

Radiolabeling of Some Pharmaceutical Compounds with Technetium-99m for Diagnostic Purposes

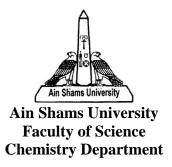
Thesis Submitted By Ashgan Fouad Mahmoud Hassan

M.Sc. (Inorganic Chemistry)
Assistant Lecturer /Radioisotopes Production Facility
Egyptian Second Research Reactor
Atomic Energy Authority

For the Fulfillment of the degree of Ph.D in Chemistry

Submitted to

Chemistry Department Faculty of Science Ain Shams University Cairo, Egypt



Radiolabeling of Some Pharmaceutical Compounds with Technetium-99m for Diagnostic Purposes

Thesis Submitted by Ashgan Fouad Mahmoud Hassan

For the Fulfillment of the degree of Ph.D in Chemistry

Supervised by

Prof. Dr. M. S. Abdel-Mottaleb

Prof. of Inorganic Chemistry, Faculty of Science, Ain Shams University

Prof. Dr. Salah E.Soliman

Prof. of Materials sciences Hot Labs Center, Atomic Energy Authority

Dr. Ismail T. Ibrahim

Ass.Prof. of Radiopharmaceutical chemistry, Hot Labs Center, Atomic Energy Authority



Approval Sheet

The Thesis Entitled

Radiolabeling of Some Pharmaceutical Compounds with Technetium-99m for Diagnostic Purposes

Submitted by/ **Ashgan Fouad Mahmoud Hassan** (M. Sc. Inorganic and Physical Chemistry 2009) **For the Degree of Doctor of Philosophy in Science** (Chemistry).

This thesis has been approved by supervisor committee **SUPERVISORS**

emistry,

Head of Chemistry Department

Prof.Dr.Hamed Ahmed Younes Derbala

<u>Acknowledgements</u>

First of all, I want to thank God, for enlightening my way and strengthening my well to produce this work.

I would like to express my deep gratitude and appreciation to *Prof. Dr. Mohamed Sabry Abdel-Mottaleb*, Professor. of Inorganic chemistry,
Faculty of Science, Ain Shams University, for his valuable scientific supervision for this thesis and his helpful guidance during the scope of the work.

It is my pleasure to give my deepest gratitude to *Prof. Dr. Salah Eldin Soliman*, Professor of Materials sciences, Hot Labs Center, Atomic Energy Authority, for suggesting the research plan of this thesis and his valuable supervision, instructions, advices and indispensable help during the course of this work.

I would like to express my deep gratitude to *Dr. Ismail Taha Ibrahim*, Assistant Professor of Radiopharmaceutical chemistry, Labeled Compounds Department, Atomic Energy Authority, for his interest and valuable help in all experimental part and kind help in revising this work.

I am deeply indebted to *Dr. Mahmoud El-taoosy*, Assistant Professor of Radiochemistry, Labeled Compounds Department, Atomic Energy Authority, for his support, cooperation, encouragement and kind help in the practical part of this thesis.

Finally, I am indeed very thankful to all the staff members of the Atomic Energy Authority for their valuable support.

Radiolabeling of Some Pharmaceutical Compounds with Technetium-99m for Diagnostic Purposes

This work has been carried out to study the labeling of some anti-inflammatory pharmaceuticals (diclofenac and dipyrone) and antibiotics (amikacin) with technetium-99m to produce radiolabeled compounds and study their imaging ability of the septic and aseptic inflammation.

^{99m}Tc-amikacin was successfully labeled with Technetium - 99m by direct labeling technique. A radiochemical yield of 95±3% was obtained by adding ^{99m}Tc to 250μg amikacin in the presence of 25μg SnCl₂.2H₂O at pH 5 for 15 min. Biodistribution studies in mice were carried out in experimentally induced septic and aseptic inflammation in the left thigh using *E.coli*, and turpentine oil, respectively. ^{99m}Tc-amikacin discriminates well between septic and a septic foci showing high T/NT ratio (7.5±0.3) in the infectious lesion and abscess to normal muscle ratio when compared with the uptake of septic foci at 2 hour post injection.

^{99m}Tc-diclofenac was successfully labeled with Technetium-99m by direct labeling technique. A radiochemical yield of 96±2% was obtained by adding ^{99m}Tc to 100μg diclofenac in the presence of 50μg SnCl₂.2H₂O at pH7 for 30 min. Biodistribution studies in mice were carried out in experimentally

induced septic and aseptic inflammation in the left thigh using E.coli, and turpentine oil. ^{99m}Tc-diclofenac is a good inflammation imaging agent. Higher uptake (T/NT=3.7 \pm 0.02) in the sterile inflamed muscle injected with turpentine oil than that of infected muscle injected with bacteria was observed.

^{99m}Tc-dipyrone was prepared at pH 5 with a radiochemical yield of 95.5±2% by adding ^{99m}Tc to 100μg dipyrone in the presence of 25μg SnCl₂.2H₂O for 30 min. Biodistribution studies in mice were carried out in experimentally induced septic and aseptic inflammation in the left thigh using *E.coli* and turpentine oil. ^{99m}Tc-dipyrone is a good inflammation imaging agent as its accumulation in the inflammation sites remained with *T/NT* nearly (5.5±0.03) up to 1 hour in the sterile inflamed muscle injected with turpentine oil which was higher than that of infected muscle injected with bacteria.

CONTENTS	Page
LIST OF FIGURES	I
LIST OF TABLES	\mathbf{V}
AIM OF WORK	VII
CHAPTER I	
INTRODUCTION	
I.1. General considerations	1
I.2. Nuclear medicine	3
I.2.1. Nuclear medicine radiotherapy	4
I.2.2. Nuclear medicine imaging	4
I. 3. A radiopharmaceutical	5
I.3.1. Therapeutic radiopharmaceuticals	6
I.3.2. Diagnostic radiopharmaceuticals	8
I.3.3. Characteristics of ideal Diagnostic radiopharmaceutical	9
I.4. Factors Influencing the Design of New Radiopharmaceuticals	11
I.5. Characterstics of radionuclides used for diagnosis in nuclear	
Medicine	14
I.5.1. Chemistry and radiochemistry of technetium-99m	16
I.6. Labeling of pharmaceutical compounds with technetium-99m	24
I.6.1. Direct labeling method	27
I.6.2. Transchelation (ligand exchange) labeling method	27
I 6.3. Labeling with hifunctional chelating agents	28

I.7. 99mTc-radiopharmaceuticals	31
I.7.1. Pertechnetate ion (99mTcO4)	31
I.7.2. 99mTc-labeled colloids and particulates	31
I.7.3. 99mTc-complexes for skeletal imaging	32
I.7.4. 99mTc-complexes for renal imaging	32
I.7.5. 99mTc-complexes for hepatobillary imaging	33
I.7.6. 99mTc-complexes for myocardial imaging	36
I.7.7. 99mTc-complexes for lung imaging	36
I.7.8. 99mTc-complexes for brain imaging	38
I.7.9. 99mTc-complexes of proteins	40
I.7.10. 99mTc-complexes for inflammation and infection scintigraphic detection	40
I.7.10.1. Inflammation	41
I.7.10.2. Non Steroidal Anti Inflammatory Drugs (NSAIDs)	42
I.7.10.3. Antibiotics	42 43
I.10.7.4. Scintigraphic detection of infection and inflammation	44
I.8. Nuclear medicine imaging of inflammation and bacterial infection	50
I.8.1. Characteristics of the ideal radiopharmaceutical for	
infection imaging	52

CHAPTER II

EXPERIMENTAL

II.1. Chemicals	53
II.2. Equipment	54
II.3. Animals	55
II.4. Radioactive Material	55
II.5. Bacteria	55
II.6. Labeling of ligands (Amikacin, Diclofenac and dipyrone)	56
II.6.1. Preparation of stock solution of SnCl ₂ .2H ₂ O	56
II.6.2. Kit preparation and labeling	56
II.6.3. Analysis of the reaction mixture (radiochemical purity	
and labeling yield)	57
II.6.3.1. Ascending paper chromatography analysis	57
II.6.3.1.1. Determination of free pertechnetate (99mTcO4)	57
II.6.3.1.2. Determination of reduced hydrolyzed-99mTc	58
II.6.3.1.3. Determination of ^{99m} Tc-ligand complex	58
II.6.3.2. Electrophoresis analysis	60
II.6.3.3. HPLC analysis	61
II.6.3.3.1. HPLC for ^{99m} Tc-Amikacin	61
II.6.3.3.2. HPLC for ^{99m} Tc- diclofenac	61
II.6.3.3.3. HPLC for ^{99m} Tc-dipyrone	61
II.6.4. Study of the factors affecting the percent labeling yield of	
99mTc-ligand complexes	62
II.6.4.1. Effect of pH of the reaction mixture	62
II.6.4.2. Effect of SnCl ₂ .2H ₂ O content	62
II.6.4.3. Effect of ligand amount	63

63

63

64

II.7. Biological evaluation of ^{99m} Tc-ligand complexes	64
II.7.1. Induction of infectious foci	64
II.7.2. Induction of non-infected (sterile) inflammation	64
II.7.3. Study of the biological distribution	64
II.7.3.1. In normal mice	64
II.7.3.2. In inflammation bearing mice	65
II.8. Statistical analysis	66
CHAPTER III	
RESULTS AND DISCUSSION	
III.1. Labeling of the ligands	67
III.2. Factors affecting the percent labelling yield of 99mTc-Amikacin,	
^{99m} Tc-Diclofenac, and ^{99m} Tc-dipyrone complex	69
III.2.1. Effect of pH of the reaction medium	69
III.2.1.1. Amikacin	69
III.2.1.2. Diclofenac	69
III.2.1.3. Dipyrone	72
III.2.2. Effect of SnCl ₂ .2H ₂ O content	72
III.2.2.1. Amikacin.	74
III.2.2.2. Diclofenac.	75
III.2.2.3. Dipyrone	78
III.2.3. Effect of ligand amount	7 9

II.6.4.4. Effect of reaction time.....

II.6.4.5. In-vitro stability study.....

III.2.3.1. Amikacin	81
III.2.3.2. Diclofenac	81
III.2.3.3. Dipyrone.	84
III.2.4. Effect of reaction time	84
III.2.4.1. Amikacin	86
III.2.4.2. Diclofenac	86
III.2.4.3. Dipyrone	86
III.2.5. In-vitro stability study	90
III.2.5.1. Amikacin	90
III.2.5.2. Diclofenac	90
III.2.5.3. Dipyrone	91
III.2.6. Electrophoresis analysis	91
III.2.6.1. Amikacin	91
III.2.6.2. Diclofenac	91
III.2.6.3. Dipyrone	94
III.2.7. HPLC analysis of ^{99m} Tc-ligands	94
III.2.7.1. HPLC analysis of 99mTc-amikacin	94
III.2.7.2. HPLC analysis of 99mTc-diclofenac	94
III.2.7.3. HPLC analysis of 99mTc-dipyrone	98
III.3. Inflammation imaging study	100
III.3.1. 99mTc-amikacin	101
III.3.1.1. In normal mice	101
III.3.1.2. In sterile inflammation bearing mice	102
III.3.1.3. In bacterial inflammation bearing mice	105
III.3.2. 99mTc-diclofenac	105
III.3.2.1. In normal mice	105
III.3.2.2. In sterile inflammation bearing mice	108
III.3.2.3. In bacterial inflammation bearing mice	111

III.3.3. ^{99m} Tc-dipyrone	114
III.3.3.1. In normal mice	114
III.3.3.2. In sterile inflammation bearing mice	114
III.3.3.3. In bacterial inflammation bearing mice	116
Summary and conclusion	122
REFERENCES	130

List of Figures

		Page
Figure (1)	Selected technetium chemical reactions	18
Figure (2)	Decay scheme of technetium-99m	21
Figure (3)	Decay scheme of molybdenum-99	22
Figure (4)	Decay scheme of ⁹⁹ Mo and ^{99m} Tc radionuclides	23
Figure (5)	The strategy for labeling of proteins with metal ions using bifunctional chelating agent	30
Figure (6)	Structures of some pharmaceuticals for kidney imaging and renal function study after labeling with ^{99m} Tc	34
Figure (7)	Structures of some iminodiacetic acid (IDA) analogues suggested as hepatobillary imaging agents after labeling with \$^{99m}Tc	35
Figure (8)	Structures of some ^{99m} Tc-radiopharmaceuticals used for brain perfusion imaging	39
Figure (9)	Chemical structure of amikacin	48
Figure (10)	Chemical structure of diclofenac	49
Figure (11)	Chemical structure of dipyrone	49

Figure (12)	The reported structure of ^{99m} Tc-ciprofloxacin complex	52
Figure (13)	Radiochemical analysis pattern	59
Figure (14)	Effect of pH on the labeling yield of ^{99m} Tc-amikacin complex	70
Figure (15)	Effect of pH on the labeling yield of ^{99m} Tc-diclofenac complex	71
Figure (16)	Effect of pH on the labeling yield of ^{99m} Tc-dipyrone complex	73
Figure (17)	Effect of Sn(II) content on the labeling yield of ^{99m} Tc-amikacin complex	76
Figure (18)	Effect of Sn(II) content on the labeling yield of ^{99m} Tc-diclofenac complex.	77
Figure (19)	Effect of Sn(II) content on the labeling yield of ^{99m} Tc-dipyrone complex	80
Figure (20)	Effect of amikacin amount on the labeling yield of ^{99m} Tc-amikacin complex	82
Figure (21)	Effect of diclofenac amount on the labeling yield of ^{99m} Tc-diclofenac complex	83