MDP aliquots were stored under ambient conditions (defined as the room temperature that existed within our laboratory during the research period and which ranged from 25°C to 35°C) until the 18th day, while one MDP aliquot was stored refrigerated, and another one was kept frozen for a duration of 28 days.

Radiochemical Purity Test

Radiochemical purity (RCP) testing was conducted daily on a total of 36 MDP aliquots stored under ambient conditions ranging from 1 to 18 days. Additionally, testing was performed on a total of four¹³ MDP aliquots stored under refrigerated and frozen conditions on the 28 day. This RCP test involved labeling the aliquots with 600 to 1,200 MBq of technetium solution following a fresh elution.²⁰

This was done by dropping 0.05 mL of technetium-labeled MDP aliquots applied to two Whatman chromatographic paper strips. These were then submerged in two distinct mobile phases (NS and methyl ethyl ketone (MEK)) to determine the concentrations of radiochemical impurities and expected form of the radiochemical compound. Free technetium pertechnetate ($^{99m}\text{TcO}_4^-$) and the colloid also known as hydrolyzed reduced pertechnetate ($^{99m}\text{TcO}_2$) in ^{99m}Tc labeled complexes were found as radiochemical impurities. Poor-quality pictures and an extra dosage of radiation for the patient come from the presence of high level of radiochemical contaminants in a RP.

The Whatman paper was employed as the immobile phase to detect free $^{99m}\text{TcO}_4^-$, while MEK and NS were utilized as the mobile phase. Free $^{99m}\text{TcO}_4^-$ is soluble in both NS and MEK; as a result, it migrated with the mobile phase as the paper got wet until the expected mark and the reference values were noted. Whatman paper number 1 served as an immobile phase and MEK served as a mobile phase to determine the colloid, $^{99m}\text{TcO}_2$. Sodium chloride is insoluble in the colloid ($^{99m}\text{TcO}_2$) and required RP. To do this, a strip of 0.9 cm × 8.5 cm Whatman paper was cut, marked with a pencil to indicate the origin at 1 cm from the bottom, and marked with a pencil to indicate the solvent front line at 6.5 cm from the origin.

The two chromatography paper strips were immediately put in the developing chamber for chromatography with a lid containing MEK and NS and allowed to develop once a drop (0.05 mL) of the mixture was detected on the origin line. The strip was removed and left to dry when the solvent got to the solvent front line. The amount of ^{99m}Tc -MDP, free $^{99m}\text{TcO}_4^-$, and $^{99m}\text{TcO}_2$ colloid were typically measured on chromatograms made from RCP using the dosage calibrator. According to the World Health Organization (WHO), ^{99m}Tc -MDP should only be used if the RCP is at least 90%.

Results

The MDP was reconstituted according to the manufacturer's instructions and then subjected to different storage temperatures and time period. As indicated in **Table 1**, the storage

Table 1 RCP of ^{99m}Tc -MDP aliquots made from the two streams of NS stored at ambient conditions (NS and NSA) and those kept at recommended conditions

Aliquot stream	No of aliquots	Aliquot age (days)	Storage temp. ($^{\circ}\text{C}$)	RCP (%)	WHO (>90)
NS	1	28	-23 (frozen)	100	Passed
	1	28	3 (refrigerated)	99	Passed
	18	18	27 (ambient)	98	Passed
NSA	1	28	-23 (frozen)	100	Passed
	1	28	2 (refrigerated)	99	Passed
	18	18	27 (ambient)	99	Passed

Abbreviations: NSA, normal saline aliquot; RCP, radiochemical purity; ^{99m}Tc -methylene diphosphonate; WHO, World Health Organization.

time ranged from 18 to 28 days at room temperature ranging from 25 to 42 $^{\circ}\text{C}$ for ambient stored MDP aliquots and from 2 to -4 $^{\circ}\text{C}$ for MDP aliquots stored at recommended conditions. The RCP that was our dependent variable showed a reduction of 1 or 2% from the one stored at recommended conditions. The reduction, however, passed the WHO standards. The RCP findings comparison between the NSA and NS stream is shown in **Fig. 1**.

Discussion

The goals of this research were to determine the effect of storage conditions and additives on the RCP. This section discusses the outcomes that were achieved.

Radiochemical Purity (RCP) of Aliquots of ^{99m}Tc -MDP

Table 1 provides the RCP findings of ^{99m}Tc -MDP aliquots held at various settings. Both streams of normal saline (NS and NSA) surpassed the WHO's recommended criterion of at least 90% for RCP, despite differences in storage duration and temperature. The small but statistically significant decrease in RCP seen across all examined time periods suggests stability as seen in **Table 2**.

Fig. 1 clearly exhibits the comparison of RCP results between different streams, illustrating the constancy in RCP throughout the streams. **Figs. 2** and **3** indicate a comparison of NS and NSA MDP aliquots with those stored at recommended conditions. The findings from this research

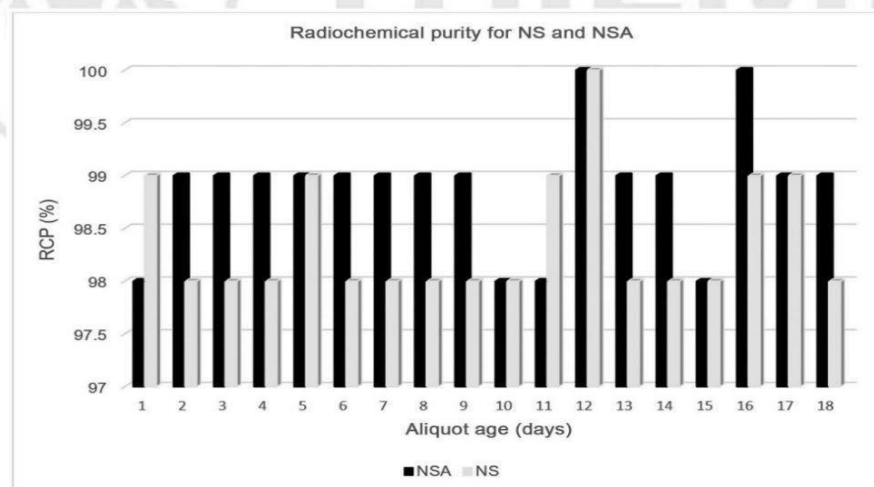


Fig. 1 Radiochemical purity (RCP) of ^{99m}Tc -methylene diphosphonate aliquots reconstituted with normal saline aliquot (NSA) and with normal saline (NS), only those stored at ambient conditions. The NSA stream is represented by the black color, whereas the NS stream is represented by the color gray.

Table 2 Statistical inference of NS and NSA streams of MDP aliquots

Normal Saline (NS) RCP (%)	Argon purged NSA					Total
0.98	3 0.1	9 0.3	0 1.3	0 0.6		12 2.3
0.99	1 0.0	3 0.0	1 0.4	0 0.3		5 0.7
1.00	0 0.2	0 0.6	1 7.6	0 0.1		1 8.5
NS RCP	0 0.2	0 0.6	0 0.1	1 17.1		1 18.0
Total	4 0.5	12 1.5	2 9.4	1 18.0		19 29.4
	Pearson $\chi^2(9) = 29.4500$		Pr = 0.001			

Abbreviations: MDP, methyl diphosphonate; NSA, normal saline aliquot; RCP, radiochemical purity.

revealed that MDP aliquots stored at room temperature remained viable for almost a month with RCP of 98 to 100%. Thus, MDP aliquots stored at ambient temperatures have acceptable quality control results and may be used to diagnose cancer and infections, especially in low-resource countries with cold chain concerns.

Thokchom et al studied how MDP kit fractionation affects RP RCP and biodistribution using vial and syringe storage

methods. The aliquots were kept at -20°C . In their study, the efficacy and RCP of $^{99\text{m}}\text{Tc}$ -MDP were compared with those of a traditional process without fractionation.²⁰ Fractionated $^{99\text{m}}\text{Tc}$ -MDP in the vial and syringe had more than 95% RCP although by day 8, RCP had dropped to 83.6 and 88%, respectively. Biodistribution studies were assessed in a cohort of 100 patients. Dhingra and colleagues evaluated the Radiochemical Purity (RCP) of MDP, Di Mercapto Succinic Acid (DMSA), and

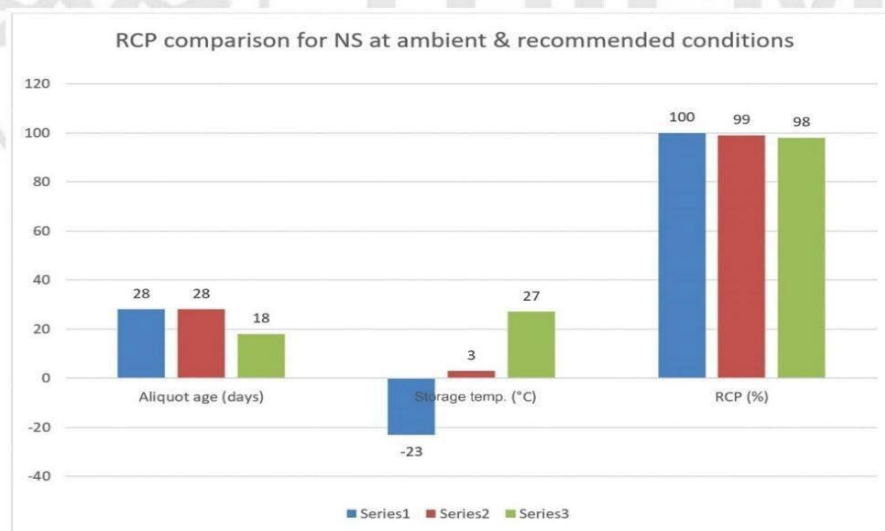


Fig. 2 Radiochemical purity (RCP) comparison for $^{99\text{m}}\text{Tc}$ -methylene diphosphonate aliquots made from the normal saline (NS) stream under different storage conditions: Series 1 (-23°C), Series 2 ($2-8^{\circ}\text{C}$), and Series 3 (ambient conditions, $25-35^{\circ}\text{C}$).

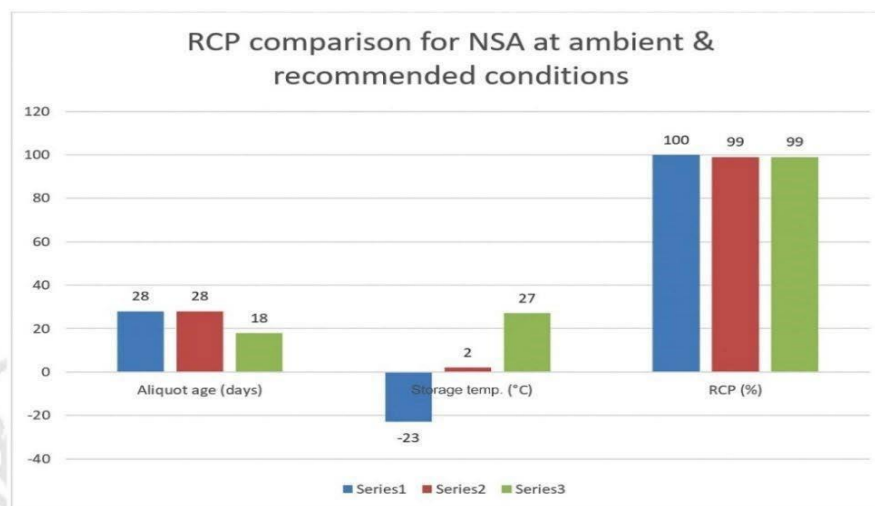


Fig. 3 Radiochemical purity (RCP) comparison for ^{99m}Tc -methylene diphosphonate aliquots made from normal saline aliquot (NSA) and stored under various conditions: Series 1 (-23°C), Series 2 ($2-8^{\circ}\text{C}$), and Series 3 (ambient, $25-35^{\circ}\text{C}$).

Diethylenetriamine Penta Acetate (DTPA) kits, with average RCP values of 95%, 91%, and 95%, respectively.²¹ They examined 90 samples altogether (30 of each) after adding NS and refrigerating the aliquots in evacuated vials at -20 to -30°C for 1 to 15 days. Bayomy et al studied Egyptian approaches to lower MDP kit prices. After 30 days, the RCP was tested upon which it was discovered that the aliquots were effectively maintained with an average RCP of 98.56% for fractions maintained between -20 and -28°C in nonoxygenated vials and 97.15% for fractions maintained between 0 and $+4^{\circ}\text{C}$.²² Successful biodistribution studies localized in the region of interest were seen in the human subjects.

Consequences and Prospects for the Future

Ambient stored aliquots of ^{99m}Tc -MDP RP were studied for their stability and behavior under various storage circumstances. The stable RCP concentrations provide evidence for the stability of the RP product. To better understand how ambient stored MDP aliquots, researchers should dig further into the particular processes patterning to their biodistribution patterns as this will also help generalize and apply the findings to a wider context.

Conclusion

In conclusion, this study successfully investigated the stability of ambient stored MDP RP aliquots. The RCP results

demonstrated the resilience of MDP aliquots, with both streams meeting the WHO's recommended threshold of at least 90% RCP. Despite minor reductions in RCP levels, well within an acceptable margin, the RP maintained its integrity over the tested duration and temperature ranges.

The statistical analysis using Stata version 14 added robustness to the findings, ensuring a reliable interpretation of the data. This study adhered to ethical standards and obtained necessary approvals, further validating the credibility of the results.

These findings contribute to the understanding of ambient stored MDP aliquots behavior, laying the groundwork for potential further future research.

As a concluding remark, this research not only provides insights into the specific context of ^{99m}Tc -MDP but also sets the stage for further investigations into the broader implications of storage conditions and additives on RP performance, ultimately advancing the field of nuclear medicine.

This study evaluated the use of ambiently stored MDP aliquots, a radiopharmaceutical employed in diagnosing cancer and infectious diseases. Future research could compare the cost-effectiveness of using fractionated versus unfractionated MDP. Additionally, further studies could investigate the stability of ambiently stored MDP aliquots over periods longer than eighteen days, particularly in hotter regions of Zambia.

Compliance with the Declaration of Helsinki
 Authority to conduct this research was obtained from all the relevant authorities and these include the Zambia National Health Research Authority, Management of the University Teaching Adult Hospital, and ERES Converge Ethical Committee. To further safeguard the safety of researchers, the general public, and the environment, Standard Operating Procedures for the use of RPs were adhered to the Zambia Radiation Protection Authority standard.

Conflict of Interest
 None declared.

Acknowledgments

Authors wish to thank the Africa Centre of Infectious Diseases in Human and animals (ACEIDHA) and Ministry of Higher Education (MoHE) for the financial support, and also the University of Zambia Staff, University Teaching Adult Hospital Management and Members of Staff for their support. More appreciation goes Tionenje Daka, Calvin Nachuma, and Mando Kolala who helped with data collection and laboratory procedures.

References

- 1 Weber WA, Johannes C, Carolyn JA, et al. The future of nuclear medicine, molecular imaging, and theranostics. *J Nucl Med* 2020; 2(61):2635–2725.
- 2 Vallabhajosula S, Berna DP, John WB. Molecular imaging of prostate cancer: radiopharmaceuticals for positron emission tomography (PET) and single-photon emission computed tomography (SPECT). *Precision molecular pathology of prostate cancer* 2018:475–501.
- 3 Dougherty DD, Rauch SL, Fischman AJ. Positron emission tomography and single photon emission computed tomography. *Essentials Neuroimaging Clin Pract* 2004;13(10):75–82.
- 4 St James S, Bednarsz B, Benedict S, et al. Current status of radiopharmaceutical therapy. *Int J Radiat Oncol Biol Phys* 2021; 109(04):891–901.
- 5 Bombardieri E, Bonadonna G, Gianni L. Breast cancer: nuclear medicine in diagnosis and therapeutic options. *Springer Science & Business Media*; 2007 Dec 22.
- 6 Shende P, Gandhi S. Current strategies of radiopharmaceuticals in theranostic applications. *J Drug Deliv Sci Technol* 2021; 64:102594.
- 7 Urbain JL, Scott AM, Lee ST, et al. Theranostic radiopharmaceuticals: a universal challenging educational paradigm in nuclear medicine. *J Nucl Med* 2023;64(06):986–991.
- 8 Assaad T. New amino bisphosphonate compound for skeletal imaging: Comparison study with methylenediphosphonic acid (MDP) and (1-hydroxyethane-1, 1-diyl) diphosphonic acid (HEDP). *Nukleonika* 2016;61(01):69–74.
- 9 Chopra A. 99mTc-Methyl diphosphonate. *Mol Imaging Contrast Agent Database* 2009;24:1 (MICAD).
- 10 Schillaci O, Pilippi L, Manni C, Santoni R. Single-photon emission computed tomography/computed tomography in brain tumors. In *Seminars in nuclear medicine*. WB Saunders 2007;01(37):34–47.
- 11 Lange R, Schreuder N, Hendrikse H. Radiopharmaceuticals: Practical Pharmaceutics: An International Guideline for the Preparation, Care and Use of Medicinal Products. Cham: Springer International Publishing; 2023:531–550.
- 12 Oikarinen SM. Recent developments of prostate-specific membrane antigen (PSMA)-specific radiopharmaceuticals for precise imaging and therapy of prostate cancer: an overview. *Clin Transl Imaging* 2019;7:189–208.
- 13 Vallabhajosula S, Lilja S, Brigitte V. A broad overview of positron emission tomography radiopharmaceuticals and clinical applications: what is new? *Seminars in nuclear medicine WB Saunders* 2011;4(41):246–264.
- 14 Bayomy T, Abdulrazzak M, Moustafa H, Khalil WM, Pant GS. Radiopharmaceutical, original article different models for cost-effective fractionation of bone radiopharmaceuticals: methylene diphosphonate (MDP) & hydroxy methylene diphosphonate (HDP). *Egypt J Nucl Med* 2009;2(02):74.
- 15 Fleming WK, Jay M, Ryo UV. Reconstitution and fractionation of radiopharmaceutical kits. *J Nucl Med* 1992;33(10):1915.
- 16 Negi M, Dhingra M, Dhingra VK. Quality of radiochemical purity in multiple samples of various fractionated cold kits: testing a cost & time effective technique. *Hell J Nucl Med* 2019;22(03):200–205.
- 17 Bhatia N, Dhingra VK, Watts A, et al. Fractionation of common cold kits as a cost-saving method in a low-volume nuclear medicine department. *Indian J Nucl Med* 2013;3(28):536.
- 18 Penglis S, Tsopelas C. 99Tcm-tetrofosmin: evaluation of fractionated cold kits and two new methods of quality control. *Nucl Med Commun* 2000;21(05):469–472.
- 19 Bayomy T, Abdulrazzak M, Moustafa H, Khalil WM, Pant GS. Radiopharmaceutical, original article different models for cost-effective fractionation of bone radiopharmaceuticals: methylene diphosphonate (MDP) and hydroxymethylene diphosphonate (HDP). *Egypt J Nucl Med* 2009;2:74.
- 20 Thokchom AK, Kumar R, Bharati S, Vasumathi. Quality assessment of Tc-99m methylene diphosphonate (MDP) radiopharmaceutical prepared using different cold kit fractionation methods. *Indian J Nucl Med* 2022;37(01):7–11.
- 21 Decristoforo C, Siller R, Chen P, Riccabona G. Radiochemical purity of routinely prepared 99Tcm radiopharmaceuticals: a retrospective study. *Nucl Med Commun* 2000;21(04):349–354.
- 22 Waight LA, Cunnane CM, O'Brien LM, Millar AM. Effect of 99Tc on the radiochemical purity of 99mTc radiopharmaceuticals. *Nucl Med Commun* 2011;32(08):752–756.

APPENDIX D2: Articles presented at 4th IPET IAEA Conference on 10th October, 2024.

- a) Optimization Methylene Diphosphonate Radiopharmaceutical to enhance Affordability and Accessibility in Resource-Limited Settings
- b) Exploring the In Vivo biodistribution of ambient-stored Technetium-99m Labeled Methylene Diphosphate (^{99m}Tc-MDP) Radiopharmaceutical aliquots in Infected Rat Models

APPENDIX E: ELUTION PROCESS OF ^{99m}Tc FROM A ^{99}Mo MOLYBDENUM (^{99}Mo)

The elution process of ^{99m}Tc from a ^{99}Mo generator is a crucial step in nuclear medicine for producing radiopharmaceuticals used in various diagnostic imaging procedures. This process enables accurate diagnosis and treatment of various medical conditions.

Below is a detailed description of the elution process:

1. **Generator Setup:** The $^{99}\text{Mo}/^{99m}\text{Tc}$ generator typically consists of a column containing a parent isotope, ^{99}Mo , which decays into the daughter isotope ^{99m}Tc . The ^{99}Mo is typically bound to a column of alumina or other suitable material.
2. **Priming:** Before elution, the generator is primed to allow the buildup of ^{99m}Tc . This involves allowing sufficient time for ^{99}Mo to decay and produce the desired ^{99m}Tc . The time required for priming depends on the activity of the generator and the half-life of ^{99}Mo , which is about 66 hours.
3. **Preparation:** Prior to elution, the elution system is prepared. This involves ensuring that all necessary equipment and supplies are ready and that the elution apparatus is properly sterilized to maintain the sterility of the final radiopharmaceutical products.
4. **Elution Setup:** The elution apparatus typically consists of a shielded container with lead shielding to reduce radiation exposure to the operator. The generator column is connected to tubing that leads to a syringe or collection vial. The elution process is performed under strict aseptic conditions to prevent contamination of the final product.
5. **Elution Procedure:** The elution process begins by introducing a suitable eluting agent, often a saline solution containing reducing agents such as stannous chloride (SnCl_2), into the generator column. The eluting agent flows through the column, displacing the ^{99m}Tc from the alumina or other binding material. The ^{99m}Tc , being a technetium cation (TcO_4^-), is carried along with the eluent. The eluent containing ^{99m}Tc is collected in a sterile vial or syringe. The volume of eluent collected depends on the desired activity and concentration of the final radiopharmaceutical product.

6. Quality Control: After elution, quality control tests are performed to ensure the purity, radiochemical purity, and activity of the eluate. This may include measuring the pH, radioactivity, and conducting tests for the presence of any impurities.
7. Radiopharmaceutical Preparation: The eluate containing ^{99m}Tc is then used to prepare radiopharmaceuticals. Depending on the specific radiopharmaceutical being produced, the eluate may be mixed with other pharmaceutical ingredients to form the final product. Common radiopharmaceuticals include technetium-based compounds used for imaging various organs and tissues in the body.
8. Patient Administration: Once prepared, the radiopharmaceutical is administered to the patient for diagnostic imaging. The dosage and administration route depend on the specific procedure being performed and the patient's condition.
9. Disposal: Proper disposal procedures are followed for any unused or waste materials to minimize radiation exposure and environmental impact.

APPENDIX F: LABELLING OF MDP (METHYLENE DIPHOSPHONATE) RADIOPHARMACEUTICAL WITH ^{99m}Tc

The labelling process of MDP (Methylene Diphosphonate) radiopharmaceutical with ^{99m}Tc is a crucial step in nuclear medicine imaging for bone scans. In summary, the labelling process of MDP radiopharmaceutical with ^{99m}Tc involves reconstituting the MDP kit, preparing the ^{99m}Tc eluate, combining the two components, performing quality control tests, and administering the labelled radiopharmaceutical to the patient for nuclear medicine imaging.

Below is a detailed discussion of the process:

1. **Preparation of MDP Kit:** MDP kits typically consist of lyophilized (freeze-dried) MDP, which is a phosphonate compound with a high affinity for bone mineral. The MDP is formulated with various excipients and stabilizers to ensure stability and compatibility with the labeling process.
2. **Reconstitution of MDP:** Prior to labeling, the MDP kit is reconstituted with a suitable sterile solution. This is usually done using sterile saline or another appropriate solvent. The reconstitution process involves adding the solvent to the vial containing the lyophilized MDP and gently swirling or mixing until the powder is completely dissolved. Care must be taken to maintain sterility throughout this process.
3. **Preparation of ^{99m}Tc :** The ^{99m}Tc eluate, obtained from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator through the elution process described earlier, is prepared for labeling. The eluate typically contains $^{99m}\text{TcO}_4^-$ in a suitable solvent such as saline.
4. **Labeling Procedure:** Once both the MDP solution and the ^{99m}Tc eluate are prepared, they are combined for labeling. A portion of the ^{99m}Tc eluate is withdrawn using a shielded syringe or dispensing system. The withdrawn ^{99m}Tc eluate is added to the vial containing the reconstituted MDP solution. The amount of ^{99m}Tc added is typically calculated based on the desired radioactivity dose for the patient and the specific labeling efficiency of the kit. The vial containing the MDP solution and ^{99m}Tc eluate is gently mixed to ensure thorough mixing of the radiotracer with the MDP compound.

5. Quality Control: After labeling, quality control tests are performed to ensure the radiochemical purity and stability of the labeled product. Common quality control tests include thin-layer chromatography (TLC) to assess the radiochemical purity and sterility testing to ensure the product meets appropriate standards for injection.
6. Patient Administration: Once the labeling process is complete and quality control tests are passed, the labeled MDP radiopharmaceutical is ready for patient administration. The labeled MDP solution is typically drawn into a shielded syringe or vial for injection into the patient. The dosage and administration route are determined based on the specific imaging protocol and the patient's condition.
7. Imaging Procedure: Following administration, the patient undergoes nuclear medicine imaging, usually in the form of a bone scan. The radiopharmaceutical accumulates in areas of increased bone turnover or pathology, allowing for the detection of bone abnormalities such as fractures, tumors, infections, or metastases.
8. Disposal: Proper disposal procedures are followed for any unused or waste materials to minimize radiation exposure and environmental impact.