STUDIES ON THE LABELLING OF SOME ORGANIC COMPOUNDS WITH TECHNETIUM – 99m FOR RENAL IMMAGING

Thesis

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سبحانك لا علم لنا إلا ما علمتنا
إنك أنت العليم الحكيم
صدق الله العظيم
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To my (mother,brothers)
My (hospand)
my sons (Aiya, Ahmed and Yara)
my Friends.

Aim of the work

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Radiopharmaceuticals have wide spread application for diagnosis and therapy in nuclear medicine. Among the various types of radiopharmaceuticals used for kidney function studies are the technetium-99m labelled aminohippuric acid derivatives. ^{131}I-ortho-iodohippuric acid is the gold standard for kidney function studies. It has disadvantages of imparting a relatively high absorbed radiation ($t_{1/2}$= 8.03 d, γ = 340 KeV, β^-= 0.61 MeV) dose to the patient at low diagnostic doses. Although ^{123}I-ortho-iodohippuric acid lowers the radiation dose ($t_{1/2}$= 13.2 h, γ = 159 KeV) to the patient, however, it is not available at a reasonable coast for routine use. Technetium-99m has ideal physical and nuclear properties for many applications in nuclear medicine, by its virtue of its short half life and favorable radiation characteristics ($t_{1/2}$= 6.02, γ = 140 KeV). It is difficult to label ortho-amino-iodohippuric acid by technetium-99m due to the lack of donor atoms or chelating groups to bind ^{99m}Tc.

To prepare ^{99m}Tc aminohippuric acid derivatives for renal function studies, chelating moiety, iminodiacetic acid (IDA) can be attached to aminohippuric acid molecule to bind with ^{99m}Tc.

The aim of the present work is to synthesize the aminohippuric acid derivatives:-

(1)ortho aminohippuric acid iminodiacetic acid

(2)meta aminohippuric acid iminodiacetic acid

(3)para aminohippuric acid iminodiacetic acid

The synthesised compounds will be characterized using different analytical techniques (elemental analysis, IR, ^{1}H-NMR and mass spectroscopy) and then labelled with technetium-99m by direct labelling

method. The thesis also aims to study the factors affecting on the percent labelling yield of AHIDA derivatives with technetium-99m. These factors include, the amount of AHIDA derivatives, the Sn(II) content, pH, and the reaction time. The ^{99m}Tc-AHIDA complexes will be evaluated biologically in the experimental animals (mice).

Summery of the work

SUMMARY

Radiopharmaceuticals are preparations of adequately constant composition, radiochemical and radionuclidic purity and uniformity of physiological action for use in nuclear medicine as a diagnostic or therapeutic agent. Nearly about 80% of all radiopharmaceuticals used in nuclear medicine are ^{99m}Tc-labelled compounds. The reason for such a permanent position of technetium-99m in clinical use is its extremely favourable physical and radiation characteristics. The six hours, physical half life and the free of beta particles permit the administration of millcurie amounts of ^{99m}Tc-radioactivity without significant radiation dose to the patient. In addition, the monochromatic 140 KeV photons are readily collimated to give images of superior spatial resolution. Technetium-99m is readily available in a sterile, pyrogen free state from ^{99}Mo/^{99m}Tc generator.

The goal of this study is the organic synthesis of ortho, meta and para-amino hippuric acid iminodiacetic acid analogs and labelling them with technetium-99m which used as renal function agents.

The factors affecting the labelling yield were studied and the labelled complexes were evaluated radiochemically and biologically. This thesis was divided into two chapters, chapter one includes the general introduction, chapter two includes the organic synthesis of three iminodiacetic acid (IDA) derivatives and characterization of the synthesized compounds, chapter three includes labelling of the synthesized AHIDA derivatives with technetium-99m and its biological distribution.

The results obtained from this study can be summarized as follows:

Chapter I

It deals briefly the following topics:

Radionuclide produced in a reactor, in a cyclotron and radionuclide generator. Among the important radionuclide produced in a reactor is technetium-99m isotope. The chemical and nuclear properties of technetium-99m and its availability from $^{99}Mo/^{99m}Tc$ generator led to the preparation of different groups of ^{99m}Tc-radiopharmaceuticals used in diagnostic procedures in nuclear medicine.

Chapter II

Synthesis, characterization and evaluation of iminodiacetic acid derivatives ortho, meta and para-aminohippuric acid analogs as renal function agents. Several substituted amino hippuric acid iminodiacetic acid had gained much popularity in the recent years as an important class of imaging agents. A number of studies have been performed to evaluate its chemical properties and also to improve its biological behavior. Because AHIDA derivatives are not available commercially it is necessary to synthesis them locally.

The following AHIDA derivatives were synthesized:

1- ortho aminohippuric acid iminodiacetic acid

2- meta aminohippuric acid iminodiacetic acid

3-para aminohippuric acid iminodiacetic acid

The above compounds were synthesized following Burn's method which depends on the condensation reaction between nitrilotriacetic acid monoanhydride and ortho aminohippuric acid, meta aminohippuric acid and para aminohippuric acid in pyridine to give reaction yield 79%, 65% and 69% respectively. The different analytical techniques such as m.p.,

I.R, ^{1}H-NMR and mass spectroscopy were used for characterization the synthesized compounds.

Chapter III

Labelling of iminodiacetic acid derivatives of ortho, meta and para aminohippuric acid analogs as renal function agents with technetium-99m. AHIDA derivatives were labelled with technetium-99m using tin reduction method in which the chelats react with the reduced technetium-99m at pH 5.7. The percent labelling yields for all the synthesized AHIDA derivatives were found equal to 95.8%, 96.3% and 98.9% for OAHIDA, MAHIDA and PAHIDA respectively. The different parameters affecting the labelling yield were studied such as the amount of substrate Sn(II) content, the pH of reaction mixture, and reaction time. The optimum conditions for the preparations of ^{99m}Tc-ortho, meta and para-AHIDA complexes were as follow:

Exactly weigh 10 mg of o, m and p-aminnohippuric acid analogs , 0.25mg $SnCl_2.2H_2O$, pH of reaction mixture 5.7 and 15 min reaction time. The radiochemical purity and labelling yield of ^{99m}Tc-AHIDA complexes were estimated using instant thin layer chromatography (ITLC-SG) in acetonitrile : water (3:1) and saline as eluants. These ^{99m}Tc-AHIDA complexeswere found stable for 6h after labelling with ^{99m}Tc (> 95%). The biodistribution of the in-house formulated AHIDA derivatives after labelling with technetium-99m were evaluated in mice. The percent administrated dose measured in urine of mice for ^{99m}Tc-o, m and p-AHIDA complexes were equal to 60, 56 and 87 respectively after 60 min post injection. The organ distribution studies in mice confirmed that ^{99m}Tc-p-AHIDA complex has excellent renal excretion characteristics compared to the other derivatives.

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Chapter III :

Labeling of ortho, meta , and para –amino hippuric acid derivatives by ^{99m} Tc for use in renal funcion studies.

General Introduction

Radiopharmaceuticals are medicinal products that are radioactive. They are used for both diagnosis and therapy as reviewed by Vera–Ruiz 1998 in nuclear medicine. Radiopharmaceuticals vary from inorganic salts to large organic molecules and complexes.

The radionuclides incorporated into radiopharmaceuticals are produced either as a result of nuclear fission of heavy nuclides such uranium-235 or in a reactor or in a cyclotron. In choosing a production route, the manufacturer considering factors such as the yield, the radionuclidic purity of the product and whether the outcome of the process is a carrier-free radionuclide. A radionuclide is described as " carrier-free "when every atom of the element is present as the radionuclide and therefore no other isotopes of the element are present.

The carrier-free state can only be achieved when the production process leads to the formation of a new element. By the use of a carrier-free radionuclide only a trace amount of the element is administered to the patient. Free from carrier is particularly important in radiopharmaceuticals, which contain toxic elements such as the ^{201}Tl isotope of thallium and the ^{67}Ga isotope of gallium.

In the synthesis of a radiolabeled compound, the success of labeling reaction may depend upon the radionuclide being in a carrier-free state.

1.1Radionuclide produced in a reactor:

Most of the radionuclides used in nuclear medicine are produced in a nuclear reactor as reviewed by Poggenburg (1974).

In this process, a target element is inserted into the core of the reactor where it is bombarded by neutrons. When a neutron enters the nucleus of a target atom, the nucleus undergoes a rearrangement and a

new isotope of the target element is produced. In the most common reaction of this type the capture of the neutron is accompanied by emission of a γ-ray. This process is known as (n,γ) reaction, is the route by which most reactor–produced radionuclides are prepared and results in the formation of radionuclides with the excess of neutrons. An example of the reaction is the production of chromium-51, the reaction is described as ^{50}Cr (n,γ) ^{51}Cr. As this type of reaction is not 100 % efficient, the product contains both ^{50}Cr and ^{51}Cr.

Chemical separation of these isotopes is not possible and therefore ^{51}Cr can not be obtained as a carrier–free product when prepared by this route. The important radionuclide produced by this reaction are ^{32}P, ^{59}Fe, ^{75}Se, ^{113}Sn and ^{198}Au. In the reactor, two types of interactions with thermal neutrons are of considerable importance in the production of various useful radionuclides:

Neutron capture or (n,γ) reaction and fission of heavy elements

I.1.1 Neutron capture or (n,γ) reaction:

A variation of the (n,γ) reaction occurs when the radionuclides produced decays to a daughter radionuclide.

The reaction is an important means for the production of ^{131}I.

A tellurium target irradiated in the reactor to form ^{131}Te, which disintegrates by β^- emission to form ^{131}I.

$$^{130}Te\ (n,\gamma)\ ^{131}Te \xrightarrow[25\ min]{\beta^-} {}^{131}I$$

Same examples of neutron capture reactions are: -

$^{98}Mo(n,\gamma)\ ^{99}Mo$, $^{196}Hg(n,\gamma)\ ^{197}Hg$ and $^{50}Cr(n,\gamma)\ ^{51}Cr$.

I.1.2 Fission or (n,f) reaction :

As already mentioned, fission is a breakup of heavy nucleus into two fragments of approximately equal mass. When a target of heavy elements is inserted in the reactor core, heavy nuclei absorb thermal neutrons and undergo fission.Fission heavy elements are ^{235}U,^{239}Pu,^{237}Np,^{233}U,^{232}Th, and many others having atomic numbers greater than 92. Nuclides produced by fission may range in atomic number from about 28 to nearly 65. These isotopes of different elements are separated by appropriate chemical procedures that involve precipitation, solvent extraction, ion exchange, chromatography and distillation. The fission radionuclides are normally carrier-free or NCA, therefore isotopes of high specific activity are available from fission. Many chemically useful radionuclides such as ^{131}I,^{99}Mo,^{133}Xe,and ^{137}Cs are produced by fission from ^{235}U.

An example of thermal fission of ^{235}U is presented as follows: -

$$^{235}_{92}U + ^{1}_{0}n \longrightarrow ^{236}_{92}U \longrightarrow ^{131}_{53}I + ^{102}_{39}Y + 3\,^{1}_{0}n$$

$$\longrightarrow ^{99}_{42}Mo + ^{135}_{50}Sn + 2\,^{1}_{0}n$$

$$\longrightarrow ^{133}_{54}Xe + ^{101}_{38}Sr + 2\,^{1}_{0}n$$

$$\longrightarrow ^{137}_{55}Cs + ^{97}_{37}Rb + 2\,^{1}_{0}n$$

$$\longrightarrow ^{155}_{62}Sm + ^{78}_{30}Zn + 3\,^{1}_{0}n$$

Besides, other radionuclides are also produced in the example.

- **Iodine-131:**

For chemical separation of ^{131}I from irradiated ^{235}U target, the latter is dissolved in 18 % NaOH by heating, and hydroxides of many metal ions are precipitated by cooling. The supernatant containing sodium iodide is acidified with sulfuric acid in a distillation system. Iodide is oxidized to iodine by the acid, and iodine is collected in NaOH solution by distillation.

- **Molybdenum-99:**

For ^{99}Mo separation, the irradiated uranium target is dissolved in nitric acid and solution is adsorbed on an alumina (Al_2O_3) column.The column is then washed with nitric acid to remove uranium and other fission product cations. Molybdenum-99 is then eluted with ammonium hydroxide, and ultimately used for ^{99}Mo -^{99m}Tc generator. The ^{99}Mo radionuclide produced by fission is carrier-free or NCA and its most common contaminants are ^{131}I and ^{103}Ru.

I.2. Radionuclides produced in cyclotron: -

In a cyclotron, charged particles such as protons, deuterons, α particles, ^{3}He particles, and so forth are accelerated in circular paths in dees under vacuum by means of an electromagnetic field. These accelerated particles can possess a few kiloelectron volts (KeV) to several billion electron volts (BeV) of energy depending on the design and type of the cyclotron.

When targets of stable elements are irradiated by placing them in the external beam of the accelerated particles or at a given radius in a cyclotron, the accelerated particles irradiate the target nuclei and the nuclear reactions take place. In a nuclear reaction, the incident particle may leave the nucleus after interaction, leaving some of its energy in it, or it may be completely absorbed by the nucleus, depending on the incident particle.

In either case a nucleus with excitation energy is formed and the excitation energy is disposed of by the emission of nucleons(i.e., protons and neutrons). Each nuclear reaction for the production of a nuclide has a definite threshold or Q energy, which is either absorbed or released in the reaction. This energy requirement arises from the different between the masses of the target nucleus plus the irradiating particle and the masses of the product nuclide plus the emitted particles.

In nuclear reactions requiring the absorption of energy, the irradiating particles must possess energy above the threshold energy; otherwise the nuclear reaction would not take place.

An example of a simple cyclotron – produced radionuclide is ^{111}In, which is produced by irradiating ^{111}Cd, with 12-MeV protons in a cyclotron. The nuclear reaction is written as follows:-

$$^{111}Cd(p,n)\ ^{111}In$$

As another example, relatively high energy nuclear reactions induced in ^{89}Y irradiation with 40-MeVprotons are listed below: -

$$^{89}Y+P(40MeV) \longrightarrow {}^{89}Zr + n$$
$$\longrightarrow {}^{89}Y + P$$
$$\longrightarrow {}^{88}Zr + 2n$$
$$\longrightarrow {}^{88}Y + Pn$$
$$\longrightarrow {}^{88}Sr + 2P$$
$$\longrightarrow {}^{87}Zr + 3n$$
$$\longrightarrow {}^{87}Y + P2n$$

Although all reactions mentioned in the above example are feasible, the most probable reactions are (P,3n) and (P,P2n) reactions with 40-MeV protons. The target material for irradiation must be pure and preferably mono isotopic or at least enriched isotope, in order to avoid the production of extraneous radionuclides.

The energy and type of the irradiating particle must be chosen so that contamination with undesirable radionuclides resulting from extraneous nuclear reaction can be avoided. Since various isotopes of different elements may be produced in a particular irradiating system, it is necessary to isolate isotopes of a single element; this can be accomplished by appropriate chemical methods such as solvent extraction, precipitation, ion exchange, and distillation. Cyclotron

produced radionuclides are usually neutron deficient and therefore decay be β^+ emission or electron capture. Methods of preparation of several useful cyclotron-produced radionuclides are described as follows: -

- **Gallium-67:-**

Gallium-67 ($t_{1/2}$-78h) can be produced by several nuclear reactions such as $^{66}Zn(d,n)\ ^{67}Ga$,$^{68}Zn(p,2n)^{67}Ga$,$^{64}Zn(\alpha,p)^{67}Ga$. A pure natural zinc target or enriched zinc isotope in the form of oxide is irradiated with 20MeV, 8MeV deuterons or 23MeV α particles in a cyclotron at a certain beam current for a specified time after irradiation the target is dissolved in 7NHCl and carrier-free ^{67}Ga are extracted with isopropyl ether. The organic phase is then evaporated to dryness in water bath and the residue is taken up in dilute HCl for supply as gallium chloride. It may be complexed with citric acid to form gallium citrate, which is most commonly used in nuclear medicine.

- **Iodine-123:-**

Iodine-123 has gained considerable importance in nuclear medicine because it has good radiation characteristics such as decay by electron capture, half-life of 13.2h and γ ray emission of 159 KeV, it is produced directly or indirectly in a cyclotron by several nuclear reactions. Direct nuclear reactions are those reactions whereby ^{123}I is produced directly and likely to be contaminated with other iodine isotopes such as ^{124}I and ^{125}I depending on the type of target and irradiating particle.

Examples of such reactions are $^{121}Sb(\alpha,2n)^{123}I$,$^{123}Te(p,n)^{123}I$ $^{122}Te(d,n)^{123}I$ and $^{124}Te(p,2n)^{123}I$. Depending on the target composition, and energy of the irradiating particles, other side reactions obviously may produce various radioisotopes of iodine.

In the direct methods, after irradiation the target is dissolved in mineral acid and iodine is collected into dilute sodium hydroxide (NaOH). In the indirect method, the nuclear reaction is so chosen that ^{123}Xe is produced initially which then decays with a half-life of 2.1h to produce ^{123}I with a half-life of 13.2h. These reactions allow the production of ^{123}I free of other radioisotopes of iodine. Various reactions include $^{122}Te(\alpha,3n)^{123}Xe$ using 42-46 MeV α particles, $^{122}Te(^{3}He,2n)^{123}Xe$ using 20-30 MeV ^{3}He particles.

- **Indium-111:**

Indium-111 is produced by the $^{111}Cd(P,n)^{111}In$ and $^{109}Ag(\alpha,2n)^{111}In$ reaction. After irradiation with 15-MeV protons, the cadmium target is dissolved in mineral acid, the acidity is made in HCl. The solution is passed through anion-exchange resin (Dowex-I). Indium-111 is removed by elution with 1NHCl, Leaving cadmium on the column.

- **Thallium-201:**

Thallium-201 is primarily produced by the $^{203}Tl(p,3n)^{201}Pb$ reaction, whereby ^{201}Pb decays to ^{201}Tl with a half-life of 9.4h. Thallium-201 obtained in this way is pure and free of other contaminants. After irradiation with 35-45 MeV protons, the natural thallium target is dissolved in a mineral acid, and ^{201}Pb are isolated by the ion-exchange method. The lead radionuclides are then adsorbed on another ion-exchange column, and sufficient time is allowed for decay ^{201}Pb to ^{201}Tl. Thallium-201 is then eluted as thallous chloride in NCA form.

I.2.1. Short-lived radionuclides:

Considerable interest has developed for the production of short-lived radionuclides and their clinical uses because of the availability of the positron emission tomography (PET) imaging system. Among them are the key radionuclides such as ^{11}C,^{13}N,^{15}O, and ^{18}F which decay by positron emission. These positron emitters are useful in imaging by

(PET). Because they have very short half-lives, a cyclotron or a medical cyclotron must be located on site in the laboratory.

- **Carbon-11:**

Carbon-11 has a half-life of 20.4 min. and can be produced by $^{10}B(d,n)^{11}C$, $^{11}B(p,n)^{11}C$, and $^{14}N(p,\alpha)^{11}C$ reactions in the cyclotron. In the first two reactions, B_2O_3 is the target, and nitrogen gas in the third, both ^{11}CO and $^{11}CO_2$ are produced in boron targets, which are then flushed out by neutral gases. Both ^{11}CO and $^{11}CO_2$ are commonly used as precursors in the preparation of various clinically useful compounds, such as ^{11}C-Palmitate for myocardial perfusion imaging by (PET).

- **Nitrogen-13:**

Nitrogen-13 has a half-life of 10 min. and is commonly used as NH_3. It is produced by the $^{12}C(d,n)^{13}N$ reaction by bombarding Al_4C_3 or methane with 6-7 MeV deuterons, or by the $^{16}O(p,\alpha)^{13}N$ or $^{13}C(p,n)^{13}N$ reaction. In the latter two reactions, a target of slurried mixture of ^{13}C powder and water is used for irradiation with 11-12 MeV protons. Nitrogen –13 is converted to NH_3 in a aqueous medium.

$^{13}NH_3$ in the form of NH_4^+ ion is used primarily for myocardial perfusion imaging by (PET) $^{13}NH_3$ is also used to label glutamine and asparagine for assessment of viability of tissues.

- **Oxygen-15:**

Oxygen-15 has a half-life of 2 min. and is produced by the $^{14}N(d,n)^{15}O$ reaction by deuterons irradiation of gaseous nitrogen or by the $^{15}N(p,n)^{15}O$ reaction by proton bombardment of enriched ^{15}N target.

$^{15}O_2$ is then passed over activated charcoal heated at 600°C. in order to convert it to $C^{15}O$ and $C^{15}O_2$, which are used for labeling heamoglobin and for clinical investigation of pulmonary and cardiac malfunctions.

- **Fluorine-18:**

Fluorine-18 ($t_{1/2}$ = 110 min.), is produced by the $^{18}O(p,n)^{18}F$ reaction on ^{18}O-water target. ^{18}F is recovered from water by passing the mixture through a column of quaternary ammonium resins, and ^{18}O-water can be reused as the target. Fluorine-18 is used primarily to label glucose to give ^{18}F-labeled fluorodeoxyglucose (FDG) for cerebral metabolic studies.

I.3. Radionuclide generator:

The use of short-lived radionuclides has grown considerably, because larger doses of these radionuclides can be administered to the patient with only minimal radiation dose and excellent image quality. This increasing appreciation of short-lived radionuclides has led to the development of radionuclide generators that serve as convenient sources of their production. A generator is constructed on the principle of decay-growth relationship between a long-lived parent nuclide and its short-lived daughter radionuclide.

The chemical property of the daughter nuclide must be distinctly different from that of the parent nuclide, so that the former can be readily separated. In a generator, basically a long-lived parent nuclide is allowed to decay to its short-lived daughter nuclide and the latter is then chemically separated. The importance of radionuclide generators lies in the fact that they are easily transportable and serve as sources of short-lived radionuclides in institutions far from the site of any cyclotron or reactor facility. A radionuclide generator consists of a glass or plastic

column fritted at the bottom with a fretted disk. The column is filled with adsorbent material such as cation or anion-exchange resin, alumina, and zirconia, on which the parent nuclide is adsorbed.

The daughter radionuclide grows as a result of the decay of the parent until either a transient or a secular equilibrium is reached within several half-lives of the daughter, after which the daughter appears to decay with the same half-life as the parent. Because there are differences in chemical properties, the daughter activity is eluted in a carrier-free state with an appropriate solvent, leaving the parent on the column.

After elution the daughter activity starts to grow again in the column until an equilibrium is reached in the manner mentioned above; the elution of activity can be made repeatedly. The first commercial radionuclide generator was the ^{132}Te (t 1/2 =78h)$\longrightarrow$$^{132}I$ (t 1/2 = 2.3h) system developed at the Brookhaven National Laboratory in the early 1960s. Since, then a number of other generator systems have been developed and tried for use in nuclear medicine. They are the ^{99}Mo-^{99m}Tc, ^{113}Sn-^{113m}In, ^{87}Y-^{87m}Sr, ^{82}Sr-^{82}Rb, ^{81}Rb-^{81m}Kr, and ^{68}Ge-^{68}Ga system.

I.4. Technetium:

In 1937 the element of the atomic number 43 was discovered by Segre and Perrier who showed that radioactivity obtained by irradiation of molybdenum with deuterons was due to isotopes of missing element eckamanganese.

The metastable isomers ^{95m}Tc and ^{97m}Tc had been produced by **the** following nuclear reactions: -

$^{94}Mo(d,n)^{95m}Tc$ and $^{96}Mo(d,n)^{97m}Tc$ .....(1)

Perrier & Segre (1947) suggested the name technetium, since it was the first element to be prepared artificially from ^{99}Mo, as reported by Seaborg et al (1939). About twenty isotopes and numerous with have lives ranging between one second and several million years have been

shown in Tab.(1), The isotope with the longest half-lives are $^{97}Tc(2.6\times 10^{6}$ y), $^{98}Tc(\ 4.2\ x10^{6}$ y) and $^{99}Tc\ (\ 2.1\ x10^{5}$ y), In the range of the mass numbers of long half life isotopes, stable isobars of the neighbouring elements molybdenum and ruthenium are known.

Table(1) : Isotopes and isomers of technetium

Nuclide	Half-Life	Decay	Nuclide	Half-Life	Decay
^{91}Tc	3.2 m	B^+ , γ	^{99m}Tc	6.0 h	γ,IT
^{92} Tc	4.4 m	B^+ , γ	^{99}Tc	2.1x105 y	B^-
^{93m} Tc	43.5 m	EC , γ	^{100}Tc	15.8 s	B^- , γ
^{93} Tc	2.7 h	EC,B^+,γ	^{101}Tc	14 m	B^- , γ
^{94m} Tc	53 m	B^+ , γ	^{102}mTc	4.3 m	B^- , γ
^{94} Tc	4.9 h	EC,B^-,γ	^{102}Tc	6.3 m	B^- , γ
^{95m} Tc	60 d	EC,B^+,γ	^{103}Tc	50 s	B^- , γ
^{95} Tc	20 d	EC , γ	^{104}Tc	18.0 m	B^- , γ
$^{96 m}$Tc	52 m	EC , γ	^{105} Tc	7.6 m	B^- , γ
^{96}Tc	4.3 d	EC , γ	^{106} Tc	36 s	B^- , γ
^{97m}Tc	91 d	γ	^{107} Tc	21 s	B^- , γ
^{97}Tc	2.6x10^{6}y	EC	^{108} Tc	5.0 s	B^- , γ
^{98}Tc	4.2x10^{6}y	B^- , γ	^{109} Tc	1.0 s	B^- , γ
			^{110} Tc	0.83 s	B^- , γ

1.4.1. Properties of Technetium: -

Technetium was the first element to be produced artificially. Minute amounts have been isolated by Perrier & Segre (1937), Technetium is a metallic element belonging to the transition elements of group VIIB of the periodic table. The chemistry of the element lies between that of manganese and rhenium. Its electronic configuration is $4s^2,4p^6,4d^6,5s^1$ or $4s^2,4p^6,4d^5,5s^2$. It forms compounds in all states of oxidation from 1- to 7+ but the most stable being those 4+ and 7+ states as reviewed by Deutsch(1983), It has different oxidation states between 1-and 7+ but the oxidation states of 5+ and 6+ are important in some

analytical applications and in the chelate compounds. The Tc^{5+} and Tc^{6+} species frequently disproportionate into Tc^{4+} and Tc^{7+} states:-

$$3Tc^{5+} \longrightarrow 2Tc^{4+} + Tc^{7+}$$

$$3Tc^{6+} \longrightarrow Tc^{4+} + 2Tc^{7+}$$

Technetium of oxidation state 7+ is present as the pertechnetate ($^{99m}TcO^-_4$) which is the most stable. Chemical form of technetium followed by the tetravalent state (TcO_2) which is the other oxidation states gains stability through complex formation. $^{99m}TcO^-_4$ is the the starting material of the preparation of ^{99m}Tc radiopharmaceuticals. Technetium is a silver gray metal it has a melting point of 2200°C, and a boiling point of 4973°C. Metallic technetium dissolves in acids that are oxidants such as nitric acid, aquaregia, and concentrated sulfuric acid.

Metallic technetium dissolves in bromine water and also in neutral and alkaline solutions of hydrogen peroxide. First type of technetium oxide which is the volatile Tc_2O_7 was prepared by burning the technetium in excess of oxygen at 500°C. Other type of technetium oxide is the relatively TcO_2 which can be obtained by reduction of aqueous solutions of pertechnetate with zinc and hydrochloric acid.

As reviewed by Boyd (1959), Technetium forms two sulfides the first one was technetium heptasulfides Tc_2S_7 which is prepared by passing hydrogen sulfide through acid solution of pertechnetate and the other one was technetium disulfides TcS_2 which can be obtained by heating Tc_2S_7 with elemental sulfur in an autoclave for 24 h. at 1000°C.

Technetium forms compounds with halogens at different oxidation states. When technetium metal was reacted with excess of fluorine in a closed nickel vessel for 2h at 400°C, technetium hexaflouride(TcF_6) was formed.

When technetium metal is fluorinated directly technetium pentaflouride(TcF_5) is formed also, when gaseous chlorine is passed over

technetium metal at 200°C, a reaction begins at 400°C which takes place rapidly, with the formation of $TcCl_6$ which is very unstable, it decomposes even at room temperature to $TcCl_4$.

$TcCl_4$ can also be obtained by reacting technetium heptaoxide with carbon tetrachloride in autoclave at 400°C. Some chemical relationships of technetium are presented in Fig (1)

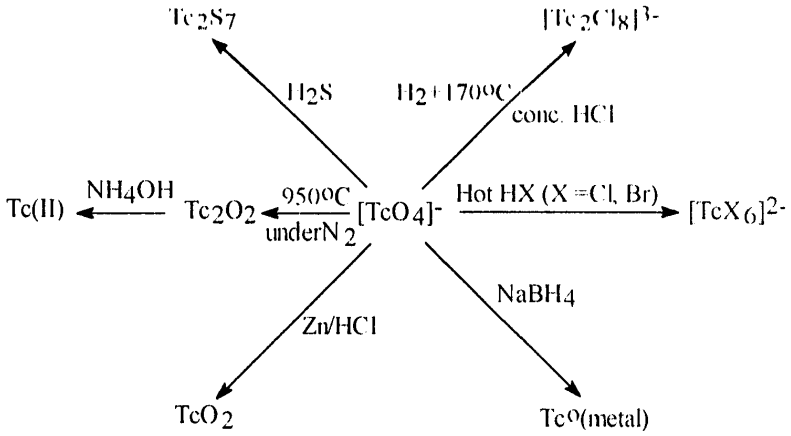


Fig.1: Selected relationships of technetium chemistry.

The radionuclide ^{99m}Tc has a half-life of 6h and decays to ^{99}Tc by isomeric transition or γ-transition of 140 keV. Approximately 10% of these transitions are via internal conversion. The ground state ^{99}Tc has a half-life of 2×10^5 years and decays to stable ^{99}Ru by β^--emission (Fig.2). These excellent physical and nuclear properties of ^{99}Tc are nearly ideal for a current generation of imaging devices such as single photon emission computed tomography.

(SPECT) or gamma camera, which applied in nuclear medicine as, reported by Dechiara (1987). Technetium-99m is considered as one of the most useful radionuclides used in diagnostic nuclear as suggested by

Richards (1960). It is not expensive and ready availability to all hospitals via $^{99}Mo/^{99m}Tc$ generators. The ^{99}Mo radionuclide has a half-life of 67h and decays by β^-emission (87%) to the metastable ^{99m}Tc and has the remaining (13%) to the ground state ^{99}Tc (Fig 3). Molybdenum 99 has photon transition of 740KeV and 780 KeV.

^{99m}Tc–Radiopharmaceuticals are currently used for imaging brain, liver, kidneys, skeleton and blood pool as reported by Eckelman et al (1977), Recently, many substances of interesting behaviour labeled with ^{99m}Tc are used for characterizing the morphology and function of different human organs as presents by Johannsen (1991).

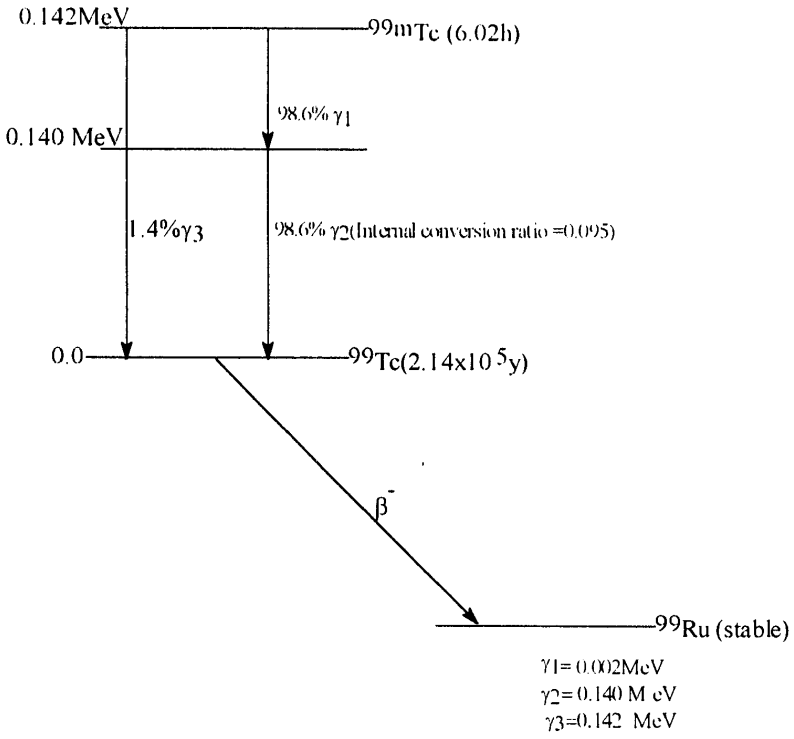


Fig.2: Decay scheme of technetium-99m

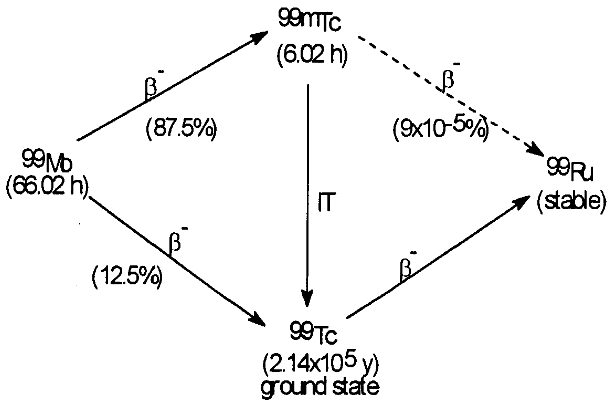


Fig.(3): Decay scheme of ^{99}Mo - ^{99m}Tc generator

I.4.2. Source of technetium: -

The isotope ^{99m}Tc is produced by the β^--decay of ^{99}Mo which is obtained either from uranium fission products or from neutron irradiated $^{98}MoO_3$ according to the reaction.

$^{235}U \xrightarrow{(n,f)} {}^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc$ ....(3)

$^{98}Mo \xrightarrow{(n,\gamma)} {}^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc$ ....(4)

Technetium-99m is the radioactive daughter nuclide of ^{99}Mo as shown from the Fig (3)

I.4.3. Methods of ^{99m}Tc separation:

Various Methods for the separation of ^{99m}Tc from ^{99}Mo are available, and the most important one is the alumina column chromatography on witch the ^{99}Mo/^{99m}Tc generators are based as reported by Boyed (1982), Marques et al (1987).

Three methods of technetium separation are in current use. These are: -

(i) Elution from ^{99}Mo adsorbed on an aluminium oxide ion-exchange column.

(ii) Extraction with methyl-ethyl-ketone from ^{99}Mo-sodium molybdate in sodium hydroxide solution.

(iii) Distillation (sublimation) from ^{99}Mo-molybdenum trioxide.

I.5. ^{99}Mo / ^{99m}Tc generator systems:

I.5.1. Chromatographic generator:

This system is the most widely used as pointed by Tucker et al(1958). The technique is based on the relative difference in distribution coefficients between the two radionuclides on aluminum oxide for both the anion molybdate and pertechnetate.

The molybdate anion is strongly adsorbed on alumina while the pertechnetate is easily eluted by saline. The passage of physiological saline through the alumina bed containing adsorbed molybdate /pertechnetate will result in the elution of the pertechnetate component leaving the molybdate on the bed. Two kinds of chromatographic generators namely, The wet generator and the dry one were developed.

Regarding to the wet generator, the radiation emitted from ^{99}Mo on the column will induce radiolysis of water with the formation of hydrogen peroxide (H_2O_2) and other free (OH) radicals these species are highly oxidant and in presence of ^{99m}Tc eluate, they will interact with technetium, on the other hand in a dry column generator, after routine elution, the left over saline in the column drawn out by using an evacuation vial without adding any more saline.

The suggestion for a dry column generator a high elution efficiency of ^{99m}Tc.(Fig (4)).

- **Advantages of AL_2O_3 column chromatography:**

1- Ability to incorporate the column in a closed system for maintenance of sterility.

2- Simple operation.

3- Less radiation dose to the operator.

4- Less time consuming and hence less decay of ^{99m}Tc.

5- ^{99m}Tc can be obtained more than one in a day resulting in more efficient use.

I.5.2. Solvent extraction generator: -

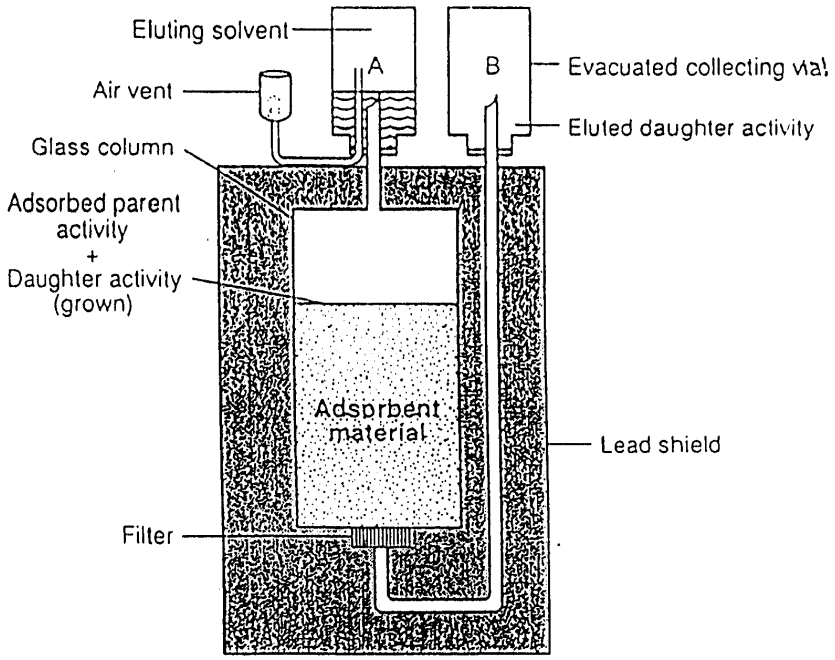
The ^{99}Mo–^{99m}Tc generator was first introduced at brook haven National Laboratory by Tucker et al (1958). Before the development of this generator, the ^{99m}Tc radioactivity is used to be extracted with methyl–ethyl ketone (MEK) from 20% NaOH solution (pH~10–12) of ^{99}Mo.

After extraction, the organic phase was evaporated and the $^{99m}TcO_4^-$ dissolved in isotonic saline for clinical use. This method of solvent extraction has been employed to construct the liquid–liquid extractor type of generator. ^{99}Mo radioactive alkaline solution was kept in a glass column and then letting MEK flow through the column from the bottom, MEK will extract $^{99m}TcO^-_4$ leaving ^{99}Mo in the aqueous solution, Repeated elutions of the column can be made after or before the transient equilibrium between ^{99}Mo and ^{99m}Tc system.

The advantage of this generator is that the cost of ^{99m}Tc is low, but the disadvantage is that it needs a lot of manipulations in the overall method, It is rarely used in nuclear medicine (Fig (5)).

I.5.3. Sublimation generator:

In this type of generators, ^{99m}Tc are separated at increased temperature from suitable ^{99}Mo compounds by sublimation with carrier gas or air. The starting material, neutron–activated $^{99}MoO_3$ is placed into an electrically heated quartz furnace, $^{99m}TcO_7$ is volatilized at some hundreds C^0 transferred by carrier gas, usually by carrier air, into cooling



Fig(4):Column chromatographic generator.

trap. ^{99m}Tc is dissolved with isotonic NaCl solution and sterilized by membrane filtration to obtain a ready–to–inject solution.

Sublimation generators have not found wide applications due to their insufficient yield.

1.5.4. Gel generator:

The gel generator system for ^{99m}Tc combines the advantages of chromatographic column generator and using non polluting $(n,\gamma)^{99}Mo$. This generator is based on eluting the ^{99m}Tc from a column of ^{99}Mo MoO_4^{2-} obtained either by converting ^{99}Mo to an insoluble molybdate or by irradiation of an insoluble molybdate neutrons. In view of its close functional similarity with the alumina column generator, considerable efforts have been made towards preparation of gel generators and under standing the various factors critical for obtaining ^{99m}Tc with good yield and purity. Since the gel generator is only recently introduced as a source of ^{99m}Tc, experience in its patient use is limited as reported by El-Kollaly (1996).

(a)Gel generators based on converting ^{99}Mo into gel matrix:

^{99m}Mo produced by(n,γ) is precipitated as zirconium or zirconium molybdophosphate (molybdate) under carefully controlled conditions and separated by filtration. The precipitate is dried carefully, powdered and packed into a column over an inactive bed. The column is eluted with 0.9% NaCl to get ^{99m}Tc. The drying conditions and the water content of the dried precipitate are critical to get good ^{99m}Tc yield. The same purity of ^{99m}Tc obtained from generators is comparable to ^{99m}Tc from other generators.

(b)Gel generators based on neutron activation of metallic molybdates. A metallic molybdate, prepared under carefully controlled conditions, is irradiated in the nuclear reactor and directly used in a

column for eluting ^{99m}Tc. This method would probably offer the simplest method for making column type ^{99m}Tc generators.

A variety of metallic (Sn, Ti, Ce) molybdate can be evaluated as suitable target for irradiation.

The ^{99m}Tc yield depends mostly on the conditions of preparation of the metallic molybdate gel, and its final water content.

The ^{99m}Tc yield were also found to depend on irradiation conditions such as neutron flux and the temperature of the target during irradiation.

1.6. ^{99m}Tc – Radiopharmaceuticals: -

1.6.1. Labeling with ^{99m}Tc:

Labeling a molecule by ^{99m}Tc is a complex formation, where the pertechnetate in oxidation state VII, is reduced to reactive forms of Tc(V),Tc(IV) or Tc(III). After reduction of pertechnetate, the radionuclide can be complexed by suitable chelating ligands. Radio pharmaceutically used complexing agents (chelating ligands) are compounds, which contain oxygen, nitrogen, phosphorus and sulphur in donor sites as shown in Table (2).

Table (2): Essential groups present in many chelating agent used for ^{99m}Tc-complexation

Ligands	Function groups (donor groups)
Cirate	OH,COOH
Glucoheptonate	OH,COOH
Gluconate	OH,COOH
DTPA (diethylenetriaminepentaacetic acid)	COOH
DMSA (dimercaptosuccenic acid)	SH,COOH
Penicillamine	SH,NH2,COOH
HEDP(hydroxyethylene diphosphonate)	OH,PO3H
MDP (methylene diphosphonate)	PO3H
HIDA {N-(2,6-dimethylephenyl carpamoyl methyl) iminodiacetic acid}	NH,COOH
DIARS (phenyldimethyl arsine	As
TMP (trimethyl phosphine)	P
Mercapto acetyl triglycine	SH/NH
Ethyl cysteinat dimer	SH/NH
Ethyl cysteinate	SH/NH
2-Methoxy isobutylisonitrile	CN

Recent developments in ^{99m}Tc labeled myocardial radiopharm-aceuticals rely on substituted arsine and phosphine chelating sites as mentioned by katti et al (1992).

The ligands currently used contain hydroxyl, carboxyl, phosphate isonitrile, amino or mercapto sites as shown in the above Table(2).

These obviously essential groups occur in many chelating agents such as carbohydrates, proteins, hydrophil drugs, mercapto acids and

molecules and thus drastically alters the original reactivity and other important properties of the ligand.

The following are the possible changes of the ligand properties as a result of ^{99m}Tc–complex formation: -

1- Partially or completely blocked functional sites.
2- Inevitably increased molecular weight.
3- Altered size form and shape.
4- More or less changed electrical charge.

Thus the change of biological behaviour can be expected to be caused by considerably different properties of the free ligand and labeled complex. At least small ^{99m}Tc–labeled molecules cannot be considered representative for unlabelled substances as it is partially possible for compounds labeled by ^{3}H, ^{14}C or radioiodine they deviate essentially from one another. A halogen atom is a covalent bond atom and thus forms a molecular analogue of similar size, shape and electron configuration, whereas the complex formation yields a substantially altered molecule that mainly concerns. Of course, low molecular substances but not high-molecular substances which can advantageously be labeled by ^{99m}Tc. Generally the following substance can be labeled with ^{99m}Tc as reviewed by Dewanjee et al(1990).

Chelating agents such as methylene diphosphonate (MDP), colloids (include proteins and particles,monochlonal antibodies and receptor binding agents, blood elements, and diethylene triamine pentaacetic acid.

1.6.2 Reduction of $^{99m}TcO_4^-$

The chemical form of ^{99m}Tc available from the ^{99}Mo/^{99m} Tc generator is sodium pertechnetate (Na ^{99m} TcO_4^-).

The pertechnetat ion, $^{99m}TcO^-_4$ having the oxidation state 7+ for ^{99m}Tc, resembles the permanganate ion, MnO^-_4, and the pertechenat ion, ReO_4^-.

It has a configuration of a pyramidal tetrahedron with Tc^{7+} located at the center and four oxygen atoms at the apex and corners of the pyramid.

Chemically, $^{99m}TcO_4^-$ is a rather non-reactive species and does not label any compound by direct addition.

In ^{99m}Tc labeling of many compounds, prior reduction of ^{99m}Tc from the 7+ state to a lower oxidation state is required. Various reducing systems that have been used are stannous chloride ($SnCl_2 2H_2O$), stannous citrate, stannous tartrate, concentrated HCl, sodium borohydride ($NaBH_4$), dithionite, and ferrous sulfate as reviewed by Saha (1992). Among these, stannous chloride is the most commonly used reducing agent in acidic medium in most preparations of ^{99m}Tc–labeled compounds. Another method of reduction of $^{99m}Tc^{7+}$, involves the electrolysis of a mixture of sodium pertechnetate and the compound to be labeled using an anode of zirconium .

The chemical reactions that occur in the reduction of technetium by stannous chloride in acidic medium can be stated as follows:-

$$3Sn^{2+} \rightleftharpoons 3Sn^{4+} + 6e^- \quad \ldots\ldots (5)$$

$$2\ ^{99m}TcO4^- + 16\ H^+ + 6e^- \rightleftharpoons 2\ ^{99m}Tc^{4+} + 8\ H_2O \quad \ldots\ldots (6)$$

adding the two equations, one has

$$^{99m}TcO_4^- + 16\ H^+ + 3Sn^{2+} \rightleftharpoons 2\ ^{99m}Tc^{4+} + 3Sn^{4+} + 8\ H_2O \ldots\ldots (7)$$

Equation (6) indicates that $^{99m}Tc^{7+}$ has been reduced to $^{99m}Tc^{4+}$. Other reduced states such as $^{99m}Tc^{3+}$ and $^{99m}Tc^{5+}$ may be formed under different physicochemical conditions.

It may also be possible for a mixture of these species to be present in a given preparation. Experiments with milimolar quantities of ^{99}Tc have shown that Sn^{2+} reduces ^{99m}Tc to the 5+ state and then slowly to the 4+ state in citrate buffer at pH 7 as investigated by Steigman et al (1975). Technetium –99 is reduced to the 4+ state by Sn^{2+} in acidic mediums.

The amount of ^{99m}Tc atoms in the ^{99m}Tc– eluate is very small (~ 10^{-9} M), and therefore only a minimal amount of Sn^{2+} is required for reduction of such a small quantity of ^{99m}Tc. However, enough Sn^{2+} is added to ensure complete reduction. The ratio of Sn^{2+} ions to ^{99m}Tc atoms may be as large as 10^6.

I.6.3. Labeling methods:

There are three methods for labeling compounds with ^{99m}Tc:

I.6.3.1. Direct labeling: -

The reduced ^{99m}Tc species are chemically reactive and combine with a wide variety of chelating compounds. A schematic reaction would be represented as follows:

Reduced ^{99m}Tc + chelating agent $\rightleftharpoons$ ^{99m}Tc-chelate

The chelating agent which contain donar atoms such as S, P, N, As, O, usually donates lone pairs of electron to form coordinate covalent bonds with ^{99m}Tc. Chemical groups such as – COO, - OH ,- NH_2 and – SH are electron donars in compounds such as DTPA, gluceptate and various protein which mentioned before in Table (2).

However, Eckelman et al (1977), reported several compounds labeled with ^{99m}Tc by this method such as DTPA , N [N' (2,6 – dimethylphenyl carbamoylmethyl]imminodiaacetic acid (HIDA), methylene diphosphonate (MDP), pyrophosphate (PYP), hydroxy ethylidene diphosphonate (HEDP) and gluconate, ethyl cysteine (EC), 2-methoxy isobutyl isonitril (MIBI).

I.6.3.2. Ligand exchange method:

The ligand exchange method, also termed the transchelation, involves first forming a ^{99m}Tc–complex with a weak ligand in aqueous media and then allowing the complex to react with a second ligand that is forming more relatively stable complex. Because of the difference

instability of the two ligands, a ligand exchange occurs, forming a more stable ^{99m}Tc–complex with the second ligand. For example, in the preparation of ^{99m}Tc–labeled mercaptoacetylglycylglyclglycine(MAG_3) by Fritzberg et al (1986), ^{99m}Tc–tartrate , ^{99m}Tc–gluconate is first formed by reduction of $^{99m}TcO_4^-$ with stannous ion in the presence of sodium tartrate or gluconate. Subsequent heating with MAG_3 results in ^{99m}Tc–MAG_3.

The following are the sequences of reactions for ^{99m}Tc MAG_3:

$$^{99m}Tc\ O^-_4 + Sn^{+2} \rightleftharpoons \text{reduced } ^{99m}Tc + Sn^{+4} \quad \ldots\ldots(8)$$

$$\text{Reduced } ^{99m}Tc + \text{tartrate} \rightleftharpoons ^{99m}Tc - \text{tartrate} \quad \ldots\ldots(9)$$

$$^{99m}Tc - \text{tartrate} + MAG_3 \rightleftharpoons ^{99m}Tc - MAG_3 + \text{tartrate} \quad \ldots\ldots(10)$$

These reactions normally occur when the solubility of the stronger chelate and the stability of the Sn(II)complex are lower in aqueous media gluconate, tartarate, citrate, and EDTA are weaker ligands.

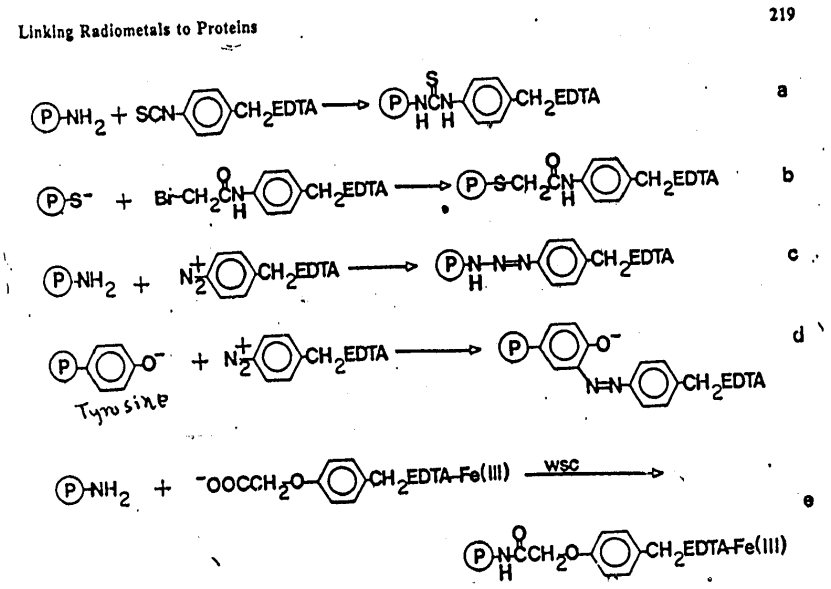
I.6.3.3. Bifunctional chelation method:

In general, chelating agents are compounds that comprise both powerful metal chelating group and a second functional group that is usually chemically reactive in nature.

The metal chelators are often derived from polyamino carboxylic acids such as ethylene diamine tetraacetic acid (EDTA) , diethylene tetra amine penta acetic acid (DTPA) , or imino diacetic acid (IDA), because of there large formation constants with a variety of metal ions and their relative ease of synthestic manipulation as reported by Meares et al(1983). One functional group is always a multidentate metal chelating ligand, but the other functional group can be of various types. It can be a reactive moiety of forming covalent bonds with biological molecules or, hydrophobic aliphatic chain likely to incorporate into biological membrane; a hepatic molecules with affinity for an antibody.

Many of the most interesting applications of this technology are found in medicine Radioactive metal ions attached by chelation to small molecules, peptides, or proteins such as monoclonal antibodies have been used clinically for diagnosis of cancer as reviewed by Vera–Ruiz (1998). and for study various organs. With the availability of monoclonal antibodies which had been developed by Kohier and Milslein in the (1975), which bind with great selectivity to biological molecules.

These antibodies can be used for in-vitro for analysis of hormones and other biological compounds, or they can be used for diagnosis and treatment of disease. One of the principal reasons for the development of bifunctional chelating agents for radiolabeling is the availability of radionuclides with convenient half–life and useful radiation, for example ^{99m}Tc. Fig.(6) Shows some examples of bifunctional, chelating agents that commonly used.



Bifunctional chelator is first conjugated to the antibody and then reduced ^{99m}Tc is allowed to couple to the chelator DTPA, EDTA, dithiosemicarbazone and N_2S_2 which have been used as chelating agents.

I.7. Groups of ^{99m}Tc–radiopharmaceuticals:

These are compounds, which contain chelating group to bind the reduced technetium, and are concentrated in the organs of choice depending on the ability of that organs to remove foreign substance from the blood circulation as reviewed by Eckelman et al(1977). Recently, the field of nuclear medicine imaging has viewed as the portrayal of regional physiology and biochemistry as presented by Narasimhan, Johannsen (1992).

The following are the different classes of ^{99m}Tc– radiofarmaceuticals:

I.7.1. Pertechnetate ion ($^{99m}TcO_4^-$):

Technetium-99m eluted directly from $^{99}Mo/^{99m}Tc$generator as the $^{99m}TcO_4^-$ ion, which was first evaluated by Harper et al (1962) as possible biological tracer. This work led to the present wide spread use for brain tumor localization and for thyroid imaging.

I.7.2. ^{99m}Tc–Labelled colloids and particulates:

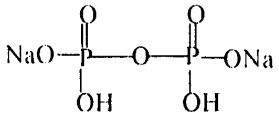
^{99m}Tc–sulfur colloid is prepared by heating mixture of $^{99m}Tc\ O_4^-$ and sodium thiosulphate in acidic medium for 5 to 10 min in boiling water bath. Gelatin is added before the reaction with the acid in order to stabilize sulphur in the colloid state.

It is used for scintigraphy of the recticuloendothelial lemph system as tried by Harper et al (1964). Other ^{99m}Tc–labeled colloids e.g. $^{99m}Tc(OH)_4$, $Sn(OH)_2$, ^{99m}Tc antimony sulphide ^{99m}Tc–Sb_2S_3 colloid, and ^{99m}Tc microparticulates of denatured albumin are used for imaging the resident pool of macrophage in the recticuloendothelial system as reviewed by

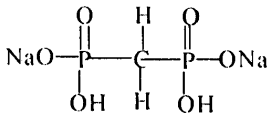
Eckelman et al(1977).The ^{99m}Tc–antimony sulphide colloids have an narrow particles size distribution (5 to 15 μm) than ^{99m}Tc sulphide colloid (300 to 900 μm) and the former migrate much faster after interstitial administration facilitating regional lympho–scintrography less erythema at the sites of infection. Some of these colloids were also used for labeling polymorphonuclear neutrophilic (PMN) granulocytes, which are used for diagnosis of infection as carried by Moke et al (1987).

I.7.3. ^{99m}Tc–chelates for skeletal imaging: -

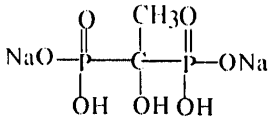
These ^{99m}Tc– chelates are used for skeleton scintigraphy such as MDP, HEDP, HMDP, pyrophosphates and polyphosphates as tried by Subramanian (1971). The structure of these chelating agents are presented in Fig.(7). Diphosphonates chelating agents are analogue to pyrophosphate whose P–O–P structure is replaced by P–C–P which is more stable against enzymatic decomposition by phosphatase enzyme. The labeling of ^{99m}Tc–phosphonate and pyrophosphate complexes by addition of pertechnetate to a freeze kit has been reported by Subramania et al (1971). This revolutionized bone scanning by providing the most sensitive method for surveying skeletal abnormality.



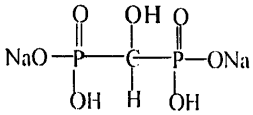
Disodium pyrophosphate(PYP)



Disodium methylenediphosphonate (MDP)



Disodium hydroxyethylenediphosphonate(HEDP)



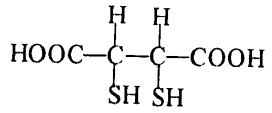
Disodium hydroxymethylenediphosphonate (HMDP)

Fig.(7): Structures of some pharmaceuticals used for skeletal imaging

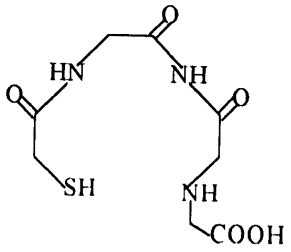
I.7.4. ^{99m}Tc–Chelates for renal imaging: -

Dewenjee et al (1990) mentioned that several ^{99m}Tc–chelates, like a variety of organic acids and bases are filtered by the glomerulus. A few of them may be partially secreted by the proximal tubular reabsorption passively depending on their pk_a's lipid solubility, and pH of the tubular fluid. The tubular cells retain a variety of metal ions by chelations with thiol groups present in these proteins. Numerus ^{99m}Tc–complexes are frequently used for kidney function study such as ^{99m}Tc iron – ascorbate, which reported, by Harper et al (1966), diethylene triamine pentaacetic acid glucoheptonate and dimercaptosuccenic acid reviewed by El Asrag et al(1988). These ^{99m}Tc–complexes are prepared by reduction of pertechnetate in presence of Sn(II) salts.

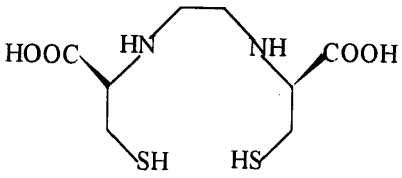
Several ^{99m}Tc–labeled compounds had been prepared and tested for the measurement of effective renal plasma flow (ERPF) and to find a replacement for radioiodohippuran. Fritzberg et al (1981, 1986) synthesized,N,N–bis(mercaptoacetyl)2,3-diaminopropanoate(CO_2DADS) N_2S_2 and mercaptoacetylglycylglycylglycin (MAG_3)N_3S. ^{99m}Tc MAG_3 was prepared by ligand exchange method in which the technetium is reduced in the presence of pro–ligand to form ^{99m}Tc–weak complex and in the presence of MAG_3 a ligand exchange occurs and a more stable ^{99m}Tc– MAG_3 complex was formed. Some ^{99m}Tc–complexes for renal imaging are in Fig.(8).



Dimercaptosuccinic acid (DMSA)



Mercaptoacetyltriglycine (MAG



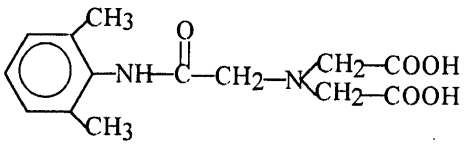
Ethylenedicysteine diacid (EC)

Fig.(8):Some Radiopharmaceuticals for kideny imaging and renal function study.

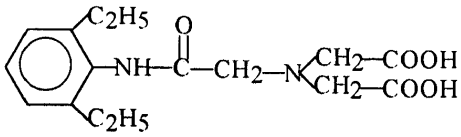
I.7.5. ^{99m}Tc–Chelates for hepatobillary imaging :-

The derivatives of iminodiacetates (IDA) are excellent chelating agents for ^{99m}Tc. The first complex was developed by Loberg et al (1976) and involved the ligand 2,6–dimethylacetanilidoiminodiacetate. ^{99m}Tc–IDA derivatives are prepared by the direct reduction of pertechnetate with Sn(II) in presence of the ligand (IDA) where a ^{99m}Tc–IDA complex is formed. A variety of ^{99m}Tc–chelate of IDA derivatives were evaluated clinically, the trimethyl bromo, iodo and tertiary butyl derivatives of IDA were found to be excellent ligands as reported by Mitta et al (1982) and

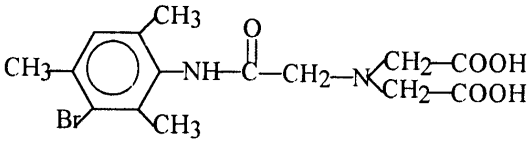
Arguelles et al (1988). The structure of some ^{99m}Tc–IDA derivatives are presented in Fig.(9).



N(2,6–dimethylacetanilido) iminodiacetic acid (HIDA)



N(2,6–diethylacetanilido) iminodiacetic acid



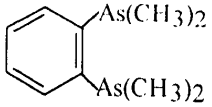
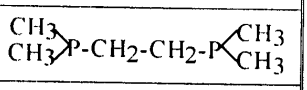
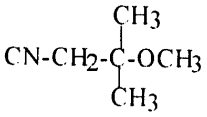
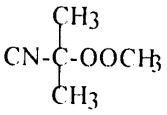
N(3-bromo, 2,4,6–trimethylacetanilido) iminodiacetic acid

Fig(9): Structures of some ^{99m}Tc– IDA complexes suggeste as hepatobillary imaging agents.

I.7.5.1. ^{99m}Tc– Chelates for myocardial imaging:

Deutsch et al(1981) first demonstrated that cationic complexes of ^{99m}Tc(III) with ligands of arsine and phosphine, like the monovalent alkali metal ions, localize in the muscle cells. The ligands used for myocardial imaging reviewed by Dewaujee et al (1990). Some of ^{99m}Tc–cationic complexes used for myocardial perfusion imaging are shown in Table(3).

Table (3): The structures of some ligands used for myocardail imaging

Ligand	Abbreviation	structure
Phenyldimethyldiarsine	**DIARS**	
Dimethyl Phosphinoethane	DMPE	
2-Methoxyisobutylisonitril	MIBI	
Carbomethoxyisopropyl-isonitril	CPI	

I.7.6. ^{99m}Tc–Complexes for brain perfusion imaging:

^{99m}Tc-Propylene amine oxime (PnAO) was reported by Trounter et al (1984), as brain SPECT imaging agent. Several derivatives of PnAO were synthesized by different methyl substitutions on the amine oxime backbone. One of these derivatives is d,l–hexamethyl propylene amine oxime (d,l-HMPAO). ^{99m}Tc–HMPAO is neutral lipophilic chelate used for measurement of cerebral perfusion of brain by SPECT technique as developed by Ballinger et al(1988). Walovitch et al(1987), demonstrated that oxo-^{99m}Tc(v) complexes of amine derivatized diamine dithiol (DADT) ligands cross the blood brain barrier, permitting measurement of cerebral perfusion. The ^{99m}Tc complex of N, N′-1,2- ethylenediyl bis l. cysteine ester (L,L–ECD) demonstrated cerebral uptake and longer retention time in brain as reported by Harris et al(1992). The structure of some ^{99m}Tc chelates used for brain imaging are presented in Fig.(10).

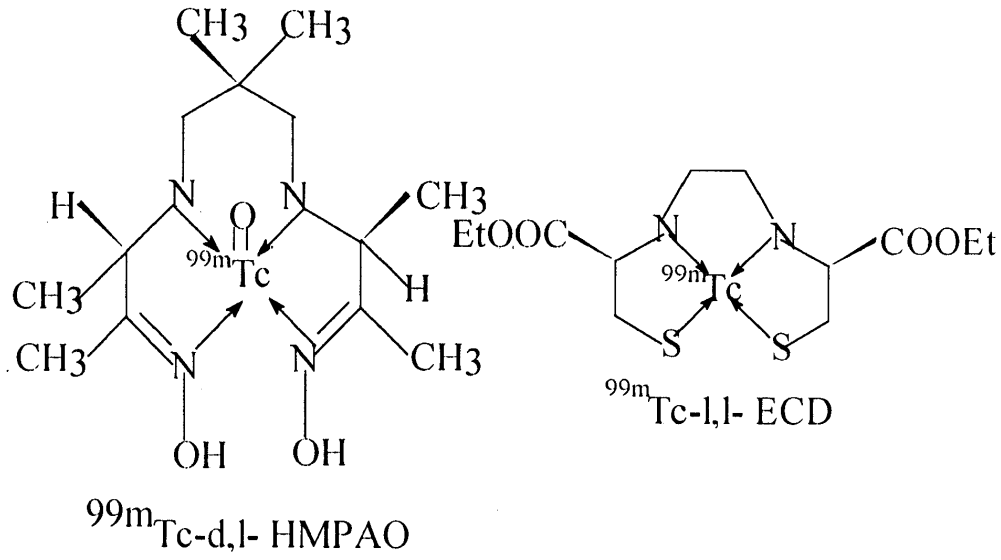


Fig (10): Some ^{99m}Tc-chelates for brain imaging

I.7.7. ^{99m}Tc–Complexes of proteins:

Several proteins (human albumin, neoga–lactoalbumin, fibrmigen, monoclonal and polyclonal antibodies) have been labeled with ^{99m}Tc radionuclide as reported by Hnatowich et al (1987).

The labeling was accomplished by direct chelation of macromolecules (which contain large number of binding sites) with reduced ^{99m}Tc (pertining method) as reported by Eckleman (1990) or by indirect conjugation of ^{99m}Tc complex (pre-complexing agent method) to protein via bifunctional chelating agent as developed by Hnatwisch et al(1987). ^{99m}Tc Human serum albumin has been used as blood pool imaging agent. Monoclonal antibody fragments $(Fab)_2$ and Fab have been labeled with ^{99m}Tc for radioimmun scintigraphy as reported by Rods (1991). The labeling of these fragments (MoAb) is performed with a variety of bifunctional chelating agents.

^{99m}Tc is coordinated to N_2S_2(diaminodithiol), EDTA (ethylen diaminetetracetic acid) or (DTPA) which in turn conjugated with MoAb fragments as reported by Bridoo et al (1990) and Rods et al(1991).^{99m}Tc–MoAb is used for diagnosis of tumers and infraction and thrombosis as reviewed by Baum (1989).

I.7.8. ^{99m}Tc–Blood elements: -

^{99m}Tc–Red blood cells are frequently labeled in vivo by pretining method as reported by Eckelman (1991) method. It is used for measurements of cardiac output, ejection fraction and regional motion. White blood cells and platelets are labeled by ^{99m}Tc using lipid soluble ^{99m}Tc-HMPAO and evaluated in the diagnosis of thrombosis and injection by Dewanjee et al (1989).

I.8. Kit preparation: -

The kits are prepacked sets of sterile pyrogen free reagents (organic chelates of divalent tin) in the lyophilized form, upon addition of

$^{99m}TcO^-_4$ to the kit, labeled complex is formed. These ^{99m}Tc-complexes have been used as diagnostic agents for the different organs of the body such as DTPA for kidney function, phytate for liver scanning and phosphate for bone scanning as reported by Eckelman et al (1977).

The production of kits for ^{99m}Tc-radiopharmaceuticals is a highly sophisticated process. The composition of such kits has to provide a complete conversion of pertechnetate into the desired ^{99m}Tc-radiopharmaceuticuls which must be stable for several hours.

To protect the highly diluted Sn(II) solution from oxidation, the whole process is carried out under purified nitrogen gas. The obtained solution of Sn.(II) chelating agent is immediately dispensed, quickly frozen by liquid nitrogen and instantly lyophilized. After lyophilization the lyophilizator is floaded by oxygen-free nitrogen and the vials are closed under protective gas. The sealed vials can be stored at 2–8°C

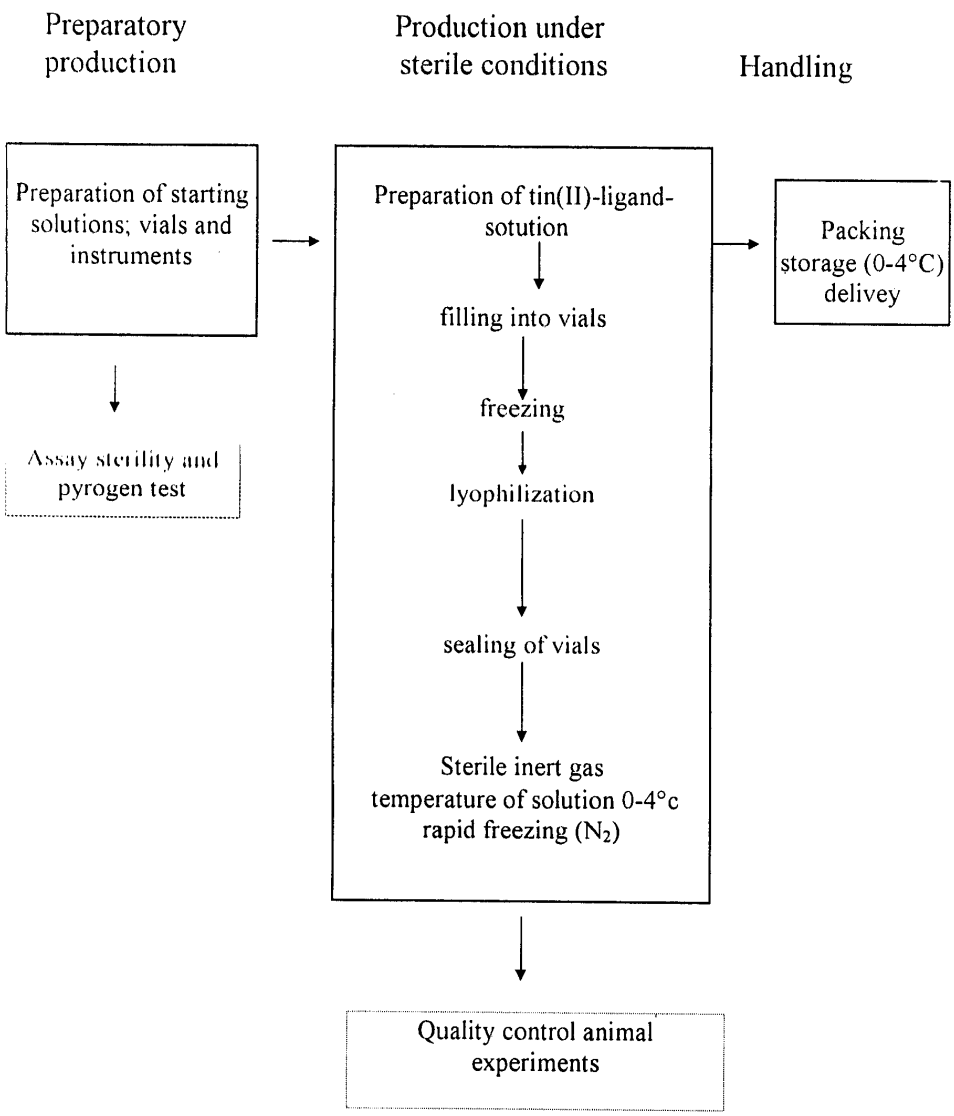


Fig.12:Scheme for the production of kits for the preparation of ^{99m}Tc - radiopharmaceuticals

For each kit the following points must be involved in its production using the freeze drying process.

- Choice of the suitable parameters that affects the formulation of the kit such as pH, Sn^{2+} content, active ingredients and addition of stabilizing agent.
- Labeling with ^{99m}Tc to determine the labeling yield and biological distribution.
- Quality control has to be performed, stability of kit upon storage and application in nuclear medicine has to be studied to find its eligability for human application.

I.9. Freeze drying:

Freeze-drying is a very important technological process of great relevance to the drug, pharmaceutical industry and food preservation industry. There have been other novel applications also in the preparation of very fine metal powders, preservation of old manuscripts, biological specimens. This technique is primarily used for preserving over a long period of time under ambient temperature conditions of material which otherwise will bio–degrade. Practically the technique involves freezing the specimen below its eutectic point and then drying it by removal of moisture from the condensed phase to vapour phase by sublimation as reported by Goldblith et al (1975).

The removal of water vapour from frozen solution by sublimation forms the basis of freeze-drying technique.

The first consideration in freeze drying of any solution is the temperature at which it must be held for sublimation to occur from the solid state in the absence of dissolved solid, the solution must be cooled below the triple point temperature and the pressure is reduced in the drying chamber to a value below the pressure equivalent to the triple–

point. But when the solution of solids are dried, the depression of freezing point of water by solutes must be considered.

It is essential that the temperature is brought below the eutectic point so that no liquid phase is present. When the eutectic point is not known, freezing the product to about –40°C is usually sufficient.

The production of freeze dried material may be considered in three stages.

I.9.1. Preliminary freezing:

A definite quantity of solution are introduced into the final container and cooled below its eutectic point or to about -40°C as follows:

(1) Freezing by contact with cooled surface: -

This is a static freezing technique where the refrigerating device must be capable of adjusting the freezing rate between 1°C and 4°C per minute. A final temperature of -50°C will normally be sufficient to meet all requirements.

(2) High speeds vertical spin freezing:

This is a dynamic freezing technique which is used wherever larger quantities of a liquid product are to be frozen and dried, e.g. plasma or serum, the bottles are spun on either vertical axes at between 750 and 1000 rpm. The liquid is there by distributed uniformly inside the bottle round the periphery, leaving a conical air space in the center reaching to the bottom of the bottle. The liquid after super–cooling freezes suddenly in this position.

I.9.2. Vacuum evaporation:

The maintenance of the contents in the solid frozen condition during application of the vacuum is virtually important. If a liquid were formed as a result of partial thawing, a considerable amount of degassing

would occur with probable loss of the material, which would float over the sides of the container. Air is exhausted by double–stage, high–vacuum until a suitable vacuum is obtained (a vacuum of 0.01 mm Hg is possible). A condensing coil at the top or the bottom of the chamber is maintained at -50°C. When the pressure is sufficiently low, ice evaporates.

I.9.3. Heat requirement for evaporation:

Latent heat being required for evaporating the residual moisture of frozen material. The solution would cool still further a small source of radiant heat is therefore placed in the chamber head and evaporation takes place rapidly with the frozen material remaining at -20°C until all the ice has evaporated.

Freeze dried powder has many properties:

1. No concentration occurs and the evaporated solid occupies practically the same volume as that of the original frozen solution.
2. No denaturation occurs
3. Easily soluble.
4. Low moisture content and so increased shelf–life.

I.10. Quality assurance of ^{99m}Tc–radiopharmaceuticals: -

^{99m}Tc–Radiopharmacenticals are subjected to quality control tests, and measurements to assure safety, sterility, apyrogenicity, radiochemical purity and suitability for the purpose intended, These criteria are applied to those newly developed products for clinical application.

I.10.I. Moisture content: -

The moisture content in the freeze dried kits intended for ^{99m}Tc–radiopharmaceuticals was determined by heating the freeze dried solid in vacuum oven at 50°C for 7 days. All the kits have ≤ 2% water content of the dry residue as reported by El–Sayed (1989).

I.10.2. Sn (II) Content:

The Sn(II) of the freeze-dried kits intended for reduction of $^{99m}TcO^-_4$ was determined by iodometric titration using standard KIO_3 solution. This method is a modification of iodometic titration previously reported by El–Kolaly(1983) in using indefinitely stable iodate solution instead of the iodine solution. The accuracy and reproducibility of KIO_3 method gives the actual Sn(II) content used in most of ^{99m}Tc–radiopharmaceuticals as reported by El-Asrag et al(1988).

I.10.3. Radiochemical purity:

It is the proportion of total activity in the desired form, ^{99m}Tc-radiofarmaceuticals contains ^{99m}Tc in the following states: -

(1) ^{99m}Tc-chelates : ^{99m}Tc (+4) bound to the chelate .

(2) ^{99m}Tc-reduced and hydrolyzed(^{99m}Tc-RH), ^{99m}Tc(+4) unbound to the chelate .

(3) Tin colloid (Tc): stannous hydroxide $Sn(OH)_2$ formed by hydrolysis of stannous chloride binds for reduced ^{99m}Tc(+4) to form insoluble stannous hydroxide complex .

(4) Sodium pertechnetate (Na $^{99m}TcO_4^-$): state of ^{99m}Tc before reduction or produced after reoxidation by air.

Different chromatographic methods are used to determine the radiochemical purity of ^{99m}Tc-radiopharmaceuticals as reviewed by Robbins (1984).

Chromatography with paper or thin layer (silica gel) with aqueous solution of 0.9% NaCl is used for checking the presence of non–migrating ^{99m}Tc–labeled particulate materials ^{99m}Tc–chelates and RH ^{99m}Tc. Organic solvents (e.g. methyl alchol, methyl ethyl ketone (MEK) and acetone) used to check the presence of free ^{99m}Tc pertechnetate where the water-soluble complex is retained at the origin and pertechnetate

migrates with the solvent front. For lipid soluble complexes such as ^{99m}Tc–HMPAO ethyl alchole with paper media is used for chromatography, where lipid soluble ^{99m}Tc–HMPAO migrates with solvent front. HPLC is used for separation of labeled stereoisomers from pertechnetate e.g. d,l–HMPAO as described by Ballinger et al (1988). It was reported by Phan etal(1981), that the radiopharmaceuticals preparation should contain more than 95% in the ^{99m}Tc–chelate and less than 5% in the form of tin colloid and free $^{99m}TcO_4^-$.

I.10.4. Sterility, apyrogenicity and undue toxicity:

^{99m}Tc–Radiopharmaceuticals are used for human diagnosis by intravenous injection. These products must be sterile–pyrogen free Sterilization of base kits is done by millipore filtration.

Distribution of the filtrate is done in a laminar flow clean bench. All the base kits for ^{99m}Tc-labeling are tested for sterility with thioglycolate medium and for pyrogen in rabbits according to the U-S-Pharmacopoeia (1985) procedures. The undue toxicity test is performed by injection of ^{99m}Tc– complex in the tail vein of mice. No death or abnormal reaction was observed after injection. This test is a quality control to assure that the product is safe for human application and non-toxic.

Chapter II

Synthesis of ortho, meta and para-amino hippuric acid analogs for kidney functions studies

II.1 Introduction

Renal structure can be investigated in great detail using ultrasound and X-Ray methods, but functional evaluation of the kidney and urinary tract needs complementary radionuclide methods. Many different radionuclides and techniques used in renal function studies in nuclear medicine are urinary or plasma clearance measurements, renal imaging using the gamma camera at different time intervals after a single dose injection or dynamic scintigraphy, i.e. recording the change of radioactivity in kidneys with time. Renal functions involve excretory, regulatory and endocrine processes. The physiology of renal excretory functions in rather complex and stills the target of intensive research. It consists of a varity of discrete processes glomular filtration, tubular secretion, tubular reabsorption at different refraining level as shown in Fig(11).

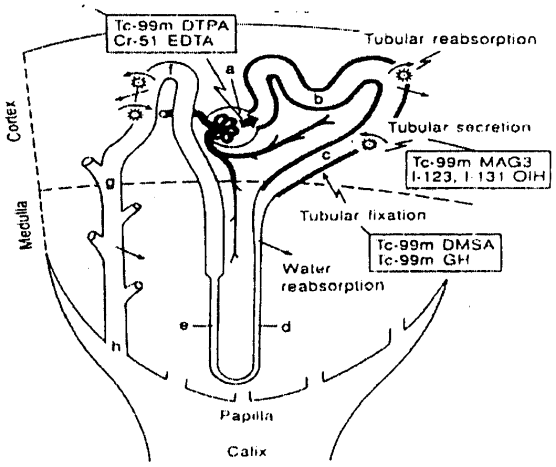


Fig (11): Schematic drawing of the nephron, indicating some features of renal function

The radionuclides in clinical use provide information about these discrete processes since they are handled in the kidney by different mechanisms and at different levels of the nephron.

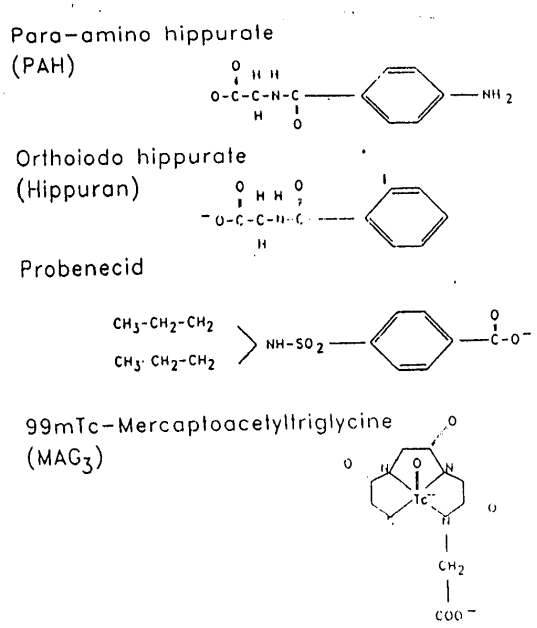
Renal radionuclides can be divided into four main groups:

1-Those which are used to measure renal plasma flow.

2-Those which are used for measuring glomerular filtration.

3-Those which are used for studying renal mass by tubular fixation of traces in the parenchyma.

4-Those with special immunological and other features, the excretion mechanism of radionuclides used in studies of renal function are not always completely known. For example, hippuran (OIH), para amino hippuric acid (PAH) and mercaptoacetyltriglycine (MAG_3) are excreted both by glomerular filtration and tubular secretion at different rates for each of the particular substances as shown in Fig(12).



Fig(12): Structure formula for some organic anions secreted by the renal tubules.

PAH was first shown to be excreted by the kidney so effectively that there is very low residual concentration in the renal venous blood. This high renal extraction fraction of PAH makes it suited to measure the renal plasma flow (RPF), so it is used as a replacement of hippuran to a certain extent. Loberg et al (1976) reported the importance iminodiacetic acid (I) as a chelating agent capable of binding reduced technetium-99m and incorporating it into biologically active molecules. This resulted in the preparation of new radiopharmaceuticals especially the ^{99m}Tc-N(2,6-dimethylacetanilido)iminodiacetic acid (HIDA) complex which used for hepatobillary system studies. Para-aminohippuric acid (PAH) is a chemical analogue of hippuran, which used to measure the renal plasma flow. Hippuran labeled by radioiodine (^{131}I,^{123}I) was used for kidney function studies. It is difficult to label hippuran by ^{99m}Tc, due to the lack of donner atoms or chelating groups to bind ^{99m}Tc.

A completely different approach to ^{99m}Tc-labeled renal function agent was the development of iminodiacetic acid derivatives of para aminohippuric acid for ^{99m}Tc-labeling as prepared by Zmbova et al (1989). The iminodiacetic acid (IDA), a side chain was coupled to aminohippuric acid molecule to bind to technetium-99m. The recommended method for coupling IDA side chain to amino-hippuric acid was reported by Burns's et al (1978) and Nunn et al (1983). Iminodiacetic acid derivative of para-aminohippuric acid ligand synthesized by the condensation of equimolar amount of nitrilotriacetic acid with p-amino hippuric acid in the presence of acetic anydride in pyridine solvent as it has been reported by Chervu et al (1984). Nitrilotriacetic monoanhydride is the acylating species produced in situ by the reaction between acetic anlydride and nitrilotriacetic acid. The scheme of synthesis were shown in Fig (13).

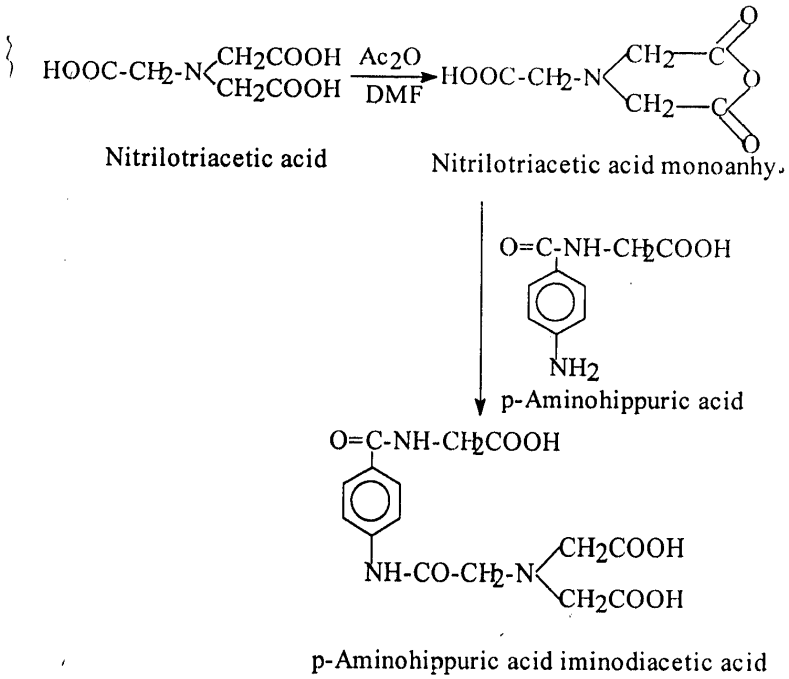


Fig (13): Scheme of synthesis of p-[(bis carboxymethyl)-aminomethyl carbamino]hippuric acid (PAHIDA)

In the present study, the synthesis of o, m and p-amino-hippuric acid analogs is described. The purity of the synthesized products is characterized by physicochemical methods such as the determination of m.p, I.R, ^{1}HNMR, and mass spectroscopy.

II.2 Experimental:

II.2.1 Chemicals and reagents: -

Unless otherwise stated, all chemicals and laboratory reagents used in this work are of the highest purity grade. In all cases, the water used is deoxygenated bidistelled water. They are arranged alphabetically as follows: -

1. Acetone, BDH product.
2. Benzoyl chloride, 98%, Aldrich chemicals.
3. Ethyl Alcohol, 96%, Cambanion chemicals.
4. Glycine, Aldrich chemicals.
5. Hydrochloric acid, AR reagent 35%, BDH product.
6. Methyl ethyl Ketone (MEK), Reidel De Han.
7. Nitrilotriacetic acid, Sigma product.
8. Pyridine, 79%, Merck, Germany.
9. Sulphuric acid, AR reagent 96%, BDH product.
10. Sodium chloride, AR Prolabs, Paris.
11. Nitric acid A.R., 70%, BDH product.
12. Para-nitrobenzoic acid and ortho-nitrobenzoic acid, Sigma product.
13. Stannous chloride., granular, 20 mesh-Mallinckrodt

II.2.2 Equipment: -

1-Infrared spectrophotometer FT-IR BOMEM Hart man Braun model: MBB 157, Canada.

2-Nuclear magnetic resonance; ^{1}H-NMR, Jeol Ex- 270 using tetra methylsillane (TMS) as an internal standered.

3-Mass spectrometer: GCMS-QP 100Ex.Schimadzu gas chromatography MS.

4-Gamma counter SR-7 Nuclear enterprises Ltd.

5-pH meters, Orion Research Incorporated, model 230 A, Boston, USA.

6-Sensitive Balance: Precision Electronic Balance, model: HA 120 MA A&D company, limited Japan.

7-Shaking water bath: Model – 127, Fisher Scientific, USA.

II.3. Methods: -

II.3.1. Synthesis of benzoylglycine (hippuric acid):

All chemicals synthesis was carried out in fume cupboard, hippuric acid was prepared according to Vogel's method (1986) as follows: -

Dissolve 25g (0.33 mol) of glycine in 250 ml of 10% sodium hydroxide solution contained in a conical flask. Exactly 54g (45ml, 0.385mol) of benzoyl chloride was weighed then added in five portions to the solution. Stopper the vessel and shake vigorously after each addition until all the chloride has reacted then the solution was transferred to a beaker and rinsed the conical flask with a little water. A few grams of crushed ice was placed in the solution and concentrated hydrochloric acid was added slowly with stirring until the mixture was acid to congored paper. Collect the resulting crystalline precipitate of benzoyl glycine, (hippuran) which was contaminated with a little of benzoic acid, upon a buchner funnel, and washed with cold water and drain well. Place the solid in a beaker with 100 ml of carbontetrachloride. The beaker was covered with a watch glass and boil gently for 10 minutes.

This extract any benzoic acid, which may be present as impurities. The mixture was allowed to cool slightly then filter under gentle suction and wash the product on the filter with 10-20ml carbontetrachloride. Recrystallise the dried product from boiling water (about 500ml) with the addition of a little decolourizing charcoal, filter through a hot water funnel and allow to crystallise. Benzoylglycine (hippuric acid) was collected in a buchner funnel. A white crystals were obtained upon drying in an oven.

II.3.2.Synthesis of ortho, meta and para-nitrohippuric acid:

II.3.2.1. Synthesis of ortho and para-nitrohippuric acid:

Ortho and para-nitrohippuric acid derivatives were prepared according to the method reported by Chervu et al (1984).

II.3.2.1.1 Ortho-nitrohippuric acid:

The method of preparation can be summarized as follows:

A suspension of o-nitrobenzoic acid (6.685g) in 40 ml of benzene was refluxed with 4ml thionylchloride (SO_2Cl_2) for 4.5h, the clear solution was flashed evaporated and the resulted yellow liquid was added dropwise to a solution of glycine (3.75g) and sodium carbonate (5.3g) in 40ml of H_2O. The mixture was stirred at room temperature for 4 h and diluted with 50ml of H_2O. Solution was filtered and the filtrate was acidified using conc. HCl. The formed solid was filtered off and crystallized from ethylacetate - pentane.

II.3.2.1.2 Para-nitrohippuric acid:

The method of preparation can be summarized as follows:

A suspension of p-nitro-benzoic acid (6.685g) in 40 ml of benzene was refluxed with 4ml thionylchloride (SO_2Cl_2) for 4.5h, the clear solution was flashed evaporated and the resulted yellow liquid was added dropwise to a solution of glycine (3.75g) and sodium carbonate (5.3g) in 40ml of H_2O. The mixture was stirred at room temperature for 4h and diluted with 50ml of H_2O, solution was filtered and the filtrate was acidified using conc. HCl. The formed solid was filtered off and crystallized from ethyl acetate-pentane

II.3.2.3. Synthesis of meta- nitrohippuric acid:

m-Nitrohippuric acid was prepared from methylhippurate as reported by Vogel's method (1986), because m-nitrobenzoic acid was not available commercially. Methylhippurate was prepared from hippuric acid by estrification as follows:

In a 500 ml round bottomed flask, a mixture of 44.02 g (0.246 mol) hippuric acid, 80g (101ml, 2.5 mol) of absolute methanol and 5g (2.7 ml) of concentrated sulphuric acid were placed. A few small chips of porous porcelain were added. Attach a reflux condenser and boil the mixture gently for 4 h. The excess of alcohol was distilled off on a water bath. Allow the mixture to cool. The residue was poured into about 250 ml of water contained in a separatory funnel and rinse the flask with a few mls of water which are also poured into the separatory funnel. Add 10-15 ml of carbon tetrachloride and shake the mixture in the funnel vigorously; upon standing the heavy solution of methylhippurate ester was separated from the water layer. Run off the lower layer carefully containing the ester and reject the upper aqueous layer. Return the methylhippurate ester to the funnel and shake it with a strong solution of sodium hydrogen carbonate until all free acid is neutralized and no further evaluation of carbon dioxide occurs. Wash once with water, and dry by pouring into a small dry conical flask containing about 5 g of magnesium sulfate to remove the residual water content. Stopper the flask, shake for about 5 min and allow to stand for at least half an hour with occasional shaking. Filter the methylhippurate ester solution through a small filter paper directly into a round bottomed flask fitted with a still-head carrying a 360 ^{o}C thermometer and an air condenser. Add a few boiling chips and distilled from an air bath, raise the temperature slowly at first until all carbontetrachloride has passed over and then heat more strongly. Collect the methylhippurate.

Nitration of methylhippurate:

In a litre round-bottomed three necked flask fitted with a mechanical stirrer and a thermometer, 16.79 g (94 ml, 0.75 mol) of pure methylhippurate was placed. A nitration mixture of 15.6 ml of concentrated sulphuric acid and 62.5 ml of concentrated nitric acid was prepared in a dropping funnel. The flask cooled in an ice bath to 0-10 °C and then run in the nitrating mixture with stirring whilst maintaining the temperature of the reaction mixture between 5 and 15 °C. The addition required about one hour, the stirring was continued for 15 min and after that reaction was poured upon 700 g of crushed ice.The crude, methyl m-nitrohippurate was filtered off and washed with cold water. The solid transferred to a 500ml bolt-head flask and stirred it with 100 ml of ice-cold methanol in order to remove a small amount of the ortho isomer and other impurities. The cooled mixture was filtered with suction then washed with 50 ml of ice-cold methanol and dried in the air. The practically colorless methyl m-nitro hippurate was obtained which was converted to m-nitrohippuric acid according to Vogel's method (1986).
The procedure of de-estrification can be summarized as follows:

Place 132.8 g (0.5 mol) of methyl m-nitrohippurate and 40 g of sodium hydroxide in 160ml water in an one litre round-bottomed flask equipped with a reflux condenser. Heat the mixture to boiling until the ester has disappeared. Dilute the reaction mixture with an equal volume of water. The diluted reaction mixture was left to cool and poured with vigorous stirring into 125 ml of concentrated hydrochloric acid. Allow to cool to room temperature, filter the crude acid and wash it with a little water. Upon drying at 100 °C the crude m-nitrohippuric acid was obtained. The product was purified by recrystallization from 1% of hydrochloric acid to obtain the pure acid.

II.3.3. Synthesis of o, m and p-aminohippuric acid:

All derivatives of aminohippuric acid were prepared by reduction of nitro group of o, m and p-nitrohippuric acid using paladium/charcoal (Pd/C).

II.3.3.1. Reduction of nitro group using Pd/C method:

o, m, and p-derivatives of amino hippuric acid were prepared following the method reported by Bhargava et al (1988) where the nitro group was reduced by hydrogen gas using Pd/C as catalyst.

The method was carried out as follows:

A solution of nitro-hippuric acid (4.48g) in 100 ml of ethanol was hydrogenated at 30-Psi pressure over 0.2g Pd/C as catalyst. The reaction was completed after two hr and the catalyst was removed by filtration. The filtrate was evaporated and crystallized from alcohol to obtain white crystals of amino-hippuric acid.

II.3.4. Synthesis of iminodiacetic acid derivatives of ortho, meta and para-aminohippuric acid analogs: -

II.3.4.1 Mitta's method:

Iminodiacetic acid derivatives of amino hippuric acid were synthesized according to the procedure of Mitta et al (1982) and it can be summarized as follows:

Nitrilotriacetic acid 5g (26.2m mol) was suspended in 70 ml pyridine in a 250 ml round bottomed flask fitted with nitrogen inlet water condenser coupled to calcium sulphate drying tube and a thermometer. The flask was heated in an isomental provided with a regulator. The suspension was warmed till the nitrilotriacetic acid was completely dissolved. After cooling, 3.3 ml of acetic anhydride was rapidly introduced and the content was heated at 100 °C for 1h. To get

nitrilotriacetic acid monoanhydride intermediate. After cooling to about 50°C, 4ml of amino-hippuric acid derivatives was added and the contents were then heated at 100 °C for another 1h. The temperature must not exceed 100 °C. The reaction mixture was cooled and pyridine was evaporated under reduced pressure. The yellow oily residue was taken in 30 ml of double distilled water and washed several times with diethyl ether. The resulting solution was filtered under suction through a bed of activated charcoal. The solution was acidified with conc HCl and the precipitate obtained was separated by centrifugation. The dissolution and reprecipitation steps were repeated several times. The final precipitate obtained was recrystallized from 85% ethanol to give amino hippuric acid iminodiacetic acid (AHIDA) derivatives.

II.3.4.2. Burns's method: -

This method reported by Burns's et al (1978). Hot solution of 0.485g (2.5 m mol) aminohippuric acid derivatives in 100 ml acetonitrile was added to a solution of 0.649 g (3.75m mol) freshly prepared nitrilotriacetic acid monoanhydride (NTAA) in 5 ml of acetonitrile. The reaction mixture was refluxed for 3h, and then cooled. The separated solid was filtered off to give aminohippuric acid iminodiacetic acid (AHAIDA) derivatives.

II.3.Results and discussion

Ortho, meta and para-aminohippuric acid iminodiacetic acid (O, M and PAHIDA) analogs have been synthesized by the coupling of o, m and p-aminohippuric acid with nitrilotriacetic acid monoanhydride. The characteristics of the synthesized products were carried out using different analytical techniques in order to insure and guarantee that the products were of the highest purity. These products are used for in-vivo diagnostic applications for renal functions measurements after being labelled with ^{99m}Tc. A high labelling yield and in-vitro stability are achieved by reduction of heptavalent $^{99m}TcO_4^-$ following by complexation of reduced technetium with o, m and p-aminohippuric acid iminodiacetic acid analogs.

The characteristics of the synthesized iminodiacetic acid derivatives are gained much attention since these products will injected i.v. after labelling with ^{99m}Tc, therefor the characteristics of the end products are studied in details. Different analytical techniques are used in the characterization studies such as:

- Melting point,
- Elemental analysis,
- I.R spectroscopy,
- ^1H-NMR spectroscopy,
- Mass spectroscopy.

II.3.1 Characterization of the synthesized benzoyl glycine (Hippuric acid).

This compound was synthesized by reacting benzoyl chloride with glycine. This reaction was took place according to the following scheme:

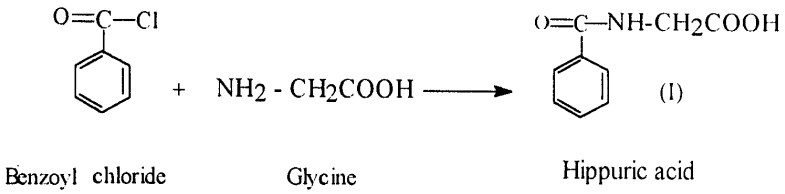


Fig (14): Scheme of synthesis of benzoyl glycine (Hippuric acid)

The desired hippuric acid analog was obtained in 71.5% yield. The structure of hippuric acid analog was confirmed by elemental analysis, melting point, infrared, ^{1}H-NMR and mass spectroscopy. Melting point was found to be 189^{0}C which is incomplete agreement with the data reported by Vogel (1986). The chemical formula can be presented as follows: $C_9H_9NO_3$, M.Wt. 179 and the following is the result of elemental analysis of the synthesized hippuric acid :

	C%	H%	N%
Calculated	60.33	5.03	7.82
Found	60.01	5.00	7.70

IR spectroscopic investigation of hippuric acid analog (Fig 15) showed the absorption vibration band as follow:

3200-3400 cm^{-1} (broad OH of carboxylic acid group)
3309 cm^{-1} (NH group)
3010 cm^{-1} (C-H of aromatic ring)
1700 cm^{-1} (carbonyl of carboxylic acid group)
1660 cm^{-1} (CONH group)
1535 cm^{-1} (C=C aromatic)

The ^{1}H-NMR spectrum of hippuric acid analog (DMSO) expressed in δ ppm scale showed the protons of methylene groups appeared at 3.5ppm {s, 2H, CH_2-COOH}, signal of aromatic protons appeared at 7.1-7.3ppm as multiplet {m, 5H, aromatic protons } and signal of NH group appeared at 9.5 ppm {s, 1H, NH} as represented in Fig(16).

The mass spectrum of hippuric acid analog showed the molecular ion peak at m/z 179, 100% which is the base peak , which losses hydroxyl radical to give the molecular ion (b) at m/z 162 {M^+,$C_9H_8NO_2$,50.3%}, cation (b) losses carboxyl group (CO) to give cation (c) at m/z 134 { M^+ ,C_8H_8NO, 27.5% } which losses methylene group to give cation (d) at m/z 120 {M^+ ,C_7H_6NO,11.2%}. Cation (d) loss NH group to give cation (e) at m/z 105{M^+, C_7H_5O, 25.3%} as shown in Fig (17,18).

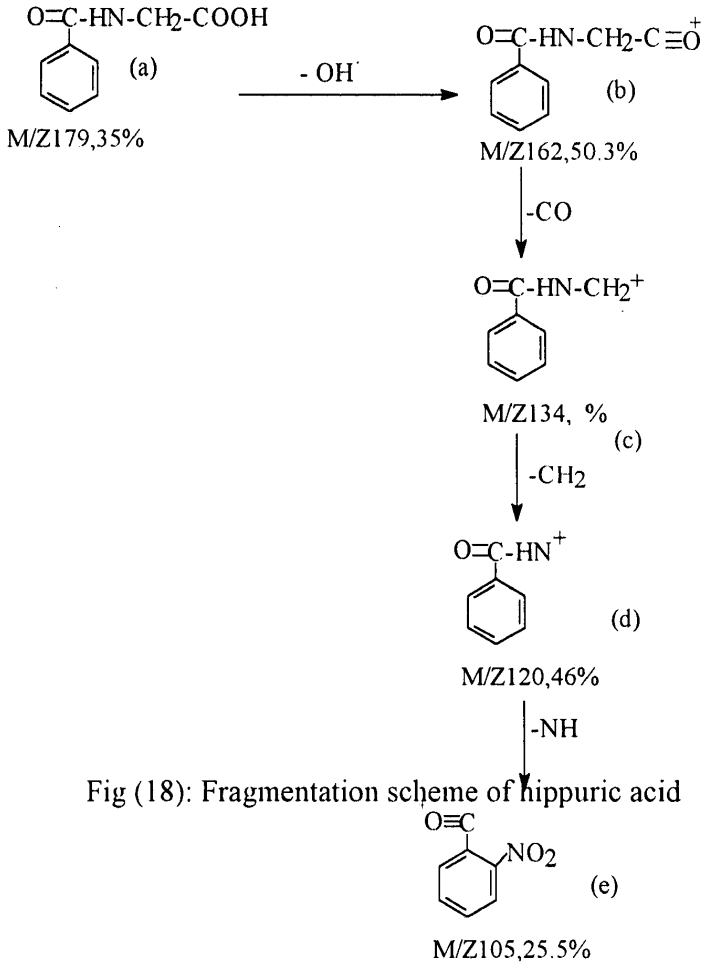


Fig (18): Fragmentation scheme of hippuric acid

II.3.2. Characteristics of the synthesized ortho, meta and para-nitrohippuric acid analogs.

II.3.2.1 ortho-Nitrohippuric acid analog.

ortho-Nitrohippuric acid was synthesized by the reaction of ortho-nitrobenzoic acid with glycine in presence of thionyl chloride. This reaction was took place according to the following scheme:

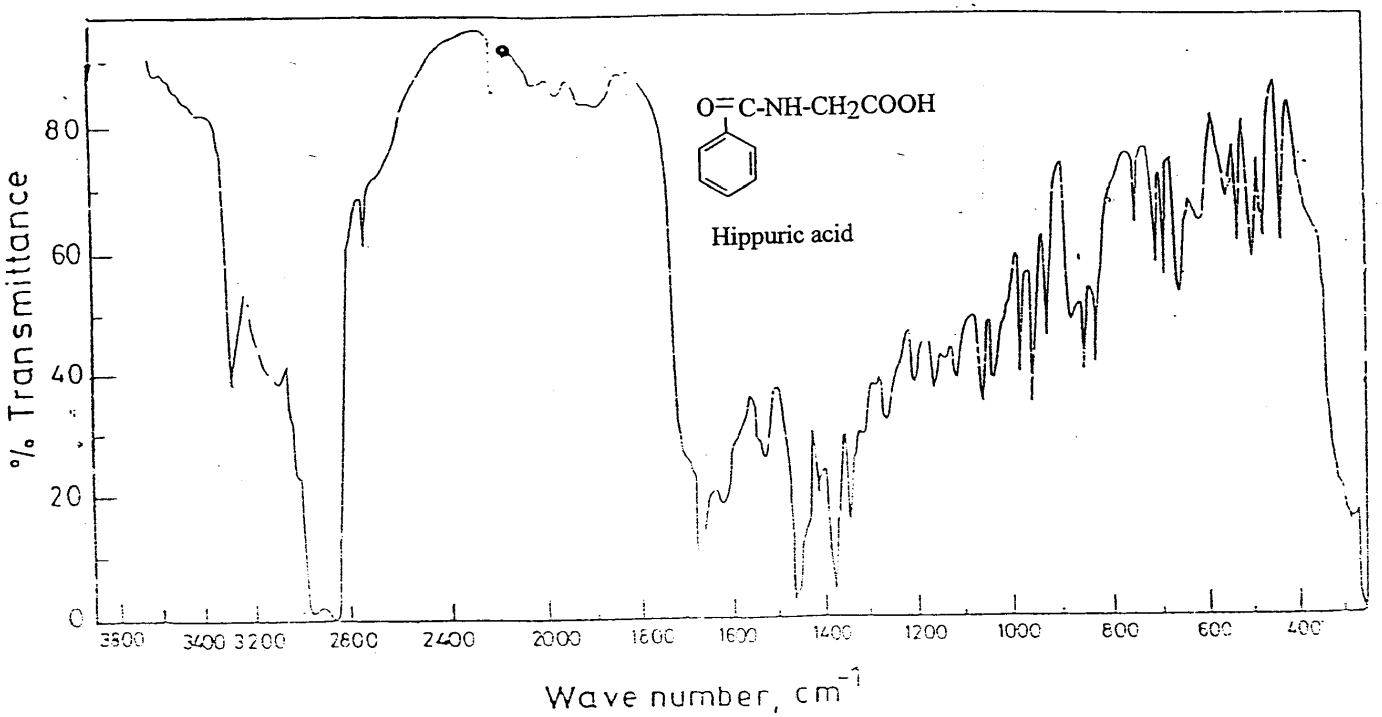


Fig (15): IR spectrum of hippuric acid

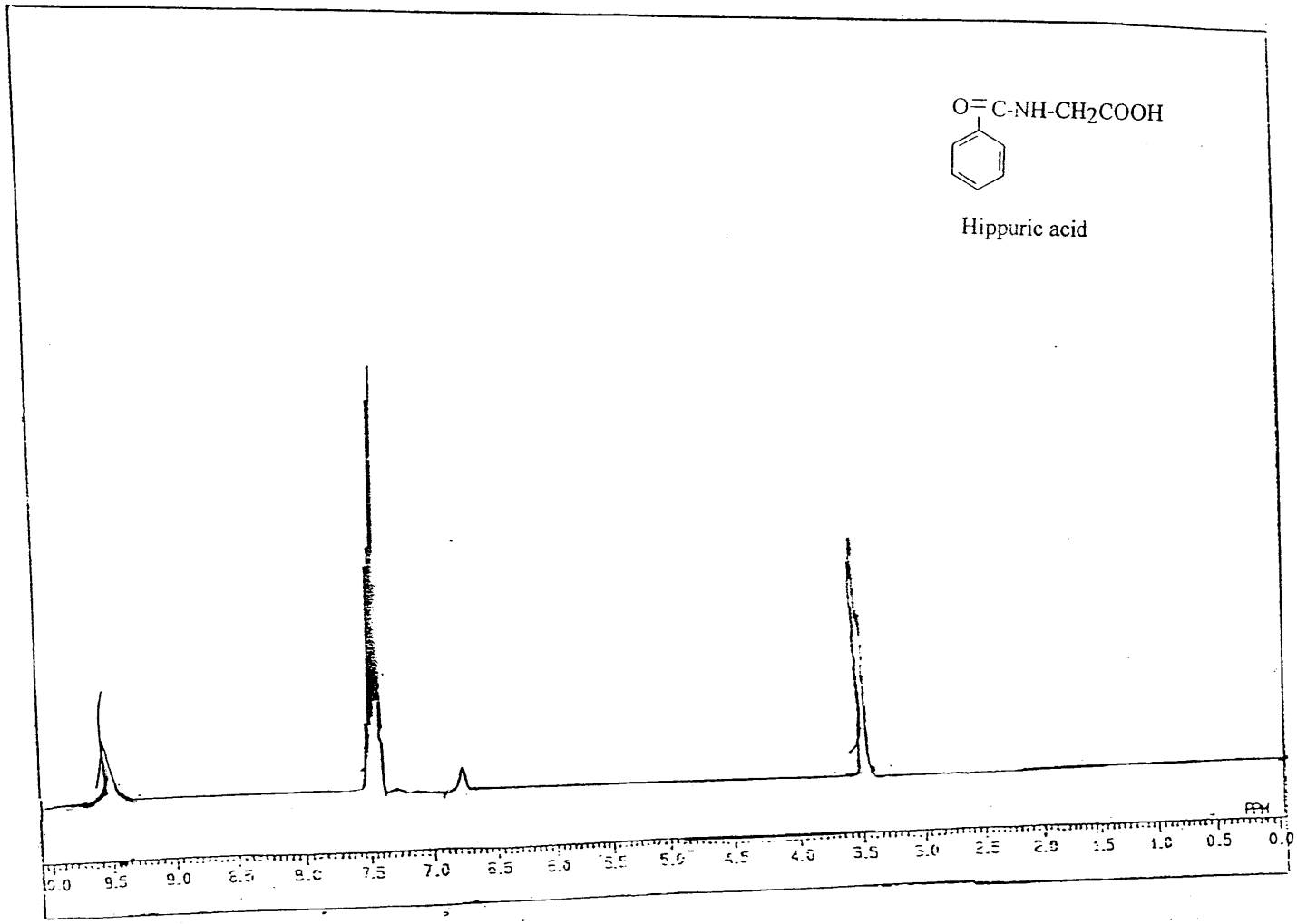


Fig (16) : H, NMR spectrum of lippuric acid.

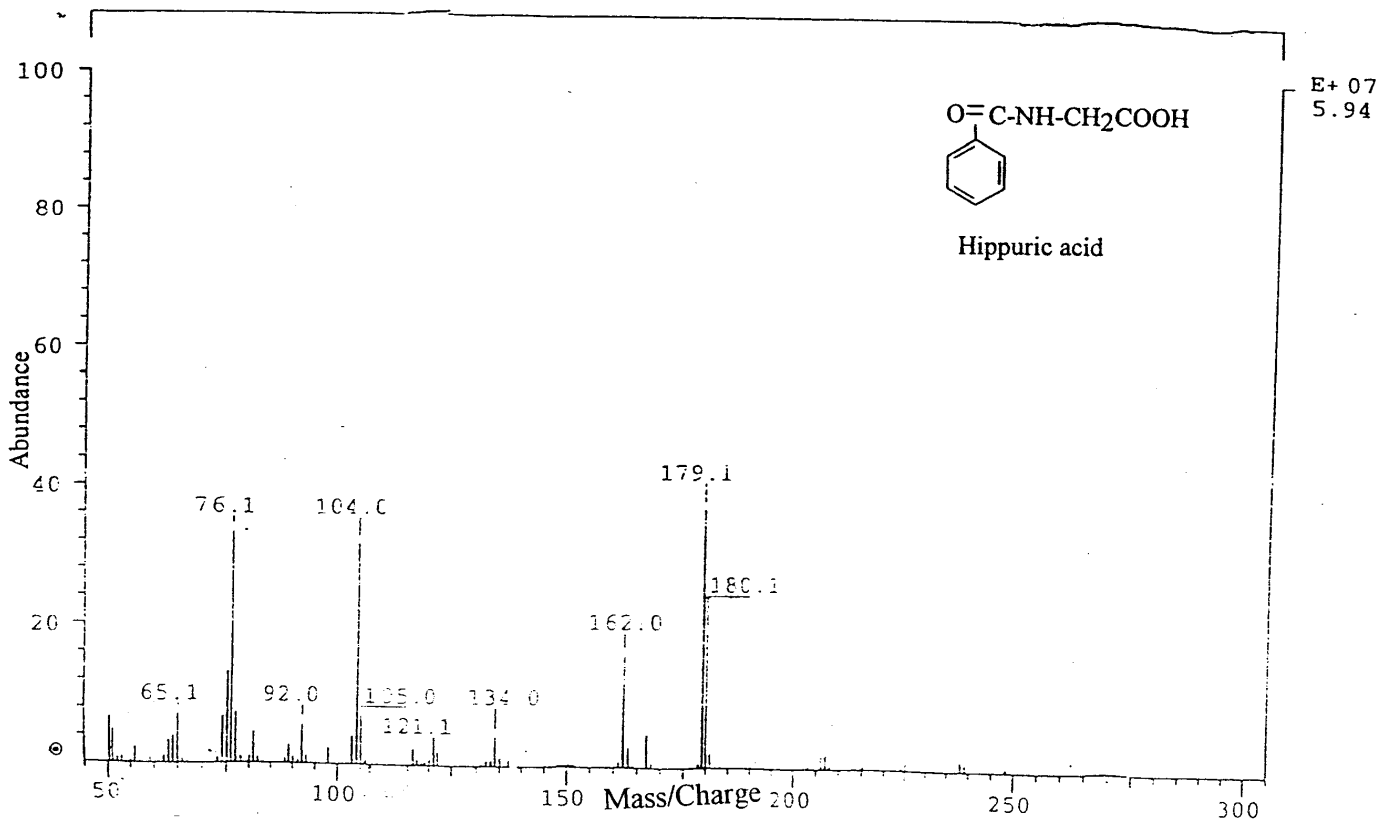
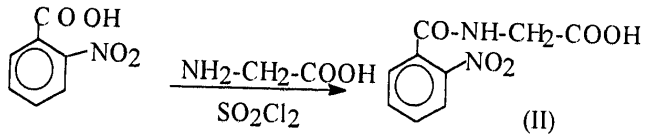


Fig (17): Mass spectrum of hippuric acid



Ortho-nitrobenzoic acid

Ortho-nitrohippuric acid

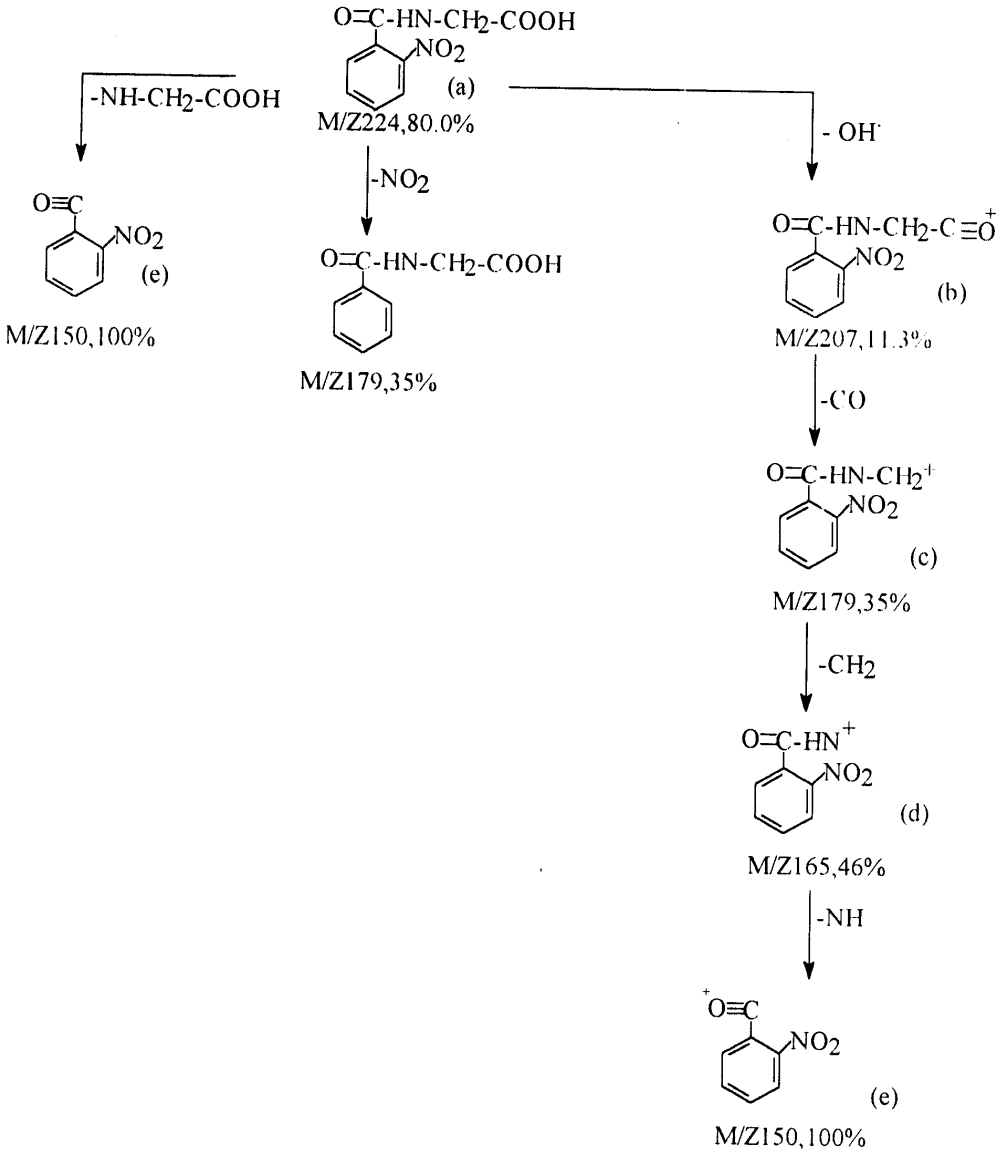
Fig(19): Scheme of synthesis of ortho-nitrohippuric acid analog.

The desired ortho-nitrohippuric acid analog was obtained in 55% yield as white crystal , its melting point equal 192^{0}C which is in complete agreement with the data published by Bhargava et al (1988). The chemical formula can be presented as follows: $C_9H_8N_2O_5$, M.Wt. 224, and the following is the result of elemental analysis of the synthesized ortho-nitrohippuric acid analog:

	C%	H%	N%
Calculated	48.21	3.57	12.5
Found	48.20	3.51	12.5

The mass spectrum of ortho-nitrohippuric acid analog (II) showed the molecular ion peak at m/z 224.1, 80% which losses hydroxyl radical to give cation (b) at m/z 207 {M^+,$C_9H_7N_2O_4$,11.31%} that losses carbonyl group to give cation (c) at m/z 179 {M^+,$C_8H_7N_2O_3$,35.1%}. Cation (c) losses methylene group to give cation (d) at m/z 165 {M^+,$C_7H_5N_2O_3$,46.3%} which losses NH radical to give radical cation (e) at m/z 150 {M^+,$C_7H_4NO_3$,100%} which is the base peak. o-Nitrohippuric acid analog (II) can losses nitro group to give a molecular ion at m/z 179

{M^+,$C_9H_9NO_3$,35.1%} and can also losses NH-CH_2-COOH to give cation (e) at m/z 150 {M^+,$C_7H_4NO_3$,100%} as presented in Figs (20)



Fig(20):Fragmentation scheme of ortho nitrohippuric acid

II.3.2.2 m-Nitro-hippuric acid analog:

Because m-nitro-benzoic acid is not available locally, m-nitro-hippuric acid analog was synthesized by nitration of methyl hippurate as reported by Vogel (1986). This reaction was took place according to the following scheme:

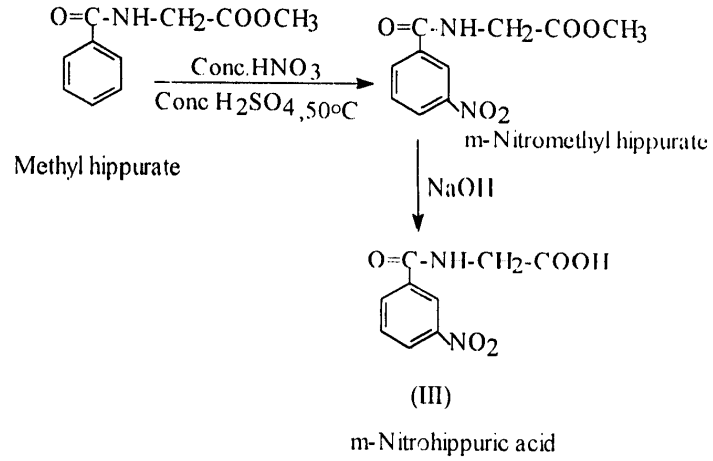
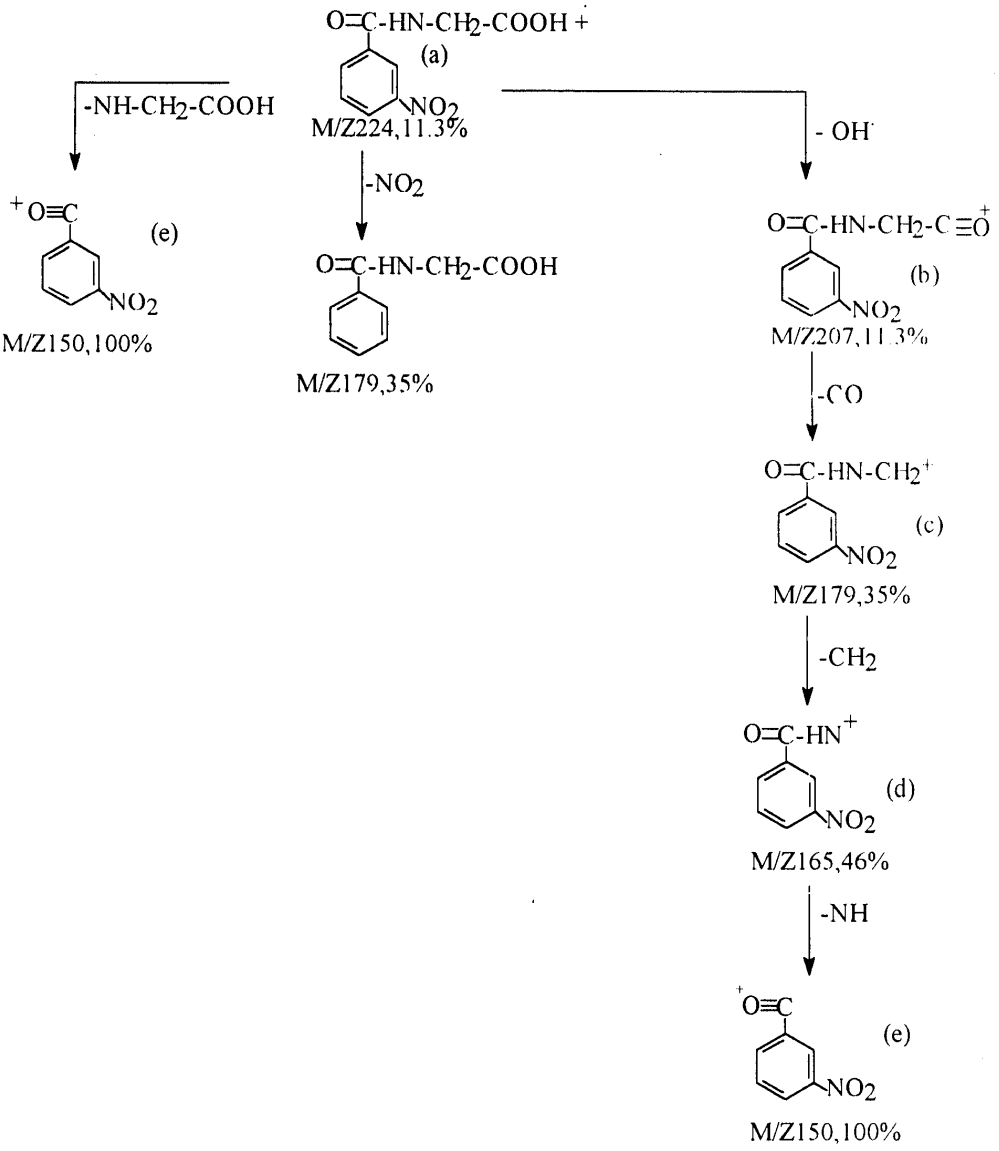


Fig (22) Scheme of synthesis of m-nitrohippuric acid analog

The yield of synthesized of m-nitrohippuric acid analog was found to be 81%. The structure of m-nitrohippuric acid (III) was confirmed by elemental analysis, melting point and mass spectroscopy. Melting point was measured and found to be 161^{0}C which is in agreement with the reported data by Bhargava et al. (1988). The chemical formula can be presented as follows: $C_9H_8N_2O_5$, M.Wt. 224 and the following is the results of elemental microanalysis of the synthesized of m-nitrohippuric acid analog

	C%	H%	N%
Calculated	48.21	3.57	12.50
Found	48.23	3.50	12.54

The mass spectrum of m-nitrohippuric acid is similar to o-nitrohippuric acid as shown in Fig (23,24).



Fig(23):Fragmentation scheme of m-nitrohippuric acid

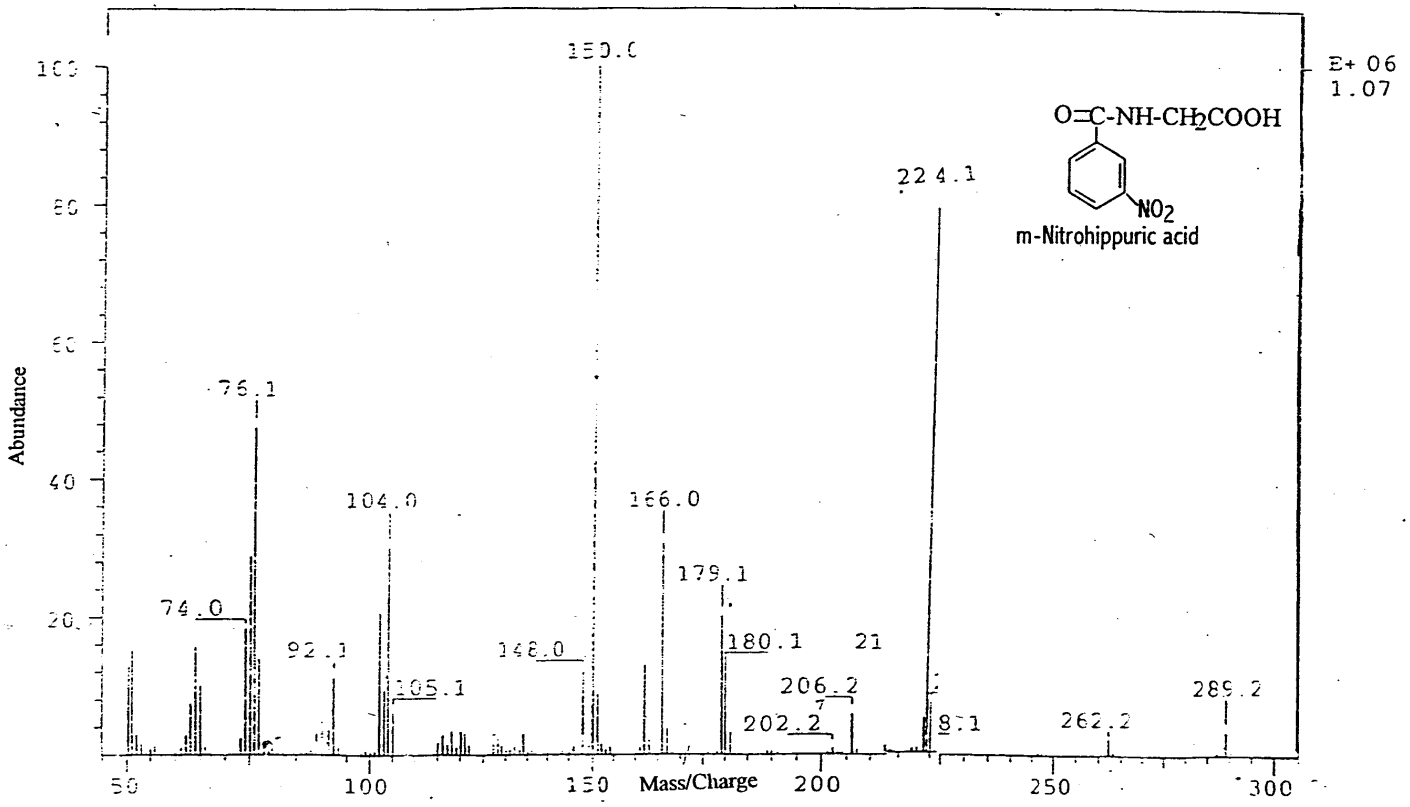
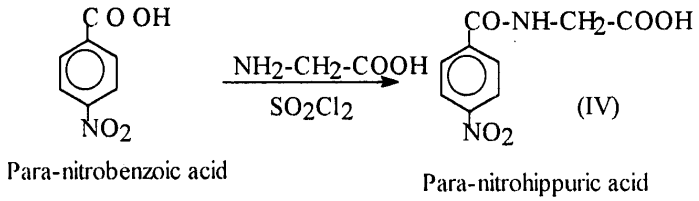


Fig (24) : Mass spectrum of meta – nitro hippuric acid analog.

II.3.2.3 para-Nitrohippuric acid analog:

Para-nitrohippuric acid was synthesized by the reaction of p-nitrobenzoic acid with glycine in presence of thionyl chloride. This reaction was took place according to the following scheme:

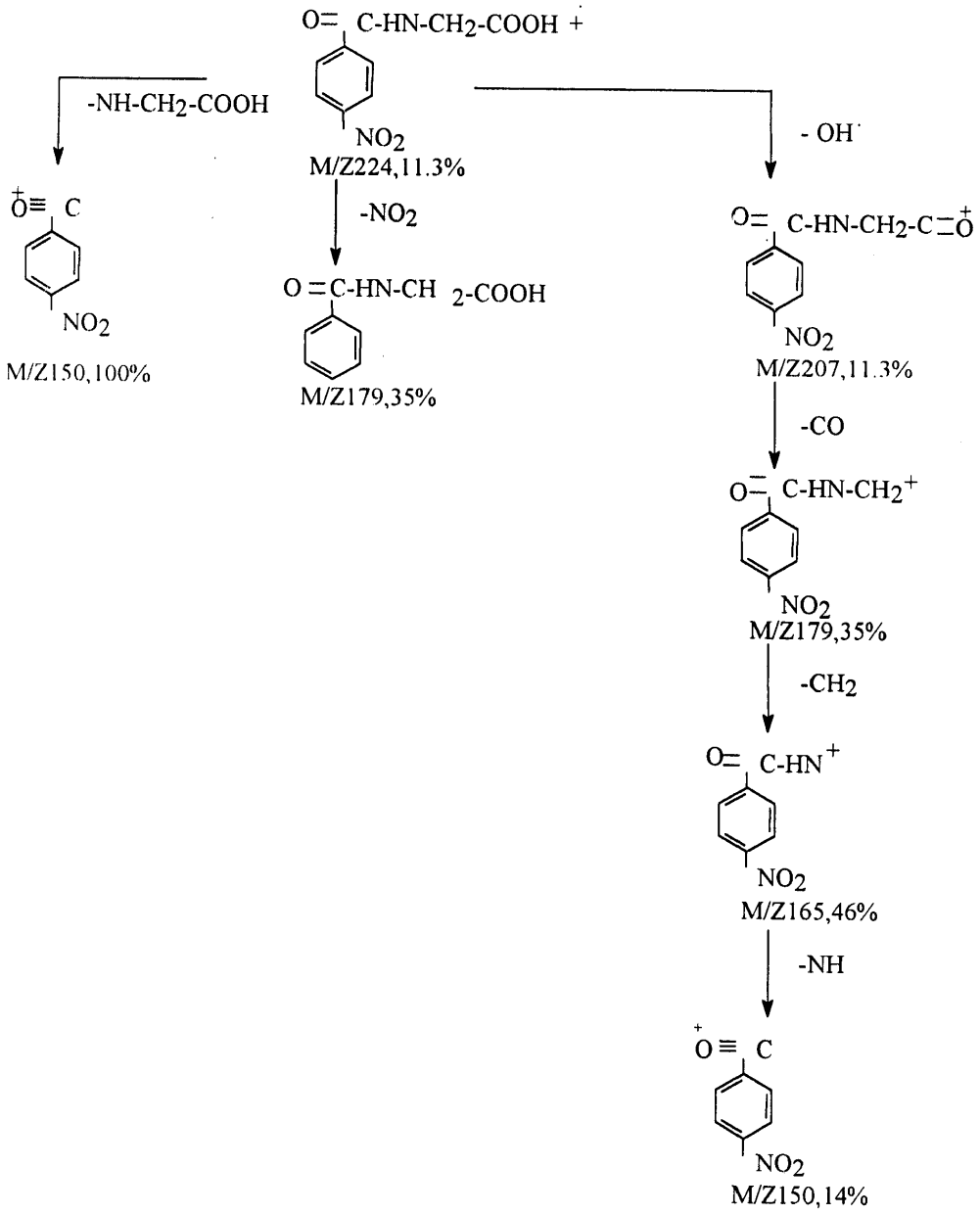


Para-nitrobenzoic acid

Para-nitrohippuric acid

Fig (25): Scheme of synthesis of p-nitrohippuric acid

The yield of synthesized of p-nitrohippuric acid (IV) was equal to 62% as a white crystals. The structure of p-nitrohippuric acid (IV) was confirmed by elemental analysis, melting point and mass spectroscopy. Melting point was fount to be 162^0C which is in agreement with the reported data by Zombova et al.(1989).It was found that the elemental analysis, mass spectrum are similar to o and m-nitrohippuric acid analog as shown in Fig(26,27).



Fig(26):Fragmentation scheme of p-nitrohippuric acid

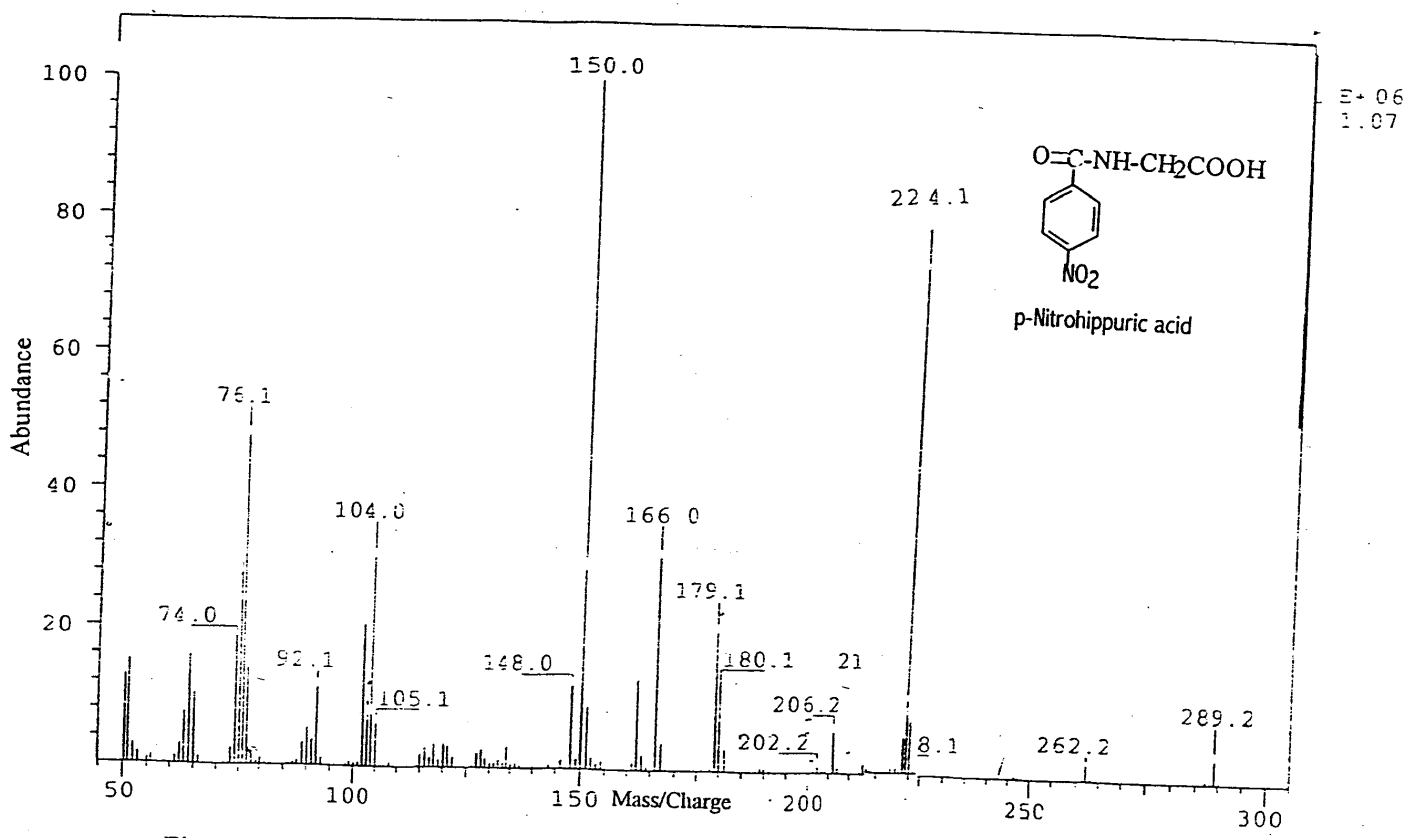
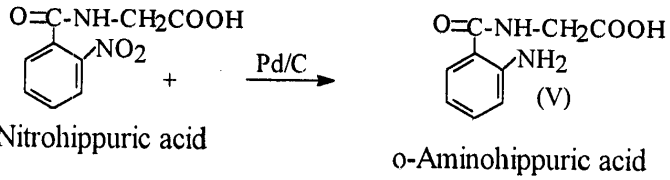


Fig (27) : Mass spectrum of para – nitro hippuric acid analog.

II.3.3 Characterization of the synthesized ortho and meta-aminohippuric acid analog:

III.3.3.1 o-Aminohippuric acid analogs:

ortho-Aminohippuric acid analog was synthesized by reduction of o-nitrohippuric acid by paladium/charcoal, (Pd/C) mixture. The synthesis was carried out according to the following scheme:



o-Nitrohippuric acid

o-Aminohippuric acid

Fig (28): Scheme of synthesis of ortho-aminohippuric acid

The desired ortho-aminohippuric acid analog was obtained in 88% yield. The structure of ortho-aminohippuric acid was confirmed by the determination of elemental analysis and melting point of the obtained product. The measured melting point was equal to 137^{0}C which is compatible with the reported data by Bhargava et al (1988). The chemical formula can be presented as follows: $C_9H_{10}N_2O_3$,M.Wt. 194. Elemental analysis of the compound (V) was carried out and the obtained results are summarized as follow:

	C%	H%	N%
Calculated	55.67	5.15	14.43
Found	55.68	5.16	14.44

The IR spectrum of ortho-aminohippuric acid (V) showed absorption bands at :

3474 cm^{-1} (OH of carboxylic group)

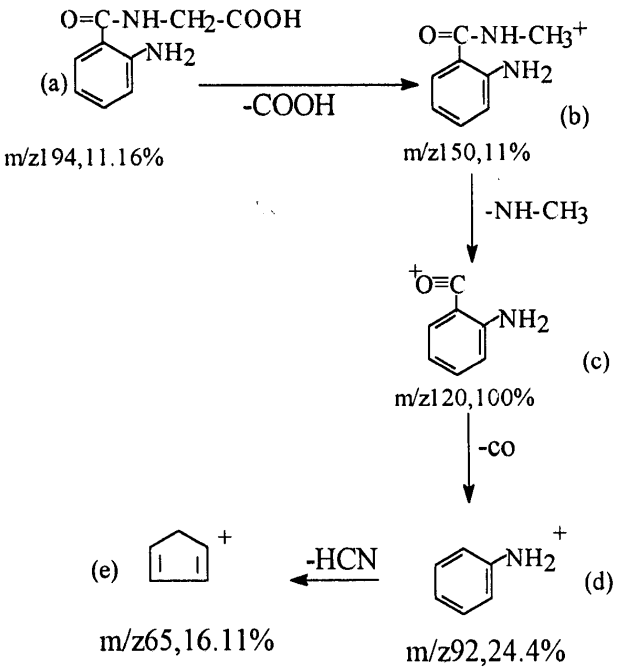
3000 cm^{-1} (C-H aliphatic bond)

1738 cm^{-1} (acidic carbonyl group)

1650 cm^{-1} (amidic carbonyl group)

Fig(29) clearly shows the results of IR analysis of ortho-aminohippuric acid analog (V) .

The mass spectrum of ortho-aminohippuric acid analog (V) showed the molecular ion peak (a) at m/z 194, 11.16% which losses carboxylic acid radical to give cation (b) at m/z 150 {M^+,$C_8H_{10}N_2O$,11%}.Cation (b) losses $NHCH_3$ group to give cation (c) at m/z 120 {M^+,C_7H_6NO,100%} which is the base peak. Cation (c) losses carbonyl group to give cation (d) at m/z 92{M^+,C_6H_6N, 24.2%} which losses HCN to give cation (e) at m/z 65 {M^+,C_5H_5,16.11%}. The results of fragmentation of ortho-aminohippuric acid analog are shown in Fig (30,31).



Fig(30): Fragmentation scheme for ortho-aminohippuric acid

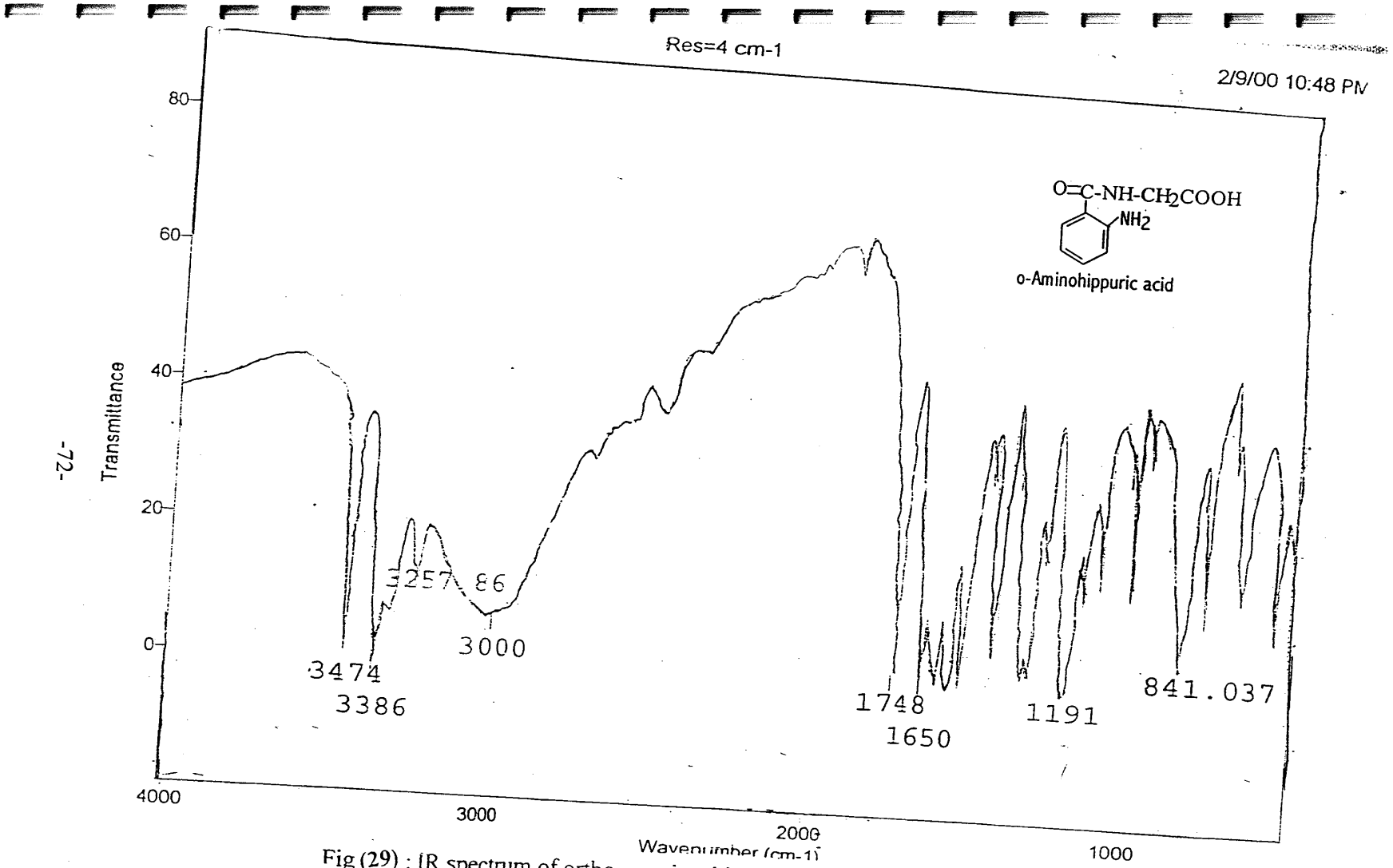


Fig (29) : IR spectrum of ortho – amino hippuric acid.

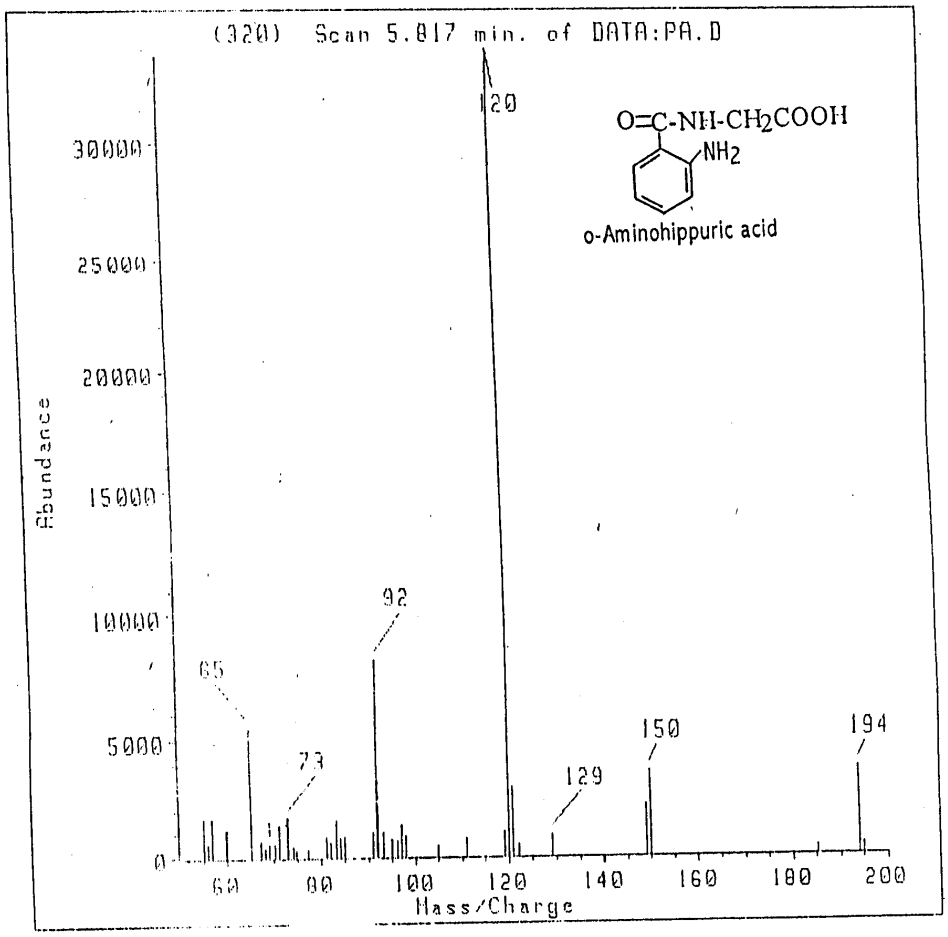
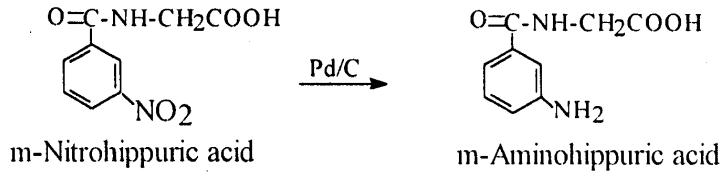


Fig (31) : Mass spectrum of o-amino hippuric acid.

II.3.3.2 meta-Aminohippuric acid analog:

This compound was synthesized by the reduction of m-nitrohippuric acid analog using Pd/C. The reduction took place according to the following scheme:



m-Nitrohippuric acid m-Aminohippuric acid

The desired meta-aminohippuric acid analog was obtained in 75% yield as a white crystal by following the procedure described by Bhargava et al. (1988). The structure of meta-aminohippuric acid analog was confirmed by different analytical techniques. Melting point of the obtained product was found to be 196°C which is similar to the published data reported by Bhargava et al (1988). IR and mass spectra are similar to ortho-aminohippuric acid.

II.3.3.3 para-Aminohippuric acid:

para-Aminohippuric acid was synthesized by the reduction of p-nitrohippuric acid using Pd/C mixture. The reduction can be presented by the following scheme:

p-Nitrohippuric acid p-Aminohippuric acid

Fig. (22): Scheme of synthesis of p-aminohippuric acid

The desired p-aminohippuric acid analog was obtained in 58% yield as white crystals following the procedure described by Zmborva et

al (1989). Melting point of p-aminohippuric acid was found to be equal to 223°C. The synthesized p-aminohippuric acid was identified by IR and mass spectroscopy. Our data are similar to the results of o-amino hippuric acid analog.

II.3.8 Characterization of the synthesized ortho, meta and para aminohippuric acid iminodiacetic acid analogs

II.3.8.1 ortho-Aminohippuric acid iminodiacetic acid analog

The desired o-aminohippuric acid iminodiacetic acid analog (OAHIDA) was synthesized by following a modified procedure of Burn's et al (1978) which involves a condensation reaction between nitrilotriacetic acid momoanhydride and o-aminohippuric acid in pyridine. This reaction was carried out according to the following scheme:

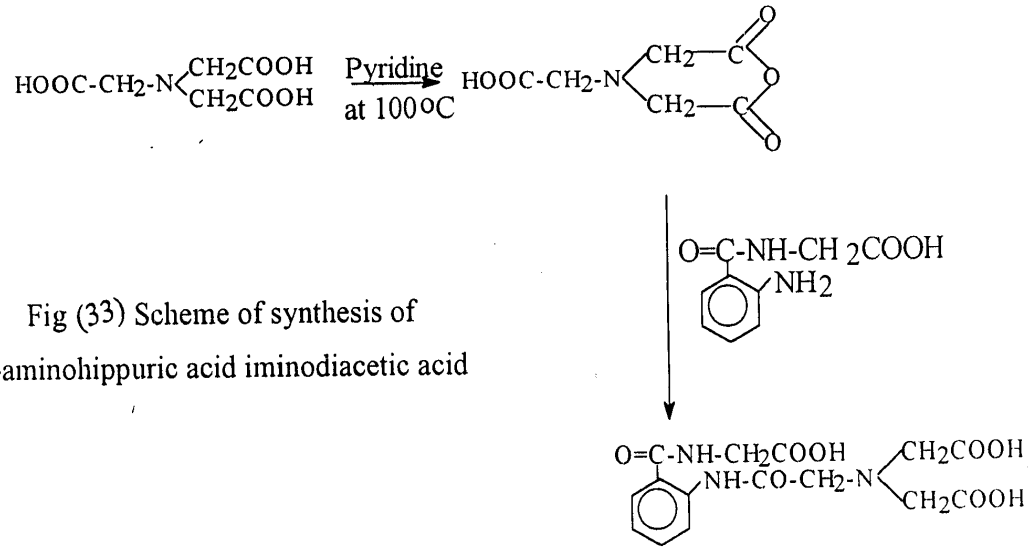


Fig (33) Scheme of synthesis of o-aminohippuric acid iminodiacetic acid

The yield of this reaction was found to be 79%. The previously synthesized OAHIDA was analyzed by different analytical techniques such as the determination of melting point, elemental analysis , ^{1}H-NMR, IR and mass spectroscopy. Melting point was measured and found to be 181°C which is in fair agreement with the previous work reported by Bhargava et al(1988).

^{1}H-NMR spectrum of OAHIDA (DMSO) expressed in δ ppm scale showed the protons of methelene group at 3.4 (s, 2H, NH-CO-CH_2N), protons of other methylene group appeared at 4(s,4H,N(CH_2-COOH)$_2$) and 3.9(s,2H,CO-NH-CH_2COOH), protons of benzene ring appeared at 7-7.5(m, 4H, aromatic protons) and the proton of NH appeared at 10 (s, 1H, NH) as shown in Fig (34)

Microanalysis results of $C_{15}H_{17}N_3O_8$, ortho-aminohippuric acid iminodiacetic acid analog (OAHIDA) was as follows :

	C%	H%	N%
Calculated	49.05	4.60	11.44
Found	48.90	4.58	11.28

The IR spectrum presented in Fig (35) showed the absorption bands as follow:

3433 cm^{-1} (OH of carboxylic group)
3041 cm^{-1} (C-H aromatic)
3000 cm^{-1} (C-H aliphatic)
1738 cm^{-1} (carbonyl of carboxylic group)
1650 cm^{-1} (amidic carbonyl group)

The mass spectrum of OAHIDA analog showed the molecular ion peak at m/z 367, 76% which losses water molecule to give radical cation (b) at m/z 349, {M^+,$C_{15}H_{16}N_3O_8$,53%} which undergo cleavage at x axis to give cation (c) at m/z 236 {M^+,$C_{11}H_{12}N_2O_4$,22.3%}.Cation (c) losses methyl radical to give radical cation (d) at m/z 221{M^+,$C_{10}H_9N_2O_4$,35.1%} which losses carbonyl group to give radical cation (e) at m/z 193 {M^+,$C_9H_9N_2O_3$,21.3%}. Radical cation (e) losses NH radical to give cation (f) at m/z 179 {M^+,$C_9H_9NO_3$, 18.9%} .The radical cation (a) undergo cleavage at y axis to give cation (g) at m/z 293 {M^+,$C_{13}H_{13}N_2O_6$,59%}. Radical cation (a) losses carboxylic acid radical to give cation (h) at m/z 322{M^+,$C_{14}H_{16}N_3O_6$, 31.6%}. Cation (h) losses methylene group to give cation (i) at m/z 308 {M^+,$C_{13}H_{14}N_3O_6$,29.6%} as shown in Figs (36,37).

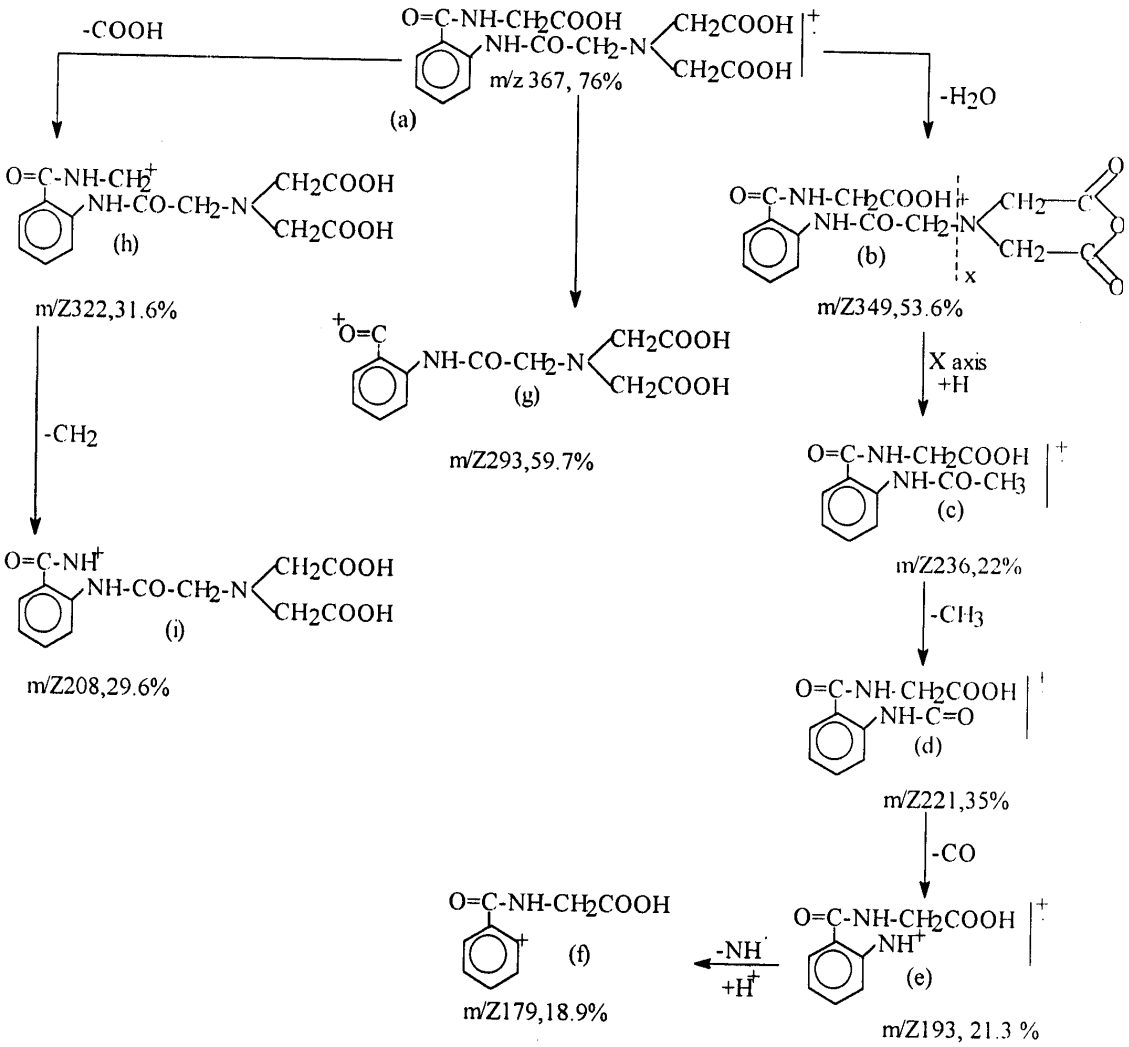


Fig (36): Fragmentation of OAHIDA analog.

Fig (34) : H NMR spectrum of OAHIDA.

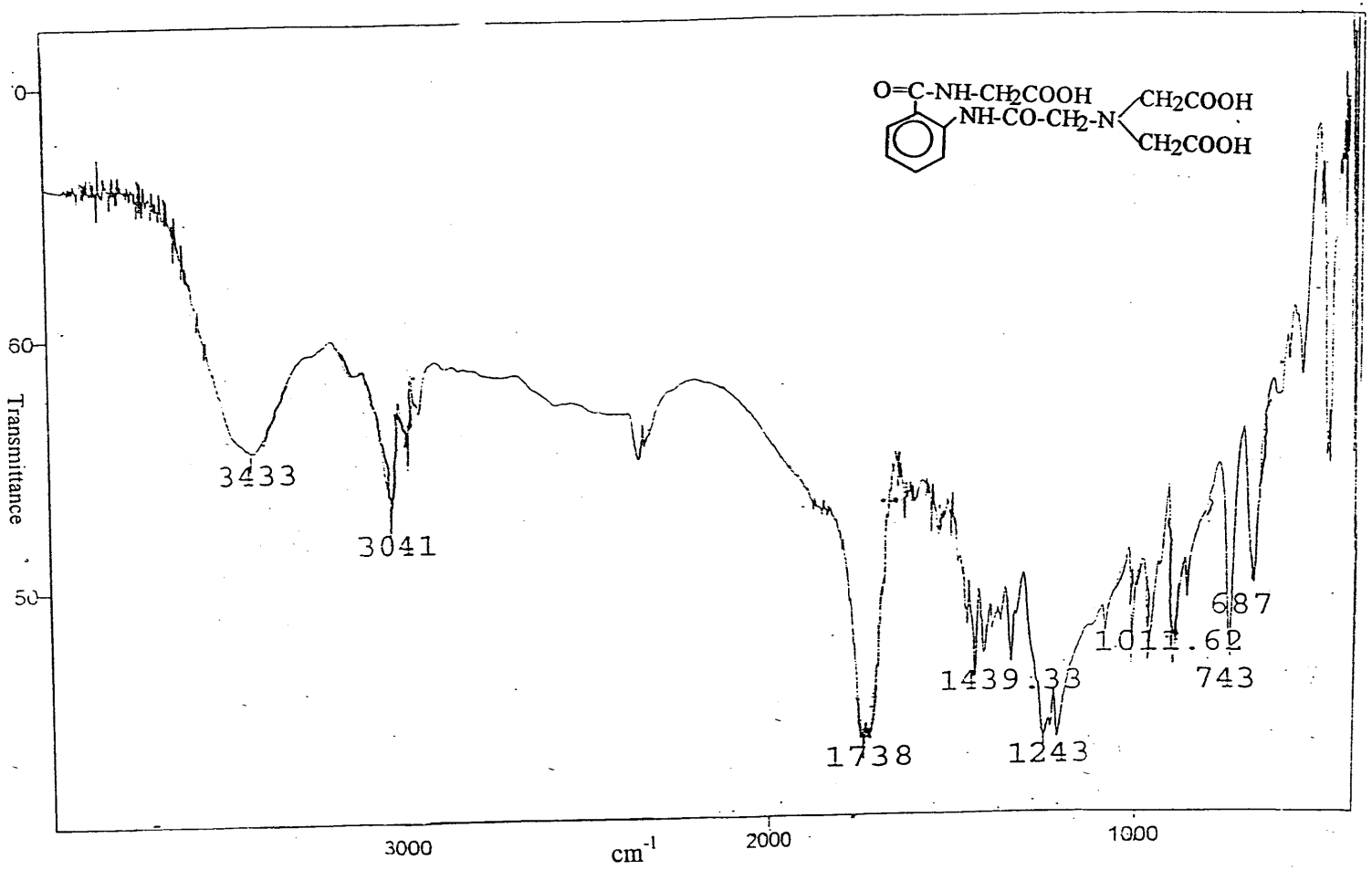


Fig (35) IR spectrum OAHIDA.

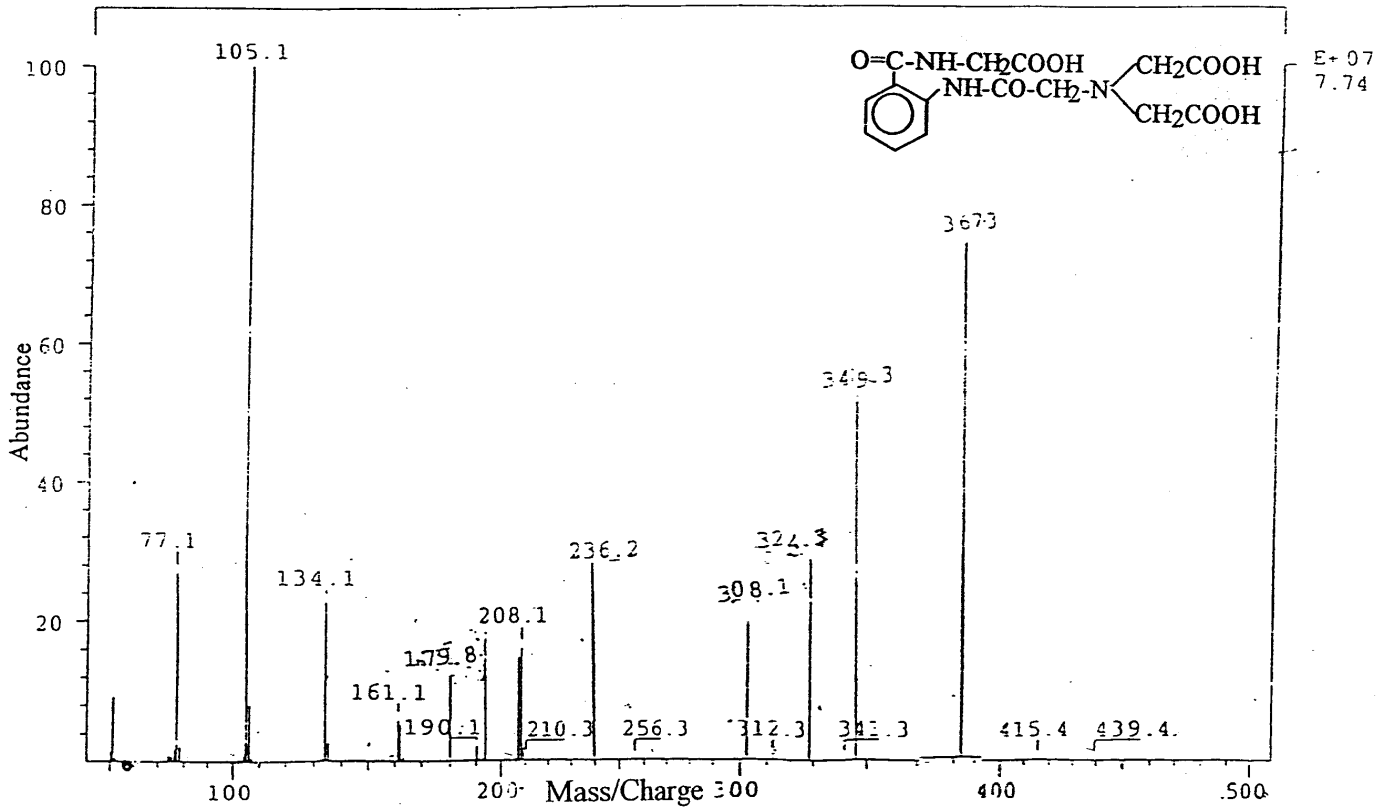
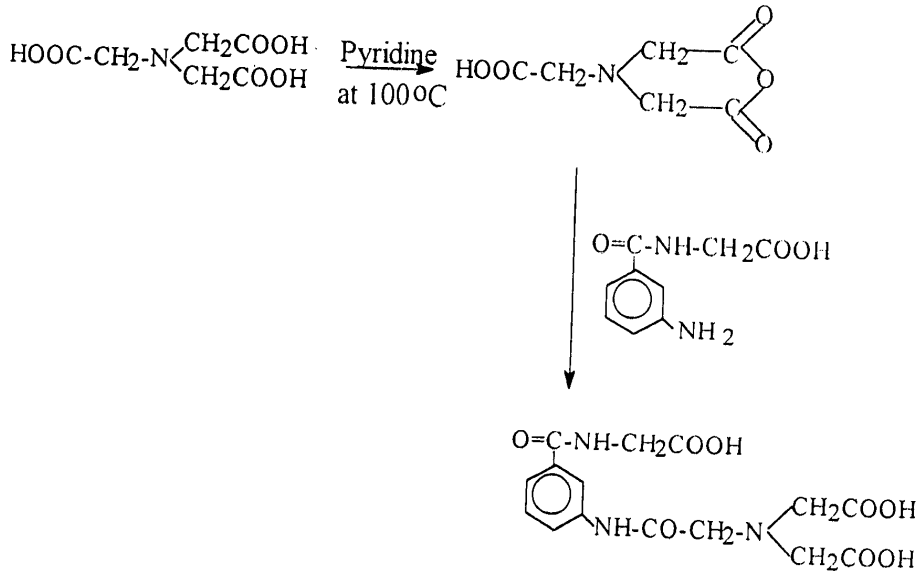


Fig (37) Mass spectrum of OAHIDA.

II.2.8.2 meta-Aminohippuric acid iminodiacetic acid analog:

meta-Aminohippuric acid iminodiacetic acid analog (MAHIDA) was synthesized following the method of Burn et al (1978) which involves condensation reaction between nitrilotriacetic acid momoanhydride and m-aminohippuric acid in pyridine. This reaction was proceeded according to the following scheme:



Fig(38) Reaction scheme of synthesis of meta-aminohippuric acid iminodiacetic acid analog (MAHIDA).

The yield of this reaction was equal to 65%. The prepared MAHIDA analog was analyzed by different analytical techniques such as the determination of melting point, IR, ^{1}H-NMR and mass spectroscopy. Melting point was identified of the product to insure the purity and the measured was found to be 181°C which is similar to the previous work. ^{1}H-NMR spectrum of MAHIDA (DMSO) expressed in δ ppm scale: 3.5(s, 2H,-NH-CO-CH_2N), 4(s,4H,N(CH_2-COOH)$_2$), 3.86(s,2H,CO-NH-

-83-

CH_2COOH),6.7-7.5(m,4H, aromatic protons) and at 10.0 (s, 2H, 2COOH) as shown in Fig (39).

Micro-elemental analysis results for the structure of molecule $C_{15}H_{17}N_3O_8$ (MAHIDA) was as follows :

	C%	H%	N%
Calculated	49.05	4.60	11.44
Found	48.93	4.59	11.24

The mass spectrum of MAHIDA analog showed the molecular ion peak at m/z 367, 79% which losses water molecule to give radical cation (b) at m/z 349 {M^+,$C_{15}H_{16}N_3O_8$,59%} which undergo cleavage at x axis to give cation (c) at m/z 236 {M^+,$C_{11}H_{12}N_2O_4$,25.9%}.Cation (c) losses methyl radical to give radical cation (d) at m/z 221{M^+,$C_{10}H_9N_2O_4$,35.1%} which losses carbonyl group to give radical cation (e) at m/z 193 {M^+,$C_9H_9N_2O_3$,24.3%}. Radical cation (e) losses NH radical to give cation (f) at m/z 179 {M^+,$C_9H_9NO_3$, 18.1%} .The radical cation (a) undergo cleavage at y axis to give cation (g) at m/z 293 {M^+,$C_{13}H_{13}N_2O_6$,59%}. Radical cation (a) losses carboxylic acid radical to give cation (h) at m/z 322{M^+,$C_{14}H_{16}N_3O_6$, 31.6%}. Cation (h) losses methylene group to give cation (i) at m/z 308 {M^+,$C_{13}H_{14}N_3O_6$,29.6%} as appeared in Fig(40,41). The IR spectrum of MAHIDA analog in Fig (42) showed absorption vibration bands similar to OAHIDA analog.

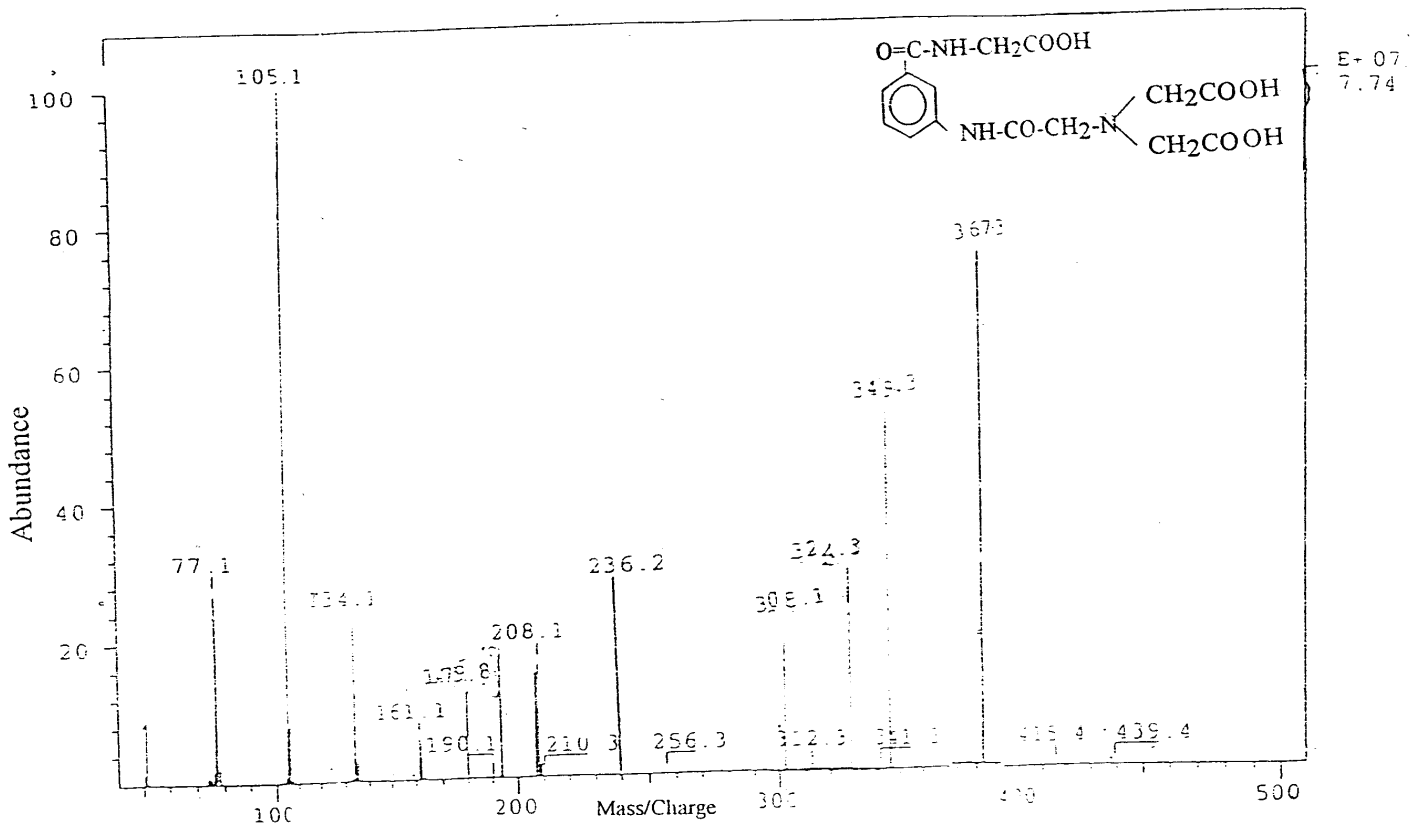
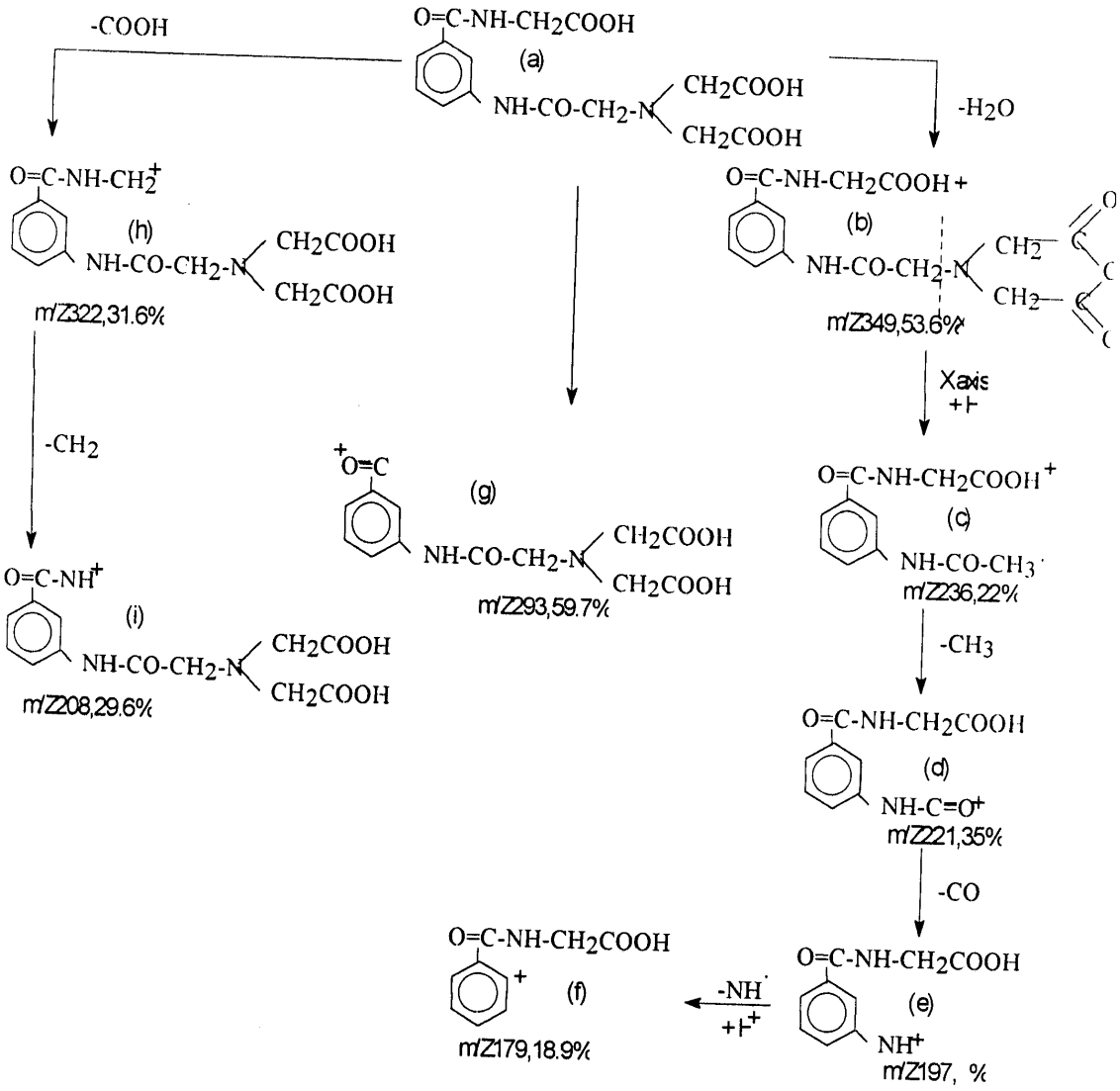


Fig (40) : Mass spectrum of MAHIDA



Fig(41): Fragmentation of m-AHIDA analog

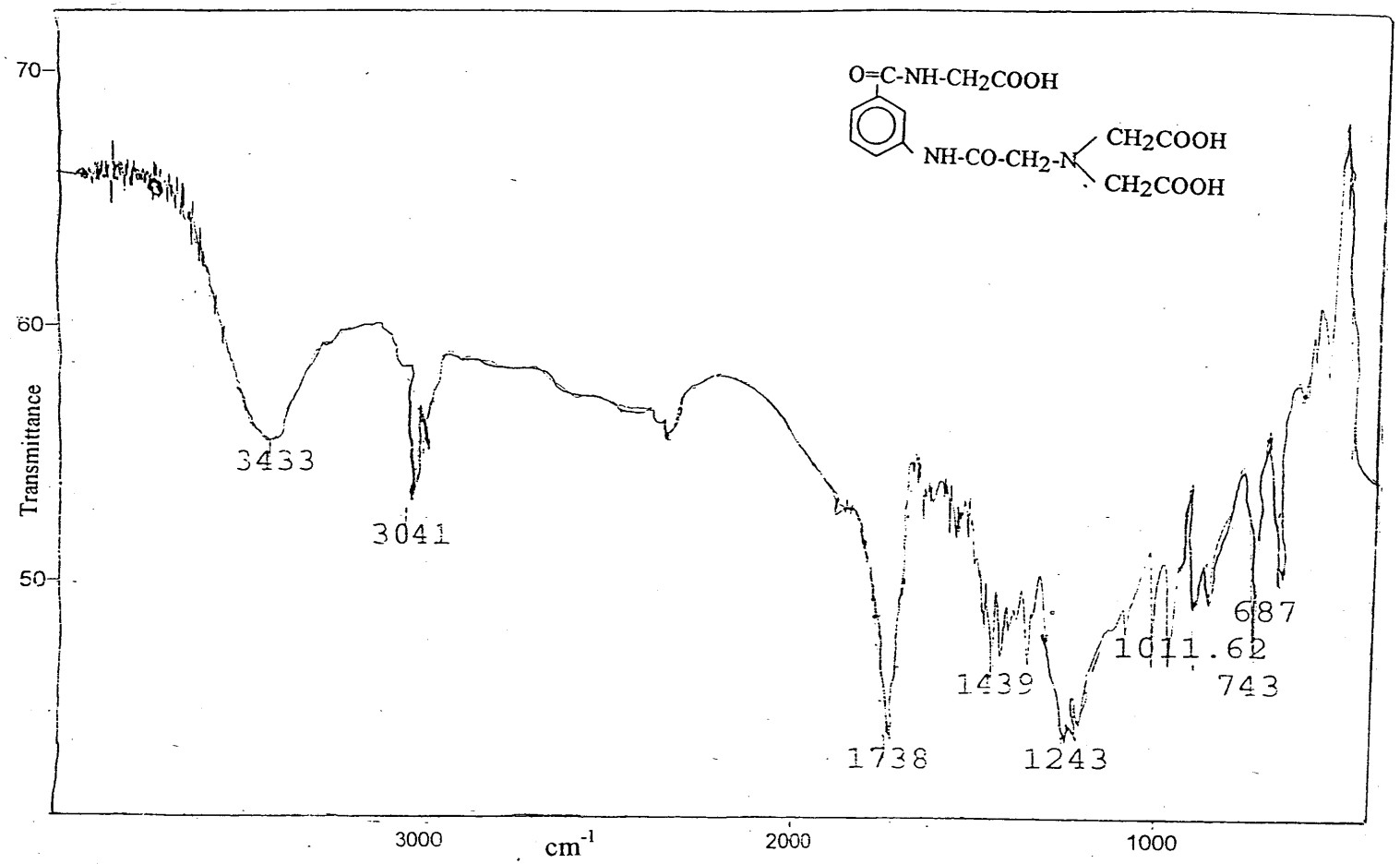


Fig (42) IR spectrum of MAHIDA.

II.2.8.3 para-Aminohippuric acid iminodiacetic acid analog

para-Aminohippuric acid iminodiacetic acid analog (PAHIDA) was synthesized following method of Burns et al.(1978) which involves condensation reaction between nitrilotriacetic acid momoanhydride and p-aminohippuric acid in pyridine. The reaction was carried out according to the following scheme:

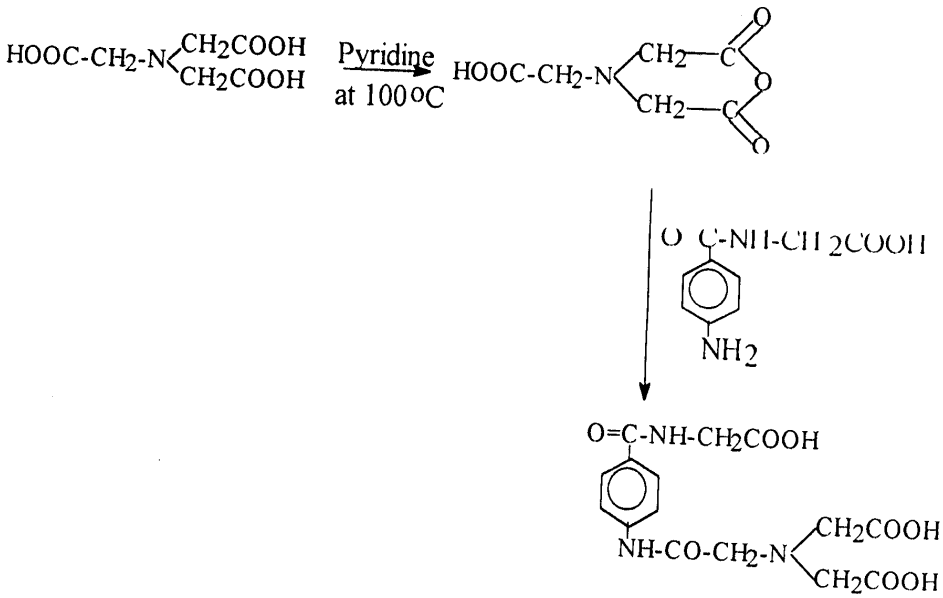


Fig (43) Reaction scheme of synthesis of para-aminohippuric acid iminodiacetic acid analog (MAHIDA)

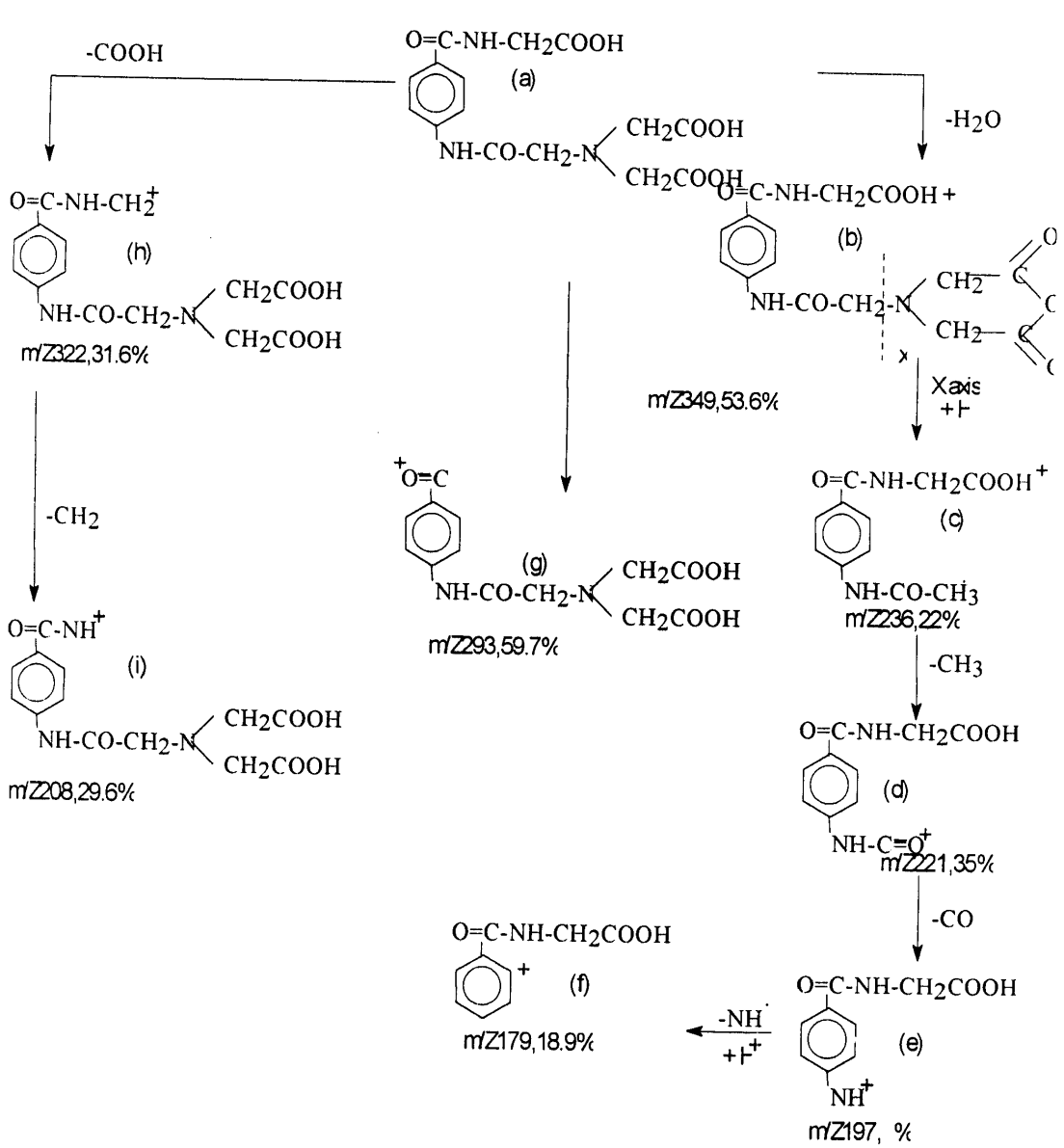
The yield of PAHIDA analog was found to be 69% as a white crystals. The synthesized PAHIDA analog was analyzed by different analytical techniques such as the determination of melting point,elemental analysis, IR, ^{1}H-NMR and mass spectroscopy. Melting point was measured and found to be 220°C. The structure of this compound, PAHIDA, could be presented as $C_{15}H_{17}N_3O_8$, M.Wt 367 and was confirmed by elemental microanalysis as follows :

	C%	H%	N%
Calculated	49.05	4.60	11.44
Found	48.94	4.7	11.51

The IR spectrum of PAHIDA analog showed absorption vibrational bands as presented in Fig (44) ,the IR data is shown similar to that obtained before in IR analysis of OAHIDA analog.

^{1}H-NMR spectrum of PAHIDA (DMSO) expressed in δ ppm scale showed the protons of methelene group at 3.4 (s, 2H, NH-CO-CH_2N), protons of other methylene group appeared at 4(s,4H,N(CH_2-COOH)$_2$) and 3.9ppm (s,2H,CO-NH-CH_2COOH), protons of benzene ring appeared at 7-7.5(m, 4H, aromatic protons) and the proton of NH appeared at 10 (s, 1H, NH) as shown in Fig (45).

The mass spectrum of PAHIDA showed the molecular ion peak at m/z 367.3,73.2% which losses water molecule to give radical cation (b) at m/z 349, {M^+,$C_{15}H_{16}N_3O_8$,51%} which undergo cleavage at x axis to give cation (c) at m/z 236 {M^+,$C_{11}H_{12}N_2O_4$,27.1%}.Cation (c) losses methyl radical to give radical cation (d) at m/z 221{M^+,$C_{10}H_9N_2O_4$,35.1%} which losses carbonyl group to give radical cation (e) at m/z 193.1 {M^+,$C_9H_9N_2O_3$,29.1%}. Radical cation (e) losses NH radical to give cation (f) at m/z 179 {M^+,$C_9H_9NO_3$, 21.4%} .The radical cation (a) undergo cleavage at y axis to give cation (g) at m/z 293 {M^+,$C_{13}H_{13}N_2O_6$,59%}. Radical cation (a) losses carboxylic acid radical to give cation (h) at m/z 322{M^+,$C_{14}H_{16}N_3O_6$, 31.6%}. Cation (h) losses methylene group to give cation (i) at m/z 308 {M^+,$C_{13}H_{14}N_3O_6$,29.6%} as shown in Figs(46,47).



Fig(47): Fragmentation of PAHIDA analog.

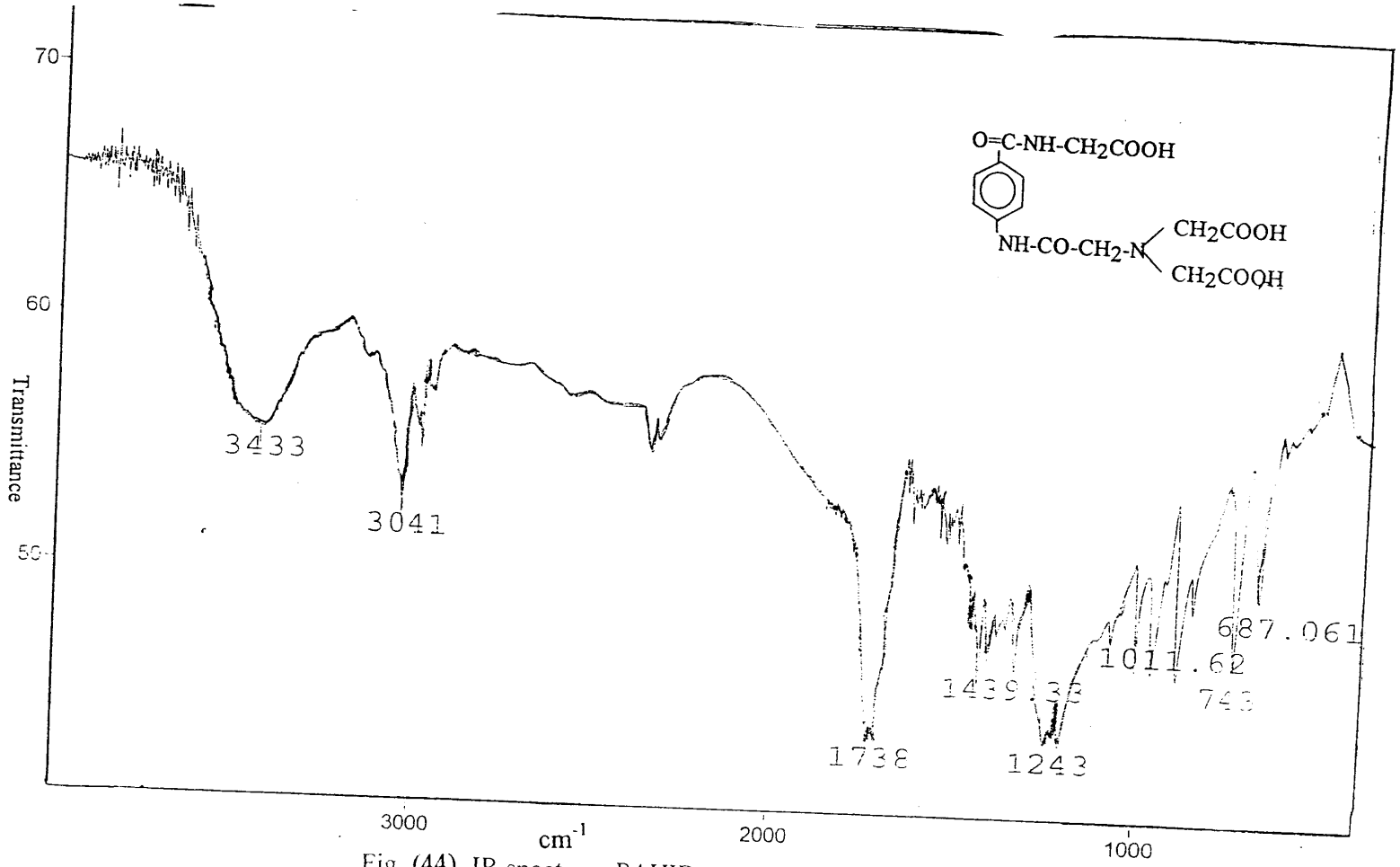


Fig (44) IR spectrum PAHIDA.

Fig (45) : NMR spectrum PAHIDA.

Fig (46) Mass spectrum of PAHIDA.

Chapter III

Labeling of ortho, meta and para-amino hippuric acid derivatives by ^{99m}Tc for use in renal function studies

3.1 Introduction:

Numerous radiopharmaceutical agents composed of radioactive metal ions bound to the chelating compounds have been developed as organ-imaging agents in nuclear medicine. Generally, these agents consist of a combination of γ-emitting radionuclide cations with the existing organic compounds, which have established complexing properties.

Different chelating agents labeled with ^{99w}Tc were proposed for the study of kidney functions or morphology as reported by Arnold et al (1975). These chelates are generally water soluble, low molecular weight and have low protein binding which are rapidly excreted via the kidney, these chelates fall into three classes, those that are filtered, those that are secreted, and those that exhibit a mixed behaviour of filtration, secretion and binding in the kidneys as shown in Table (4), and reviewed by Eckelman et al (1982).

Table (4) Chelates agents for ^{99m}Tc Kidney agents for glomerular filtration.

DTPA	Diethylenetriaminepenta acetic acid
EDTA	Ethylenediaminetetra acetic acid for tubular secretion
DADS	N,N- bis (mercapto-acetamido)- ethylenediamine
MAG_3	Mercaptoacetyltriglycine

Mixed function (Filtration, secretion, reabsorption localization): Mannital, citrate glusheptouate, tetracycline, ascorbate, gluconate, DMSA, and dimercapto succinic acid.

Radionuclidic technique may provide assessment of glomular filtration rate (GFR), effective renal plasma flow (ERPF), or individual renal function as investigated by Chervu et al(1984). Several agents are currently available for determination of these parameters but the formulation of a suitable ^{99m}Tc- labeled agent for the determination of ERPF has investigated. The tracer of choice for the clinical evaluation of renal tubular function is OI^{131}-iodohippurate. It has disadvantage of imparting a relatively high absorbed radiation ($t_{1/2}$ = 8.03d, γ =340keV,β^- = 0.61MeV) dose to the patient at low diagnostic doses, Although ^{123}I–labeled OIH lowers the radiation dose ($t_{1/2}$ =13.2h, γ =159KeV) to the patient, It is not available at a reasonable cost for routine use. The presence of varying amounts of free radioiodine in OIH preparation often poses problems for accurate quantitable assessment of function. Technetium-99m has ideal physical and nuclear properties for many applications in nuclear medicine, by virtue of its short half-life and favourable radiation characteristics. The low radiation dose permits the administration of large amounts of activity within short time intervals for serial measurements. Several ^{99m}Tc-agents have been reported for use in renal imaging and perfusion studies as reported by Chervu et al(1984). ^{99m}Tc-DTPA is widely used in clinical nuclear medicine for GFR measurements. EDTA or DTPA complexes of ^{99m}Tc are excreted solely through the slower process of glomerular filtration, and their slow rate of excretion, relative to that of compounds that are actively excreted, is a serious disadvantage. ^{99m}Tc–labeled agents that are cleared rapidly by active tubular excretion, with the minimal or preferably no reabsorption from the tubular lumen, would provide significant advantages over the

agents mentioned above.They would be excreted within a short time interval yielding low target to background ratio and minimal radiation dose. Various compounds belong to different complex forming ligand classes such as dimercaptodiamides (N_2S_2), mercapto triamides (N_3S) and substances bearing an iminodiacetic aid (IDA) moiety were developed and tested. Within these ^{99m}Tc labeled compounds, evidence for efficient tubular secretion was first observed with ^{99m}Tc–N,N-bis(mercaptoacetyl) ethylene diamine (^{99m}Tc-DADS), the renal excretion of DADS is greater than that of ^{99m}Tc-DTPA but lower than that of OIH in rodents, but its significant biliary excretion in rats and humans represents a major limitation to its use as evaluated by McAfee et al (1985). A large number of derivative of ^{99m}Tc– DADS were then evaluated by Kasina et al (1986), of which ^{99m}Tc-N,N-bis (mercaptoacetyl) –2,3 diaminopro-panoate (^{99m}Tc-CO_2 DADS) showed the most favorable renal excretion characteristics as investigated by Kumi et al (1985). However, labeling of CO_2-DADS with ^{99m}Tc always results of stereoisomers with clearly different biological properties as reported by Bormaus et al (1990). The complicated preparatory procedure for this agent, requiring heating and a high performance liquid chromatography. (HPLC) purification step to separate the component that shows optimal renal excretion kinetics precludes its application in routine clinical setting. The most appropriate and successful ^{99m}Tc-labelled chelate for renal function studies up to the present is ^{99m}Tc-mercaptoacetyl triglycine (^{99m}Tc-MAG_3), It is based on a N_3S donor ligand system and does not suffer from the problem of isomers with different biological behaviour in humans as reported by Fritzberg et al (1986). ^{99m}Tc-MAG_3extracted efficiently from the plasma by the kidneys and excreted rapidly in the urine, which results in renograms very similar to those obtained with OIH as reported by Szabo et al (1989). ^{99m} Tc MAG_3 has been approved by the Food and Drug Administration as a

radiopharmaceutcal for renal function studies. It has already been in clinical use for several years. The superior physical characteristics of ^{99m}Tc-labeled and its attractive biological properties make ^{99m}Tc-MAG_3 is the agent of choice, especially for the evaluation of transport kidney and tubular necrosis and kidney function in general.

Nevertheless, ^{99m}Tc-MAG_3 is still not the ideal replacement for OIH, and improvements are still possible. The plasma-protein binding of ^{99m}Tc-MAG_3 is very high as investigated by Bubeck et al (1990) and its plasma clearance in humans is no higher than about 60-65% of the OIH value. Therefore, accurate determination of the effective renal plasma flow (ERPF) is rather difficult using ^{99m}Tc–MAG_3. For these reasons, the development of a ^{99m}Tc-labeled renal function agent that approaches hippuran more closely than MAG_3 would be continued.

Para-aminohippuric acid (PAH) is the gold standard for the measurements of tubular cell function and its clearance is a measurements of effictive renal plasma flow (ERPF). Sundberg et al (1974) have coupled an EDTA derivative to proteins for the purpose of binding radioactive metals (^{99m}Tc, ^{111}In) to a macromolecule bound chelating agents. Incorporation of a chelating moiety into a relatively low-molecular-weight drug analogue may create an agent capable of binding with technetium-99m, while retaining the biological actions and tissue distribution characteristics of the parent drug. Iminodiacetic acid (IDA) is a metal complexing moiety, which strongly binds to transition metals (^{99m}Tc) its relatively small size and can be readily incorporate into organic molecules. Loberg et al (1976) reported the importance of the iminodiacetic acid chelating moiety in the development of ^{99m}Tc radiopharceuticals is well recognized. ^{99m}Tc-N(2.6-dimethyl phenyl) carbamoylmethyliminodiaceticacid (^{99m}Tc-HIDA) containing the chelating moiety and its derivatives have found wide clinical acceptance

as hepatobillary agent. Also, since the affinity of para-amino hippuric acid (PAH) towards renal tubular transport enzyme is not altered after attachment of iminodiacetic acid (IDA) group to PAH, subsequently radiolabeling the ligand with ^{99m}Tc was performed by Chervu et al (1984) and zmbova et al (1989).

It appears that this chelating moiety is suitable for the synthesis of receptor based on ^{99m}Tc-radiopharmaceuticals. Chatterjet et al (1991) synthesized substituted monomilides and esters of hydroxy compounds with nitrilotriacetic acid, which gained much popularity as an important class of ligands for ^{99m}Tc-radiopharmaceutical preparations used in liver imaging and function studies.

The present work describes labeling of iminodiacetic acid (IDA) derivatives of ortho-aminohippuic acid (OAHIDA), meta-aminohippuric acid (MAHIDA) and para-aminohippuric acid (PAHIDA) with ^{99m}Tc. The different parameters affecting the labeling yield such as ligand amount. Stannous content, pH and reaction time have to be studied. The biological distribution ^{99m}Tc-O,M and PAHIDA has to be investigated in mice to validate the suitability of prepared complexes for the intended uses in renal function measurements.

III.2 Experimental

III.2.1 Materials

III.2.1.1 Radioactive material

Technetium-99m was eluted as ^{99m}Tc-O_4^- from ^{99}Mo-^{99m}Tc generator, Elutec, Brussels, Belgium. Instant thin layer chromatography silicagel (ITLC-SG) strips (15x2cm) were purchused from Gelman Sciences Inc. Ann Arbor, Michigan, USA. Thin layer chromatography silicagel (ITLC-SG) sheets were purchased from Merck Gemany.

III.2.1.2. Chemicals: -

Unless otherwise stated, all chemicals and laboratory reagents used in this work are of the highest purity grade. In all cases the water used is dexoygenated bidistelled water.

The following chemicals used were as follow:

- Locally synthesized o, m, p-aminohippuric acid iminodiacetic acid analogs,(OAHIDA, MAHIDA, PAHIDA).
- Tin metal (Sn), granular, 20 mesh, At.wt=118.70
- Hydrochloric acid, AR 35%, Riedel De Haenegseelze, M.wt=36.46.
- Sodium chloride (NaCl) A.R, M.wt = 58.44
- Sodium hydroxide pellets (NaOH) Riedal
- De Haenagseelze- Hannover, M.wt = 40

III.2.1.3. Solvents:

- Acetonitrile, CH_3CN, M.wt = 41.
- Acetone $(CH_3)_2CO$, M.wt = 58.08, Laboratory Rasayan, S.D Fine chem. LTD.
- Methanol (CH_3OH), 96%, M.wt = 32.04, Chambnien chemicals.

III.2.2. Equipments:

- pH meter, Orion Research Incorporation, Model230A, Boston, USA.
- Balance: Precision Electronic Balance, Model: HA 120M, A&D Company, limited Japan.
- Hamilton: Precion Syringe, micro measure B.v The Hague-Holland.
- Tuberculin hypodermic Syringe B.D company, USA.
- Stirring hot plate, Model 210T, Thrmix, Fisher, USA.

- Scalar Ratemeter SR 7:Nuclear Enterprises Ltd.; USA.
- Ionzation Chamber, Model CRC-15R Capintec, USA.

III.2.3. Preparation of stock solution of tin(II):

III.2.3.1. Sn(II) Solution:

Exactly weigh 100mg of tin metal was dissolved in 0.5ml conc. HCl by heating on a hot plate, then the volume was completed to 10ml using nitrogen purged double distilled water, One ml stock solution (10mg /ml) was diluted to 10ml with nitrogen purged double distilled water, One ml of this solution was dispensed in a 10ml clean penicillin vial, flushed with nitrogen gas for 15min, then stored at-20°C for further use. Then final concentration was 1mg/ml ($84.2x10^{-4}$m mol). NaCl for injection was prepared according to the British Pharmacopoeia (1993).

III.2.3.2. Sodium chloride solution (NaCl)

Sterile solution of 0.9% NaCl for injection was prepared according to the British pharmacopoeia (1993).

III.2.4. Labeling procedure of OAHIDA, MAHIDA and PAHIDA:

The required amount of OAHIDA,MAHIDA and PAHIDA was transferred to a 10ml clean penicillin vial, then the required volume of 0.01 N NaOH was added till pH reached 5.5 the vial was then closed under positive pressure of N_2 gas. After that, the required amount of sn(II) solution was added and pH of the mixture adjusted to pH 5.7. One ml of $^{99m}TcO_4^-$ eluate containing 185-370 MBq was injected into the reaction vial, shaked and incubated for the recommended time. An aliquot (1-5µl) of the vial content was withdrawn for analysis and determination of the labeling yield and radiochemical purity.

III.2.5. Analysis of ^{99m}Tc-OAHIDA,^{99m}Tc- MAHIDA and ^{99m}Tc- PAHIDA complexes:

Thin layer chromatography- silicagel (TLC- SG) and Instant thin layer chromatography-silicagel ITLC-SG strips were marked 2cm from the base and lined into fragments 1cm each up to 17cm using non pointed pencil.The sheets were activated by heating at 110°C for 30-min peroir use. A spot of ^{99m}Tc–AHIDA complex was applied using hypodermic syringe, then the sheets were developed in an ascending manner in a closed jar filled with N_2 gas to prevent oxidation of labeled ^{99m}Tc-AHIDA spot. The developing solvents, acetonitrile : water (3:1) and saline were also purged with N_2 gas as reported by Zmbova et al (1989). The sheets after complete development were dried and cut into sections 1cm each, then the sections were counted in a well type NaI (Tl) detector connected to γ-counter.

III.2.5.1. Determination of free pertechnetate: -

The percent of free $^{99m}TcO_4^-$ was determined from the distribution of activity on ITLC-SG strip using saline and acetonitrile: water (3:1) as developing solvents. Free $^{99m}TcO_4^-$ move with the solvent front to R_f 1.0 while ^{99m}Tc-AHIDA complex and colloids remain at the origin with R_f 0.0 when saline was used as a developing solvent. Reduced hydrolysed technetium (RH-^{99m}Tc) was determined when acetonitrile: water (3:1) solvent was used, the colloid remains at the origin with R_f 0.0.

III.2.6. Study of the factors affecting the labeling yield of ^{99m}Tc-AHIDA analogues:

Various factors affecting the labeling yield of ^{99m}Tc-AHIDA complex were investigated namely. AHIDA amount, tin (II) content, pH and reaction time.

III.2.6.1. Effect of AHIDA amount of o, m and p-derivatives:

The effect of AHIDA amount on the labeling yield of ^{99m}Tc-AHIDA was studied in the range of 1 to 30mg. The labeling procedure of AHIDA was carried out several times as mentioned before keeping all parameters constant except the factor studied.

III.2.6.2. Effect of tin (II) content:

The effect of tin (II) content in the form of stannous chloride on the labeling yield of ^{99m}Tc-AHIDA was studied in the range of 100 to 500µg. The same labeling procedure was repeated several, times keeping all parameters constant while changing the factor studied.

III.2.6.3. Effect of pH of the reaction medium:

The effect of pH on the labeling yield of ^{99m}Tc-AHIDA was studied in the range of 4 to 10. The labeling procedure of AHIDA was carried out as mentioned before.

III.2.6.4. Effect of reaction time:

The labeling procedure was performed as mentioned before. The reaction time was varied from 5 to 60 min and the labeling yield of ^{99m}Tc-AHIDA was determined.

III.2.7. Biodistribution study:

The biodistribution of ^{99m}Tc-AHIDA complex, o, m and p-derivatives was studied in Male Albino Swiss mice weighting 20-25g.A group of 3 mice were employed for each experiment. A volume of 0.1ml of ^{99m}Tc-AHIDA complex of o, m and p-derivatives containing (185-1850KBq) was injected intravenously in the tail vein of mice. The mice were killed with ether at the predesigned interval time, their organs

were collected and assayed for their radioactivity. The average values of % administrated dose / organ were calculated after correction for radioactivity in the tail. Blood, bone and muscels were assumed to be 7,10, and 40% of the body weight as reported by Rhodes (1974).

III.3. Results and discussion:

Various technetium–99m labeled amino hippuric acid iminodiacetic acid analogues have been reported to be useful agents for renal function studies as reported by Bhragava et al (1988). Because these materials are not available commercially so they must be synthesized locally. The synthesized amino hippuric acid iminodiacetic acid analogues should have very high purity since even trace impurities in these compounds can react with technetium leading to the formation of radiochemical impurities which have different biological path ways and can expose other organs to unnecessay radiation doses. The technetium–99m labeling procedure of AHIDA analogues which involves coordination of technetium–99m in a reduced valance state with these ligands require careful study. ^{99m}Tc-AHIDA analogues are prepared by direct reduction of technetium pertechnetate ($^{99m}TcO_4^-$) with Sn(II) in presence of excess ligand (AHIDA).

The following reactions take place during labeling as proposed by Nowotnik (1994). $^{99m}TcO_4^-$ in the VII state was reduced by Sn(II) to lower oxidation state ^{99m}Tc-(IV) which is probably combining with-IDA side chain according to the following equations:

$$^{99m}TcO_4^- \xrightarrow{Sn(II)} {}^{99m}Tc(IV) \qquad \ldots(8)$$

$$2AHIDA + Sn^{2+} + {}^{99m}TcO_4^- \longrightarrow {}^{99m}Tc(IV)\ AHIDA \qquad \ldots(9)$$

The conditions for labeling of AHIDA with $^{99m}TcO_4^-$ were investigated by varying the amount of complexing agent AHIDA and Sn (II) at pH 4-10 at room temperature within reaction time 5 to 60 min. The labeling yield was determined by thin layer chromatography, in case of (ITLC-SG) strip developed in acetonitrile : water (3:1), activity at the start point represented the reduced hydrolyzed technetium (^{99m}Tc-RH) and the front contained pertechnetate and labeled AHIDA. The other (ITLC-SG) strip developed in 0.9% NaCl contained reduced technetium and labeled AHIDA at start line and the front contained free pertechnetate. Percentage of the labeling was determined in the following way.

$$\% \text{ Free } ^{99m}TcO_4^- = \frac{\text{Activity at } R_f 1.0 \text{ (saline) x100}}{\text{Total activity}} = 1.3\%$$

$$\% \ ^{99m}\text{Tc-colloids} = \frac{\text{Activity at } R_f 0.0 \ (CH_3CN: H_2O) \text{ x100}}{\text{Total activity}} = 0.8\%$$

$\%\ ^{99m}$Tc-AHIDA=100-(% free $^{99m}TcO_4^-$ $^{99m}_{+}$Tc colloids) = 97.9 %

So the radiochemical purity of the labeled AHIDA analogues was high (~98%) which falls within the acceptable limits as shown by Zembova et al (1989), and could be represented as shown in Table (5),

Table (5): Radiochromatographic behaviour of different radiochemical species and their R_f = values.

system	support	solvents	^{99m}Tc-species	R_f
ITLC-SG	silicagel	saline(0.9% NaCl)	^{99m}Tc-R_H ^{99m}Tc-AHIDA $^{99m}TcO_4^-$	0.0 0.0 1.0
ITLC	silicagel	acetonitrile: H_2O(3:1)	^{99m}Tc -R_H $^{99m}TcO_4^-$ ^{99m}Tc-AHIDA	0.0 1.0 1.0

III.3.1. Study of the factors affecting the labeling yield:

III.3.1.1. Effect of AHIDA amount:

The effect of AHIDA derivatives content was studied in the range of 1 to 30mg for OAHIDA, MAHIDA and PAHIDA respectively. The results are shown in the Tables 6,7,8. The data presented in the previous Tables clearly show that dependence the labeling yield on the amount of ligand used in the range of 5 mg up to 10mg. In this range, ITLC analysis of the reaction mixture showed that high percent of radioactivity in the form of pertechnetate and small percent of radioactivity in the form of colloids, this can be explained as follow:

At low AHIDA content, the amount of AHIDA is not sufficient for complete formation of ^{99m}Tc-AHIDA complex and the percent-labeling yield was low. By increasing the ligand amount up to 10mg a high labeling yields were achieved 95.8% for OAHIDA, 96.3% for MAHIDA and 98.9% for PAHIDA using optimum condition. When the ligand amounts were increased above this range till 30mg, the labeling yield did not increase. Therefor, the optimum conditions for labeling of AHIDA analogues are 10mg of ligand, tin (II) content 0.25mg, pH 5.7, reaction time 15 mint and 1ml $^{99m}Tc\ O_4^-$ (185-370 MBq).

Table (6) Effect of OAHIDA amount on the percent-labeling yield

OAHIDA amount, mg	$^{99m}TcO_4^-$ %	^{99m}Tc-reduced hydrolyzed %	^{99m}Tc-OAHIDA %
1	22.5 ± 0.3	1.26 ± 0.1	77.4 ± 0.3
5	19.3 ± 0.3	1.15 ± 0.4	79.55± 1.9
10	1.3 ± 0.3	2.9 ± 0.9	95.5 ± 0.4
20	1.5 ± 0.3	2.1 ± 0.3	95.4 ± 1.3
23	1.2 ± 0.3	3.2 ± 0.3	94.6 ± 1.4
30	1.8 ± 0.3	2.5 ± 0.1	95.0 ± 1.6

Table (7) Effect of MAHIDA amount on the percent-labeling yield

MAHIDA amount, mg	^{99m}Tc,O4$^-$ %	^{99m}Tc-reduced %	^{99m}Tc-MHIDA
1	23.6 ± 0.3	0.80 ± 1.4	75.59 ± 0.3
5	20.5 ± 0.3	1.2 ± 0.7	78.5 ± 1.8
10	1.2 ± 0.3	2.0 ± 0.1	96.3 ± 1.4
20	1.8 ± 0.2	2.5 ± 0.4	96.0 ± 0.1
25	1.5 ± 2.0	2.5 ± 0.3	95.5 ± 0.3
30	1.9 ± 0.2	2.8 ± 0.2	95.8 ± 0.2

Table (8) Effect of PAHIDA amount on the percent-labeling yield

PAHIDA amount, mg	$^{99m}TcO_4^-$ %	Reduced hydrolyzed - ^{99m}Tc	^{99m}Tc-PAHIDA %
1	18.9 ± 0.3	1.25 ± 0.1	81.21 ± 0.3
5	6.4 ± 0.4	1.15 ± 0.5	92.35 ± 1.6
10	1.3± 0.3	0.8 ± 0.2	97.9 ± 0.4
20	1.14 ± 0.2	1.43 ± 0.4	97.38 ± 0.3
25	1.54 ± 0.1	0.92 ± 0.3	97.1 ± 0.2
30	2. 31 ± 0.8	1.20 ± 0.2	96.91 ± 0.6

III.3.1.2. Effect of Sn (II) content:

Technetium-99m was eluted from the ^{99}Mo /^{99m}Tc- generator as pertechnetate ($^{99m}TcO_4^-$) which has to be reduced for labeling of the synthesized AHIDA derivatives using $SnCl_2$ which is the most widely used reducing agent in the kit preparation of Tc-radiopharmacitical. When acidic solution of $SnCl_2$ is added to AHIDA analog, A complex is formed between stannous ions and amino-hippuric acid according to the following equation

Sn (II)+ AHIDA ⟶ Sn AHIDA complex .....(10)

The influence of $Sn Cl_2.2H_2O$ on the labeling yield of ^{99m}Tc-AHIDA was investigated in the range of 100 to 500µg.

The amount of $SnCl_2 . 2H_2O$ which achieves high labeling yield of ^{99m}Tc AHIDA analogs was 0.25mg / 10mg of AHIDA analogs. The amount of $SnCl_2$, $2H_2O$ higher than 0.25mg did not increase, the labeling yield as shown in Tables 9,10 and 11. Below this value (0.2mg Sn(II)), stannous chloride is not sufficient for complete reduction of pertechnetate. This leads to the presence of high percentage of free pertechnetate ranged from 25% up to 30%. These results are in complete agreement with the finding of Narasimhan et al (1984).

Table (9): Effect of tin (II) content on the %-labeling yield of ^{99m}Tc-OAHIDA

Tin(II) content (mg)	$^{99m}TcO_4^-$ %	Reduced hydrolyzed-^{99m}Tc	^{99m}Tc-OAHIDA %
0.1	30.0 ± 1.1	2.01± 0.2	67.0 ± 1.9
0.15	24.3 ± 0.3	2.8 ± 1.1	72.9 ± 2.1
0.25	1.2 ± 0.8	2.9 ± 1.2	95.8 ± 1.1
0.30	1.1± 0.3	3.9 ± 0.6	95.0 ± 3.2
0.40	1.3 ± 1.1	4.0 ± 0.3	94.9 ± 2.1
0.50	1.1 ± 0.2	5.1 ± 1.1	94.0 ± 1.9

Table (10): Effect of tin (II) content on the % labeling yield of ^{99m}Tc-MAHIDA

Tin(II) (mg)	$^{99m}TcO_4^-$ %	Reduced hydrolyzed-^{99m}Tc	^{99m}Tc-MAHIDA
0.1	35.5 ± 0.2	2.3 ± 0.1	64.0 ± 0.9
0.15	25.5 ± 0.3	3.1 ± 0.2	71.4 ±1.1
0.25	2.0 ± 0.8	2.3 ± 0.2	96.6 ± 1.2
0.3	2.3 ± 0.3	2.8 ± 1.1	95.7 ± 0.9
0.4	1.5 ± 0.1	3.8 ± 0.7	95.4 ± 1.1
0.5	1.5 ± 0.2	4.5 ± 0.1	94.8 ± 3.1

Table (11): Effect of tin (II) content on the percent-labeling yield of ^{99m}Tc-PAHIDA

Tin(II) (mg)	$^{99m}TcO_4^-$ %	Reduced hydrolyzed-^{99m}Tc	^{99m}Tc-MAHIDA
0.1	29.6 ± 0.2	2.04 ± 0.9	68.85 ± 0.2
0.15	25.7 ± 0.2	2.51 ± 0.3	70.1 ± 0.3
0.25	0.8 ± 0.7	2.3 ± 0.3	97.9 ± 1.1
0.3	1.3 ± 0.2	2.5 ± 1.1	97.1 ± 0.3
0.4	1.5 ± 1.1	2.7 ± 0.3	96.8 ± 0.2
0.5	1.7 ± 1.8	3.7 ±1.3	95.26 ± 0.8

III.3.1.3. Effect of pH of the reaction mixture:

The influence of pH on the labeling yield of ^{99m}Tc-AHIDA analogs was investigated in the range of 4 - 10. The data present in Tables 12,13 and 14 clearly show that at pH 3 colloids were formed due to less solubility of the synthesized IDA analogs in aqueous solutions at this pH value as reported by Fields et al (1978). When the, pH increased up to 4 or 5, the contents of colloids and free pertechnetate was increased up to 24% due to the protonation of the imino nitrogen of IDA group. At pH values equal to 5.7, a high labeling yield was achieved 95.4%, 97.6% and 98.6% for OAHIDA, MAHIDA and PAHIDA analogs respectively.

Ahigh pH 8and 10 the labeling yield was decreased to 82.4%,76.4% and 76.5% for OAHIDA, MAHIDA, and PAHIDA respectively. The imino groups are probably partially ionized at high pH values due to zwitter ion formation. It is likely that the protons on the ionized imines compete with ^{99m}Tc for binding to nitrogen atoms and this prevent adequate complexation of ^{99m}Tc by the tetra ligand as reported by Sumerville et al (1987).

Table (12): Effect of pH of the reaction mixture on the % labeling yield of ^{99m}Tc – OAHIDA:

pH	$^{99m}TcO_4^-$ %	Reduced hydrolyzed -^{99m}Tc %	^{99m}Tc-OAHIDA
4	0.91± 0.1	22.5 ± 1.0	76.59 ±1.9
5.7	0.9 ± 0.2	2.5 ± 1.3	95.8 ± 2.8
6.5	1.5 ± 1.0	3.0 ± 0.0	95.0 ± 3.1
8	1.6 ± 1.4	3.3 ± 2.0	95.1 ± 2.1
10	1.5 ± 2.1	4.1 ± 1.9	94.4 ± 3.1

Table (13): Effect of pH of the reaction mixture on the %-labeling yield of ^{99m}Tc- MAHIDA

pH	$^{99m}TcO_4^-$ %	Reduced hydrolyzed - ^{99m}Tc %	^{99m}Tc-MAHIDA
4	2.1 ±0.9	21.5 ± 1.8	77.6 ± 3.1
5.7	1.6 ± 0.1	2.2 ± 0.2	96.8 ± 3.1
6.5	1.9 ± 1.2	2.3 ±0.2	95.7 ± 2.1
8	1.8 ± 1.0	3.1 ± 1.8	96.0 ± 2.1
10	1.5 ± 1.2	3.6 ± 0.1	95.4 ± 1.1

Table (14):Effect of pH of the reaction mixture on the % labeling of ^{99m}Tc-PAHIDA

pH	$^{99m}TcO_4^-$%	Reduced hydrolyzed- ^{99m}Tc %	^{99m}Tc-PAHIDA %
4	2.4 ± 0.9	23.8 ± 1.1	75.27 ± 3.1
5.7	0.8 ± 0.0	1.2 ±0.2	98.2 ± 1.0
6.5	1.2 ± 1.1	2.1 ± 0.3	96.7 ± 2.1
8.	1.5 ± 0.1	2.5 ± 0.2	95.78 ± 2.1
10	1.3 ± 0.0	3.0 ± 0.3	96.58 ± 2.0

III.3.1.4.Effect of reaction time and in-vitro stability:

The results in Tables 15,16,17 indicate that the reaction of ^{99m}Tc with AHIDA analogs is very fast and a high labeling yield was achieved within 15min. Further increase of reaction time did not show any increase of labelling yield. The results also indicate that the ^{99m}Tc-complex remains stable up to 6h after labeling with ^{99m}Tc as determined by ITLC-SG chromatography, in complete agreement with the results reported by Chervu et al (1984) and Zmbova et al (1989).

Table(15): Effect of reaction time on the %-labeling yield of ^{99m}Tc-OAHIDA

Time, min	$^{99m}TcO_4^-$ %	Reduced hydrolyzed - ^{99m}Tc	^{99m}Tc-OAHIDA
5	17.5 ± 1.8	1.4 ± 0.8	81.69 ± 3.1
10	14.1 ± 0.0	13.1 ± 0.2	84.7 ± 2.8
15	2.2 ± 1.1	2.5 ± 0.3	95.4 ± 2.1
30	2.8 ± 0.2	3.1 ±0.5	95.0 ± 3.1
40	1.9 ± 0.2	2.3 ±0.6	95.4 ± 2.1
60	2.8 +0.8	3.2 ±0.1	94.0 ± 1.6
180	2.7 + 0.5	3.1 ± 0.3	93.2 ± 1.2
360	2.9 +0.2	2.2 ± 0.8	94.2 ± 1.2

Tables (16): Effect of reaction time on the percent labeling yield of ^{99m}Tc-MAHIDA

Time, min.	$^{99m}TcO_4$ %	Reduced hydrolyzed-^{99m}Tc	^{99m}Tc-MAHIDA
5	28.6 ± 2.1	0.8 ± 0.0	71.32 ±1.4
10	11.2 ± 0.8	3.1 ± 0.9	87.9 ± 2.1
15	1.7 ± 1.3	2.1 ± 0.9	96.8 ±1.8
30	2.3 ± 1.1	1.4 ± 1.1	95.3 ± 1.2
60	1.5 ± 0.3	2.1 ±2.1	94.8 ± 1.4
180	2.56 ± 0.6	2.6 ± 1.1	95.6 ± 1.5
360	2.7 ± 0.8	2.2 ± 0.9	94.1 ± 0.6

Table (17): Effect of reaction time on the %-labeling yield of ^{99m}Tc-PAHIDA

Time, min.	$^{99m}TcO_4^-$ %	Reduced hydrolyzed – ^{99m}Tc %	^{99m}Tc-PAHIDA %
5	6.2 ± 0.9	2.1 ± 1.1	91.7 ± 2.1
10	3.1 ± 1.1	1.1 ± 0.2	95.7 ± 3.1
15	1.5 + 0.7	1.3 ± 0.5	97.2 ± 1.9
30	1.4 + 0.9	2.1 ± 1.2	97.4 ± 2.1
60	2.1 ± 1.1	1.0 ± 0.8	97.9 ± 2.5
180	2.3 ± 0.6	1.5 ± 0.3	97.0 ± 1.2
360	2.2 ± 0.5	1.6 ± 0.1	96.0 ± 0.6

III.4.Biological distribution of ^{99m}Tc-AHIDA analogs:

The localization of ^{99m}Tc-complexes in particular target organs like liver, kidney, brain, bone, etc, is the bases for use of these complexes as diagnostic agent in nuclear medicine. The biodistribution of ^{99m}Tc-OAHIDA, ^{99m}Tc-MAHIDA and ^{99m}Tc-PAHIDA in mice were investigated at different time intervals. The percent administrated dose in different organs is given in Table (18). These data showed that the urinary excretion of ^{99m}Tc-AHIDA analogs was almost rapid and high value for ^{99m}Tc-PAHIDA complex than other two analogs. Both the

ortho, meta, and para amino hippuric acid derivatives showed good renal excretion but not the same degree as PAHIDA. Results given in Table (18) show high uptake of gastro-intestine (GI) o and m-amino hippuric acid iminodiacetic acid analogs than p-amino derivatives. These results are in good agreement with the obtained by Bhargava et al (1988). The organ distribution studies in mice confirmed that ^{99m}Tc-PAHIDA has excellent renal excretion characteristics compared to others derivatives.

Table(18): Biodistribution of ^{99m}Tc-OAHIDA, MAHIDA and PAHIDA in mice:

Time min.		% administratered dose in different organs and fluid in mice*				
		liver	Kidneys	Urine	GI	Blood
OAHIDA	15	5.2 ± 0.3	5.9 ± 09	41.0 ± 5.7	3.0 ± 0.8	5.1 ± 1.3
	30	4.6 ± 0.1	4.8 ± 0.1	50.2 ± 6.5	4.0 ± 0.3	1.6 ± 0.6
	60	3.4 ± 0.5	3.6 ± 1.2	59.6 ± 3.8	4.9 ± 1.8	1.1 ± 0.3
MAHIDA	15	3.5 ± 0.5	4.0 ± 0.3	45.6 ± 5.7	2.5 ± 0.5	1.4 ± 0.7
	30	1.3 ± 0.3	3.1 ± 1.2	55.5 ± 5.6	4.8 ± 1.3	0.5 ± 0.2
	60	0.7 ±0.1	0.4 ± 0.3	60.5 ±2.9	5.5 ± 1.1	0.3 ± 0.1
PAHIDA	15	0.8 ± 0.1	2.3 ± 0.1	54.5 ± 4.9	1.5 ± 0.6	3.5 ± 0.3
	30	0.5 ± 0.2	0.9 ± 0.3	85.0 ± 5.0	1.6 ± 0.2	0.7 ± 0.3
	60	0.4 ± 0.1	0.7 ± 0.1	87.2 ± 4.5	1.5 ± 0.4	0.2 ± 0.1

*Three animals for each time interval

This agent, ^{99m}Tc-PAHIDA studied here reported as superior to ^{131}I-OIH for renal imaging due to its biological properties in renal failure, Bhargava et al (1988). The agent (^{99m}Tc-PAHIDA) satisfies structural requirement for renal requirements for renal secretion as reported by

Despopoulos (1965). Due to the presence of the $CONHCH_2COOH$ grouping (carboxyglycine, CO-G) analogous to OIH (orthoiodohippuric acid). This structure entity is generally believed to be essential in these compounds for an efficacious fit with the receptor proteins of the tubular(transport system according to Despopoulos's theory (1965).

References

1-Arguelles, M.G., Canellas, C.O., et al. Acta Bioquimica Clinica. Latinco America, 22, 379 (1988).

2-Arnold. W., Subramang, McAfee. J.G.,Blatr, R.J and Thomasf, D. J. Nucl. Med. 16, 357 (1975).

3- Boyd, G.E., J. Am. Chem. Soc. 75, 5773 (1953).

4- Boyd, R.E., Radio Chem. Acta, 30, 123 (1982).

5- Balaban, A., and Polymer, A., Technical Reports Series 128, IAEA, Vienna 705 (1971).

6- Boyd, R.E., L, Arson, O.V. and Motta, E., J. Am. Chem. Soc., 82, 809 (1960).

7- Boyd, R. E. and Hetherimgton E., L., Int. J. Appl. Radiat. ISOT., 31 , 250 (1980).

8- Ballinger, J.R., Reid, R.H., and Galenchgn,K.Y., J. Nucl. Med., 29, 572 (1988).

9- Baidoo, K., Scheffel, U., et al, Cancer Res. (Suppl), So, 7995 (1990).

10-Baum,R.P. (1989) in : Monoclonal Autibodies in immunoscintigraply (Edited by Chatal J.P.) CRC Press Boca, Raton, Fla, P. 121.

11-Bormans, G, Cleynhens, J, Jose D, Hoogmartens, M, De Room, Verbruggen A. Synthesis and biological characteristics of the four stereoisomers of ^{99m}Tc-NN bis (Mercaptoacetyl) 2,3 – diamino propanoate. Nucl.Med. Biol 1990; 17: 499. 506.

12-Bubeck, B, Brandau, W., Weber, E., Kalble.T, parekh. N, Georgi P. J. Nucl Med. 1990; 31: 1285 – 1293.

13-Bhargaua K. K. Zhuangu Zhang, Chun S. B., . Raochervu L.and Blaufox,M.D., J Label Compound , Radiopharm 25 ,943 (1988) .

14- Burns H.D, Sowa D.T. and Mazzilli, L.G. J.Pharm. Sci. 67 : 1434 (1978).

15-Chrevu. L.R., Sundoro. B.M. and Blaufox, M.D.J. Nucl. Med. 25, 111 (1984).

16- Chervu, L.R, Blaufox, M.D.: Semin Nucl. Med. X II : 224 – 245 (1982).

17- Dechiara, S., Lassen, N.A. and Anderson, A.R., Am. J. Phys imag, 2, 192 (1987).

18-Deutsch, E., Radiopharmaceuticals, Society of Nucl. Med. NY 130 – 146 (1979).

19- Dewanjee, M.K., Semin, Nucl. Med., 20 (1), 5 (1990).

20- Dewanjee, M..K.: Non – invasive imaging of dystrophic calcification, in Rubin R.P., etal (eds : Calcium in Biological System, New York.

NY, Plenum, 677 (1985).
21- Deutsch, K., Libson etal., J.Nucl. Med., 22, 89, 7(1981).
22- Dewanjee, M.K., Robison, R.P et al., J Nucl. Med, 30, 737 (1989).
23- Despopoulos A J. theor Biol, 8 : 63 (1965).
24- Eckelman. and Volkert W.A. Int. J Appl, Radiat. Isot, . 33, 945 (1982).
25- El-Asrag, H.A, El-Kolaly, et al., 4th Conf–Nucl. Sc. Appl; 2 (P. 3.3.1/1). 565 (1988).
26- Eckelman, W.C, and Levenson, S.M., Int. J. Appl. Radiat. Isot; 28, 67 (1977).
27- El-Kolaly, M.T. and El-Wetery, A.S., Labelled Compds and Radio Pharm; 28, 329 (1990).
28- El-Rolay M T., Preparation of some radiopharmaceuticals Labelled with ^{99m}Tc for medical use " Ph. D. (1983) Cairo University".
29- El-Sayed, E.A "Studies on the Preparation of Lyophilized Radio-pharmaceutical Kits and their Evaluation" M.Sc. thesis 1989, Cairo University.
30- Firtzberg, A.R, Kasina, S., Eshima, D., Johnson,D.L., J. Nucl. Med. 27 : 111 – , (1986);.
31- Fields A.T Porter, Patricks. Callary, Elizabeth B. et al; J. labelled Comp 15 ,387. (1978).
32- Fritzberg ,A.R. Klingensmith ,W.C. Whitney W.P et al J. Nucl.. Med. 22,258 (1981).
33- Fritzberg ,A.R. Kuni. C.C., Klingensmith W.C et al. J.Nucl. Med. 23, 592 (1982).
34- Fritzberg, A.R, Kasina S., et al J. Nucl . Med. 27, 11 (1986).
35-Goldblith et al Freeze drying and advanced food technology, Academic Press Inc; London, Ltd, (1975).
36-Hayes, R.L., in the Chemistry of RadioPharmaceuticals by Heindel, N.D., Burns, H.D., Honda, T. and Brady L.W., Eds, Mosson, NY, USA, P. 155, (1978).
37-Harper, P.V., Andrews G. and lathrop K.A; Semi-annual report to the Atomic Energy Commission, Jocbson, L.O. Ed. ACRH, 18, September 76 (1962).
38-Harper P.V., Lathrop, K. and Gottochalk, in Radioactive Pharmaceuticals, . 335 U.S Atomic Energy Comission (1966).
39-Harries, T.D., Edmards, D.S. and Platts, S.H., J. Nucl. Med., 33, 979 (1992).
40- Hnatawich, D.J. and Mc Gaun J., Nucl. Med. Biol., 14, 563 (1987).
41-International Union of Pure and Applied Chemistry, Reagents and Reactions for qualitative Analysis. 5. th Report, Butterworths, London (1964).
42- Ikedal., Inoueo. and Kuratak. Int. J. Appl. Radiat Isot. 27, 681 (1976).

43- Ikedal., Inou, E. O. and Kurata,.J. Nucl. Med. 18, 1222 (1977).
44- Kasina, J. Nucl. Med. 28; 1180 (1986).
45-Kuni, Klingen, Smith, W.C, Fritzberg, A.R. Spitzer, V.M., Latteier J.L. clin Nucl. Med., 10, 810 – 813(1985).
46- Knox, K., Tyree S.Y., J Am . Chem. Soc., 81 ,7 (1959).
47-Kemitt, R.D.W. and Peacok; R.D. : The Chemistry of manganese, technetium and rhenium, Oxford, New York, Pargamon Press (1973).
48-Katti, K. V., Singh, P.R, et al , Int. J. Appl. Radiat. Isot., 43, 1151 (1992).
50-Loberg, M.D., Cooper, M. Harvey, E., Callory, P. and Faith W. J. Nucl. Med 17, 633 – (1976).
51-Marques, R.O., Cristinin, P.R., et al IAEA –TECDOC –515, Fission Molybdenum for Medical use 13 – 16 Oct., (1987).
52- Mock, B. H. and Knglish, D., J. Nucl. Med., 28, 147 (1987).
53-Mitta ,A.E.A., Archiprete C.and Gros F.G., J. Labelled Compd. Radiopharm, 19 , 1602 (1982).
54-Mc Afee JG, Subramanian G, Schneider RF, et al J. Nucl. Med., 26 : 375 .(1985).
55-Nowotnik. D.P., Phsico – Chemical Concepts in the Preparation of Tc Radio Pharmaceuticals, in Text book of Radiopharmacy 2nd Edution Edited by C.B Sampson, Gordon and Breach Scince Publishers NYP 41 (1994).
56-Nunn. A.D., Loberg, M.D. and Conley, R.A., A structure J. Nucl. Med. 24, 423 –430 (1983).
56- Perrier, C. and segre, E., Nature, 140, 139 (1937).
57- Perrier and Segre, E., Nature, 159, 24 (1947).
58-Phan. T., Wasnich, R., Practical Nuclear Pharmacy, Second revised edition, 54 (1981).
59-Proggenburg, J.K, Semin, Nucl.Med. 4, 229 (1984).
60-Richards, P.AEC Seminar, 6, 323 (1966).
61-Rodes, B.A., Nucl. Med. Biol., 18, 667 (1991).
62-Robbins P.J.,Chromatography of ^{99m}Tc-radiopharmaceuticals. A practical Guide (1984).
63-Summer, Vipp D.A., Pakard, A.B., Bartynski, B., Limks, Chervu L.R., Treves, S.T., Evaluation of the renal clearance. of ^{99m}Tc – PAHIDA in dogs., Nucl. Med. 28,907 (1987).
64-Srivastaua, S.C. and Richards, P., "Technetium-99m Labelled Copounds" CRC series in Radiotracer in Biology and Medicine, 1, 197 (1983).
65-Smith, E.M., J. Nucl. Med., 5, 871 (1964).
66-Schwochah, K., Radiochemic. Acta, 32, 139 (1983).
67-Seaborg, G.T., and Segre, E, Phys. Rev., 55, 808 (1939).
68-Sampson, C.R,. Text Book of Radiopharmacy Theory and Practice,

Gordon and Breach Seieuce Publishers, (1994).
69-Srivastava, S.C., Meinken, G., et al "Problem associated with stannus ^{99m}Tc-radiopharmaceuticals"in: Welch M.J. (ed.) Radiopharmaceuticals and other compounds labelled with short lived radionuclides, Pergawan Press, N.Y.P. 83 (1977).
70-Steigman, J.M., Mein Ren, G. and Richards, P., Int. J. Appl Radiat. Isot., 26, 606 (1975).
71-Szaboz, Butkuhn, B., Georgeseu, G., Mecklen. book Wosuat mad JIA, Vosberg H. Parometrische Derste llugder Nieren Funktion mit ^{99m} Tc-Merkaptoazety ptriglyzine (MAG3) Nucl Med (1989); 28 : 72. 83.
72-Taylor A, Eshimad; Fritzberg A.R., Christion P.E. etal J. Nucl. Med 27, 795 (1986).
73-Troutner D.E., Vol Rerk et al., Int. J.Appl., Radiat. Isot., 35, 467 (1984).
74- Subramanian, G. and MCA fee, Dadiology 98, 192 (1971).
75-Saha, G.B., Fundamentals of Nuclear Pharmacy, 3rd edition Springer Verlay New York, 1nc (1992).
76-U.S. Pharmacopeia, Twenty First Revision, 1156, (1985).
77-Vera-Ruiz, H., RCM on "Development of Radioactivity Labelled Cancer Ceeking Biomolecules for Targeted Radio Therapy" 20 –23 July (1998) Milan , Italy.
78-Vogel's Textbook of practical organic chemistry, forth edition BLBS long man group 1 Td 1978, 885 (1978).
79-Narasimban D.V.S,Kum P,Vanaja and Mani R.S,J.Radioanal.Nucl.Chem,Leh.,85,163(1984)
80-Rodes B.A, Semin. Nucl.Med.,4,281(1974).
81-Eckelman W.C. and Steigman J,Nucl.Med.Biol.,18,3(1991).
82-Johansen.B and Narasimhan D.V.S,IAEA-TECDOC-649(1992).
83-Tucker W.D.,Greene M.W.,WeissI.J.and Muckenhoft A.P.,Trans.Am.Nucl.Soc.1,160(1958).
84-Sunderberg M.W,Meares C.F., Goodwin D.A et al.J.Med Chem.,17,1304.(1974).
85-Walovitch, R,C, Tams, S.W., et al S. Nucl. Med. 28, 738 (1987).
86-Zmbova, D., DJOKIC, V. Bo GDANOVA, TA, DZER,. B. AJDJNOVIC[3] and M. RASTOVAC[3] Int. Appl. Radiat J. Sot., 40 , no 3 (1989) 225.
87-Eckelman and Steigman J,Nucl.Med.Biol.,18,3(1991).
88-Meares C.F and Goodain D.A,J.of protein Chem.,3,215(1984).
89-Chatterjee Mand Banerjee S,Nucl.Med.Biol.18,263(1991).
90-Kohler G.and Milstein C,Nature,256,495(1975).
91-Deutsch E.and Libson K.In Technetium in chemistry and Nuclear Medicine(Eds Deutsch E.,Nicolini M and Wagner H.N.J).(Cortina International,Verona,1983).

الملخص العربي

ملخص

أن التطور الحادث اليوم في مجال الطب النووي جاء نتيجة التقدم السريع في اســـــتخدام المركبـــات الصيدلانية المجفدة و المرقمة بالتكنيسيوم-٩٩م و نتيجة للخواص الطبيعية المتميزة للتكنيســـيوم -٩٩م فقد شاع استخدام هذا النظير المشع في مجال الطب النووي . وانطلاقا من هذا فقد جـــاء اهتمامنـــا بصياغة و تركيب مثل هذه المركبات الصيدلانية المرقمة بالتكنيسيوم-٩٩م.

تهدف هذه الرسالة إلى التخليق الكيميائي لبعض مركبات حمض الهيبيوريك امينو ثنـــــائى حـــامض الخليك مع توصيفها بالطرق الفيزوكيميائية ثم ترقيمها بالتكنسيوم-٩٩م ودراسة العوامل المؤثرة على عائد ترقيمها بالتكنسيوم-٩٩م و كذلك تعين مسارها البيولوجى فى الفئران و دراسة العوامل المؤثرة على عائد ترقيمها باستخدام التكنيسيوم-٩٩م و كذلك تعيين مسارها البيولوجـــــي في الفـــئران و تستخدم هذه المركبات في التصوير الإشعاعي للكلى.

وتنقسم هذه الدراسة إلى ثلاث أجزاء رئيسية و التي يمكن الإشادة إليها في أيجاز على النحو التالي:

الفصل الاول

يشتمل هذا الجزء على المقدمة و التي تفيد الدراسة النظرية لفهم طبيعة هذا العمل و الوقوف علـــــى معانى مصطلحاته و توضيح الآتي:

شاع استخدام التكنيسيوم-٩٩م في مجال الطب النووي وذلك لما له من خواص أهمها أشعة جامـــا النابعة عنه و المناسبة للتصوير الإشعاعي باستخدام الجاما كاميرا و التي تساوى ١٤٠ كيلو إليكـترون فولت. ينتج التكنيسيوم-٩٩م من انحلال الموليبدنيوم-٩٩ والذي بدوره يمكن الحصول عليـــه مـــن نواتج الانشطار النووي لليورانيوم-٢٣٥ أومن تشعيع ثالث أكسيد الموليبدنيوم بالمفاعل.

تحتاج المركبات التي يتم ترقيمها بالتكنيسيوم-٩٩ الى ضرورة وجـــــود عـــامل مخـــتزل لاختـــزال التكنيسيوم-٩٩ من صورته المؤكسدة الخاملة +٧ إلى الصورة النشطة و التي تكـــــون فيـــها حالـــة أكسدته تساوى +٥,+٤,+٣ وبذلك يتمكن من تكوين المعقدات مع هذه المركبات

و من أهم المواد المختزلة و التي تستخدم بكثرة هي كلوريد القصديروز المـــائي و حيـــث أن هـــذه المركبات تستخدم للحقن في الإنسان فاءنها يجب أن تكون ذات خواص معينـــة و يجـــب أن تمـــر باختبارات خاصة منها:

أن تكون مصدرة لأشعة جاما فقط و خالية من إصدار الجسيمات المشعة الأخرى مثل جسيمات البيتا و الالفا كما يجب أن تكون قصيرة العمر الإشعاعي البيولوجي و أن تكون معقمة و خالية من البيروجين و كذلك عديمة السمية.

الفصل الثاني

ويشتمل هذا الفصل التخليق الكيميائي لثلاث مركبات هي:

(١) ١(امينو حامض الهيبيوريك),٤ امينو ثنائي حامض الخليك.

(٢) ١(امينو حامض الهيبيوريك),٣ امينو ثنائي حامض الخليك.

(٣) ١(امينو حمض الهيبيوريك),٢امينو ثنائي حامض الخليك.

لقد تم تخليق هذه المركبات باستخدام طريقتين الأفضل فيها هي بيرن لأنها تعطى مركبات ذات نقاوة كيميائية عالية و في وقت اقل و تعتمد على تفاعل تكثيفي بين كل من نيتريلو ثلاثي حامض الخليك احادى اللامائى مع ٢،١مينو حامض الهيبيوريك و ٣،١ امينو حامض الهيبيوريك او ٤،١ امينو حامض الهيبيوريك في وجود البيريدين ليعطى ناتج تخليقي مقداره ٧٩ %, ٦٥%و ٦٩ % على الترتيب .

و قد تم إثبات تراكيب المركبات الناتجة باستخدام التحاليل الدقيقة للعناصر و طيف الاشعة تحت الحمراء و طيف الرنين النووي المغناطيسي و كذلك طيف الكتلة .

الفصل الثالث

في هذا الفصل شرح لترقيم المركبات التي تم تخليقها باستخدام التكنيسيوم-٩٩م لتكوين معقدات لها صفات بيولوجية محددة ومع هذا توجد بعض المشاكل التى تؤثر على كفائتها و منها عدم ثباتها بعد الترقيم و كذلك وجود الشوائب التي تتداخل في توزيعها البيولوجي .

لذلك كان من المهم دراسة العوامل التى تؤثر على عائد الترقيم للوصول الى اعلى عائد ترقيمي ، أعلى درجة نقاوة راديو كيميائية، افضل درجات ثبات للمعقدات و كذلك افضل تمركز للمعقد في العضو المراد تصويره. وجد أن افضل تمركز للمركبات التي تم تخليقها: ٢،١ امينو حامض الهيبيوريك و ٣،١ امينو حامض الهيبيوريك و ٤،١ امينو حامض الهيبيوريك امينو ثنائي حامض الخليك هي ١٠ مجم لكل منها . ووجد إن استخدام كوريد القصديروز بتركيز ٢٥،. مجم يؤدى إلى اختزال البيرتكنيتات بدرجة كافية تؤدى إلى تكوين المعقدات بنسبة عالية . عامل اخر له تأثير فعال على

ترقيم هذه المركبات و هو درجة الأس الهيدروجيني لوسط التفاعل و الذي تبين بعد دراسته أن افضل تركيز للأس الهيدروجيني هو ٥،٧ و يعطى أعلى عائد ترقيمي .

اما بالنسبة للعامل الاخير و هو الزمن فقد وجد انه عند حقن هذه المعقدات فى فئران التجارب وجد انها تتمركز بسرعة و بتركيز عالي في الكلى و البول بعد مدة ١٠ دقائق من الحقن .

فقد وجد إن المتعاقد يفرز في البول بعد الحقن بنسبة ٦٠% ,٦٥ %و٨% على التوالي وهذه النتائج تثبت أن الركب ٤،١ امينو حامض الهيبيوريك امينو ثنائى حامض الخليك عند ترقيمه بالتكنيسيوم-٩٩ تحت هذه الظروف يعطى اعلى عائد ترقيمى وهو (٩٥%) .

و يتميز هذا المركب بأن يفرز بنسبة عالية بالمقارنة بالمثيلين الآخرين.

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"دراسات على استخدام بعض المركبات العضوية المرقومة بنظير التكنسيوم-٩٩م فى التصوير الكلوى"

رسالة مقدمة من

هدى على عبد الرحمن شويتة

بكالوريوس علوم (كيمياء) ١٩٩٢

مركز المعامل الحارة

هيئة الطاقة الذرية

إلى

كلية علوم بنها-جامعة الزقازيق

للحصول على درجة الماجستير فى الكيمياء

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هيئة الطاقة الذرية

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