CHEMICAL AND BIOLOGICAL STUDIES OF TECHNETIUM NITROSYL COMPLEXES

By

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The author certifies that the work submitted in this thesis is her original work and that neither the thesis nor the original work has been submitted in full, or in part, to this or any other institution for a higher degree.

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ABSTRACT

A simple and widely applicable method for the synthesis of technetium nitrosyl complexes containing either [^{99}Tc] or [^{99m}Tc] has been developed using hydroxylamine as a reducing agent. A number of readily available complexes such as $[TcO_4]^-$ or $[TcX_6]^{2-}$ (X = Cl, Br, I) may be employed as the starting material.

From $[TcCl_6]^{2-}$ a quantitative yield of $[Tc(NO)Cl_4L]^-$ was produced. using [^{99m}Tc] generator eluant the complex has been prepared by reduction with hydrochloric acid and hydroxylamine. Using the same conditions for [^{99}Tc] pertechnetate the complex has been prepared, isolated and recrystallised as the tetrabutylammonium salt from methanol solution. The complex is found to be six coordinate and a single crystal X-ray structure determination of the crystalline form confirms the identity of $[(C_4H_9)_4N][Tc(NO)Cl_4(MeOH)]$. The anion takes the geometry of a distorted octahedron where the four chlorine atoms lie in the equatorial plane while the methanol ligand is trans to the nitrosyl. The nature of the sixth ligand may be varied readily due to the trans labilising effect of the nitrosyl and in vivo is likely to be replaced.

The synthesis is a multistage one but affords a single complex readily amenable to exchange of the chloride ligands by a series of compounds. In particular reactions with ammonia, alkylamines, acetylacetone, 1,2-bis(diphenylphosphine)ethane, o-phenylenebis-(dimethylarsine), 2,2'-bipyridine, 1,10-phenanthroline and t-butylisonitrile have been studied and the products isolated and characterised.

A novel series of potential radiopharmaceuticals has thus been prepared containing the technetium nitrosyl core as an alternative to the commonly available oxo- and dioxo- complexes. The biological distribution in rats of some of the above complexes has been examined.

ABBREVIATIONS

acac	acetylacetone
bipy	2,2'-bipyridine
bipm	2,2'-bipyrimidine
cyclam	1,4,8,11-tetraazocyclotetradecane
depe	1,2-bis(diethylphosphine)ethane
diars	o-phenylenebis(dimethylarsine)
diphos	o-phenylenebis(dimethylphosphine)
DMF	N,N'dimethylformamide
dmpe	1,2-bis(dimethylphosphine)ethane
dppe	1,2-bis(diphenylphosphine)ethane
DTPA	diethylenetriaminepentaacetate
EDTA	N,N,N',N'-ethylenediaminetetraacetate
en	1,2,-diaminethane
Et	ethyl
HBPz	hydrotris(pyrazolyl)borate
HEDP	1-hydroxyethylidene-1,1-diphosphonate
HIDA	N-(2,6-dimethylphenylcarbamoylmethyl)-iminodiacetate
HM-PAO	hexamethyl PnAO
MAG3	mercaptoacetyltriglycine
MDP	methylenediphosphonate
Me	methyl
phen	1,10-phenanthroline
PnAO	[3,3'-(1,3-propanediyldiimino)bis(3-methyl-2-butanone oximato)$^{-2}$]
py	pyridine
terpy	2,2':6'2"-terpyridine
t-butyl-	tertiary-butyl-
tu	thiourea

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CHAPTER 1

INTRODUCTION

1.1. Isolation of technetium and its general properties.

1.1.1. Discovery.

By the end of the first quarter of the twentieth century the element with the atomic number 43 was still missing from the periodic table, despite the prediction of the existence of ekamanganese by Mendeleev in 1869. A series of errors in the discovery of the new element followed and attempts to find element number 43 in nature were unsuccessful. In 1925 the Germans Noddack, Tacke and Berg published a report (1) on the discovery of new elements called masurium and rhenium. Further experiments characterised rhenium but not masurium (2).

Failure of scientists to find technetium in the earth's crust can be explained by the absence of this element in nature as only radioactive isotopes of technetium exist with a half-life much shorter than the age of the earth. With the advance of nuclear physics, conditions were created for the detection and preparation of technetium.

In 1937 element 43 was the first element to be produced artificially in trace amounts (10^{-10}g). Called technetium, from the Greek word *technetos* (artificial), by the Italian physicists Segre and Perrier who correctly identified the radiations from a molybdenum plate which had been bombarded by deuterons as the missing element (3).

1.1.2. Occurrence in nature.

After some of the properties of technetium had been investigated attempts were again made to find naturally occurring technetium.

Early reports suggested the presence of technetium on the sun (4) (later disputed) and in the atmosphere of certain stars (5,6,7). Following this researchers attempted to isolate primordial technetium in samples of molybdenum blende with high rhenium contents, isolating the technetium by multiple distillations (8). The first isolation of an appreciable amount of natural technetium was achieved by Kenna and Kuroda (9) who obtained (10^{-9}g) of ^{99}Tc from 5.3kg of pitchblende mined in the Congo.

1.1.3. Isotopes and nuclear properties of technetium.

Over 30 radioactive isomers and isotopes of technetium are known with mass numbers ranging from 92 to 107 decaying by a number of routes - β^- decay, electron capture or isometric transition. Half-lives vary from the longest lived ^{99}Tc - 2.12 x 10^5 years through

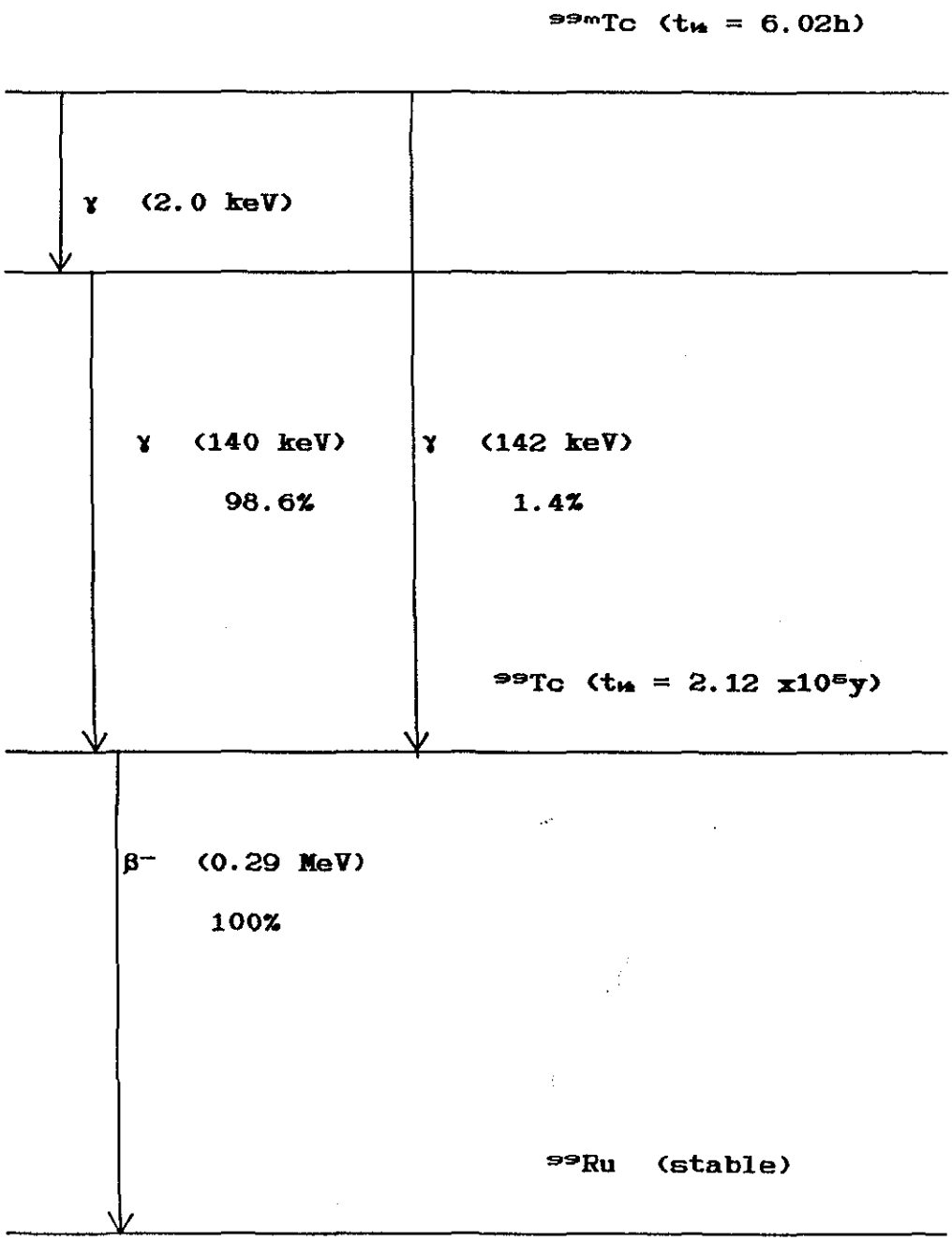
^{97m}Tc - 90.5 days, ^{95}Tc - 60 days, ^{99m}Tc - 6.02 hours and ^{100}Tc - 15.8 seconds. Full tables of these isotopes and isomers may be found in the relevant texts (10).

Most chemical studies of technetium are performed using the longest lived isotope of technetium, ^{99}Tc, which is now produced in kilogram amounts in nuclear reactors mainly by fission of ^{235}U by thermal neutrons in approximately 6.2% yield (11). This isotope presents only a minor external radiation hazard although care must be exercised to minimise the more serious production of Bremsstralung during manipulation. Concentration of technetium with a long half-life within an organism is dangerous since it may lead to lesion of the tissues by β^- irradiation. Therefore when working with technetium it is necessary to use a fume hood to prevent the spread, contamination and ingestion of volatile compounds.

The metastable isomer ^{99m}Tc has found extensive use in diagnostic nuclear medicine because of its nuclear properties. It is produced by irradiation of stable ^{98}Mo which becomes unstable ^{99}Mo by neutron capture. The ^{99}Mo decays by β^- emission ($t_{½}$=67 hours) to the short-lived ^{99m}Tc which in turn decays to ^{99}Tc. This decays to stable ruthenium (Figure 1.1.)

Figure 1.1.

Decay scheme of the isotopes ^{99m}Tc and ^{99}Tc



1.1.4. Applications of technetium.

Technetium has many properties which make it valuable in many research fields.

In contrast to the analogous perrhenate ion the pertechnetate ion exhibits exceptional anticorrosive properties. Studies on the anticorrosive effect of $[MO_4]^-$ type ions showed that $[TcO_4]^-$ is the most effective inhibitor of the corrosion of iron and steel. This effect is exhibited even at high temperatures such as 250°. Thus technetium compounds can be used effectively to protect homogeneous reactors, parts of submarines, etc from corrosion.

At low temperatures, metallic technetium is a super-conductor and some of its alloys also show this property. Thus it is possible to use technetium in rocket technology, in inertial guiding systems, in electronic computers as a storage element and to create high voltage magnetic fields in thermonuclear installations (11).

As mentioned earlier the isotope ^{99m}Tc is widely used in diagnostic nuclear medicine because of the nuclear properties of the isotope and the diverse chemistry of the element. It is the exploration into the inorganic chemistry of technetium that permits the development of radiopharmaceuticals.

1.1.5. General chemical properties.

Technetium is a second row transition metal in Group VII of the periodic table. It would be expected that its chemistry resemble that of its immediate neighbours molybdenum and ruthenium but because of the lanthanide contraction its chemistry is more like that of rhenium rather than the higher first row congener manganese, despite similarities in the stoichiometries of some compounds, for example, the series $[MnO_4]^-$, $[TcO_4]^-$ and $[ReO_4]^-$.

The most stable state for manganese is the (II) oxidation state dominated by the chemistry of the high spin Mn^{2+} cation. Technetium has extensive chemistry in the (IV), (V) and (VII) oxidation states although the chemistry of the lower oxidation states, (I), (II) and (III) is now being more fully explored. A characteristic feature of rhenium(III) in its halides is to form metal-metal bonds. Technetium does this to some extent whereas manganese forms no such compounds (12).

Elemental technetium has the electronic configuration $(Kr)4d^65s^1$ and compounds in all oxidation states from (-I) d^{10} to (VII) d^0 have been isolated and characterised displaying coordination numbers ranging from 4-9. Selected examples are given in Table 1.1.

Table 1.1.

Selected examples of technetium complexes

Complex	Oxidation State	Reference
Tc_2O_7	7	(13)
$KTcO_4$	7	(14)
$TcCl_6$	6	(15)
$TcOF_4$	6	(16)
$[TcOCl_4]^-$	5	(17,18)
$TcOCl_3(phen)$	5	(19)
$[TcO_2(en)_2]^+$	5	(20)
$[Tc(diars)_2Cl_4]^+$	5	(21)
TcO_2	4	(22)
$[Tc(acac)Cl_4]^+$	4	(23)
$Tc(acac)_2Cl_2$	4	(23)
$TcCl_6^{2-}$	4	(24)
$[Tc(dmpe)_2Cl_2]^+$	3	(25)
$[Tc(acac)_2(PPh_3)Cl]$	3	(23)
$[Tc(dppe)_2Br_2]^+$	3	(26)
$[Tc(NO)Cl_4]^-$	2	(27)
$[Tc(NO)(NCS)_5]^{2-}$	2	(28)
$Tc(diars)_2I_2$	2	(29)
$[Tc(NO)(NH_3)_4(H_2O)]^{2+}$	1	(30)
$Tc(C_5H_5)(CO)_3$	1	(31)
$Tc_2(CO)_{10}$	0	(32)
$[Tc(CO)_5]^-$	-1	(33)

The perrhenate and pertechnetate ions are well known in aqueous solution although they are much less oxidising than permanganate. Pertechnetate is the starting material of choice for most synthetic chemistry and nuclear medicine applications and although it is stable in alkaline solution it can be reduced by HCl, HBr or HI to generate two other classes of convenient starting material. The technetium(V) complexes $[TcOX_4]^-$ (X = Cl, Br) can be prepared by reaction of pertechnetate with cold HX (17,18) while, with hot HX, the technetium(IV) species $[TcX_6]^{2-}$ (X = Cl, Br, I) can be prepared (24).

The synthesis of technetium complexes generally involves the reduction of the technetium centre and/or the substitution of a ligand.

All nuclear medicine preparations start with $[^{99m}TcO_4]^-$ which is then reduced in the presence of the ligand or particle that is to be labelled. A wide variety of reducing agents have been used to reduce pertechnetate. Tin(II) in the form of tartrate, chloride and sulphide, tin foil (34,35,36,37), sodium borohydride (38,39), sodium dithionite (40,41), sodium bisulphite (42,40), hypophosphorus acid (41), formamidine sulphinic acid (40,42), hydrazine (40,43), hydroxylamine (40,44), sodium azide (45,46), chromium(II) (47), molybdenum(III) (48) and concentrated hydrochloric acid (17,18,24,49) amongst others.

The pertechnetate will eventually be reduced to the tetravalent, insoluble TcO_2xH_2O unless a complexing ligand is present to stabilise the lower oxidation state. For example the reduction of $[TcO_4]^-$ from technetium(VII) to technetium(I) by sodium dithionite is stabilised in the presence of isonitriles (40,41,50) and the reduction of technetium(VII) to technetium(V) by tin(II)tartrate is performed in the presence of ethylenediamine (34).

Some ligands have the ability to reduce the technetium and complex simultaneously such as phosphines (25,51,26,27) arsines (51,29), thiols (52,53) and thiourea (54).

Substitution reactions have been carried out mainly on the starting materials $[^{99}TcOX_4]^-$ and $[^{99}TcX_6]^{2-}$ (X = Cl^-, Br^-) but for radiopharmaceuticals these are not easily prepared. However, complexes such as ^{99m}Tc-gluconate, ^{99m}Tc-citrate, ^{99m}Tc-DTPA, and ^{99m}Tc-glucoheptonate have been used as starting materials in substitution reactions (55,56).

1.1.6. Bonding in technetium complexes.

Elemental technetium has the electronic configuration $(Kr)4d^65s^1$ as shown in Figure 1.2. In complex formation the d electrons are generally in a low spin state, as expected for a second row transition element.

Figure 1.2.

Electronic configuration of elemental technetium

^{43}Tc $1s^2$ $2s^2$ p^6 $3s^2$ p^6 d^{10} $4s^2$ p^6 d^6 $5s^1$

5s

$4d_{e_g}$

$4d_{t_{2g}}$

Technetium compounds have been characterised in all oxidation states from 0 to 7 and which oxidation state is stabilised is a function of the ligands surrounding the central metal atom. Table 1.2. lists examples of hard and soft bases. Soft bases are π or p acid ligands which accept electron density and stabilise low oxidation states whilst the hard bases are electron donors and stabilise high oxidation states.

Table 1.2.

Hard and soft bases

Hard Bases	H_2O, R_2O, ROH, NH_3, RNH_2, OH^-, OR^-, Cl^-, NO_3^-, SO_4^-, CO_3^{2-}, PO_4^{3-}
Soft Bases	R_2S, RSH, R_3P, R_3As, RS^- I^-, SCN^-, CN^-, H^-, R^-
Borderline	Pyridine, Br^-, N_3^-, NO_2, SO_3^{2-}

A characteristic feature of the d group transition metals is their ability to form complexes with a wide variety of neutral molecules such as carbon monoxide, isocyanides, substituted arsines and phosphines, nitric oxide and various molecules with delocalised π orbitals such as pyridine, 2,2'-bipyridine and 1,10-phenanthroline. In many of these complexes the metal atoms are in low-positive, zero or negative formal oxidation states. It is characteristic of these π-acceptor or π-acid ligands that they can stabilise low oxidation states. This property is associated with the fact that these ligands possess vacant π-orbitals in addition to lone pairs. The vacant π-orbitals accept electron density from filled metal orbitals to form a type of π-bonding that supplements the σ bonding which arises from lone pair donation. High electron density on the metal atom is therefore delocalised onto the ligands.

Since the π-acceptor ligand forms bonds to the metal using σ orbitals and exercise their π-acidity by using π orbitals whose nodal planes include the axis of the σ-bond, the metal lies along the axes of the linear ligands or in the plane of planar ones.

The most important π-acceptor ligand is CO and the NO molecule is very similar except that it contains one more electron which occupies a π* orbital. This electron is easily lost to form the NO^+ ion where the vacant π-orbitals are available to accept electron density from occupied metal orbitals. Bonding of this type is generally associated with linear CO or NO groups. Low

oxidation state complexes with these and other π-acceptor ligands such as CN^- and RCN are described later in this chapter. In general the metal has filled or partially filled t_{2g} molecular orbitals available for backbonding from the metal to the ligand. The extent of π-bonding and the influence of other ligands in the coordination sphere on electron density is reflected in the infra red absorptions of the N-O stretching frequency.

High oxidation states are stabilised by π-electron donors such as Cl^-, N^{3-} and O^{2-} where the largely unoccupied t_{2g} metal 'd' orbitals of the same symmetry as the ligand's p nonbonding orbitals.

1.2. Technetium in nuclear medicine.

1.2.1. Suitability and availability.

Radiopharmaceuticals may be used as diagnostic or theraputic agents by virtue of the physical properties of their constituent radionuclides. Thus, their utility is not based on any pharmacologic action. Clinically used technetium radiopharmaceuticals are diagnostic agents which, because of the physical or metabolic properties of the coordinated ligands, localise in a specific organ after intravenous injection. A gamma camera is used to detect the distribution of the radiation emitted by the the radioactive molecules and produces images which reflect organ structure or function.

In the early sixties it was suggested that [^{99m}Tc]technetium had potential application in nuclear medicine and was first used for this purpose in 1962. It is now the isotope of choice for diagnostic nuclear medicine because of its radiochemical properties:

(i) A short half life ($t_{½}$ = 6.02 hours), it has a high specific activity and decays quickly enough not to present a serious dose hazard;

(ii) There are no α or β emissions which minimises absorbed dose to non-target organs;

(iii) It decays to a daughter isotope, ^{99}Tc, which gives negligible radiation dose because it remains in minute amounts;

(iv) It is a monoenergetic γ emitter of sufficient energy (140keV) to be detected through layers of tissue and which lies within the optimum detection range of commercial gamma cameras;

(v) It has the ability to form many and stable complexes with a wide variety of ligands, thus enhancing its versatility;

(vi) It is readily and inexpensively available from commercial generators;

The first ^{99}Mo/^{99m}Tc generator was developed in 1965 in the USA. the ^{99m}Tc is produced by the decay of ^{99}Mo adsorbed onto an alumina column. Separation is usually achieved by aqueous elution,

usually with physiological saline, of the ^{99m}Tc as sodium pertechnetate.

The column must fulfil certain requirements - it must be stable to radiolysis, elution of nephrotoxic Al^{3+} ions must be minimal and reliable elution of pertechnetate must be guaranteed.

1.2.2 Technetium radiopharmaceuticals.

Many technetium imaging agents are available, usually supplied in the form of a freeze dried or 'cold' kit which consists of a reductant in the presence of a complexing agent and is reconstituted on the addition of generator eluant (57). These radiopharmaceuticals consist of two broad classes (58):

(a) Those where the technetium is 'tagged' to a large molecule and essentially is such a small addition that the normal biodistribution of the molecule is unaltered. Examples of these include.

(i) *Human serum albumin (HSA)* for blood pool assessment and lung imaging;

(ii) Human erythrocytes for blood pool imaging;

(iii) Sulphur colloids for imaging of the reticuloendothelial systems of the liver, spleen and bone;

(iv) Micro and microaggregated spheres of HSA for evaluation of the lung arteriolar capillary bed;

In the above systems the technetium is essentially a tracer and monitors the in vivo distribution of the parent substance.

(b) Those where the biodistribution depends on the properties of the technetium complex itself. Examples of these compounds include:

(i) Kidney function agents Tc-DTPA, Tc-EDTA, Tc-MAG3 (116);

(ii) Kidney structure agents Tc-gluconate, Tc-glucoheptonate, Tc-Fe-ascorbate, Tc-dimercaptosuccinic acid;

(iii) Infarct visualisation agents Tc-pyrophosphate, Tc-glucoheptonate, Tc-tetracycline and Tc-HEDP (hydroxyethylidene)diphosphonate;

(iv) Hepatobiliary agents - iminodiacetate derivatives, acetanilido-IDA ligands such as HIDA, also Tc-isomercaptobutyric acid and Tc-pyridoxylideneglutamate;

(v) Bone agents e.g. polyphosphates and phosphonates such as Tc-MDP;

(vi) Brain agents Tc-HMPAO, Tc-ECD, Tc-MRP20 (117). Tc-HMPAO has also been noted for use as a tumor blood flow agent (118);

(vii) Tc-isonitrile series for heart imaging (50).

In addition, direct use of pertechnetate itself is advantageous in imaging some types of lesions particularly of the thyroid (59) and brain (60).

Early radiopharmaceutical kits produced mixtures of two or more complexes (61) but modern kits attempt to produce only a single species. Because of the concentration of technetium in most generator eluants (10^{-6} to 10^{-8}M) it is not possible to identify by direct methods the complexes produced. Instead, the radiopharmacist has to rely on other methods such as chromatography and electrophoresis to confirm the desired product. However, generator eluant contains a mixture of $^{99m}TcO_4^-$ and $^{99}TcO_4^-$ because of the continuous decay of firstly ^{99}Mo and then ^{99m}Tc. The exact concentration depends upon a number of factors but particularly on the length of time that has elapsed since the last elution. These concentrations are much greater than those usually encountered in carrier free radiochemical solutions, mainly because the naturally occuring daughter ^{99}Tc acts as an innate carrier.

^{99m}Tc derived from $^{99}Mo/^{99m}Tc$ generators is referred to as 'no carrier added' to indicate the presence of variable amounts of ^{99}Tc. Inorganic chemistry performed on milligram amounts of ^{99}Tc is therefore referred to as 'carrier added' but can be readily transferred to the no carrier added levels encountered in radiopharmaceutical synthesis.

Technetium chemistry and the development of technetium radiopharmaceuticals is therefore inter-linked and there appears to be an ideal correlation between the chemical behaviour of the two isotopes. In practice, it is usual to formulate the complex

using ^{99}Tc and modify the complex, if necessary, to reach no carrier added levels. However, it has been observed that at carrier added concentration levels the formation of dimeric and polymeric species can cause the chemical behaviour to be rather different than at no carrier added levels where monomeric species form (62).

1.3. Technetium chemistry.

As stated above, technetium is known to form complexes in oxidation states ranging from -1 to 7. This work is primarily concerned with the synthesis and characterisation of technetium (I) and (II) nitrosyl complexes with several different classes of ligand and this review of the chemistry will be confined as far as possible to those oxidation states. However, where appropriate, reference will be made to higher oxidation states.

1.3.1. Technetium(I) and (II) nitrosyl complexes.

Early work by a Russian group led to the preparation of the neutral complex $Tc(NO)Br(py)_3$ from the reaction of $[Tc(NO)Br_4]^-$ in neat pyridine (63).

Following this work Orvig and Davison reacted NO with freshly precipitated TcO_2xH_2O in 4M HBr at 75°C and isolated the tetrabutylammonium salt of $[Tc(II)(NO)Br_4]^-$ (27). The chloro-analogue was prepared by simple halide substitution on this

material. Both complexes show molar conductances in CH_3CN consistent with their formulations as 1:1 electrolytes. The complexes exhibit 10 line electron spin resonance spectra at room temperature in methanol solution as expected for low spin d^5 technetium(II) complexes. The 10 line pattern is the result of electron-technetium nuclear hyperfine interaction (^{99}Tc, $I=^9/_2$) (64).

$[Tc(NO)I_4]^-$ was prepared and isolated by Kirmse et al. by halide substitution of $[Tc(NO)Br_4]^-$ with HI in acetone (65). The ESR spectrum was subsequently reported (66) with a view to getting more insight into the bonding properties of the Tc-ligand bonds. In comparison to the corresponding Tc-nitrosyl compounds with Cl^- or Br^- ligands, more covalent bonds are to be expected. The room temperature spectrum was poorly resolved, due to strong overlapping lines resulting from the hyperfine interactions with the ^{99}Tc and ^{127}I ($I = ^5/_2$) nuclei. Even the spectrum of the frozen solution was not well resolved, but a comparison of the data obtained shows a considerable increase in covalency for the equatorial ligand bonds if going to the heavier halogenide donors.

$[Tc(NO)Br_4]^-$ has been used as a precurser for other complexes containing the $Tc-(NO)^{3+}$ core. $[Tc(II)(NO)(NCS)_5]^{2-}$ has been synthesised by the reaction of $[Tc(NO)Br_4]^-$ with NH_4NCS in methanol (27). The technetium(I) complex $[Tc(I)(NO)(NCS)_5]^{3-}$ is formed rapidly by the reduction of the inky blue solution of $[Tc(II)(NO)(NCS)_5]^{2-}$ with hydrazine. Electrochemical studies in

acetonitrile on both of the isolated tetrabutylammonium salts show that a simple reversible one electron couple exists between the two species.

The *ESR spectrum of* $[Tc(II)(NO)(NCS)_5]^{2-}$ has been recorded and the data analysed to describe the Tc-ligand bonds. The frozen solution spectrum shows well resolved ^{99}Tc multiplets in the parallel and perpendicular parts of the spectrum indicating that the complex is axially symmetrical. The calculations suggest that there is a large extent of covalency in the Tc-N bond due to the π-bonding properties of the NCS^- ligand (119).

Mixed nitrosyl/isonitrile complexes have also been prepared by different methods developed by Linder et al. (68).
$[Tc(NO)(CNt\text{-}butyl)_5]^{2+}$ is prepared firstly by the reaction of $[Tc(CNt\text{-}butyl)_6](NO_3)$ and nitric acid in glacial acetic acid. Yellow needles of $[Tc(NO)(CNt\text{-}butyl)_5](PF_6)_2$ are precipitated on the addition of Na_2PF_6. The second method of preparation is by the reaction of $[Tc(CNt\text{-}butyl)_6](NO_3)$ with $NOPF_6$ in acetonitrile.

$[Tc(NO)Br_2(CNt\text{-}butyl)_3]$ was prepared by the same group from the reaction of $[(C_4H_9)_4N][Tc(NO)Br_4]$ with t-butylisonitrile by refluxing in methanol. The product was separated by flash chromatography and purple crystals grown by evaporation of an aqueous ethanolic solution. The crystal structure of the complex was also determined and found to have distorted octahedral geometry with the ligands cis to the nitrosyl bending out of the

equatorial plane and away from the nitrosyl. The Tc-N bond recorded as 1.726Å and the Tc-N-O angle at 175.9° indicating an essentially linear nitrosyl. Interestingly, there is an isonitrile group trans to the nitrosyl with the two bromides trans to each other and the remaining two isonitrile groups in the equatorial plane. It would be expected that the isonitrile ligand trans to the nitrosyl would be better able to compete with the nitrosyl for electron density of the technetium. This is reflected in the bond length for N-O of 1.136 Å which is somewhat shorter than the N-O bond length of 1.203Å recorded for trans-$[Tc(NO)(NH_3)_4(H_2O)]^{2+}$ (where the water molecule is trans to the nitrosyl) (69). This indicates that π backbonding, which is expected to lengthen the N-O bond distance is less important in $Tc(NO)Br_2(CNt\text{-}butyl)_3$. As a result of this, the two isonitrile ligands cis to the nitrosyl have slightly longer Tc-C bond lengths than are seen in $[Tc(CNt\text{-}butyl)_6]^+$ (average Tc-C bond lengths of 2.08 and 2.03 Å respectively) (74). The Tc isonitrile bond trans to the nitrosyl is longer still at 2.137(22)Å. This lengthening is consistent with the structural trans influence expected at a position axial to the π backbonding nitrosyl.

Eakins et al first isolated a technetium(II) complex formulated as $[Tc(NH_2OH)_2(NH_3)_3]Cl_2$ synthesised from the reaction of hydroxylamine with $[TcCl_6]^{2-}$ (44). Investigation by Armstrong and Taube showed that it was trans-$[Tc(NO)(NH_3)_4(H_2O)]Cl_2$, a six co-ordinate technetium(I) species (30). The crystal structure of the complex was subsequently reported by Radonovich and Hoard

(69). The cation has a distorted octahedral geometry and the bond lengths and angles again indicate a linear nitrosyl.

Taube also described the preparation and characterisation of $[Tc(I)(NO)(NH_3)_2(phen)(H_2O)]^{2+}$, its one electron oxidation product and $[Tc(I)(NO)(NH_3)(phen)_2]^{2+}$

The technetium(II) complex trans-$[Tc(NO)(NH_3)_4(H_2O)]^{3+}$ was obtained by oxidation of trans-$[Tc(NO)(NH_3)_4(H_2O)]^{2+}$ by potassium dichromate or ceric sulphate by Yang et al. and the ESR spectrum of the complex observed (70). When the technetium(II) complex was warmed in hydrochloric acid $[Tc(NO)Cl_5]^{2-}$ was formed. ESR spectra of both complexes showed ten line splitting, as expected. It was also concluded that by altering the oxidation state of the technetium from (I) to (II) the NH_3 ligands become more labile and are consequently replaced in concentrated HCl solution.

More recently, the ESR spectra of the bromo and iodo analogues of $[Tc(NO)Cl_5]^{2-}$ together with the mixed halogenate complexes of the type $[Tc(NO)X_{4-p}Y_pZ]^{2-}$ where X, Y = Cl, Br, I and Z = axially coordinated solvent molecule or halide have been analysed. The ESR parameters measured have been shown to be clearly correlated to the composition of the coordination sphere and can be used to characterise the mixed ligand complexes (121).

$Tc(II)(NO)Cl_3(PMe_2Ph)_2$ was prepared by bubbling NO through a solution of $TcCl_3(PMe_2Ph)_3$ in boiling benzene. A blue green

solution formed and after removal of the solvent black-green crystals of $Tc(NO)Cl_3(PMe_2Ph)_3$ formed from CH_2Cl_2/methanol solution (71). ESR spectrum at room temperature was as expected for the d^5 low spin Tc^{2+} configuration.

The bromo analogue has been prepared by refluxing $Tc(NO)Cl_3(PMe_2Ph)_2$ in acetone with HBr. The product, $Tc(NO)Br_3(PMe_2Ph)_2$, was obtained as green crystals from a mixture of chloroform and n-hexane. Study of the ESR spectrum of the bromo-complex and comparison with that of the chloro-complex suggested that the two phosphine ligands were in equatorial positions although whether these are trans or cis to each other cannot be determined from the ESR spectra (120).

$Tc(II)(NO)Cl_3(PEt_2Ph)_2$ was prepared by bubbling NO through a refluxing solution of $TcCl_3(PEt_2Ph)_2$ in benzene and characterised by Fergusson (72). Green and purple crystals were obtained and over a period of some weeks the purple crystals changed to green but recrystallisation from CH_2Cl_2 gave back the purple crystals. The purple product had an X-ray powder photograph identical to $Re(NO)Cl_3(PEt_2Ph)_2$ whilst that of the green crystals was different. It was concluded that the green and purple products were isomers.

The bright yellow technetium(I) complex $Tc(NO)(PPh_3)_3(H)_2$ has been prepared by the reaction of either $Tc(NO)(PPh_3)_3Cl_2$ or $[(C_4H_9)_4N][Tc(NO)Cl_4]$ with sodium borohydride in ethanol with an excess of triphenylphosphine (122). Evidence from IR and NMR spectra

indicated that a hydrogen ligand was most probably trans to the nitrosyl with the three triphenylphosphine ligands and the remaining hydrogen in the equatorial plane.

Other nitrosyl dihydrides can be synthesised, for example, when $Tc(NO)(PMe_2Ph)_2Cl_3$ is reacted with sodium borohydride and an excess of triphenylphosphine, a yellow compound is obtained which is $Tc(NO)(PMe_2Ph)_2(H)_2(PPh_3)$. The proposed structure is with a hydrogen trans to the nitrosyl and the two dimethylphenylphosphine ligands cis to each other in the equatorial plane (122).

A technetium(III) nitrosyl compound has also been prepared and characterised (123). Upon the reaction of $[Tc(NO)Cl_4]^-$ with 2,3,5,6-tetramethylbenzenethiol (Htmbt) the neutral compound $Tc(NO)Cl(tmbt)_3$ is obtained. The IR spectrum shows a band at 1798 cm^{-1} from the linear nitrosyl group, formally NO^+, establishing that the metal oxidation state is +3. ^1H NMR and crystal structure data confirm that the tmbt ligands are bound in the equatorial plane in the "two up one down" arrangement seen in analogous trigonal bipyramidal compounds. The other two ligands occupy the axial positions.

Oxidation of Tc(II) to Tc(III) was found to be facile in the presence of a limited quantity of air or a stoichiometric quantity of the disulphide of Htmbt.

The crystal structure of the complex establishes that one of the thiolate ligands is directed toward the chloride ligand and the remaining two toward the nitrosyl. This reflects the fact that the chloride is larger than the nitrosyl and the sterically hindered thiolate ligands are adopting the least encumbered formation. The Tc-N-O bond angle of 176.8° confirms the linear nature of the nitrosyl. The Tc-N and N-O bond lengths of 1.767(6) and 1.15(7)Å respectively are longer than those recorded for $[Tc(NO)(NH_3)_4(H_2O)]^{+}$ indicating less π backbonding between the central technetium and the nitrosyl group and more competition for electron density from the thiolate ligands (π acceptors).

Infra-red absorptions of the above technetium nitrosyl complexes where available are given in Table 1.3. below and are indicative of the linear NO^{+} character and the extent of π backbonding in the complexes.

Table 1.3.

Infra-red absorption frequencies of ν(NO) in technetium nitrosyl complexes

Complex	ν(NO) cm^{-1}	Reference
$[Tc(III)(NO)Cl(tmbt)_3]$	1798	(123)
$[Tc(II)(NO)(NH_3)_4(H_2O)]^{3+}$	1830	(30)
$[Tc(II)(NO)Cl_5]^{2-}$	1803	(30)
$[Tc(II)(NO)(NCS)_5]^{2-}$	1785	(27)
$[Tc(II)(NO)Cl_3(PEt_2Ph)_2]$	1775	(72)
$[Tc(II)(NO)Br_3(PMe_2Ph)_2]$	1779 and 1794	(120)
$[Tc(II)(NO)Cl_3(PMe_2Ph)_2]$	1795 and 1770	(71)
$[Tc(I)(NO)(NH_3)_4(H_2O)]^{2+}$	1690	(30)
$[Tc(I)(NO)(NCS)_5]^{3-}$	1690	(27)
$[Tc(I)(NO)(NH_3)(phen)_2]^{2+}$	1712	(30)
$[Tc(I)(NO)(CNt\text{-butyl})_5]^{2+}$	1865	(68)
$[Tc(I)(NO)Br_2(CNt\text{-butyl})_3]$	1775	(68)
$[Tc(I)(NO)(PPh_3)_3H_2]$	1636	(122)

1.3.2. Technetium (I) isonitrile complexes.

Hexakis(t-butylisonitrile)technetium(I) hexafluorophosphate has been prepared by the reaction of red hexakis(thiourea-S)technetium(III) with t-butylisonitrile in refluxing methanol (73).

The complexes $[Tc(CNR)_6]PF_6$ (where R = t-butyl-, methyl-, cyclohexyl- and phenyl-) have also been prepared by the reduction of pertechnetate with aqueous sodium dithionite in the presence of the alkylisonitrile ligand (50). All of the complexes crystallise as white, or in the case of the phenyl derivative pale yellow, crystals. The crystal structure for the hexakis(t-butylisonitrile technetium(I) cation and its rhenium analogue have been determined and have O_h symmetry (74)

The pertechnetate reduction preparation translates well to the no carrier added concentrations giving a quantitative and radiochemically pure preparation. The potential application of these complexes for lung perfusion studies, imaging of normal myocardial tissue, the detection of vascular emboli and the labelling of cells, platelets and liposomes has been reported (73,75,76).

Considerable excitement was generated by this series of complexes and they have been extensively studied clinically (77,78,79) and further derivatives of the parent t-butyl complex have been

prepared in order to overcome the high lung and liver uptake of this complex. These include $[Tc(MIBI)_6]^+$ (MIBI = methoxyisobutyl isonitrile) although even this is not ideal (80,81).

As the high lipophilicity of the complexes makes them potentially useful as white blood cell labelling agents, several derivatives have been prepared and tested including t-butylisonitrile, n-butylisonitrile and cyclohexylisonitrile (82).

The hexakis t-butyl-, n-butyl-, cyclohexyl- and phenyl- isonitrile complexes have also been prepared from the reaction of Tc-acetate or Tc-formate in an aqueous buffer and the isonitrile in dichloromethane. The reaction mixtures were shaken and the product obtained from the organic layer (124).

A further advance in the tailoring of structure of the complexes with radiopharmaceutical activity is the preparation of hexakis isonitrile derivatives with two different isonitrile ligands in the coordination sphere. Complexes of the type $[Tc(CNR^1)_k(CNR^2)_{6-k}]^+$ where k = 0 - 6 and R^1 and R^2 may be t-butyl-, cyclohexyl- or (ethoxycarbonyl)methyl- are prepared by refluxing $[Tc(tu)_6]Cl_3$ with 100 fold excesses of the two isonitrile ligands in the molar ratios 3:1, 1:1 and 1:3. The products of the reaction were monitored by ^{99}Tc NMR (126).

The technetium(II) complex $[Tc(CNt\text{-}butyl)_6]^{2+}$ has been prepared by one electron oxidation of the technetium(I) compound by cyclic

voltametry in acetonitrile (68). The technetium(II) complex is not particularly stable and it appears to be formed prior to the formation of $[Tc(I)(NO)(CNt\text{-butyl})_5]^+$ noted above (1.3.1.). In the reaction between $[Tc(CNt\text{-butyl})_6]X$ (X = PF_6) with nitric acid in glacial acetic acid the unstable dication precipitates from the reaction mixture before formation of the nitrosyl is complete. Where X = NO_3^- or BPh_4^- the dication remains in solution and the reaction to the nitrosyl pentaisonitrile goes to completion.

The mixed isonitrile-phosphine technetium(I) complex $[^{99m}Tc(CNR)_2(depe)_2]^+$ was tested on three animal species and a human. The cation was found to give good images of myocardium in animals. However, the same complex in humans was found to bind to blood proteins and act as a blood pool imaging agent (83, 84).

Other mixed ligand-isonitrile complexes have been prepared including the series of complexes of the type $[Tc(I)(CNt\text{-butyl})_x(PPh_3)_{6-x}]^+$ where x = 4 or 5 (125). The complexes are prepared by refluxing $[TcO_4]^-$ in ethanol with triphenylphosphine and t-butylisonitrile. By controlling the amount of isonitrile the final product can be varied. However, very low yields of the complexes were obtained which precluded their preparation at tracer level for radiopharmaceuticals.

A series of technetium(I) isonitrile compounds with bidentate aromatic amine ligands have been prepared by either photolysis of

$[Tc(CNR)_6]PF_6$ in the presence of excess ligand or by reduction of NH_4TcO_4 in the presence of excess ligand and 4 molar equivalents of isonitrile (127). A single crystal X-ray structure determination carried out on the complex $[Tc(CNt\text{-butyl})_4(bipy)]PF_6$ indicates the the cation is a distorted octahedron. The most significant feature is that one of the isonitrile ligands trans to the bipyridine ligand is bent. A number of bent isonitrile ligands are known and can be explained by considering the resonance forms of the isonitrile:

$$Tc^{I}-C\equiv N-R \leftrightarrow Tc^{III}=C=\ddot{N}$$
$$\backslash$$
$$R$$

This is thought to be caused by the σ donating property of the bipyridine ligand which increases the electron density at the technetium centre. This in turn allows the technetium to donate electron density into the Tc-C bond, which causes the bend at the nitrogen atom. The Tc-C bond of the bent isonitrile (1.90(2)Å) is somewhat shorter than in $[Tc(CNt\text{-butyl})_6]^+$ (average Tc-C bond length 2.029(5)Å). The complex is described as having Tc(III) character as a result of the internal oxidation of the Tc(I).

Descriptions of the synthesis of the series $[Tc(CNt\text{-butyl})_4(NN)]^+$ where NN is Me_4phen, Me_2bipy, bipy, phen, NO_2phen are also given.

1.3.3. Technetium (I) carbonyl and organometallic compounds.

Most of these complexes can be considered to be derivatives of $Tc_2(CO)_{10}$ a structurally characterised complex of technetium in the (0) oxidation state (32). A range of mixed ligand Tc(I) complexes of the types $[Tc(CO)_5]X$, $[Tc(CO)_4X]_2$ and $Tc(CO)_{5-n}L_n$ have been prepared where X = Cl, Br, I and L is a monodentate organic ligand such as py, PR_3, or AsR_3 (85,86,87).

The mixed ligand carbonyl complexes $Tc(I)Cl(CO)_2(PMe_2Ph)_3$ and $Tc(I)Cl(CO)_3(PMe_2Ph)_2$ have been prepared by Mazzi et al together with the bromide derivatives (88)

Several porphyrin complexes of the type porphyrin-$[Tc(CO)_3]_2$ have been prepared together with their rhenium analogues. The crystal structure of the technetium complex with meso-tetraphenyl porphyrin has been reported (89,90).

The reaction of technetium tetrachloride with sodium cyclopentadienidein THF yields $Tc(C_5H_5)_2H$ analogous to the rhenium derivative (91). π-cyclopentadienyltechnetium(I)tricarbonyl, $Tc(C_5H_5)(CO)_3$, has been formed by the action of technetiumpentacarbonyl chloride on sodium cyclopentadiene (31). Previously this complex was prepared by irradiation of $Mo(C_5H_5(CO)_3)$ by thermal neutrons (92).

$Tc(C_5H_5)(CO)_3$ undergoes acylation in the ligand ring with benzyl chloride in the presence of $AlCl_3$ to give $Tc(C_5H_5COC_6H_4)(CO)_3$ (93).

$[Tc(C_5H_5)_2]PF_6$ has been prepared from the reaction of $TcCl_4$, aluminium powder, $AlCl_3$ and benzene and is shown to be analogous to the rhenium compound (94). $[Tc(C_6(Me)_6)_2]PF_6$ can be prepared from the similar reaction with hexamethylbenzene instead of benzene (95).

1.3.4. Complexes with phosphorous ligands.

Early work by Fergusson led to the preparation of the complex $[Tc(III)Cl_2(diphos)_2]Cl$ from the treatment of $TcCl_4$ with diphos. The analogous bromine compound $[Tc(III)Br_2(diphos)_2]Br$ was prepared by adding LiBr to the chloro complex and refluxing in ethanolic solution or by treating $TcBr_4$ with diphos. The technetium(II) complexes $[Tc(II)Cl_2(diphos)_2]$ and $[Tc(II)Br_2(diphos)_2]$ are produced by reduction of the corresponding tervalent complexes with borohydride. These are soluble in benzene and DMF but are readliy oxidised to the tervalent complexes in solution (26,96). The structure of trans-$[Tc(II)(diphos)_2Cl_2]$ has been reported.

Subsequent work by Deutsch resulted in the preparation of the series of technetium(III) complexes of the type $[TcL_2X_2]^+$ where L = ditertiary phosphine or arsine ligands and X = Cl or Br by the

reaction of the ligand with $[TcX_6]^{2-}$ in acidic ethanol/water mixtures. Pertechnetate at the carrier free level can also be used as the starting material where the phosphine ligands act as both the reductant and chelating agent (25). The structure of $[Tc(III)(dmpe)_2Cl_2]F_3CSO_3$ is also reported as being representative of this class of complexes in which the cation shows a trans octahedral arrangement of the ligands around the metal ion.

The $[^{99m}Tc(III)(dmpe)_2Cl_2]^+$ and $[^{99m}Tc(III)(depe)_2Cl_2]^+$ cations gave excellent images of the heart in animals but poor results in humans (99,100). It is thought that this is because the $[^{99m}Tc(III)(dmpe)_2Cl_2]^+$ and analogous complexes undergo reduction in human plasma to give neutral species which wash out of the heart (101). Electrochemical studies have confirmed one electron reduction to the neutral technetium(II) species and have confirmed the effects on the ease of reduction of varying the phosphine and halide and pseudo halide ligands (102). This line of thought has resulted in a series of papers on the preparation and redox chemistry of some of these phosphine complexes since some Tc(III) cations are taken up by the heart whilst some of the neutral Tc(II) species cross the blood-brain barrier. Neutral complexes of the general formula trans-$[^{99m}Tc(II)D_2X_2]$ where D represents a chelating ditertiary phosphine or arsine ligand and X represents a halide or pseudo halide have exhibited significant brain uptake in rats with the largest uptake where D = diars and X = Cl (133)

The technetium(I) cation $[Tc(dmpe)_3]^+$ has been prepared and characterised (103) and its oxidation to the technetium(II) species studied (104).

The technetium(II) and (III) complexes trans-$[Tc(II)(dppe)_2Cl_2]$ and trans-$[Tc(III)(dppe)_2Cl_2]NO_3.HNO_3$ have been prepared and characterised and the kinetics of the Tc(III)/Tc(II) redox couple studied (128).

The potential utility of trans-$[^{99m}Tc(III/II)D_2X_2]^{+/o}$ as organ imaging agents has been limited by the range of X ligands that can be sucessfully incorporated. Until recently only complexes with X = Cl, Br, or -NCS have been available. A series of thiolato technetium complexes of the type trans-$[Tc(III/II)D_2SR_2]^{+/o}$ have been prepared where R = Me and D = dmpe or depe. Crystal structures of the technetium(III) cations have been solved. Results from cyclic voltammetry indicate that the thiolato complexes are much harder to reduce than the corresponding halide analogues primarily because of the strong σ donating character of the thiolato ligand which stabilises the higher oxidation state of the technetium (129).

Further electrochemical studies of the $[Tc(III/II)D_2X_2]^{+/o}$ series of complexes have been undertaken in aqueous and aqueous micellar solutions (130,131).

The nitride precurser $[TcNCl_4]^-$ reacts with excess dmpe in methanol under reflux to give $[TcNCl(dmpe)_2]^+$. The structure of the complex revealed that the nitride and chloride were trans to each other with the four phosphorous ligands co-planar. It was anticipated that complexes of the type $[TcNX(dmpe)_2]^+$ should be significantly harder to reduce than the technetium(III) species $[TcCl_2(dmpe)_2]^+$ and accordingly the ^{99m}Tc analogue was prepared and the biodistribution studied. Some heart uptake was observed but there was rapid washout of the radioactivity suggesting reduction to the technetium(IV) species. This unexpected ease of reduction was ascribed to rapid formation of $[TcN(dmpe)_2]^{2+}$ in solution by loss of the trans-chloride ligand labilised by the trans-effect (136).

The synthesis and characterisation of a mixed phosphine/phosphine oxide technetium(I) complex has been reported. The reaction of $TcNCl_2(PMe_2Ph)_3$ with excess S_2Cl_2 yields the technetium(II) complex $Tc(NS)Cl_3(PMe_2Ph)_3$, whereas an equimolar reaction mixture yields the technetium(I) complex $Tc(NS)Cl_2(PMe_2Ph)_3$. The crystal structure of the former complex shows that the three chloro ligands are coordinated meridionally cis to the thionitrosyl group. The phosphine oxide ligand is trans to NS^+ which is virtually linear. The complex is slightly distorted from octahedral geometry as the four ligands cis to the thionitrosyl bend out of the equatorial plane away from the thionitrosyl (132).

A dinitrogen technetium(I) complex $HTc(I)N_2(diphos)_2$ has been prepared and the crystal structure determined. The hydrogen and dinitrogen group are trans to each other with the four phosphorous atoms of the two diphos groups in the equatorial plane (147).

As noted above, mixed carbonyl-phosphine complexes of technetium have also been prepared.

Several complexes with mixed phosphine and acac ligands have been prepared by Mazzi et al. Complexes of the type $PPh_4[Tc(IV)X_4(acac)]$, $Tc(IV)Br_3(acac)PPh_3$, $Tc(IV)X_2(acac)_2$, $Tc(III)X_2(acac)(PPh_3)_2$, $Tc(III)X(acac)_2PPh_3$ where X = Cl, Br have been reported and the crystal structure of $Tc(III)Cl(acac)_2(PPh_3)$ determined (97,98).

1.3.5. Complexes with arsenic ligands.

In 1959 Fergusson reported the preparation of the technetium(III) complex $[Tc(diars)_2Cl_2]$ by following the preparation for the analogous rhenium complex. The complex $[Tc(III)D_2Cl_2]Cl$ is prepared by refluxing $[TcCl_6]^{2-}$ in aqueous HCl with an excess of the diarsine (D). This can be transformed to the bromo and iodo complexes by refluxing in alcohol with lithium bromide or lithium iodide respectively. Treatment of these complexes with sulphur dioxide or in hypophosphorous acid solution or refluxing in ethanol leads to the technetium(II) complexes $[TcX_2Cl_2]$ (29, 105).

The $[Tc(III)(diars)_2Cl_2]^+$ cation was crystallised both as the chloride (dark red) and perchlorate (yellow-orange) salts and their crystal structures determined (106). The six coordinate complexes have trans chloride ligands with the two diars ligands almost co-planar.

The $[^{99m}Tc(III)(diars)_2Cl_2]^+$ cation has been prepared (25) and its myocardial uptake studied (51).

As noted above, the neutral complex trans-$[^{99m}Tc(II)(diars)_2X_2]$ (X = Cl) has exhibited significant brain uptake in rats (133). Complexes where X = $SCH_3C_6H_5$, $SCH_2COOHCH_3$ or thiocholesterol have also been injected into rats and found to pass through the blood-brain barrier (134).

Oxidation of the $[Tc(diars)_2Cl_2]ClO_4$ complex by introducing molecular chlorine into an alcoholic solution of the complex results in the formation of an eight coordinate technetium(V) complex $[Tc(V)(diars)_2Cl_4]ClO_4$. The stability of this complex is probably largely due to the chelating effect of the diars ligands (21).

1.3.6. Complexes with nitrogen donor ligands.

Until recently there has been a distinct lack of low valent complexes with nitrogen donor ligands such as bipy and phen and the only complexes reported were the cations

$[Tc(NO)(NH_3)_2(phen)(H_2O)]^{2+}$ and $[Tc(NO)(NH_3)(phen)_2]^{2+}$ as noted above (30). In 1989 a number of technetium(III) and technetium(II) polypyridyl complexes were reported.

The technetium(II) anion cis-$[Tc(II)(N)Br(bipy)_2]^+$ is prepared from the reaction of $[TcNBr_4]^-$ with excess bipy in refluxing methanol (135). The nitride and bromide ions are cis reflecting the steric difficulties imposed by arranging the two bipridyl ligands in a plane and their relatively small bite angle.

The technetium(II) cation $[Tc(bipy)_3]^{2+}$ has been prepared from the reaction of $[TcCl_3(MeCN)(PPh_3)_2]$ with excess bipy in refluxing methanol. The overall geometry about the technetium is found to be distorted octahedral and general bond distances and angles similar to those found in $[Ru(bipy)_3]^{2+}$ (136).

A series of complexes with mixed phosphine chloride and nitrogen-donor ligands where the technetium is in either (III) or (II) oxidation state have been reported. Technetium (III) complexes of the type $TcCl_3(P)(NN)$ and $[TcCl_2(PMe_2Ph)_2(NN)]^+$, technetium(II) complexes in the series $TcCl_2(PMe_2Ph)_2(NN)$ and the technetium(II) species $[TcCl(PMe_2Ph)_3(bipy)]^+$ have been prepared where P = PMe_2Ph or PPh_3 and NN represents the N-donor ligand bipy, phen or bipm (137).

The crystal structure of $TcCl_3(PPh_3)(bipy)$ shows the complex to have a distorted ocahedral geometry with two of the chloride

ligands trans to the nitrogens of the bipy in the equatorial plane and the PPh_3 and remaining chloride trans to each other.

The bond distances are in close agreement with those obtained for three related complexes, namely, $[TcCl_2(PMe_2Ph)_2(bipy)]BPh_4$, $[TcCl_2(PMe_2Ph)_2(phen)]BPh_4$ and $[TcCl_2PEtPh_2)_2(bipy)]SO_3CF_3$ which were prepared as part of a series of technetium(III) complexes of the type $[TcCl_2(P)_2(NN)]^+$ P = PMe_2Ph or $PEtPh_2$ and NN = bipy, phen or Me_2bipy (138) The geometry of all three complexes contains the technetium in an octahedral environment with two trans phosphine ligands and two cis chlorine atoms which are both trans to the nitrogen atoms of the coordinating bipy or phen ligand.

In both of the above papers (137, 138) the complexes are prepared by ligand substitution of the $TcCl_3(P)_3$ starting material and the method is basically an extension of the preparation of the analogous rhenium complexes.

The redox chemistry of this series of complexes has subsequently been studied with the conclusion that the combination of ligands dictates the preferred oxidation state of the technetium (139). As the extensive research into the ditertiary phosphine series of complexes has demonstrated the ability to control the redox potentials of technetium based couples, especially the Tc(III)/(II), is important in the development of technetium radiopharmaceuticals.

Ligand variation obviously plays a major part in this aspect of the design of the radiopharmaceutical.

Complexes in other oxidation states have been variously reported as follows:

The technetium(IV) complex $Tc(bipy)Cl_4$ is obtained on refluxing $[TcCl_4]$ and bipy (96).

The technetium(VII) compounds $TcO_3Cl(bipy)$, $TcO_3Br(bipy)$ and $TcO_3Cl(phen)$ have been prepared by adding first the ligand (bipy or phen) and then hydrohalic acid to an ethanolic solution of pertechnetate. These complexes are very unusual for technetium(VII) which is normally reduced in the presence of organic ligands. The analogous rhenium complex $ReO_3Cl(bipy)$, originally prepared from ReO_3Cl, can be prepared similarly from $NaReO_4$ except that the reaction must be refluxed (19).

In refluxing ethanolic HX, either NH_4TcO_4 or TcO_3XL (L = bipy, X = Cl, Br or L = phen, X = Cl) was reduced to the technetium(V) state. The rhenium analogue $ReO_3Cl(bipy)$ cannot be reduced to $ReOCl_3(bipy)$ under these conditions but requires a more powerful reducing agent such as H_3PO_2. $Tc(V)O\,X_3(bipy)$ (X = Cl, Br) can also be synthesised from the reaction of $[TcOX_4]^-$ and bipy in ethanolic HX. If HCl is omitted from the reaction of $[TcOCl_4]^-$ with bipy then the ethoxy complex $[TcOCl_2(OEt)(bipy)]$

precipitates. Treatment of this compound with acetone/HCl converts it to $TcOCl_3(bipy)$ (19).

Complexes with nitrogen donor ligands other than polypyridyl ligands have been reported in higher oxidation states only. Technetium(V) complexes with ethylenediamine $[TcO_2(en)_2]X$ where X = Cl, I and cyclam $[TcO_2(cyclam)]ClO_4.H_2O$ have been synthesised and structurally characterised (20,107) . $[TcO(HBPz_3)Cl_2]$ where $HBPz_3$ = hydrotris(1-pyrazolyl)borato has been prepared by the reduction of $[TcO_4]^-$ in aqueous HCl in the presence of $KHBPz_3$ (108), or by substitution of $HBPz_3^-$ on the technetium(V) complex $[TcOCl_4]^-$ (109). The analogous dibromo complex has been prepared by substitution of $HBPz_3^-$ onto $[TcOBr_4]^-$ (109).

1.3.7. Complexes with oxygen donor ligands.

Apart from the range of technetium(III) and (IV) complexes containing acac and triphenylphosphine noted above (1.3.4.) very few other complexes containing acac have been characterised.

The neutral complex $Tc(acac)_3$ was first prepared by refluxing $TcCl_4(PPh_3)_2$ in neat acetylacetone (97) and a later preparation from $[TcO_4]^-$ has since been described (110, 111). The complex is found to be inert to substitution. The technetium(II) complex $Tc(acac)_2$ has been generated by (d,xn) reaction in a mixture of molybdenum and cobalt(II) acetate (111).

More recently $Tc(III)(acac)_3$ has been prepared from the reaction of $[Tc(III)(tu)_6]^{3+}$ with acetylacetone in ethanol in the presence of several reductants including hydrazine hydrochloride and hydroxylamine. However, sodium dithionite, sodium borohydride or lithium tetrahydrate aluminate were found to give the best yields. Analogous complexes containing benzoylacetone and 2-thenoyltrifluoroacetone were also prepared (140).

$[Tc(V)OCl(acac)_3]^-$ has also been prepared from the reaction of acetylacetone with $[TcOCl_4]^-$ in refluxing methanol. A mixture of geometrical isomers are produced which are separated by column chromatography. Both dibenzoyl- and benzoylacetonato complexes were synthesised in a similar manner (140).

Complexes with other oxygen donor ligands such as hydroxycarboxylato compounds like citrate (36), gluconate (114), glucoheptonate and mannitol (115) have been reported. As previously noted some of these complexes are used as precursers for radiopharmaceutical preparations. A technetium complex with salicylaldehyde has been prepared (112).

1.4. Conclusion.

It can be concluded from this review of the chemistry of technetium(I) and (II) that, compared to other transition metals, the subject is poorly developed not only in the number and variety of ligands which have been used to form complexes of technetium

but also in respect of finding alternative cores to the oxo and dioxo cores which dominate both the inorganic and radiopharmaceutical chemistry of technetium(V).

Substitution reactions on the $[Tc(VI)NX_4]^-$ anion where X = Cl or Br or $[TcNCl_2(PPh_3)_2]$ have been found to provide a general route to complexes containing the Tc(V)≡N core. Many nitrido complexes containing this core have now been prepared and in some cases the biological activity studied (43, 45, 46, 141, 142, 143, 144, 145, 146). The nitrido ligand (N^{3-}) is isoelectronic with the oxo ligand (O^{2-}) and is a powerful π electron donor that tends to stabilise metals in high oxidation states, however, reactions producing low valent complexes have been noted above (1.3.4, 1.3.5).

Another alternative core which is also unexplored is the thionitrosyl. A number of complexes have been prepared which tend to be either technetium (III) or (II) the lower oxidation state being stabilised by the NCS^- ligand (148, 149, 150, 151). An example of a technetium(I) thionitrosyl is noted above (1.3.4).

The aim therefore of this work is threefold. Firstly, using [^{99m}Tc]technetium, to develop a series of low valent technetium radiopharmaceuticals containing the Tc-NO core as an alternative to the oxo and dioxo cores common to most radiopharmaceuticals. Secondly, to prepare and characterise these complexes using

[^{99m}Tc]technetium and thirdly to study the biological activity of the complexes.

CHAPTER 2

GENERAL EXPERIMENTAL

2.1. Synthesis of the no carrier added [^{99m}Tc] complexes.

2.1.1. General synthetic conditions.

All reactions were carried out in 20ml glass vials (Pierce) which were purged with nitrogen and sealed using a rubber seal and metal clasp (Pierce). The reactants were introduced through the rubber seal using a syringe fitted with a needle. After introduction of reactants was complete, a negative pressure was produced in the vial by withdrawing air with the syringe and needle. This action being particularly important before heating the vial. Heating was performed in a Gallenkhamp portable autoclave AUX 200G (pressure cooker). As a general precaution vials containing [^{99m}Tc] solutions were contained in lead pots, and never handled directly. For heating purposes, 5mm holes were drilled in the lead pots to allow more efficient heating.

2.1.2. Materials.

Sodium pertechnetate was obtained by elution of an Amertec II

$^{99}Mo/^{99m}Tc$ generator kindly donated by Leicester Royal Infirmary. Concentrated hydrochloric acid was standard laboratory grade. Solutions of hydroxylamine hydrochloride (A.R. grade, Fisons) were prepared freshly every time using triply distilled water. Organic solvents were not purified before use. Tetrabutylammonium chloride was obtained as a 75% aqueous solution (Alfa). Ligands were not recrystallised or further purified before use.

2.1.3. Synthesis of the nitrosyl complexes.

The synthesis of each complex is most appropriately described in the relevant chapter.

2.2 Experimental methods for the analysis of the no carrier added [^{99m}Tc] complexes.

2.2.1. Paper chromatography.

All stages of the reactions were monitored by paper chromatography. Separation of the reaction mixture into complexed technetium, unreacted pertechnetate and hydrolysed, colloidal TcO_2 was achieved by standard chromatographic separation. The support medium employed was Whatmann 3MM chromatography paper, supplied in a strip 50mm wide. The chromatogram, 50mm x 95mm, was marked 15mm from the bottom as the application point and 10mm from the top as the solvent front. The distance moved by the solvent in developing the chromatogram being 70mm. The chromatograms were

developed in Seprachrom tanks using isotonic saline (0.9% NaCl (Fisons) in H_2O) and butan-2-one (Fisons) as the mobile phases.

A spot of the reaction mixture was applied to each chromatogram via a syringe fitted with a 25G needle. This was allowed to dry and then placed in the tank ensuring that the solvent level was below the applied spot. The chromatogram was developed to the marked solvent front and the paper was removed from the tank, dried and scanned on a Panax RTLS - 1A scanner (Loughborough) or a multichannel analyser (Amersham). The application point and solvent front were marked and the R_f value of the complex calculated. The paper strip was then cut lengthwise into 19 x 5mm portions, each portion placed in a scintillation tube and counted with an empty tube for background subtraction on a Phillips PW4800 automatic gamma counter. The data were processed on a Commodore PET microcomputer using a chromatogram plotting program developed at Loughborough. Chromatography performed at Amersham was automatically processed for calculation of the R_f and percentage product by the computer controlling the multichannel analyser.

Pertechnetate has R_f values of 0.92(3) in butan-2-one and 0.73(4) in saline, TcO_2 has R_f values of 0.00 in both of these solvents. The complexes of technetium studied have differing R_f values in these chromatographic systems, hence reactions can be compared.

2.2.2. Electrophoresis.

Two different support media were employed - paper and gel. In both cases the experiments were carried out in a Shandon Southern 602 tank connected to a Volkam 400/100 power unit. The buffer used was monohydrogen phosphate/dihydrogen phosphate at pH 7.4. The relative proportions of each component was found from the Ciba-Geigy book of scientific tables (152).

Paper electrophoresis was performed using Whatmann number 1 paper which was cut into strips 20mm x 250mm and the application point marked in the middle and cathode and anode marked on either end in pencil. The strips were soaked in the buffer for 30 minutes before use and blotted on paper towel before application of the reaction mixture as previously described. As a standard, a small spot of bromophenol blue indicator powder was placed on another strip of paper using a syringe needle. This compound is known to move as an anion at half the rate of pertechnetate. The strips were placed in position before the power was switched on. The system was usually run at 300 Volts for two hours. The strips were removed, dried, scanned and finally cut into 5mm portions and counted on the gamma counter as before. The movement relative to pertechnetate was calculated.

Gel electrophoresis was performed using agarose A powder for electrophoresis (Pharmacia) made up as follows. 100mg of agarose was dissolved in 10ml of the phosphate buffer used as the mobile

phase by heating on a water bath. The hot agarose mixture was quickly poured and spread over a hot glass plate, 50mm x 200mm, and allowed to cool. When cool, a well was made in the middle of the plate by extracting a piece of the gel with the end of a syringe. The plate was placed on the supports in the tank and soaked wicks of Whatmann number 1 paper placed on the edges of the gel ensuring that they made contact with the buffer in the tank. A small spot of bromophenol blue was applied onto the gel, in line with the well but near the edge of the plate, a drop of the complex was applied into the well. The power was switched on, 300 Volts for 30 to 45 minutes (determined by the movement of the bromphenol blue). On completion, the gel was placed in a cellophane packet and scanned using the multichannel analyser. The electrophetic movement was again compared to that of pertechnetate.

2.2.3. Octanol/saline partition experiments.

0.1 ml of the complex was diluted to 5 ml with saline and added to 5ml of octanol in a sealed 20ml vial. The two phases are immiscible with the octanol layer floating on the saline. The vial was shaken for 10 minutes and the phases separated by centrifugation (2000G for 10 minutes). The phases were separated into different tubes and re-centrifuged. 50µl samples were taken, in triplicate, using an automatic pipette. The samples were counted in the gamma counter and the average value used.

2.2.4. Protein binding studies.

This study was performed by incubating the complex with whole rat blood for 20 minutes at 37°C. The plasma was separated by centrifugation and aliquots transferred to an Amicon MPS - 1 micropartition system using a YTM membrane. After centrifugation for 25 minutes the proportions of retained (by the membrane) and non-retained species were measured. Corrections were applied for the retention of the pure complex in the filter unit by using non-protein containing solutions as controls.

2.2.5. HPLC conditions.

High performance liquid chromatography was performed at Amersham International plc. The system consisted of two Gilson pumps, a Valco injection valve, and an Apple II controller. Radioactivity detection was by an X-ray scintillation probe connected to an ESI ratemeter model 5140. A PRP-1 (Hamilton) column was used throughout and the mobile phase consisted of 100% 0.02M phosphate buffer rising by gradient to 75% THF over 30 minutes at a flow rate of 2ml per minute.

2.2.6. Biodistribution studies.

The biodistribution studies were performed by the staff of the Animal Physiology Laboratories of Amersham International plc. All experiments were performed in triplicate or at least in duplicate.

For the gamma camera images, two male Wistar rats were used (weight 150 to 250g), anaesthetised with urethane (IP administration of a 15% w/v aqueous solution) and placed side by side on the high resolution collimator of a gamma camera (Ohio Nuclear Sigma 400). 0.1ml of complex solution (18.5 MBq) was injected intravenously via the tail vein to each rat. They were studied for 1 hour, scintiphotographs were taken at 0, 10, 20, 30, and 60 minutes post injection. After the final image had been taken the animals were sacrificed, dissected and samples of blood, muscle and bone collected in pre-weighed containers while other major organs were removed intact. The urine and injection site were also collected and along with the residual carcass were assayed for radioactivity in a large volume, twin-crystal, automatic gamma counter. In most cases, in accordance with the Amersham protocol for potential brain/heart agents, two minute dissections were also performed

2.3. Synthesis of the carrier added [^{99}Tc] complexes.

2.3.1. General reaction conditions.

Reactions were carried out in 20ml sealed glass vials as for the no carrier added reactions (2.1.1.) or were refluxed under nitrogen. Since ^{99}Tc is a weak β emitter there is minimal radiation hazard although care must be taken during manipulations to avoid the Bremsstrahlung radiation and as a general precaution,

manipulations were confined to a fume hood to minimise spread of radiation.

2.3.2. Materials.

Ammonium pertechnetate was obtained as a 0.15mM solution in 0.1M ammonium hydroxide, kindly donated by Amersham International plc. Hydrochloric acid, hydroxylamine hydrochloride and tetra-butylammonium chloride as before (2.1.2.). Solvents were dried over molecular sieves or sodium wire as appropriate and deoxygenated prior to use. Ligands were used without recrystallisation or further purification.

2.3.3. Synthesis of the carrier added [^{99}Tc] nitrosyl complexes.

The synthesis of each of the complexes is described in the relevant chapter.

2.4. Analysis of the carrier added [^{99}Tc] complexes.

2.4.1. Paper chromatography.

The chromatograms were prepared and developed as described in 2.2.1. The dried chromatograms were not scanned but cut immediately into 19 x 5mm sections which were placed in plastic scintillation vials. 10ml of scintillant (unisolve E) was added and the vials (including a vial containing scintillant only for

background subtraction) counted using an LKB1215 Rackbeta liquid scintillation counter. The data was processed as previously described.

2.4.2. Electrophoresis.

Whatmann number 1 paper was the only support medium used for carrier added [^{99}Tc] complex electrophoresis experiments. The electrophoretograms were developed as described earlier (2.2.2.). The dried strips were not scanned but immediately cut into 5mm sections which were placed in plastic scintillation vials, scintillant added and the radioactivity determined in the scintillation counter.

2.4.3. Infra-red spectra.

The samples were prepared as KBr discs, 5mm in diameter, pressed in an evacuable minidie (Beckman-RIIC Limited). Grinding of the powders and loading of the die was performed in a fume cupboard to minimise spread of contamination. The spectra were recorded on Perkin-Elmer 257 and 999 spectrophotometers, calibrated with a polystyrene standard.

2.4.4. Mass spectra.

Fast Atom Bombardment mass spectra were kindly observed by Dr. Alex Lawson of the Clinical Mass Spectrometry Unit at Northwick

Park Hospital using a Varian Mat 731 spectrometer operating at 10kV. The matrix employed was thioglycerol/erythritol / dithioerythritol.

2.4.5. Electron Spin Resonance spectra.

The ESR spectra were kindly observed by Dr. Ron Pearlstein of the Massachusetts Institute of Technology using a Varian E-line X band spectrometer with an E-9 magnet, E-234 cavity and E-101 microwave bridge. The samples were dissolved in ethanol and placed in a 5mm OD quartz tube. All measurements were relative to a DPPH standard. The simulation of the low temperature spectrum was carried out using the POWDER simulation program (161).

2.4.6. NMR spectra.

Samples for ^1H NMR spectrometry were transported to the University of Birmingham and made up as follows. 5mg of sample was dissolved in 0.3ml deuterated chloroform ([TcNO(diphos)$_2$Cl] and [TcNO(diars)$_2$Cl]) or 0.3ml deuterated methanol ([TcNO(CNCMe$_3$)$_4$Cl]). The sample was then transferred via a syringe fitted with a long (200mm) needle to a polythene NMR tube liner. The liner was stoppered and placed in a 5mm glass NMR tube. The spectra were observed on a Jeol JNM-GX270 FT NMR Spectrometer, kindly operated by Mr. Clive Jeynes. Dr. Wasif Hussain assisted with the preparation of the samples.

2.4.7. Microanalysis.

Microanalyses were provided by Butterworths Ltd.

2.4.8. Biodistribution studies.

The biodistribution studies were performed, as before, by the staff of the Animal Physiology section at Amersham International plc. following a protocol devised by Dr. Brian Higley.

There are no images available from these experiments which rely wholly on dissection data. The experiments were performed in triplicate at two time intervals post-injection, 2 minutes and 1 hour. Male Wistar rats weighing on average 200g were anaesthetised with urethane to prevent mobility because of the difficulty in collecting urine samples on tissue paper. 1-2μCi of activity in 0.1ml was injected into each animal via the tail vein. All major organs were collected as before with the exception of the remaining carcass. The tissue samples were homogenised where necessary and digested, scintillant was added and the samples counted in an LKB Rackbeta.

2.5. X-Ray crystallography.

All crystal structures described were solved at Loughborough University under the supervision of Dr. David Brown with the exception of the chloronitrosyl-bis(o-phenylene)-bis-

(dimethylarsine)technetium(I)chloride tetrabutylammonium chloride complex which was solved by Miss Hilary Banbery and Dr. Tom Hamor of the University of Birmingham. The crystals for this latter study were prepared by the author as described in chapter 6. A summary of the experimental method and data is given in Appendix IV.

Crystals suitable for X-ray diffraction studies were selected in the first instance by examination under an optical microscope. The single crystal selected was then mounted in a 0.05mm or 0.1mm Lindeman tube depending on the size of the crystal. The crystal was attached to the side of the tube with the minimum amount of petroleum jelly and mounted, as closely as possible, with an axis aligned along the length of the tube. The tube was then sealed at both ends so that the radioactive crystal was fully enclosed.

The tube was then mounted in a piece of wax on a goniometer head which was in turn attached to a Nonius Weissenberg camera. The crystal was set exactly along one axis by taking a series of oscillation photographs. The axis about which the crystal was rotated was determined, and preliminary cell dimensions calculated from zero layer Weissenberg photographs.

Once the crystal was accurately set, it was transferred to a Stoë Weissenberg diffractometer controlled by an Apple microcomputer. The diffractometer was set by programming in the preliminary cell dimensions and locating known reflexions. The cell dimensions

were determined by maximising fit of axial row reflexions. Data was collected over an appropriate range of h, k and l with reference to a standard check reflexion which was observed to ensure no significant changes were occuring. The data for each layer were transferred to the Loughborough University of Technology Honeywell Multics system and processed using the Stow program. Using the SHELX76 (153) program the Tc position was in general found by Patterson and other atoms by sucessive difference - Fourier methods. Scattering factors were obtained from International Tables for Crystallography (1974) (154) and geometry calculations carried out using the XRAY system (1972) (155) as implemented on the CDC7600 computer at the University of Manchester Computer Centre. Finally, diagrams showing the molecular structure were plotted using the ORTEP program (156) as implemented on the Honeywell Multics system at the Loughborough University of Technology.

The exact details pertaining to each crystal structure determination will be described fully in the relevant chapter.

CHAPTER 3

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF THE TETRACHLORONITROSYLTECHNETIUM(II) ANION

3.1. Introduction.

As noted in chapter 1 some low valent technetium complexes stabilised by the nitrosyl ligand have been reported. The complex prepared above has been characterised by several groups but has variously been reported as either $[TcNOCl_5]^-$ or $[TcNOCl_4]^-$ (70,27). Because of this uncertainty it was decided to undertake a crystal structure determination of the complex prepared here. In the search for novel technetium radiopharmaceuticals alternative syntheses of technetium complexes which do not contain the TcO^{3+} or the TcO_2^+ core common in high oxidation state compounds are lacking. The synthesis of $[^{99m}Tc(NO)Cl_4]^-$ *from* ^{99m}Tc *generator eluant* is described, while under the same conditions, using ^{99}Tc, it was possible to isolate and characterise the same complex. The synthesis is a multistage one, proceeding through the $[TcCl_6]^{2-}$ species, which is a disadvantage from the radiopharmaceutical aspect. This complex is the first reported example of a technetium nitrosyl complex in a form amenable to injection, at least, in animals.

The corresponding tetrabromonitrosyl and tetraiodonitrosyl technetium(II) complexes were also prepared and characterised using this synthetic method.

3.2. Synthesis of the tetrachloronitrosyltechnetium(II) anion.

3.2.1. Synthesis of *no carrier added* $[^{99m}Tc(NO)Cl_4]^-$.

To generator eluant (1 ml) was added concentrated HCl (1 ml). The mixture was heated for 30 minutes. The product of the reaction is sodium hexachlorotechnetate. The resulting solution was cooled, diluted with water (1ml), mixed with a solution of hydroxylamine hydrochloride (1ml, 2.3M) and heated for a further 30 minutes. The resulting solution contains the species $[Tc(NO)Cl_4X]^-$ (X = Cl^-, H_2O). After each stage of the reaction, the product formation was monitored by the standard chromatographic methods described earlier.

3.2.2. Preparation of the complex for biological studies.

As prepared above, the complex is not in a form suitable for injection into animals, since, because of the nature of the preparation there is an excess of hydroxylamine and chloride. This problem is solved by extracting the complex and redissolving it in a more isotonic medium. The integrity of the complex was checked by chromatographic methods as before.

By the addition of 0.1ml of 7.0% tetrabutylammonium chloride solution, quantitative extraction of the complex into dichloromethane was obtained by agitating the mixture for 10 minutes. The dichloromethane was separated and transferred to a sealed 20 ml glass vial, and evaporated to dryness by blowing nitrogen through the solution, the resultant aerosol being collected in a glass wool trap. The dried complex was redissolved in isotonic saline and used for the biological studies without further purification.

3.2.3. Synthesis of carrier added $[^{99}Tc(NO)Cl_4]^-$.

To an aqueous solution of ammonium pertechnetate (1ml, 0.15mM) was added concentrated hydrochloric acid (1ml) and the mixture heated for 30 minutes. The product of the reaction is $[TcCl_6]^{2-}$ as the yellow ammonium salt . The resulting solution was cooled, diluted with water (1ml), mixed with a solution of hydroxylamine in water (1ml, 2.3M), and heated for a further 30 minutes. The resulting green solution contains the species $[Tc(NO)Cl_4X]^-$ (X = Cl^-, H_2O) which is formed quantitatively. The anion can be isolated as the tetrabutylammonium salt by addition of tetrabutylammonium chloride solution (75% aqueous solution) and quantitative extraction of the green compound into dichloromethane. Removal of the dichloromethane allows recrystallisation of $[(C_4H_9)_4N][Tc(NO)Cl_4(CH_3OH)]$ from methanol and diethylether.

This complex readily undergoes ligand exchange with a variety of ligands (described later). It is not necessary to recrystallise the product before use.

3.2.4. Adaptation of the synthesis for the preparation of the [^{99m}Tc] tetrabromonitrosyltechnetium(II) anion.

The method is exactly as described above (3.2.1.) except HBr is substituted for HCl and hydroxylamine sulphate for hydroxylamine hydrochloride. The complex is prepared for biodistribution studies as above (3.2.2.) substituting tetrabutylammonium bromide for the corresponding chloride salt.

3.2.5. Adaptation of the method to synthesise carrier added $[^{99}Tc(NO)Br_4]^-$.

The analogous technetium tetrabromonitrosyl can be prepared as described above (3.2.3.) by substituting HBr for HCl and hydroxylamine sulphate and tetrabutylammonium bromide for the corresponding chloride salts.

The complex can be extracted, as before, into dichloromethane which can be removed to allow recrystallisation of the complex from methanol and ether to form dark red crystals.

3.2.6. Adaptation of the method to synthesise carrier added $[^{99}Tc(NO)L_4]^-$.

The complex can be prepared by adapting the basic method as for the bromide but it is not easy to recrystallise the product in the same way.

A better preparation of the nitrosyltetraiodide technetium(II) anion can be achieved by ligand exchange of the analogous chloride or bromide by the standard method of sodium iodide in acetone. On stirring, the dark green colour of the tetra-iodide forms.

3.3. Results.

3.3.1. Paper chromatography.

Paper chromatography results are summarised in Table 3.1. below.

The traces of the chromatograms obtained using both the Panax RTLS-1A scanner and the multichannel analyser (Amersham) are shown in Figures 3.1. and 3.2. respectively.

Table 3.1.

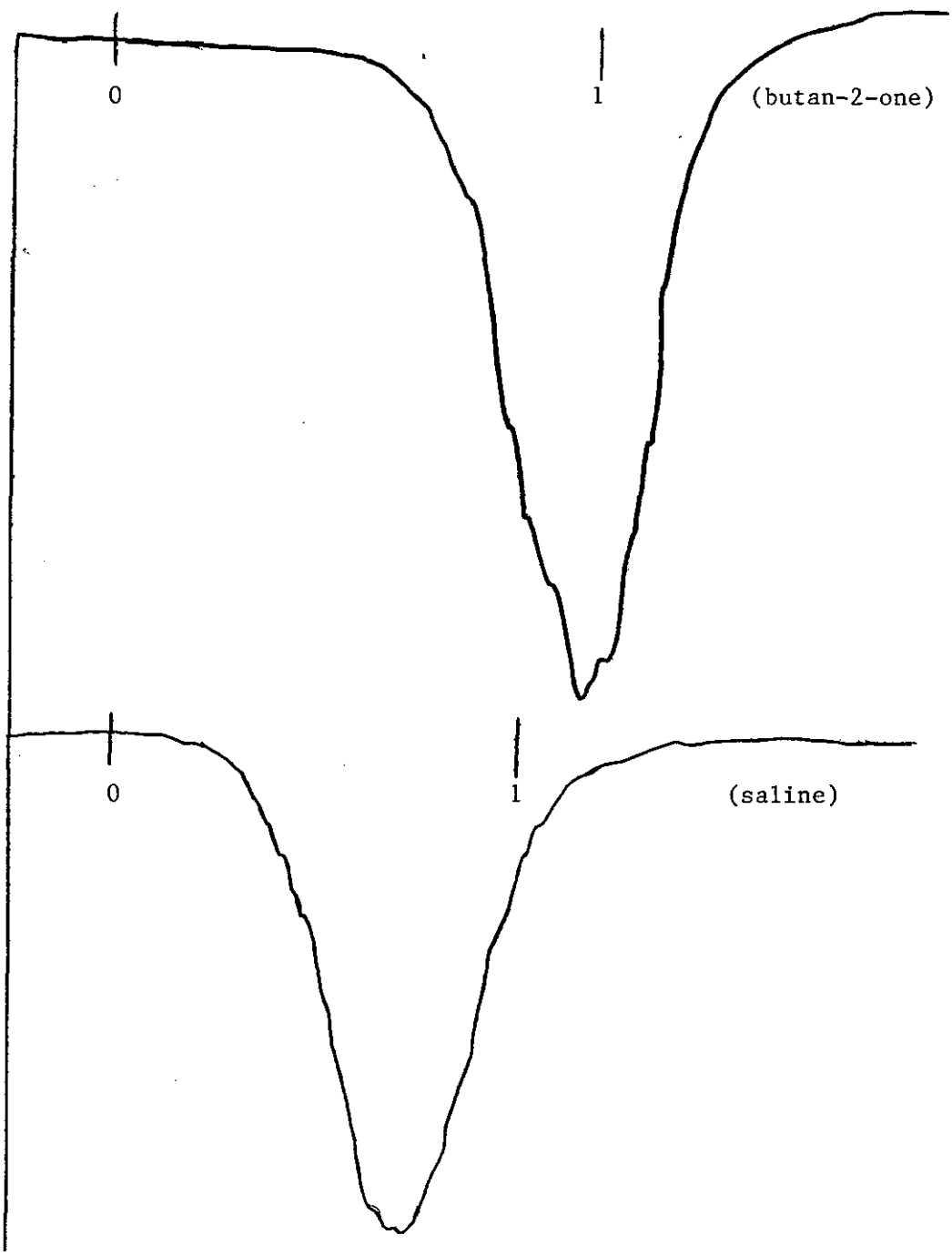
Paper chromatography results for $[TcO_4]^-$, $[TcCl_6]^{2-}$, $[Tc(NO)Cl_4]^-$ and $[(C_4H_9)_4N][Tc(NO)Cl_4]$.

Complex	R_f in butan-2-one	R_f in saline
$[TcO_4]^-$	0.92(3)	0.73(4)
$[TcCl_6]^{2-}$*	0.37(2)	0.76(4)
$[Tc(NO)Cl_4]^-$	0.70(4)	0.80(3)
$[(C_4H_9)_4N][Tc(NO)Cl_4]$	0.82(5)	0.82(3)

* For carrier added studies of $[TcCl_6]^{2-}$, disproportionation occurred to TcO_4^- and TcO_2.

Figure 3.1.1.

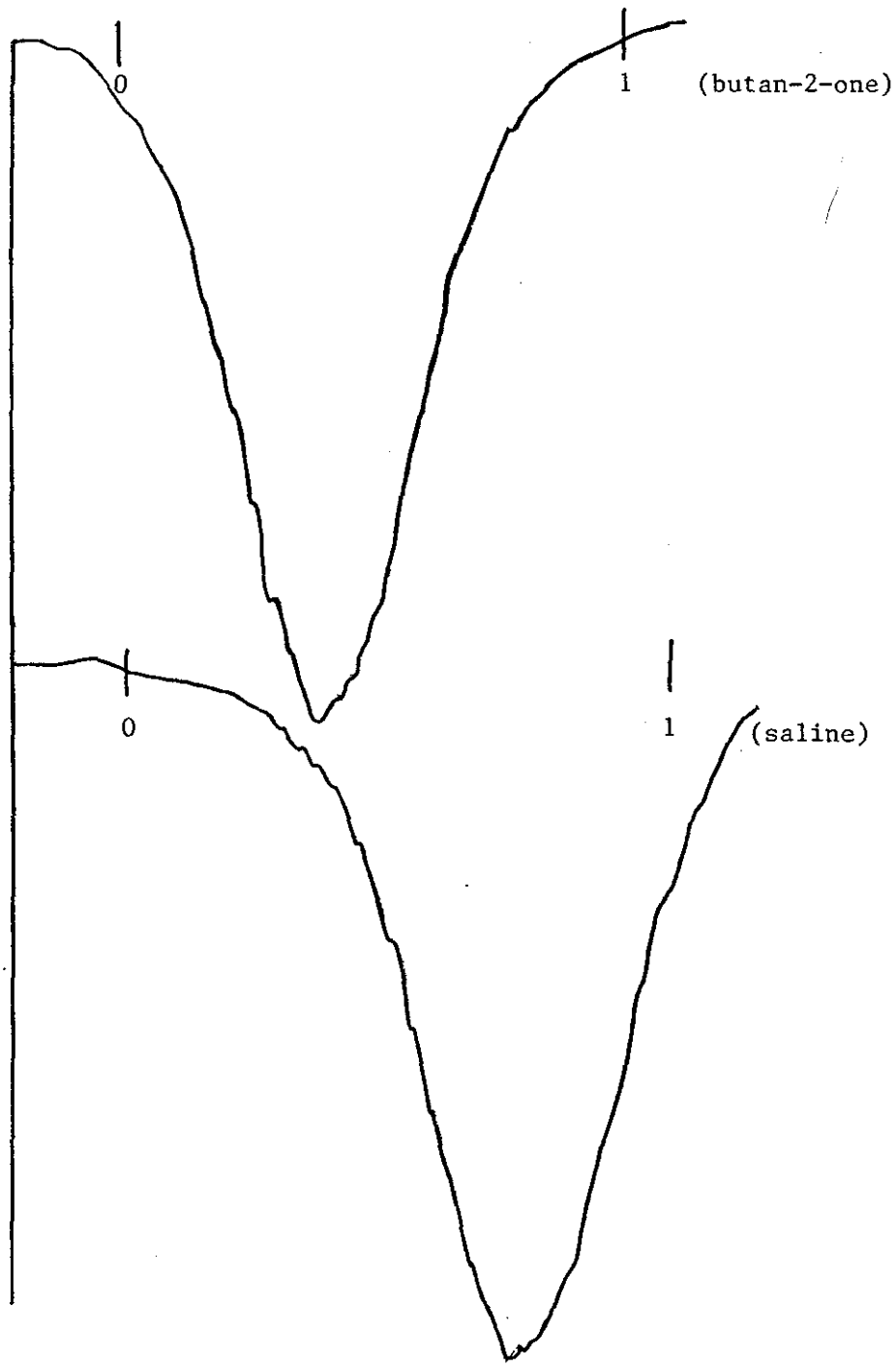
Typical chromatographic traces of $[^{99m}TcO_4]^-$ as obtained on the Panax RTLS 1A scanner.



0 = origin, 1 = solvent front

Figure 3.1.2.

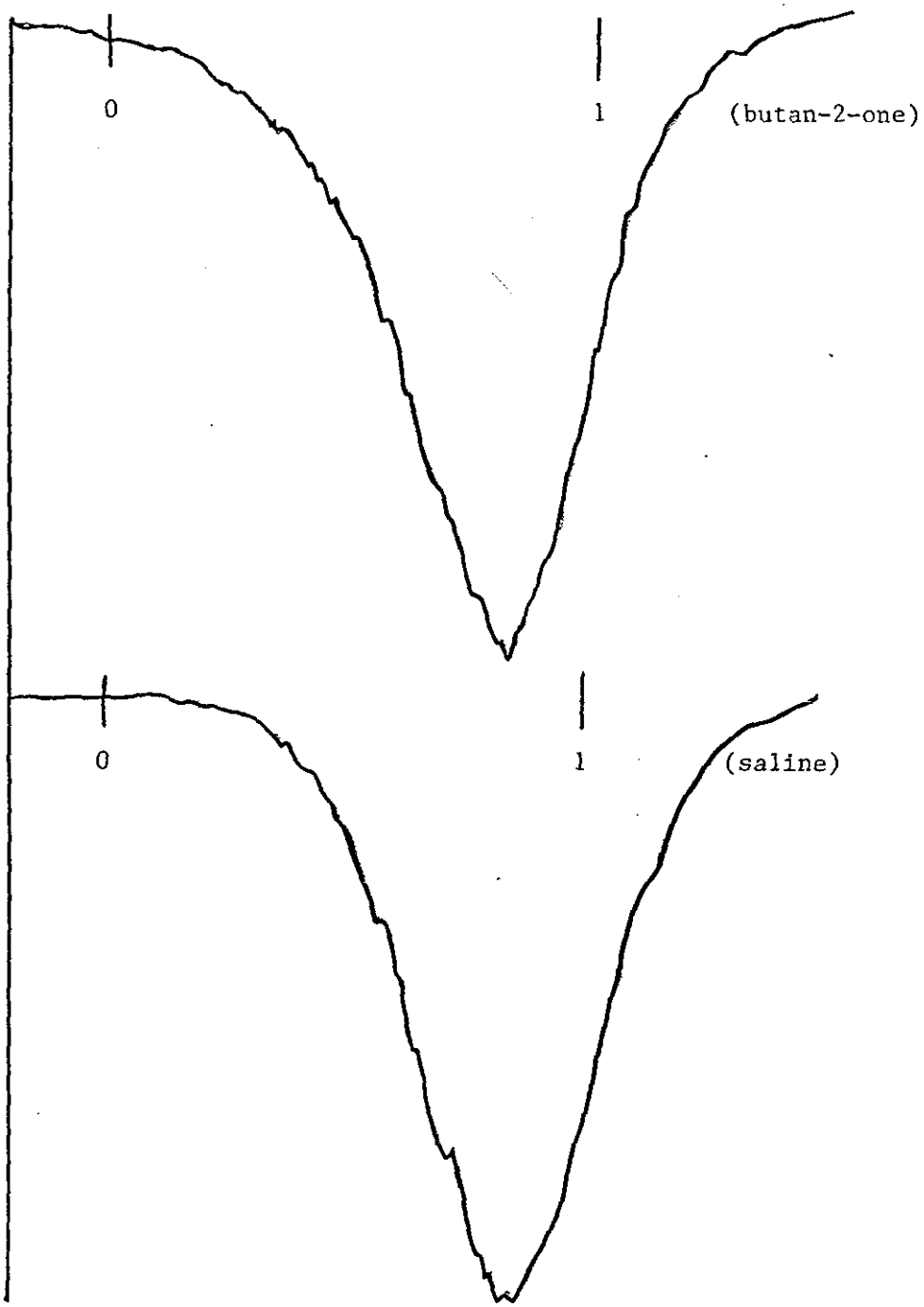
Typical chromatographic traces of $[^{99m}TcCl_6]^{2-}$ as obtained on the Panax RTLS 1A scanner.



0 = origin, 1 = solvent front

Figure 3.1.3.

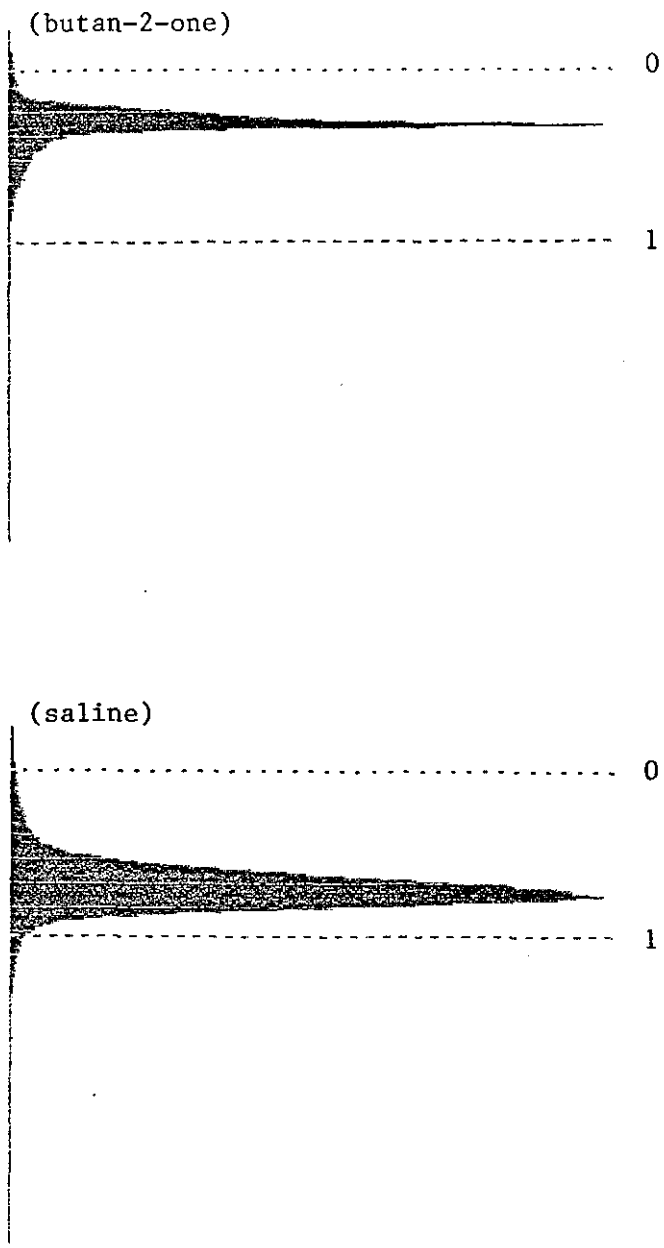
Typical chromatographic traces of $[^{99m}Tc(NO)Cl_4]^-$ as obtained on the Panax RTLS 1A scanner.



0 = origin, 1 = solvent front

Figure 3.2.1.

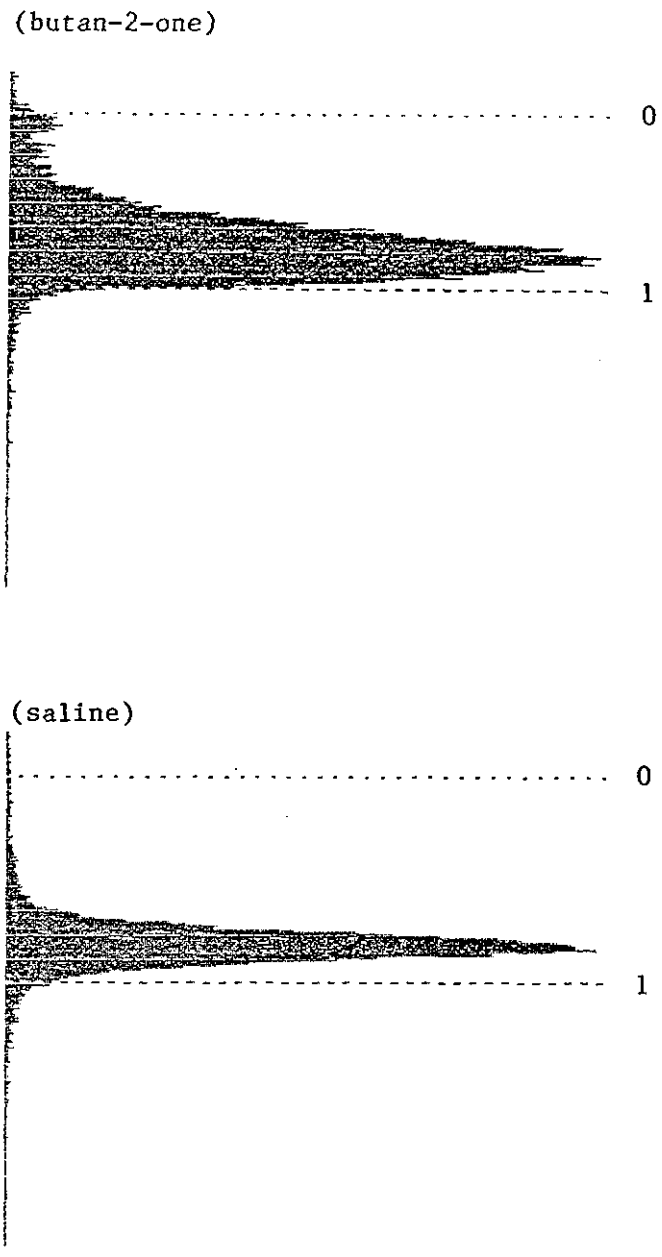
Typical chromatographic traces of $[^{99m}TcCl_6]^{2-}$ as obtained on the Amersham multichannel analyser.



0 = origin, 1 = solvent front

Figure 3.2.2.

Typical chromatographic traces of $[^{99m}Tc(NO)Cl_4]^-$ as obtained on the Amersham multichannel analyser.



0 = origin, 1 = solvent front

3.3.2. Electrophoresis.

$[Tc(NO)Cl_4]^-$ moves as an anion with a mobility of half that of $[TcO_4]^-$ using the gel system. For carrier added studies of $[TcCl_6]^{2-}$, disproportionation occurred to $[TcO_4]^-$ and TcO_2.

Traces of the electrophoretograms are shown in Figure 3.3.

3.3.3. Infra-red spectrum.

Absorptions at $1805cm^{-1}$ for $\nu(NO)$ and $326cm^{-1}$ for $\nu(Tc-Cl)$ after recrystallisation, $\nu(NO)$ is $1795cm^{-1}$ before recrystallisation.

3.3.4. Protein binding studies.

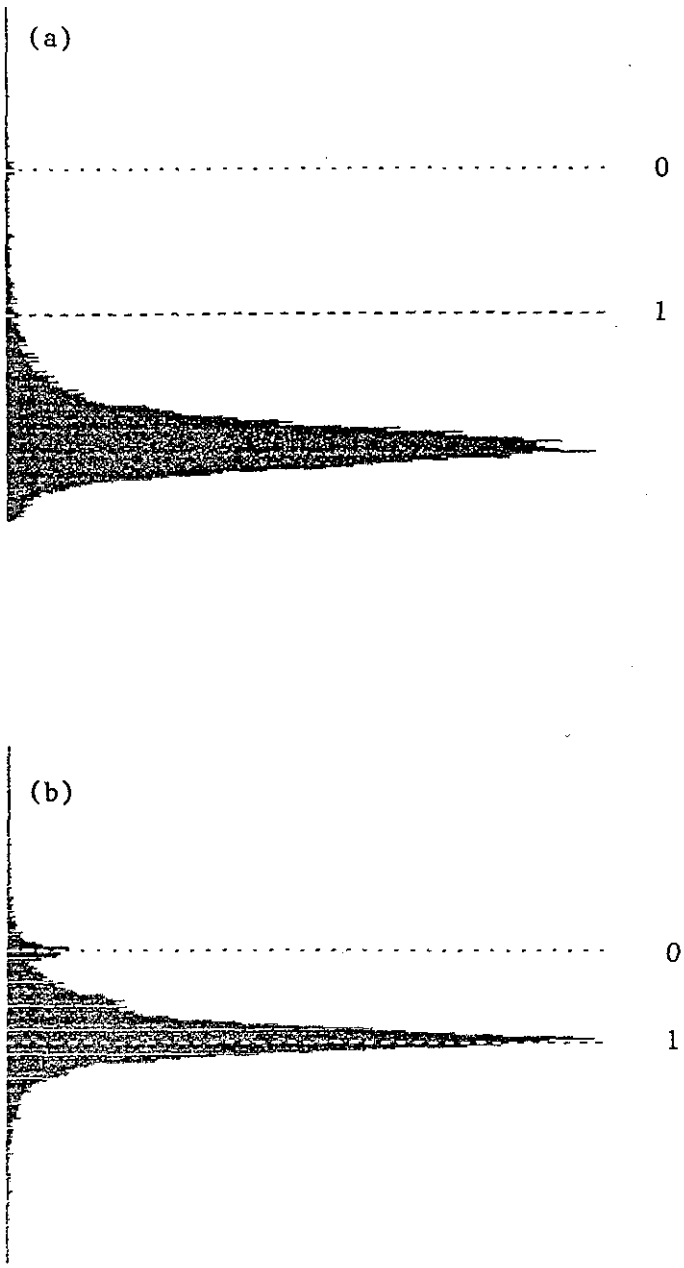
90% of the complex is associated with the initial plasma fraction with the remainder in the cellular pellet. 92% of the activity is bound to the plasma after partition.

3.3.5. Biodistribution studies.

The animal biodistribution data for $[^{99m}Tc(NO)Cl_4]^-$ and $[^{99m}Tc(NO)Br_4]^-$ are given in Tables 3.2. and 3.3. respectively. The gamma camera images of $[^{99m}Tc(NO)Cl_4]^-$ are shown in Figure 3.4.

Figure 3.3.

Typical electrophoretic traces (agarose A gel medium) of (a) $[^{99m}TcO_4]^-$ and (b) $[^{99m}Tc(NO)Cl_4]^-$ as obtained on the Amersham multichannel analyser



0 = origin, 1 = distance travelled by bromophenol blue

Table 3.2.

Animal biodistribution data for $[^{99m}Tc(NO)Cl_4]^-$

% Injected dose/organ

	2 min sacrifice		60 min sacrifice	
	mean	S.D#	mean	S.D.#
Muscle	30.35	7.96	30.41	9.06
Blood	20.32	1.77	14.94	1.83
Kidneys	5.97	0.93	3.71	1.48
Bladder	0.29	0.05	10.90	8.24
Lung	2.09	1.15	1.71	0.25
Liver	5.31	0.93	4.59	0.49
Spleen	0.21	0.06	0.20	0.02
Stomach	1.01	0.18	0.99	0.06
S.intestine	4.27	0.81	3.83	0.59
L.intestine	2.69	0.63	2.08	0.18
Heart	0.55	0.06	0.42	0.07
Thyroid	0.14	0.03	0.10	0.03
Brain	0.09	0.06	0.05	0.01
Carcass	26.70	12.48	26.07	12.94
Injection site	2.21	0.59	3.05	0.89
Counts/gram ratio				
Heart/blood	0.41	0.05	0.43	0.05
Heart/muscle	2.14	0.55	1.66	0.63
Heart/liver	1.37	0.26	0.98	0.27
Brain/blood	0.03	0.02	0.02	0.00
Brain/muscle	0.14	0.10	0.08	0.02

= standard deviation of three animals

Table 3.3.

Animal biodistribution data for $[^{99m}Tc(NO)Br_4]^-$

% injected dose/organ

	2 min sacrifice		60 min sacrifice	
	mean	S.D#	mean	S.D.#
Muscle	25.70	2.45	18.54	1.43
Blood	29.06	0.67	17.86	1.83
Kidneys	4.34	1.19	3.58	0.13
Bladder	0.25	0.10	13.85	1.28
Lung	2.89	0.24	1.67	0.13
Liver	6.84	0.29	7.79	0.77
Spleen	0.28	0.05	0.94	0.32
Stomach	0.89	0.08	1.09	0.16
S.intestine	3.62	0.34	3.67	0.45
L.intestine	2.33	0.09	1.82	0.20
Heart	0.55	0.07	0.43	0.06
Thyroid	0.64	0.05	0.11	0.02
Brain	0.14	0.02	0.08	0.01
Carcass	22.88	2.30	28.55	1.94
Injection site	2.66	0.42	9.89	9.92*
Counts/gram ratio				
Heart/blood	0.32	0.04	0.34	0.06
Heart/muscle	2.72	0.40	2.42	0.37
Heart/liver	1.08	0.10	0.58	0.02
Brain/blood	0.02	0.00	0.02	0.00
Brain/muscle	0.19	0.04	0.17	0.04

= standard deviation of three animals

* = high due to poor injection in one animal

Figure 3.4.

Gamma Camera images of $[^{99m}Tc(NO)Cl_4]^-$

Time post injection	Image No.
0 min.	1
10 min.	2
20 min.	3
30 min.	4
60 min.	5