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CHEMICAL AND RADIOPHARMACEUTICAL STUDIES OF TECHNETIUM CONTAINING COMPLEXES

By

G. F. E. Morgan B.Sc.

A Doctoral thesis submitted in partial fulfilment of the requirement for the award of Doctor of Philosophy of the Loughborough University of Technology

October 1987

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The author certifies that the work contained in this thesis is her original work and that it has not been submitted, in full, or in part, to this or any other institution for a higher degree.
DON'T PANIC

(Written in large friendly letters)

Douglas Adams (1978)

'The Hitchhiker's Guide to the Galaxy'
ACKNOWLEDGEMENTS

The course of this study has covered five years and four institutions from Chelsea College and Loughborough University through Leicester Royal Infirmary to my current post at the Royal Liverpool Hospital. During this time the help and friendship given to me by my fellow students and colleagues has been immense and makes the task of thanking them all impossible. I must, however, thank the SERC for the financial assistance of a studentship and Amersham International plc. as my collaborating company. In particular, to Dr D.P. Nowotnik for his patience and assistance throughout and to my two academic supervisors, Dr A.E. Theobald of Chelsea College and Dr J.R. Thornback who kept me going throughout! Finally, I would like to thank my colleagues at Liverpool for their unselfish cooperation with the human study described in chapter three and Bessie, without whom chapter seven would not have been possible.
ABSTRACT.

The work described within this thesis takes the form of several different but related projects. The radionuclide, $^{99m}$Tc, is widely used in nuclear medicine departments and researched in many laboratories throughout the world today and it is the intention of this thesis to contribute to the growing library of knowledge in this field.

In chapter three, the technetium complex of tiron, a disulphonato derivative of catechol, is assessed for its radiopharmaceutical efficacy using both animal and human models. The no carrier added [${}^{99m}$Tc]Tctiron preparation appears to form a single radiochemical species under the reaction conditions described, thought to be $[{}^{99m}$Tc]TcO(tiron)$_2$$^{16-}$, based on the known reaction of technetium with catechol. A small highly charged ion could be expected to clear efficiently through the renal system, an expectation upheld by the animal studies and not observed in the human studies, where it is heavily protein bound.

Chapters four and five are both concerned with thioether complexes of technetium, a donor group little studied with this particular metal. Firstly, a series of dithiadicarboxylic acids were synthesised or purchased and their subsequent reactions at both no carrier added [${}^{99m}$Tc] and carrier added [${}^{99}$Tc] levels are discussed. Secondly, a macrocycle, potentially hexadentate through a $S_2N_2$ donor set, was synthesised and derivatised and the reactions of these ligands with $^{99m}$Tc and $^{99}$Tc are examined. In both cases, comprehensive animal studies are performed using two types of rodent, which show mixed hepatobiliary and renal clearance.
Chapters six and seven are concerned with radiopharmaceuticals which, at the time of study, were both involved in clinical trials. The $^{99m}$Tc(Tc(BuNC)$_6$)$^+$ complex shows there is potential in myocardial nuclear medicine for this ligand type and is altered here by the substitution of NO$^+$ to give $^{99m}$TcNO(BuNC)$_6$.$^{2+}$. The reaction under different conditions yielded several products which were individually prepared and tested in animal and human models. Their biological behaviour proved to be interesting although none gave an image of the myocardium. Finally, the technetium complex of HM-PnA0 is known to rapidly degrade from the lipophilic species that crosses the blood/brain barrier to a hydrophilic species that does not have this property. The exact nature of this decomposition product has not been established and some HPLC analyses using $^{99m}$Tc and a $\beta$ detector are reported as part of a study, sponsored by Amersham International plc, to identify this complex.
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<td>acacH</td>
<td>acetylacetone</td>
</tr>
<tr>
<td>Bq</td>
<td>Becquerel</td>
</tr>
<tr>
<td>cyclam</td>
<td>1,4,8,11 tetraazacyclotetradecane</td>
</tr>
<tr>
<td>Ci</td>
<td>Curie</td>
</tr>
<tr>
<td>depe</td>
<td>bis(1,2 diethylphosphino)ethane</td>
</tr>
<tr>
<td>dmpe</td>
<td>bis(1,2 dimethylphosphino)ethane</td>
</tr>
<tr>
<td>dppe</td>
<td>bis(1,2 diphenylphosphino)ethane</td>
</tr>
<tr>
<td>diars</td>
<td>o phenylenebis(dimethylarsine)</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N' dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
</tr>
<tr>
<td>DMSAH₂</td>
<td>1,2 dimercaptosuccinic acid</td>
</tr>
<tr>
<td>DTPAH₂</td>
<td>diethylenetriaminepentacetic acid</td>
</tr>
<tr>
<td>EDTAHA₂</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>en</td>
<td>1,2 diaminooethane</td>
</tr>
<tr>
<td>ERPF</td>
<td>effective renal plasma flow</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>gluc</td>
<td>gluconate</td>
</tr>
<tr>
<td>HBPz₃⁻</td>
<td>hydrotris(1 pyrazolyl)borate</td>
</tr>
<tr>
<td>HEDP</td>
<td>1 hydroxyethylidenediphosphonate</td>
</tr>
<tr>
<td>HMPhAO</td>
<td>hexamethylpropyleneamineoxime</td>
</tr>
<tr>
<td>IDAH₂</td>
<td>iminodiacetic acid</td>
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<tr>
<td>Iodochippuran</td>
<td>orthoiodochippuric acid</td>
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ITLC-SG instant thin layer chromatography
silica gel

MAA macroaggregated albumin

MDP methylenediphosphonate

MEK butan 2 one

NTAH₃ nitriItriacetic acid

NPH Northwick Park Hospital

RLH Royal Liverpool Hospital

oxine 8 hydroxyquinoline

salenH₂ N,N' ethylenbis(salicylideneimine)

TDGH₂ thiodiglycollic acid

THF tetrahydrofuran

Tween 80 polyoxyethylenesorbital monooleate

tu thiourea

ORGAN LIST
(chapters 3 and 4)

1. Kidneys
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7.3. Retention times of no carrier added $[^{99}\text{Tc}]$ TcHMPnAO incubated with NH$_3$ solution.

7.4. Retention times of no carrier added $[^{99}\text{Tc}]$ TcHMPnAO with different elution systems.
CHAPTER ONE

INTRODUCTION

1.1. General preamble

1.1.1. Discovery: Occurrence and isolation

The element technetium was finally discovered in 1937 by Perrier and Segre(1) after many false claims spanning over 50 years, the most serious of which had been made by Noddack, Tacke and Berg in 1925. This group, credited with the discovery of rhenium, believed they had simultaneously identified element 43 and named it masurium(2). However, Perrier and Segre bombarded a molybdenum plate with deuterons and identified some emissions as being due to technetium isotopes produced via the reactions below.

\[ ^{94}\text{Mo}(d,n)^{95}\text{Tc} \]
\[ ^{96}\text{Mo}(d,n)^{97}\text{Tc} \]

The name technetium is derived from the greek 'technetos' meaning artificial since this was the first element, unknown in nature, artificially produced(3).

There is no stable isotope of technetium known although there are over thirty radioactive isotopes and isomers with half lives ranging through $2.12 \times 10^5$ years($^{99}\text{Tc}$), 90 days($^{99m}\text{Tc}$), 20 hours($^{98}\text{Tc}$), 6.02 hours($^{98m}\text{Tc}$) and 15 seconds($^{100}\text{Tc}$). Methods of decay are as varied as the half-lives, exhibiting electron capture, internal transition and $\beta^-$ decay(4,5,6,7). Full tables of these isotopes and isomers with nuclear...
data may be found in relevant texts(3). Despite many efforts to identify primordial technetium(9,10,11) it is now recognised that any technetium present is due to the spontaneous fission of uranium isotopes, especially by fast neutron induced fission of $^{239}$U or by slow neutron induced fission of $^{235}$U, yielding $^{99}$Tc(12,13). Kenna and Kuroda achieved the first isolation of naturally occurring technetium from pitchblende, using a solvent extraction procedure(14) and obtained $10^{-2}$µg. of technetium from over 5kg. of pitchblende. $^{99}$Tc is more abundant in spent nuclear fuel(15,16), a nuclear reactor of 100MW power produces about 2.5g. of $^{99}$Tc per day(17). The separation process to isolate the technetium involves a series of extraction and precipitation steps with a final product of radiochemically pure $[^{99}\text{Tc}]\text{TcO}_4^-$(18).

The metastable isomer $^{99m}$Tc, is produced by irradiating molybdenum trioxide, often enriched with $^{99}$Mo, with neutrons, converting stable molybdenum to unstable $^{99}$Mo(19). This undergoes $\beta^-$ decay with a half life of 66h. to give $^{99m}$Tc as shown in Figure 1.1.

1.1.2. General chemical properties of technetium

Technetium is in group VII A of the periodic table(Figure 1.2.) and is a second row transition element. It is closely aligned chemically to rhenium due to the lanthanide contraction and differs considerably from manganese(20). Manganese chemistry is dominated by the II oxidation state and the high spin Mn$^{2+}$ aquocation in particular, whilst aqueous technetium chemistry is predominantly concerned with the IV, V and VII oxidation states, and a simple Tc($\text{H}_2\text{O})_{\text{e}}^{2+}$ cation is unknown.
Figure 1.1.: The decay scheme of $^{99}$Mo
\[ {^{99}}Mo \rightarrow {^{137}}Ba, t_{\beta}=67 \text{h.} \]

17% $\beta^-$

1% $\beta^-$

1.23 MeV

82% $\beta^-$

$\rightarrow 0.002 \text{MeV}$

$\rightarrow 142 \text{keV} \gamma$

$\rightarrow 140 \text{keV}$

$\rightarrow 99mTc, t_{\beta}=6.02 \text{h.}$

$\rightarrow 0.29 \text{MeV}$

$\rightarrow ^{99}Tc, t_{\beta}=2.1 \times 10^{10} \text{y.}$

$\rightarrow ^{99}Ru, \text{Stable}$
Technetium in its elemental form has an electronic configuration of [Kr]4d\(^{8}\)5s\(^{1}\) and therefore has an extensive coordination chemistry with at least seven oxidation states available. Compounds have been proposed having oxidation states from -I to +VII although there is some doubt about the former (Table 1.1.).

<table>
<thead>
<tr>
<th>Complex</th>
<th>Oxidation State</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc(<em>{2})O(</em>{7})</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>TcO(_{4})(^{-})</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>TcO(_{4})</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>TcCl(_{6})</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>TcOCl(_{4})(^{-})</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>([\text{TcO}<em>{2}(en)</em>{2}]^{+})</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>TcO(HBPz(<em>{3}))Cl(</em>{2})</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>TcO(_{2})</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>([\text{H}<em>{2}\text{EDTA}]\text{TcO}</em>{2}\text{Tc(}\text{H}_{2}\text{EDTA})]</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>([\text{Tc(acac)}<em>{2}(\text{PPh}</em>{3})\text{Cl}])</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>([\text{Tc(dppe)}_{2}\text{Cl}]^{+})</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>Tc(dppe(<em>{2}))Cl(</em>{2})</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Tc(diars(<em>{2}))Br(</em>{2})</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Tc(A(<em>{5}^{-})-C(</em>{6})H(<em>{5}))(CO)(</em>{3})</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>([\text{Tc(A}<em>{5}^{-})-C(</em>{6})H(_{5})]^{+})</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Tc(<em>{2})(CO)(</em>{10})</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>([\text{Tc(CO)}_{6}]^{-})</td>
<td>-1</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 1.1.

SELECTED EXAMPLES OF TECHNETIUM COMPLEXES
Figure 1.2.: A selected part of the periodic table

Figure 1.3.: The electronic structure of elemental technetium
\[ ^{43}\text{Tc} \quad 1s^2 \ 2s^2 \ p^6 \ 3s^2 \ p^6 \ d^{10} \ 4s^2 \ p^6 \ d^6 \ 5s^1 \]

\[
\begin{array}{c}
5s \\
4d_{3d} \\
4d_{1d}
\end{array}
\]

\[
\begin{array}{c}
1+ \\
--- \\
1+ 1+ 1+
\end{array}
\]
Coordination numbers have been identified as diverse as 4–9, as for example \( \text{TcO}_4^- \) and \( \text{TcH}_6^{2-} \) both being technetium(VII) species.

In aqueous solution, pertechnetate is the most stable ion. It is more weakly oxidising than \( \text{MnO}_4^- \) (Table 1.2.) and is reduced, in the absence of a complexing ligand to the thermodynamically stable dioxide \( \text{TcO}_2 \cdot x\text{H}_2\text{O} \).

\[
\begin{array}{ll}
\text{Manganese} & \\
\text{E}^\circ (\text{volts}) & \\
\text{MnO}_4^-/\text{Mn}_2 & +1.695 \\
\text{MnO}_4^-/\text{Mn} & +1.781 \\
\text{MnO}_2/\text{Mn} & +0.115 \\
\text{Technetium} & \\
\text{TcO}_4^-/\text{TcO}_2 & +0.738 \\
\text{TcO}_4^-/\text{Tc} & +0.477 \\
\text{TcO}_2/\text{Tc} & +0.281 \\
\text{Rhenium} & \\
\text{ReO}_4^-/\text{ReO}_2 & +0.510 \\
\text{ReO}_4^-/\text{Re} & +0.367 \\
\text{ReO}_2/\text{Re} & +0.260 \\
\end{array}
\]

Table 1.2.

**REDOX POTENTIALS OF VIIA METALS IN AQUEOUS ACIDIC SOLUTION**
If, however, reduction of TcO$_4^-$ takes place in the presence of complexing ligands in a controlled environment other oxidation states are obtainable; for example [$^{99m}$Tc]TcO$_4^-$ is reduced from technetium(VII) to technetium(I) by sodium dithionite in the presence of isonitriles(38) and is reduced from technetium(VII) to technetium(V) by tin(II) tartrate in the presence of ethylenediamine(39).

The weakly oxidising nature of pertechnetate is reflected by the wide variety of reducing agents that have been used. Tin(II), in the form of tartrate, chloride and sulphide, tin foil(39,40,41a,41b,42), sodium borylhydride(43,44), sodium dithionite(38,45), sodium bisulphite(46,29), formamidine sulphinic acid(47), hydrazine(45), hydroxylamine(45,46), chromium(II) (49), molybdenum(III) (50), c.HCl(51,52), zirconium electrodes(53) and other electrolytic methods have all been employed with varying success(54). There is also some evidence that some ligands act as autoreductants, although the reaction time is frequently longer than normal radiopharmaceutical preparation times(55).

$^{99m}$Tc is a low energy $\beta^-$ emitter, $E_{\beta^-}=0.29$ MeV with a half life of $2.1 \times 10^8$ y., decaying to stable $^{99}$Ru. It is a pure $\beta^-$ emitter, although bremsstrahlung of secondary X-rays may produce some radiological concern. $^{99m}$Tc is a pure $\gamma$ emitter of 140 keV energy and $t_{1/2}$ of 6.02 h.

1.1.3. Bonding in technetium complexes

The electronic structure of elemental technetium([Kr]4d$^6$5s$^1$) is shown in Figure 1.3. In complex formation the d electrons are generally in a low spin state, as expected for a second row transition element.
Technetium compounds have been characterised in all oxidation states from 0-7 and which oxidation state is stabilised is a function of the nature of the ligands surrounding the central metal atom. The soft bases, listed in Table 1.3., are $\pi$ acid or $p$ acid (depending upon the nature of the empty orbitals) and stabilise low oxidation states by spreading out the high metal electron density. High oxidation states are favoured by hard ligands, such as oxygen donors, to stabilise the high metal positive charge.

<table>
<thead>
<tr>
<th>Hard Bases</th>
<th>Soft Bases</th>
<th>Borderline</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2O$, $R_2O$, ROH, NH$_2$, RNH$_2$</td>
<td>$NH_2^-$, OR$, Cl^-$, ClO$_4^-$, NO$_3^-$</td>
<td>pyridine, Br$^-$, $H_2O^-$, NO$_2^-$, SO$_3^{2-}$</td>
</tr>
<tr>
<td>$SO_4^{2-}$, CO$_3^{2-}$, PO$_4^{3-}$</td>
<td>$RS^-$, $RSH$, $R_3P$, $R_3As$</td>
<td>$NO_2^-$, SO$_3^{2-}$</td>
</tr>
</tbody>
</table>

Table 1.3.

HARD AND SOFT BASES

Low oxidation state complexes such as the hexakis(isonitrile)technetium(I) complexes, have a number of electrons with high metal character in the nonbonding $t_{2g}$ molecular orbital (Figure 1.4.). These electrons are available for backbonding from the metal to an antibonding orbital on the ligand if it possesses suitable such orbitals. For example, the nitrosyl ligand, NO$^+$, has vacant $\pi$ orbitals available to
Figure 1.4: M.O.'s in octahedral technetium complexes
accept electron density from occupied metal orbitals to form \( \pi \) bonds supplementary to the \( \sigma \) bond. This ligand, similarly \( \text{CN}^- \), \( \text{RNC} \) and \( \text{CO} \) are strong \( \pi \) acceptor ligands and stabilise the metal-ligand bonding in the manner shown in Figure 1.5.

The high oxidation states have largely unoccupied \( t_{2g} \) metal 'd' orbitals of the same symmetry as the ligand p non-bonding orbitals, for example \( \text{Cl}^- \), \( \text{S}^2- \) and \( \text{O}^2- \) and \( \pi \) bonds are formed which confer extra stability to the complex (Figure 1.6.). Chloride is a hard base, forming strong \( \sigma \) and \( \pi \) ligand-metal bonds. Bromide, however exhibits a degree of softness in character by virtue of the increased number of available empty orbitals and is a borderline base while iodide is recognised as a soft base.

Metal-metal bonding is more common to the second and third row transition metals than the first row and increasingly such complexes are being prepared in technetium chemistry (56) mainly analogous to previously known rhenium species.

This thesis is mainly concerned with oxidation states III to V and therefore the introduction will concentrate on those complexes and their reactions. However, chapter 6 does consider isonitrile nitrosyl complexes which are technetium(I) or technetium(II) species.

1.2. Technetium in nuclear medicine
1.2.1. Fundamentals

Nuclear medicine departments are usually concerned primarily with diagnosis and \( ^{99m}\text{Tc} \) is the major isotope of choice for this purpose.
Figure 1.5.: Metal-ligand bonding with strong π acceptor ligands
Figure 1.6: M.O.'s in high and low oxidation state technetium complexes
ligand
\textit{t}_{2g}
e.g. chloride

$\sigma + \pi$
bonding

Metal $\sigma$ bonding

$\Delta_{oct}$

$e_{g}$

$\Delta_{oct}$

ligand
\textit{t}_{2g}
e.g. cyanide

$\sigma + \pi$
bonding
However, injections of particulate emitters such as those in Table 1.4, are used for therapeutic purposes. Since the \( \beta^- \) or \( \alpha^{2+} \) particles have very restricted ranges in tissue it is important that the target to

<table>
<thead>
<tr>
<th>Principal Radiation</th>
<th>Examples of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclide</td>
<td>(MeV)</td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>14.3d. ( \beta^- )</td>
</tr>
<tr>
<td>(PO₄³⁻)</td>
<td></td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>2.7d. ( \beta^- )</td>
</tr>
<tr>
<td>(colloid)</td>
<td></td>
</tr>
<tr>
<td>( ^{131}I )</td>
<td>8.0d. ( \beta^- )</td>
</tr>
<tr>
<td>(NaI)</td>
<td></td>
</tr>
<tr>
<td>( ^{198}Au )</td>
<td>2.7d. ( \beta^- )</td>
</tr>
<tr>
<td>(colloid)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.4.

RADIONUCLIDES IN THERAPY

non-target ratio is high to minimise the radiation dose to healthy tissue. The energy of the \( \beta^- \) particles is not critical, although a reasonably high energy is desirable to give sufficient penetration. Radionuclidic half life is important since a short lived isotope requires
large activity levels to be administered, whilst long-lived isotopes present high dosimetry problems. In general, therapy is underdeveloped compared to diagnosis and further advances in this area are vital in the future.

Procedure | Example | Radionuclide
---|---|---
**Imaging** | | |
1. Static | Bone scan | $^{99m}$Tc
Lung scan | $^{99m}$Tc, $^{133}$Xe, $^{81}$Kr
Liver scan | $^{99m}$Tc
2. Dynamic | Brain Perfusion | $^{99m}$Tc
Hepatobiliary | $^{99m}$Tc
Renal Function | $^{99m}$Tc, $^{123}$I

**Non-imaging** | | |
1. Uptake and Retention | Thyroid Function | $^{131}$I
2. Specimen Measurements (Faecal) | Protein Loss | $^{51}$Cr
Plasma Volume | $^{125}$I
(Blood) | $^{57}$Fe
Vit B$_{12}$ Absorption | $^{58}$Co
(Urine)

**Table 1.5.**

DIAGNOSTIC PROCEDURES IN NUCLEAR MEDICINE
Diagnostic procedures subdivide into imaging and non-imaging techniques (Table 1.5.). Currently $^{99m}$Tc is considered the imaging radionuclide of choice. A study undertaken by the N.R.P.B., of nuclear medicine activity in England in 1982, showed that of a total 304,000 in vivo administrations, 8,700 were therapeutic and 257,000 involved imaging. Of the total imaging procedures, 75% contained the $^{99m}$Tc radionuclide. Further, bone scans ($^{99m}$Tc-phosphonate complexes) accounted for 24% of all the nuclear medicine procedures in the U.K. (Table 1.6.) (57).

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Radionuclide</th>
<th>Chemical</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{99m}$Tc</td>
<td>MDP</td>
<td>24.0</td>
</tr>
<tr>
<td>Liver</td>
<td>$^{99m}$Tc</td>
<td>Colloid</td>
<td>13.0</td>
</tr>
<tr>
<td>Lung</td>
<td>$^{99m}$Tc</td>
<td>MAA</td>
<td>8.2</td>
</tr>
<tr>
<td>Thyroid</td>
<td>$^{99m}$Tc</td>
<td>pertechnetate</td>
<td>4.6</td>
</tr>
<tr>
<td>Renal</td>
<td>$^{99m}$Tc</td>
<td>DTPA</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>$^{99m}$Tc</td>
<td>DMSA</td>
<td>1.15</td>
</tr>
<tr>
<td>Myocardial</td>
<td>$^{201}$Tl</td>
<td>chloride</td>
<td>1.3</td>
</tr>
<tr>
<td>Lung</td>
<td>$^{133}$Xe/$^{81}$Kr</td>
<td>gaseous</td>
<td>4.3</td>
</tr>
<tr>
<td>Tumour</td>
<td>$^{67}$Ga</td>
<td>citrate</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Table 1.6.

THE RELATIVE % OF SCAN TYPES IN 1982 IN THE U.K. (57)
1.2.2. Technetium: Radionuclide of choice (58)

There are several reasons why $^{99m}$Tc has achieved such popularity in nuclear medicine;

1) It is a monoenergetic pure $\gamma$ emitter. The presence of other radiation emissions would result in an unnecessary radiation dose to non-target organs and could interfere with the quality of the image obtained.

2) Technetium has a rich chemistry and forms many radiopharmaceuticals by incorporating the radionuclide into different complexes and formulations.

3) The half life allows for delayed imaging to take place without requiring very high activities to be administered.

4) The $\gamma$ or Anger cameras currently available have optimal efficiency of detection of $\gamma$ photons in the range 100–300 keV. The energy of the $^{99m}$Tc $\gamma$ photon falls well within this range. Furthermore, except for deep seated lesions the 140 keV photon is excellent for imaging externally without significant tissue absorption or scatter. Thus the critical organ doses and whole body doses incurred with $^{99m}$Tc radiopharmaceuticals are low compared to other radiopharmaceuticals currently favoured in nuclear medicine (Table 1.7.)

5) The progress of $^{99m}$Tc owes much to the development of the $^{99}$Mo/$^{99m}$Tc generator. Since its invention in the early 1950's at Brookhaven National Laboratories, many advances have been made to improve the performance of these generators.

The essence of generator-produced radionuclides is that the daughter nuclide (in this case $^{99m}$Tc) can be readily separated from its parent ($^{99}$Mo). Several separation techniques are possible for $^{99m}$Tc.
<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Form</th>
<th>Typical Activity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>^67Ga</td>
<td>Citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>^75Se</td>
<td>methionine</td>
<td>0.25</td>
<td>2.7</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>^99mTc</td>
<td>Pertechnetate</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver (0.9)</td>
</tr>
<tr>
<td>^99mTc</td>
<td>Sulphur colloid</td>
<td>2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver (0.7)</td>
</tr>
<tr>
<td>^99mTc</td>
<td>DTPA</td>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
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<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bladder (2.7)</td>
</tr>
<tr>
<td>^99mTc</td>
<td>MAA</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lung (0.4)</td>
</tr>
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<td>^99mTc</td>
<td>diposphonate</td>
<td>10</td>
<td>0.1</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bladder (4)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Bone (0.4)</td>
</tr>
<tr>
<td>^123I</td>
<td>sodium iodide</td>
<td>0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>&lt;0.01</td>
</tr>
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<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thyroid (2.6)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Stomach (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wall</td>
</tr>
<tr>
<td>^131I</td>
<td>sodium iodide</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thyroid (26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stomach (0.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wall</td>
</tr>
</tbody>
</table>

Table 1.7.

ABSORBED DOSES (RAD) FOR VARIOUS PROCEDURES
chromatography, sublimation and solvent extraction but the method generally employed is chromatography (59).

Most $^{99m}$Tc/$^{99m}$Mo generators currently supplied to nuclear medicine departments are 'fission' generators, where the $^{99m}$Tc is produced from the fission of $^{235}$U. High specific activity $^{99m}$Mo is produced in the fission process since the fission yield of $^{99m}$Mo is very low and so essentially all the molybdenum isolated is the desired isotope. In the traditional generators the molybdenum is produced by neutron irradiation of $^{99m}$Mo and is thus a mixture of this and the desired $^{99m}$Mo. Although the separation costs of fission produced $^{99m}$Mo are expensive the higher specific activities of $>10^4$Ci.g.$^{-1}$ obtained fully justify the extra cost.

The typical generator works on the principle of anion exchange. $^{99m}$Mo is adsorbed onto an acidified alumina column which is eluted with isotonic saline, exchanging Cl$^-$ for TcO$_4$$^-$. Figure 1.7. shows a model of a commercial generator currently supplied to nuclear medicine departments. It incorporates some of the recent advances in generator design, with reduced aerial exposure, no bacteriological contamination and choice of elution volume from 4-20ml.

Several other factors have to be considered when supplying a $^{99m}$Mo/$^{99m}$Tc generator and these relate to the chromatography column itself. It is essential that the parent nuclide remains tightly bound to the alumina throughout the working life of the generator as [${}^{99m}$Mo]MoO$_4$$^2$- eluted with the eluate will give unnecessary $\beta^+$ irradiation to the patient. The column packing material should be inert to radiolysis. The elution of Al$^{2+}$ ions from acidified activated alumina is known and must be minimised as Al$^{2+}$ is neurotoxic if present in more than trace
Figure 1.7: A model of the commercial $^{99m}$Tc/$^{99m}$Tc generator
Valve position – see elution instructions

Elute (small volume)

Evacuated

Saline remaining in secondary reservoir

Close valve

Open valve

Elute (large volume)

Open valve

Volume eluted

Close valve

Open valve
quantities\(^{60}\). Finally, the elution yields must be sufficiently high and reliable. It is improbable that 100\% of the \([{^{99m}\text{Tc}}\text{TcO}_4^-]\) available for elution will exchange with \(\text{Cl}^-\) but low or unreliable yields make a generator unserviceable in a radiopharmacy\(^{61,62}\).

The specific activity of the generator eluant is important since the vast proportion of all the technetium present in a sample usually termed carrier free from the generator is in fact \(^{99}\text{Tc}\) and not \(^{99m}\text{Tc}\). The amount of \(^{99}\text{Tc}\) present is a function of the time between elutions as there is continual decay of \(^{99m}\text{Tc}\) to \(^{99}\text{Tc}\). Thus the initial elution of a generator by a hospital on receipt may contain several orders of magnitude higher concentration of total technetium than an eluant of the same \(^{99m}\text{Tc}\) activity taken from the same generator at a later date. Most radiopharmaceutical kits are designed to be able to cope with this range of concentrations by using very large ligand and reducing agent to metal ratios.

A \(^{99m}\text{Tc}\) containing radiopharmaceutical is generally prepared by reconstitution of a freeze-dried kit\(^{63}\) containing the ligand of interest, reductant and stabilisers with generator eluate. A simple one step reaction takes place yielding technetium coordination complexes in low concentration. Early radiopharmaceuticals produced mixtures of two or more complexes\(^{64}\) but modern kits attempt to produce only a single species. Intravenous injection of the formulation will result in preferential uptake into the organ of interest or passage through the biological pathway under study with subsequent visualisation on the \(\gamma\) camera. The nature of the radioactive complexes present cannot be directly identified as the concentration of technetium in these
formulations is of the order of $10^{-9}$ M and only indirect methods of analysis are available such as electrophoretic or chromatographic behaviour. The prolific output of the radiopharmaceutical scientists is reflected in the vast number of licensed freeze-dried kits ranging from brain imaging to lymphoscintigraphy and by the volume of the research currently being explored world-wide into novel radiopharmaceuticals; optimising radiopharmaceuticals currently in use; or even a triannual international symposium devoted entirely to the subject of technetium in chemistry and nuclear medicine (65).

1.3. Technetium chemistry and radiopharmaceuticals

1.3.1. An introduction to the problem

Until recently, a paucity of information resulting from a lack of research in the subject has led to the briefest of mentions for $^{99m}$ Tc on the pages of most inorganic chemistry text books (66, 67, 68). However, since the metastable isotope $^{99m}$ Tc has become widely used in nuclear medicine the need for a comprehensive study of the coordination chemistry of $^{99m}$ Tc has become of paramount importance. There has recently been a double-volume Gmelin Handbuch published which covers very fully the developments over the past few years (69). Numerous reviews covering a number of aspects of technetium have been published; crystal structures (70), electrochemistry (71), inorganic chemistry of technetium (V) (72) and technetium (I) to technetium (IV) (73), radiopharmaceutical use (74), plus general technetium reviews by, among others, Deutsch, Seddons, Schwachau and Turp (65, 75, 76, 77, 78). The problem associated with $^{99m}$ Tc complexes is an inability to get involved with the
actual chemistry of the radiopharmaceuticals. The very nature of the no carrier added level $^{99m}$Tc concentrations makes study limited and often chemical speciation identification can only be postulated. With $^{99m}$Tc it is possible to carry out a full range of traditional chemical tests to identify the complexes.

The role of the inorganic chemist is two fold. In one respect there is a need to expand the library of technetium chemistry, which over the last six to seven years has been effectively 'carte blanche' in the field. With so little actually documented, there were many areas to explore. At the same time, there is a responsibility towards nuclear medicine to be honoured as the current intense interest in $^{99m}$Tc largely arises from the profusion of $^{99m}$Tc studies which requires a union of $^{99m}$Tc and $^{99}$Tc chemistries(79,80).

Whilst the chemistry of the isotopes should be similar there are differences which arise in the main from the difference between no carrier and carrier added concentration levels. The most common approach is to formulate the complex using $^{99}$Tc and by dilution, with the necessary modification of the complex, to reach no carrier added levels. Comparisons are then made of the chromatographic behaviour of both complexes. At carrier added concentration levels, chemical behaviour can be radically different than at the low concentrations due to the formation of dimeric and polymeric species as opposed to the monomeric species in the radiopharmaceutical formulations(81).
1.3.2. An overview of technetium(III) and technetium(IV) chemistry and radiopharmaceuticals

1.3.2.1. Tertiary phosphine containing compounds

Unlike technetium(V) the chemistry of technetium(III) and technetium(IV) includes many complexes which contain π acid ligands such as the phosphines. The chemistry of tertiary phosphine complexes of technetium does not closely mimic that of rhenium. When PPh₃ is the ligand of choice, only TcL₂X₄ (where X=halide) can be isolated, whereas ReOCl₃L₂ has been prepared under identical conditions (82). The technetium analogue of the latter has not yet been characterised. The preparation of TcX₃L₂ (where X=halide, L=dialkylphenylphosphine) is facile, while that of the rhenium analogue is a difficult and lengthy procedure. The synthesis and interconversion of some technetium(III) and technetium(IV) tertiary phosphine complexes are shown in Figure 1.8 (83). These compounds have been prepared by both the direct reaction of the ligand with TcO₄⁻ and oxidation/reduction of the presynthesised technetium(III) or technetium(IV) complexes. The relationship between technetium(III) and technetium(IV) has been studied by cyclic voltammetry in acetonitrile solvent and a mechanism via charge transfer is suggested (84).

\[ \text{TcX₃L₂} + e^- = (\text{TcX₄L₂})^- + \text{MeCN} \rightarrow \text{TcX₄L₂MeCN} + X^- \rightarrow \text{product} \]

X= halide
L= (Me₂Ph)P, (Et₂Ph)P, Ph₃P
if X=Cl⁻, in the presence of Na⁺, 1 mole of NaCl per faraday of electricity is deposited.
Figure 1.8.: Synthesis and interconversion of technetium(III)/technetium(IV) tertiary phosphine complexes
\[ \text{NH}_4\text{TcO}_4 \xrightarrow{\text{HCl}} \text{TcCl}_4(\text{PR}_2\text{Ph})_2 \]

\[ \text{Tc}:\text{L} \ 1:5 \]

\[ \text{HCl} \]

\[ \text{NaBH}_4 \]

\[ \text{CCl}_4 \]

\[ \text{Ligand} \]

\[ \text{Tc}:\text{L} \ 1:15 \]

\[ \text{HCl} \]

\[ \text{TcCl}_3(\text{PR}_2\text{Ph})_3 \]

where R=methyl or ethyl but not phenyl
These complexes are air stable and soluble in common organic solvents. However, the technetium(III) complexes, $\text{TcX}_3\text{L}_2$ (generally yellow red), are unstable in chlorinated solvents with respect to trans-$\text{TcX}_4\text{L}_2$ (generally green). The ease of preparation of the latter makes them useful reaction precursors. The labile halide ligands may be readily replaced giving rise to a large number of novel species. Trans-$\text{TcX}_4\text{L}_2$ ($\text{X}=\text{Cl}^-, \text{Br}^-$, $\text{L}=\text{PPh}_3$, $\text{PMes}_2\text{Ph}$) reacts with CO in ethyleneglycolmomonomethylether to give $[\text{TcX(CO)}_2\text{L}_2]$. Under the same conditions mer-$\text{TcX}_2(\text{PMes}_2\text{Ph})_2$ gives a mixture of trans-$[\text{TcX(CO)}_2(\text{PMes}_2\text{Ph})_2]$ and cis-$[\text{TcX(CO)}_2(\text{PMes}_2\text{Ph})_2]$, technetium(I) complexes.

A technetium(III) complex with seven coordination around the metal atom has been made, $[\text{TcCl}_3(\text{CO})(\text{PMes}_2\text{Ph})_2]\text{EtOH}$ by using ethanol as the solvent.

More recently the reaction of a technetium(V) complex $\text{TcO}([\text{OPhsal}](\text{BSB}))$ (where OPhsal = $\text{N}$(2 oxidophenylsalycylideniminato) and BSB = quinolinolato) with $\text{PET}_2\text{Ph}$ resulted in the formation of $\text{Tc}([\text{PET}_2\text{Ph})(\text{OPhsal}])\text{(BSB)}$. The $\text{PET}_2\text{Ph}$ presumably removing the oxo as the phosphine oxide.

The diphosphine ligands ($R_2\text{PR}^+\text{PR}_2$) have been studied in some depth, partly due to their potential as myocardial imaging agents.

Stable cationic technetium(III) species of the form $\text{TcX}_2\text{L}_2^+ (\text{X}=\text{halide}, \text{L}=\text{diphosphine or diarsine})$ are readily obtained from $\text{TcO}_4^-$ and the ligand with added halide in the solution. Extensive studies of the diarsine containing species $[\text{ars}^-\text{TcI}(\text{diars})_2\text{X}_2]^+ (\text{X}=\text{Cl},\text{Br})$ have been carried out for myocardial uptake, based on the current theory that a cationic
compound of technetium, possibly mimicking other unipositive cations K⁺, Tl⁺, and Rb⁺ will show myocardial uptake(89).

The dmpe and depe complexes are characterised by giving excellent images in animal models but poor human results(90,91). It has been proposed that the [⁹⁹m⁹⁷¹⁷²Tc(dmpe)₂Cl₂]+ and analogous complexes undergo reduction in human plasma to give neutral technetium(II) species which are not taken up by the myocardium(92). The technetium(I) species [⁹⁹m⁹⁷¹⁷²Tc(dmpe)₂]+ and analogues also show large species variation giving very slow blood clearance and only poor myocardial visualisation after 4 hours(93). A purported mixed isonitrile-phosphine complex of technetium shows promise as a blood pool agent(94).

To confirm the postulate of in vivo reduction of the [Tc(dmpe)₂X₂]+ extensive electrochemical studies have been carried out(95). All indeed show one electron reductions to the neutral technetium(II) species. A comparison of the ease of reduction of a series of the (TcX₂L₂)+ complexes where L is the same diphosphine but X may be SCN⁻, Cl⁻ or Br⁻ shows that the reduction potential is lower for the thiocyanate, which thus stabilises the lower oxidation state more successfully than the halides(96). As might be expected Br⁻ is better than Cl⁻ and changing from alkyl to aryl substituted phosphines increases the ease of reduction. The neutral technetium(II) species have recently been reported as potential brain perfusion agents(97).

Technetium(V) complexes are also known with these ligands. The bisoxo [TcO₂(dppe)₂]⁺ has been prepared although not exhaustively characterised(98). The oxo-hydroxo analogue containing the dmpe ligand
has also been reported (99). Neither of these species is useful as a radiopharmaceutical.

1.3.2.2. Compounds containing sulphur donor ligands

Whilst the thiolates generally form technetium(V) oxo complexes of the general form $\text{TcOL}_2^{-}$ (L=alkyldithiol) recently a neutral technetium(III) species has been prepared using a sterically hindered thiol, 2,3,5,6 tetramethylbenzenethiol which has solvent acetonitrile ligands in the remaining two occupied positions. These axial solvent molecules are labile and this complex affords good potential as a synthetic intermediate. This has not yet found use in radiopharmaceuticals (100).

However, one ligand which has found use in radiopharmaceuticals and is thought to give a technetium(III) species which is favoured in the study of kidney morphology is dimercaptosuccinic acid (DMSAH₄) (Figure 1.9.).

The reaction of DMSAH₄ with $[{}^{99}\text{TcO}_4]^{-}$ with a tin(II) reductant gives two major components neither of which have been completely characterised but one is supposedly $\text{Tc(DMSA)}^{-}$ (101). The ligands in the other positions have not been identified. When the reaction was carried out at ${}^{99m}\text{Tc}$ no carrier added concentration essentially one species is formed which is the kidney morphology agent (102, 103).

A technetium(V)oxo compound has also been reported, prepared by the ligand exchange reaction of $[\text{TcO(gluc)}_2]^{-}$ (104), although since DMSAH₄ is autoreducing (65) it is possible that the simplistic approach of mere ligand exchange may be erroneous.
Figure 1.9.: The ligand DMSAH₄

Figure 1.10.: The ligand DTPA⁻
The former complex remains an interesting problem in radiopharmaceutical chemistry but of more interest currently is the development of a new \[^{99m}Tc\]TcDMSA complex loosely assigned as technetium(V)\(^{105,106}\). This is now being used clinically in the diagnosis of thyroid medullary carcinoma\(^{105,107}\). The complex may be prepared from the normal marketed kits but reconstituted at high pH and increased ligand concentration\(^{108}\). It is not clear whether or not this complex is the second one observed in the \[^{99m}Tc\] studies.

Thiourea and \(N,N'\)-dimethylthiourea containing complexes, TcL\(_2\), have proved to be excellent starting materials for the synthesis of novel technetium complexes in low oxidation states. The starting complexes may be prepared from TcCl\(_4\) or TcO\(_4^-\)\(^{109,110}\).

1.3.2.3 Aminocarboxylate containing complexes

This ligand type illustrates precisely the mismatch between carrier and no carrier added reactions and also indicates some of the wealth of the chemistry involved in Tc-Tc dimeric species in oxidation states III and IV. These ligands are well known in nuclear medicine. A lyophilised freeze dried kit of DTPAH\(_5\) (Figure 1.10) is currently marketed by several companies, which upon reconstitution with \[^{99m}Tc\]TcO\(_4^-\) gives a complex suitable for renal/brain imaging. The same formulation is also used in liquid gastric emptying studies\(^{111}\), as are DTPAH\(_5\) complexes of \(^{111m}\)In\(^{112}\). \[^{99m}Tc\] complexes of EDTA\(_4\) have been reported as renal imaging agents\(^{113}\) and many technetium complexes have been synthesised with ligands based on IDA\(_2\) derivatives for optimised hepatobiliary imaging\(^{114}\). However attempts to isolate the monomeric technetium
complexes of DTPAH$_5$ and IDAH$_2$ have not yielded definitive results, although a mass spectroscopic study suggested the latter to be \( \text{Tc(IDA)}_2 \) (115). Judicious choice of ligand i.e. a hexadentate, strongly chelating cyclic moiety triazacyclononane \( \text{N,N',N''} \) triacetate (TCTAH$_3$) failed to form a 1:1 complex in oxidation states III and IV, preferring dimeric metal-metal bonds and oxo-bridging complexes (116). Only in the technetium(V) oxidation state were some monomeric complexes of these ligands formed but did not resemble the radiopharmaceutical formulation (assigned as technetium(III)).

Several aminocarboxylates were synthesised with mixed oxidation states technetium(III)/technetium(IV), technetium(III)/technetium(III) or technetium(IV)/technetium(IV). Of these, only the technetium(IV) compounds are stable enough to have allowed full characterisation, the technetium(III) or mixed III/IV species being either too air sensitive or labile to allow complete isolation (116). The general reaction is reduction of pertechnetate in the presence of the ligand. This method emulates radiopharmaceutical formulation but frequently is not a suitable method for carrier added reactions. If \( \text{TcO}_4^- \) is reduced with aminocarboxylates in the reaction solution, pink dimeric bis \( \mu \)-oxo complexes of the type

\[
(\text{H}_2\text{EDTA})\text{TcO}_2\text{TC}(\text{H}_2\text{EDTA})\quad (29)
\]

or

\[
\text{Na}_2(\text{NTA})\text{TcO}_2\text{TC(NTA)})\cdot 6\text{H}_2\text{O}\quad (117)
\]

are isolated, assigned technetium(IV)/technetium(IV). Under different reaction conditions a mono-oxo bridge can be prepared, also technetium(IV), with rapid hydrolysis from mono oxo to bis oxo at high pH (116).
Few aminocarboxylate complexes have been fully characterised. Several groups have now prepared complexes with EDTAH₄(29,116,118,119), ranging from a technetium(V) oxo anion with a hexadentate ligand and seven coordination around the technetium nucleus to a technetium(IV) oxo anion H₂(TcOEDTA) in which the ligand is purportedly bonded to the technetium in a tetradentate N₂O₂ fashion. Without X-ray crystallographic data this latter result must be viewed with some suspicion. DTPAH₆ and IDAH₂ complexes remain largely unidentified due to the difficulty in crystallising such complexes and therefore any characterisation, based on less precise data remains questionable. It has been claimed that yellow (TcODTPA)²⁻ may be prepared from TcBr₆²⁻(119). Complications arise in this reaction from the variety of complexes formed by subtle pH changes(Figure 1.11.). These complexes are largely discussed in terms of their colour which range from pink to purple(blue) to gold. They are all highly coloured water soluble species which appear to bear no resemblance to the radiopharmaceutical formulation. The gold coloured complex appears to be an intermediate formed during the reduction reaction before the stable end product is formed, but it is not readily isolated. Only the gold coloured intermediate in the reaction with TCTAH₆ has so far proved accessible and preliminary classification indicates it to be a mono oxo bridged dimer of technetium(III).

Interestingly this ligand, which is potentially hexadentate, reacted with [TcO(OCH₂CH₂O)₂]- to form a mixed TCTAH₆-ethylene glycolate complex coordinating only through the amino groups and not the acetates. In the bis-oxo bridged molecule the bonding is through a N₂O donor set(87).
Figure 1.11.: Some $[^{99}\text{Tc}]$ TcDTPA complexes formed during reactions in aqueous solution
\[ \text{TcO}_4^- + \text{DTPA}^- + \text{Sn}^{2+} \]
\[ \text{or} \]
\[ \text{K}_2\text{TcBr}_6 + \text{DTPA}^- \]

\( \text{pH} < 2 \)

Colourless

\( \text{pH} > 4 \)

\( \rightarrow \text{red} \quad \lambda_{\text{max}} = 500 \)

\( \text{pH} > 10 \)

\( \rightarrow \text{purple} \quad \lambda_{\text{max}} = 510 \)

\( \text{pH} < 10 \)

\[ \text{yellow} \quad \lambda_{\text{max}} = 433,500 \]

in ligand free solutions

brown
An evaluation in 1977 of the current radiopharmaceuticals available for hepatobiliary imaging indicated that the iminodiacetic acid family of ligands showed great potential with the added attraction of simple one-step reconstitution\textsuperscript{(120).} Although the IDA\textsubscript{H}, were developed as analogues of lignocaine, while the \textsuperscript{14}C IDA\textsubscript{H}, derivative of lignocaine is excreted through the kidneys the technetium complex showed hepatobiliary clearance\textsuperscript{(121,122).} To consider that the technetium complex would mimic the pure ligand biological distribution was of course illogical due to the lack of similarity in structure, charge and electronic configuration. The technetium complex has been tentatively characterised at both the carrier and no carrier added concentrations as a technetium(III) anion with two ligands around the metal, each three coordinate. However, these assignments are based on the potentiometric titration of TcO\textsubscript{4} with tin(II) in a HIDA\textsubscript{H}, buffer and a charge determination of -1 indicated by electrophoresis\textsuperscript{(123).} The stability of these complexes was examined by ligand exchange kinetics with EDTA\textsubscript{4} and their comparative inertness confirmed their potential use for hepatobiliary studies\textsuperscript{(124).} Subsequently many different derivatives of IDA\textsubscript{H}, were synthesised and at least four of these are now marketed commercially for hepatobiliary imaging, each one having slightly different biological clearance. For example, in the presence of high bilirubin levels, the diisopropylphenylcarbamoylmethyliminodiacetic acid (PIPIDA\textsubscript{H},) is preferred\textsuperscript{(125).} At normal bilirubin levels diethylacetanilideiminodiacetic acid (EHIDA\textsubscript{H},) is preferred due to its low renal excretion and rapid clearance from the blood by the hepatocytes\textsuperscript{(126).} HPLC analysis of the EHIDA\textsubscript{H}, radiopharmaceutical
subsequently revealed that at least two components are present in solution. Fortunately it has been shown that, at isotonic pH, they interconvert to a single species. In plasma this is rapid so a single radiochemical species is effectively injected(64,127).

An electrolytic study on the HIDAH₂ derivatives of ⁹⁵Tc produced a more complicated picture with multiple species being formed, the relative concentrations of which vary with reaction conditions(128). The same study revealed an equally complicated situation with DIPA'H₂ with a variety of technetium(III) and technetium(IV) species being formed. Despite the uncertainties over actual speciation, IDAH₂ derivatives play a large part in a modern radiopharmacy and research into further derivatives continues(129).

The chemistry associated with the dithiadicarboxylate ligands and discussed in this thesis has some similarities to the aminocarboxylates except that the former are not stable enough for full characterisation.

1.3.3. Technetium(V) chemistry and radiopharmaceuticals

1.3.3.1. Oxo and dioxo containing complexes

Reviews and papers have dealt with this area more comprehensively than this introduction could allow. The chemistry of the technetium(V) oxidation state is dominated by the formation of a TcO²⁺ core or its allied cores OTcO⁺ and OTcL⁻ where L may be Cl⁻, OH⁻ or MeOH. Table 1.8. lists some of the characterised compounds which contain the TcO²⁺ core. As nitrogen is a neutral donor, it appears that the OTcO⁺ core is necessary to diminish the overall charge, whereas the TcO²⁺ requires formally charged ligands such as Cl⁻.
An interesting comparison is made if the following three cyano-tecnietium complexes are considered, all are tecnietium(V) (Figure 1.12.).

The ligand trans to the oxo ligand appears to be flexible. Comparing 1. and 3., this is a function of solvent and demonstrates the route by which the OTcO⁻ forms. The intermediate prior to 1. has an aquo ligand in the position trans to the oxo. Because of the strong trans influence shown by the TcO group the O-H bond is weakened sufficiently to cause deprotonation and formation of the OTcO⁺. In the case of 3., this was made using methanol solvent and the trans influence is insufficient to cause demethylation. Complex 2. presents the intermediate position, being stable only in high cyanide concentration and being hydrolysed to the complex 1. in dilute aqueous solution. The presence of a 6th coordinated ligand depends therefore not only on the ability of the axial planar ligands to stabilise(reduce) the charge of the technetium core but also their ability to diffuse the trans influence of the oxo group. It is thought that even 'pentacoordinated' complexes in coordinating solvents such as water or alcohols, a solvent molecule weakly bound in the 6th position. The cyano complexes also demonstrate the additional principle that if the ligand is present in high concentration then it is possible to have coordination of that ligand in the position trans to the oxo. Because of the trans labilising effect then ligand exchange may readily occur.

\[ \text{TcOCl(NH}_2\text{CH}_2\text{CH}_3\text{S)}_2 + \text{NCS}^- \rightarrow \text{TcO(NCS)}(\text{NH}_2\text{CH}_2\text{CH}_3\text{S})_2 \]

The TcO⁺⁺ complexes have \( C_4v \) symmetry in square pyramidal complexes whilst the TcO⁺⁺ core, in octahedral geometry has a \( D_{4h} \) symmetry and as
Figure 1.12.: Technetium(V) cyano containing complexes; the trans effect of the oxo core
technetium (V) $d^8$ are spin paired. Figure 1.13 is a representation of the orbitals in both $C_4v$ and $D_{4h}$ point groups and it can be seen that two electrons occupy the non bonding $d_{xy}$ orbital and this configuration confers a degree of inertness to these complexes. The strength of the oxo core arises from the $\pi$ overlap between the O p orbitals and the $\pi^*_{\sigma_2\pi_2\pi_2}$ orbitals.

<table>
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<tr>
<th>Complex</th>
<th>Tc-OA</th>
<th>Tc=OA</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>$\text{Tc} (\text{dmg})_2 (\text{SnCl}_3)\text{OH}$</td>
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<td></td>
<td>130</td>
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<tr>
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<td>132</td>
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<td>$\text{TcO}(\text{HBPz}_3)\text{Cl}_2$</td>
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<td>27</td>
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<tr>
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<td></td>
<td>133</td>
</tr>
<tr>
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<td></td>
<td>134</td>
</tr>
<tr>
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<td>135</td>
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<tr>
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<td></td>
<td>136</td>
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<tr>
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<td></td>
<td>89/70</td>
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<tr>
<td>${\text{TcO}_2(\text{en})_2}^+$</td>
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<td>26</td>
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<td>$(\text{TcO}_2\text{cyclam})^+$</td>
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<tr>
<td>${\text{Tc}(\text{OH})\text{MDP}}^-$</td>
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<td>${\text{TcO}(\text{H}_2\text{O})(\text{C}_1\text{H}_2\text{N}_2\text{O}_2)}^+$</td>
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<td>1.648</td>
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<tr>
<td>${\text{TcOCl}(\text{salen})}$</td>
<td>1.626</td>
<td></td>
<td>139</td>
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Table 1.8.

SELECTED EXAMPLES OF COMPLEXES CONTAINING
TECHNETIUM-OXYGEN BONDS
Figure 1.13: M.O.'s in C4v and D2h point groups; the symmetry of technetium(V)
<table>
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<th>$o^*$</th>
<th>$a_1g$</th>
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<td>$o^*$</td>
<td>$b_1g$</td>
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<td>$xz$</td>
<td>$n^*$</td>
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<tr>
<td>$b_2$</td>
<td>$xy$</td>
<td>$n_b$</td>
<td>$b_2g$</td>
</tr>
</tbody>
</table>

$^1A_1 \text{ C}_{4v}$  \hspace{2cm} $D_{4h} \  ^1A_{1g}$
One of the advantages of single crystal structures is that it gives an opportunity to compare bond lengths between the same atoms in different structures. Table 1.8 compares the length of the Tc-O bonds in dioxo, oxo and Tc-O-R complexes. As expected the Tc=O bond is stronger in complexes where there is no trans ligand and weakest when the ligand is another oxo due to sharing of orbitals.

The marriage of technetium chemistry to radiopharmaceutical chemistry is probably best documented for the technetium(V) oxidation state. There are many examples of radiopharmaceuticals both marketed and under research that are technetium(V)oxo species, where the carrier added chemistry is directly related to the no carrier added reaction. Some of the most important are described in detail below.

1.3.3.2. Technetium complexes of propyleneamineoxime and other potential brain imaging agents

In 1984, Troutner reported a neutral lipophilic complex of $[^{99m}\text{Tc}]$technetium with 3,3' (1,3 propanediylidimino)bis(3 methyl 2 butanone) dioxime(140) from the reaction of $[^{99m}\text{Tc}]$TcO$_4$- with a reducing agent or by ligand exchange from $[^{99m}\text{Tc}]$Tccitrate(141).

The complex, formed in almost quantitative yields appears on HPLC to be a single component, stable over 24 hours at pH 6-8 and extracted into CHCl$_3$ with 97% efficiency. In vivo this was found to traverse the blood-brain barrier(142). This complex passed out of the brain rapidly and so extensive derivitisation studies were carried out to produce a complex with a suitable residence time for SPECT investigations. Over 100 derivatives were prepared but the one finally chosen was that shown
below, commonly known as hexamethylpropyleneaminoxime (HmPnAO) (143,144,145). The ligand has optically active centres and may be separated into the d,l and meso forms. The d,l HmPnAO ligand is far superior to the meso form for rCBF imaging. The complex with the latter gives only 1% cerebral uptake while the former has 2% uptake. The technetium complex has been identified by X-ray crystallography as the technetium(V) oxo compound (Figure 1.14) (143,146).

Additionally, pK_a values for some PnAO derivatives have been assigned through a novel method using HPLC techniques (147). This is relevant if pH shift mechanisms are operating in these complexes but may be useful in future studies (148).

One problem with the d,l HmPnAO containing formulation is that the neutral TcO⁸ species converts in vitro over a relatively short time to a more hydrophilic species which does not accumulate in cerebral tissue. The exact nature of this complex is apparently still unknown (149,150). Some of the HPLC information is discussed in chapter 7 of this thesis.

Although the reagent was designed as a brain imaging agent it is also excellent for labelling white blood cells. The current reagent of choice for this is [111In]In(Oxine)_3 or the tropolone analogue and a technetium containing reagent could be very useful clinically (151,152).

Following initial studies by Davison, Jones and co-workers with the bisamidebisthiol complexes of technetium (153), technetium(V) oxo complexes, a number of amino analogues have been prepared and investigated in vivo (154,155,156,157). The neutral compound, which has been fully characterised, is known to cross the blood brain barrier (158). These are commonly known as the BAT type ligands but extending to the
triaminedithiol ligands yielded disappointing results and the BAT moiety seems important (159). Replacing the $\text{Tc}=\text{O}$ by $\text{Tc}=\text{N}$ affects the biodistribution only slightly (160). Derivatisation of one of the amine groups by a piperidinylethyl group when complexed with a technetium(V) oxo core shows considerable promise over the original BAT complex (155).

1.3.3.3. Potential replacements for $^{123}$I Iodohippuran

The technetium complex currently used to assess renal function is the $^{99m}\text{Tc}$Tc-DTPA formulation, although this is inferior to $^{123}$I Iodohippuran (161). The higher extraction rate of the latter, being secreted through the renal tubular cells as well as glomerular filtration, means that there is a need for a new reagent containing technetium to measure full ERPF. The first serious candidate for this was a diamidodithiol or DADS containing complex, similar in structure to the BAT complexes above. Preliminary data showed more rapid clearance than the DTPAH$_5$ reagent above (162). A second generation reagent has been developed with the synthesis of the so-called Carboxy-DADS ($^{99m}\text{Tc}$technetiumoxo($\text{N},\text{N'}$ bis(mercaptoacetyl) 2,3 dianaminopropanoate) formulation which has improved extraction efficiency, although the formulation requires separation of two epimers, only one of which shows the desired biological activity. The complex has been fully characterised (163,164).

Simultaneously, a triamidothiol ligand has been shown to react with $\text{TcO}_4^-$ under reducing conditions to also give a technetium(V) oxo compound, isostructural with the rhenium analogue (165).

Mercaptoacetylglucylglycylglycine (MAG$_3$) and several MAG$_2$ amine
derivatives give complexes which have high renal specificity and extraction rates superior to $[^{125}\text{I}]$iodochippuran in several animal species (166,167). The $\text{MAG}_9$ complex is currently the subject of a product licence application in the U.K.

These are the major areas of interest and topical development in technetium(V) oxo chemistry. There are other smaller projects involving technetium(V) radiopharmaceuticals. One such is the technetium complex with tetraphenylporphyrindisulphonate used in the detection of deep seated infections and inflammation (168). Although the synthesis of the carrier added species is not possible by the same route the porphyrinatotechnetium(V)oxoacetates can be prepared by an alternative route. Biological studies to compare the behaviour of the carrier and no carrier added preparations are presently underway (169).

Recently, the oxo core has been replaced by a nitrido and sulphido (170) opening whole areas of technetium chemistry to study. In the first reported instance of the $\text{Tc}=\text{N}$ core, diethyldithiocarbamate and $[^{99}\text{Tc}]\text{TcO}_4^-$ were reacted together in the presence of hydrazine hydrate and the resulting yellow crystals characterised as $\text{TcN(dtc)}_2$ (171). The source of the nitrido is believed to be deprotonation of the hydrazine, as previously observed in rhenium chemistry (172). Following this the same authors have prepared a whole range of species including $\text{TcCl}_4^-$, $\text{TcBr}_4^-$, $[\text{technetium(VI)}]_2$ (173), $\text{TcN(KCS)}_2(\text{CH}_3\text{CN})(\text{PPh}_3)_2.\text{CH}_3\text{CN}$ (174) and $\text{TcNCl}_2(\text{PPh}_3)_2[\text{technetium(V)}]$ (175) and found the biological distribution of some $[^{99}\text{Tc}]\text{Tc}$nitrido radiopharmaceuticals to be significantly changed (176). In particular the $[^{99}\text{Tc}]\text{Tc}$HEDP formulation has low bone uptake whilst the normal radiopharmaceutical is used for bone imaging.
Using a modification of their procedure, technetium nitrido complexes for a series of triaminodithiol ligands, which were known to form technetium oxo complexes, were compared in vivo with the oxo analogue (160). Only a small variation on biodistribution behaviour was observed for these brain perfusion agents. The Tc-nitrido core has also been reported with a number of ligands containing sulphur donors of the general formula (TcNL₂)²⁻ and the biological distribution of the neutral complexes shows interesting brain uptake (177). Additionally, a nitrido complex of tropolone is being investigated as a blood cell labelling agent (178). However, hydrazine is toxic and it is difficult to perceive of a freeze-dried kit. Overall the nitrido complexes seem to be more substitution labile than the oxo analogues but the biological distribution of both sets is quite similar (179).

As previously noted the immense quantity of publications related to technetium(V) chemistry cannot be fully reviewed and several areas have not been discussed; technetium(V) Schiff base complexes (180,181,182,183), technetium(V) cyanide complexes (28,184), technetium(V) dithiolates and diolates (44,135,185,186,187), technetium(V) complexes with pyridine and other amines (188,189,190) and the technetium(V) halides (191,192,193).

1.3.4. Technetium(I) chemistry
1.3.4.1. Isonitrile containing complexes

Isonitrile containing complexes, low oxidation state cations, were synthesised for their potential use as myocardial imaging agents. These complexes have now formed the basis of at least three doctoral dissertations (194,195,196).
The reaction between tertiarybutylisonitrile, sodium pertechnetate and sodium dithionite yields the hexakis('butylisonitrile)technetium(I) cation\[\text{TcC'BuNC)}^+\] (Figure 1.15.) (38). The reaction at the no carrier added concentration, is quantitative, reproducible and radiopharmaceutically pure. HPLC studies of the formulation indicated a single component (197), and this has been comprehensively investigated by many nuclear medicine departments and cardiologists. In recent years there have been many clinical papers on the efficacy of these complexes for myocardial studies (198,199,200). The reaction at the carrier added level is facile and essentially quantitative. The complexes, \([^{99m}\text{Tc}][\text{Tc(RNC)}_3]^+\) are white crystalline materials, soluble in polar organic solvents and may be prepared using a variety of reducing agents or by ligand exchange from the hexakisthioureatechnetium(III) cation (38).

The methoxyisobutylisonitrile is the current ligand which is to be employed in the commercial radiopharmaceutical.

The lipophilic nature of the \([^{99m}\text{Tc}][\text{Tc(RNC)}_3]^+\) complexes makes them potentially useful as white blood cell labelling agents. Several derivatives have been tested including 'butylisonitrile (201), 'butylisonitrile (202) and cyclohexylisonitrile. The latter is purported to be the most promising so far tried (201).

Crystal structures for hexakis('butylisonitrile)technetium(I) cation and the rhenium(I) analogue have been solved and have $D_3$ symmetry (203).

Oxidation of the technetium(I)alkylisonitrile compounds by cyclic voltammetry in acetonitrile show a reversible one electron oxidation from technetium(I) to technetium(II) to give \([\text{Tc(RNC)}_3]^2+\) (204). This complex is not particularly stable but may be prepared chemically by reaction of
Figure 1.14.: The complex $^{99}\text{Tc}[(\text{OH})\text{PnAO}]^{+}$

Figure 1.15.: The complex $^{99}\text{Tc}[(\text{BuNC})_{6}]^{+}$
34

[Tc(RNC)$_n$]$_n^+$ with nitric acid. Secondary products from the reaction are
[TcNO(RNC)$_n$]$_n^+$ and TcNOBr$_2$(RNC)$_n$. The reaction with halogens gives
[Tc(RNC)$_n$Cl]$_n^+$.

1.3.4.2. Nitrosyl containing complexes

The nitrosyl ligand, as NO$^+$, is a good π acid ligand and as such
stabilises technetium in low oxidation states. Principally these have
been technetium(II) complexes such as (TcNOCl$_4$L)$^-$ where L=MeOH, Cl$^-$. These complexes may be made from the reaction of TcO$_2$, NO and HCl (205) or more simply from the reduction of TcOCl$_4^-$ or TcCl$_6^{2-}$ by hydroxylamine (206). Ligand replacement by acetylacetone gives
[TcNOCl$_3$(acac)]$^-$ (207), while reaction with NH$_3$ gives the technetium(I) species (TcNO(NH$_3$)$_4$(H$_2$O))$^{2+}$ (208). Other ligand replacement reactions
with π acid ligands such as isonitriles and phosphines gives the technetium(I) species (TcNOCl$_4$L)$^+$ (where L=monodentate ligand) (209), while NCS$^-$ gives the expected technetium(II) complex, [TcNO(NCS)$_n$]$^{2-}$ (210).

1.4. Conclusions

It is clear from the above survey of the literature that the area of
technetium chemistry associated with the development of new
radiopharmaceuticals is a particularly active one and one which is changing rapidly as new complexes are prepared and more information becomes available. It has been said that compared to organic chemists the inorganic chemist is still back in the 1930's because of the greater complexity and range of the ligands and metals employed. Technetium
chemistry is still the least developed even in inorganic chemistry and
tied in with the added problem that we are dealing with what is
essentially a whole new area of xenobiochemistry all data amassed in this
area is of interest.

In the remainder of this thesis are described a set of related
projects concerned with principally searching for novel
radiopharmaceuticals. The biological data was amassed at different times
and places during the project and for both practical and ethical reasons
it was not possible to carry out exactly the same protocol in all cases.
Comparisons of the data from various experiments may still, however, be
compared under certain constraints, which will be noted where
appropriate.
CHAPTER TWO
EXPERIMENTAL

2.1. Experimental methods for the no carrier added $^{99m}$Tc reactions.

2.1.1. Complex analysis by paper chromatography.

Separation of the complex mixture into complexed technetium, unreduced pertechnetate and hydrolysed colloidal TcO$_2$ was achieved by standard paper chromatographic separation. The support medium employed was generally Whatmann 3MM although Gelman ITLC-SG and Whatmann no.1 were both used on occasion. A chromatogram strip, 95x25mm, was marked 15mm from the bottom as the application point and again 10mm from the top as the solvent front. The distance moved by the solvent was therefore 70mm in all cases. Using "Seprachrom" chromatography tanks with mobile phases of saline (0.9%) and butan-2-one, the complexes were analysed, in triplicate, by the following procedure.

A spot of the solution was applied to the paper via a syringe fitted with a 25G needle. This was allowed to air dry and then placed in the tank with the solvent level below the applied spot. The solvent developed to the marked solvent front and the paper was removed, dried and scanned on a Panax tlc scanner. The resulting trace was then marked at the application point and solvent front and the Rf value of the complex was calculated. The paper strips were then cut into 19x5mm portions and counted, along with an empty tube for background subtraction, on a Philips automatic gamma counter PW4800 or manually using a single crystal well detector attached to a
Nuclear Enterprises SR5 ratemeter. The data was processed on a Commodore PET microcomputer using a chromatogram plotting programme. From this, % complexation was calculated. Pertechnetate has Rf values of 0.9 in butan-2-one and 0.75 in 0.9% saline, TcO₄⁻ has Rf values of 0.00 in both butan-2-one and saline whereas the complexes of technetium studied have varying solubilities in the two solvents. Where an additional solvent system was employed, for example 90% acetone or 40% methanol, pertechnetate was run as a standard.

2.1.2. Electrophoresis

Whatmann no.1 chromatography paper was used as support medium and the experiments carried out in a Shandon-Southern 602 tank connected to a Volkam 400/100 power pack. The buffers used were generally acetate/acetic acid for pH values 4 to 5, monohydrogen phosphate/dihydrogen phosphate for pH values 6 to 8 and carbonate/bicarbonate for pH values 9 or above. The relative proportions of each component were found in the GEIGY book of scientific tables (211). The electrophoresis strips were soaked in the buffer for an hour before use, patted dry with paper towel and the reaction solution applied, as before, to a premarked spot. The strips were placed in position, pertechnetate was always applied to one strip to act as a standard and the power switched on. Generally, the system was run at 10-11 V/cm. for two hours. At the end of this time the strips were removed, dried and scanned as before. Finally, they were cut into 10mm portions and counted on the gamma counter. Movement, relative to pertechnetate, was calculated and some information on the complex charge obtained.
2.1.3. Octanol/saline partition coefficients.

0.1ml of the complex was diluted to 5ml with saline and added to 5ml of octanol. The two phases are immiscible with the octanol forming a layer above the saline. This was gently agitated for ten minutes and then centrifuged to separate the two layers completely. The contact time of 10 minutes was found to be sufficient for equilibrium to be attained. The phases were separated into different tubes and centrifuged again to remove as much trace contamination as possible. Finally, aliquots were taken using an automatic Finn pipette and counted. This was performed in triplicate and the average value is quoted.

2.1.4. Protein binding experiments.

Two methods were employed. The complex was incubated, typically for half an hour with serum albumin. If available, fresh human albumin was used. In the first method of measurement, 20% trichloroacetic acid was added to the complex-albumin mixture (1:1 ratio) and the resulting precipitated protein was centrifuged for five minutes at 1000rpm. The supernatant was decanted and the protein washed with 2x1ml trichloroacetic acid. The washings were added to the supernatant and both protein and liquid samples counted. The % bound was calculated simply as the ratio of activity associated with the denatured protein to that in the aqueous phase. The second method of measurement relied on electrophoretic separation of protein-bound species from non protein-bound species. Following the method for electrophoresis outlined above in 2.1.2, two strips were run, usually in pH7 buffer, for about one hour. One strip was then stained using
Ponceau s (Sigma Chemicals) protein staining solution. The electrophoresis strip was immersed in a working solution of the stain for about ten minutes, rinsed with 5% acetic acid and finally washed with water. This removes the stain from the paper except where it has been taken up by proteins. The protein is therefore indicated by a stained pink spot on the paper. The second strip is scanned, cut and counted as before. Finally the two strips are considered together. The activity on the stained paper will frequently be washed off during the staining process; this is particularly true for very water soluble species. Therefore by comparing the two strips it is possible to determine the % activity associated with the protein stain.

2.1.5. HPLC conditions

Three different columns were used to collect the HPLC data, a Hamilton PRP-DVB (5µm mesh), a Spherisorb ODS reverse phase column and a Partisil SAX anion exchange column. The data for the dithiadicarboxylic acid containing complexes was acquired on a system of a Magnus "Minor" pump, connected via a Rheodyne 7010 injection valve fitted with a 20µl loop. Radiochemical detection was done with a NaI(Tl) well crystal connected to a Nuclear Enterprises SR-5 ratemeter and a Spectra-Physics model 4270 integrator. An alternative system, also used for the carboxylate complexes and for the S₄N₂-18 macrocyclic complexes, employed an Altex 300 pump connected via a Rheodyne valve to a Tennelec power supply, Ekco gamma counter and Tekmann chart recorder. HPLC of [⁵²⁷Tc]TcHMPnAO, described in chapter seven, was done on the Altex system using a Radiomatics Beta detector,
generously loaned by Radiomatics for the duration of the project. The dead volumes of the columns were calculated using tritiated water, which was injected onto the column and the eluted product collected in 0.2ml aliquots into scintillation vials. 5ml of a suitable scintillant, Unisolve E, was added and the vials counted on an LKB Rackbeta using the preset programme for $^3$H. Regular cleaning of the columns was done by eluting, overnight, with a dilute solution of hydrogen peroxide. This oxidised any technetium compound adhering to the column to pertechnetate and consequently was washed off the column. Equilibration periods of at least two hours were allowed when changing buffers or after the cleaning. The integrity of the anion exchange column was checked at regular intervals using pertechnetate as a standard. A guard column was generally used with the Partisil SAX column, packed 'in house' with the same material, at regular intervals when the back pressure on the pump built up.

2.1.6. Biodistribution studies.

Studies performed at Amersham International plc were carried out using male wistar rats, in duplicate. The rat was anaesthetised with urethane (ip administration of a 15% w/v aqueous solution) and injected with 20MBq in a volume of 0.1ml into the tail vein. They were studied on a Baird gamma camera for two hours with scintiphotographs taken at seven time intervals; 0, 10, 20, 30, 40, 60 and 120 minutes. At the end of the two hours, the animals were sacrificed, dissected and the major organs including injection site, were assayed in a large volume, twin crystal automatic gamma counter. In one instance, when the biological distribution on the $^{28}$Na-18
macrocycle was under investigation, a two minute dissection was
carried out in accordance with Amersham International protocol for
potential brain/heart agents.

Studies performed at Chelsea college on mice were carried out in
triplicate with sacrifice at 5, 15, 30, 60, 120 and 240 minutes. The
mice were anaesthetised with ether, weighed and injected with a
weighed (approximately 0.1ml) dose into the tail vein. The mouse was
placed in a cage lined with filter paper to collect any urine and the
syringe was reweighed. The exact weight of injected complex was
calculated. At the appropriate time, the animals were sacrificed by
cervical dislocation and dissected. Organs were quickly removed and
rinsed in isotonic saline, blotted dry and assayed by counting on a
Vilj automatic gamma counter. The heart was incised and washed free
from blood before counting, an aliquot of blood was also taken for
assay. The organs were weighed into tared counting tubes except for
the gallbladder and urine which were allocated weights of 0.01g and
1.0g respectively. All the urine passed or collected in the bladder
was blotted onto the filter paper and counted with the samples.

Standards were prepared from the original reaction solution according
to the following:

1ml (weight x) of preparation diluted to 10ml. (strong standard
S.S)

1ml (weight y) of S.S. diluted to 10 ml. (weak standard W.S.)

1ml (weight z) of W.S. diluted to 10 ml. (very weak standard
V.W.S.)

Dilution factors could then be calculated:
F(S.S.) = 10/x
F(W.S.) = 10/y \times F(S.S.)
F(V.W.S.) = 10/z \times F(S.S.)

One ml. aliquots were counted for each standard, both before and after each set of organs. The counts obtained for the standards and organs were processed using a BASIC programme written for this procedure. All counts are corrected for background and the standard counts are analysed by weighted linear regression techniques to obtain the best estimate of the slope of the counts/concentration curve. This slope was taken as the activity (cps) per gram of the injected material and therefore the actual cps injected can be computed. The activity remaining at the site of injection was subtracted from the activity administered and therefore a true representation of % dose per organ could be calculated. At the end of a biodistribution study, frequently eight hours in length, the stability in vitro of the injected material was checked by paper chromatography.

Studies performed on male adult rats at Chelsea college were done on a Nuclear Enterprises 8900 Scinticamera fitted with a low energy parallel hole collimator, type 8922. The animals were anaesthetised with ether and approximately 20MBq in a volume of 0.1ml injected into the tail vein. Scintiphotographs were acquired at various time intervals between 0 and 120 minutes. At the end of the study the animals were sacrificed but not dissected.
2.2. Synthesis and analysis of the no carrier added
\[^{99m}Tc\] complexes

2.2.1. Preparation of the reducing agents.
2.2.1.1. Tin(II) tartrate (Sigma chemicals) was prepared as a 10mM solution in tridistilled water at pH<1. This solution remained chemically active for two to three days if stored at 4°C, without any noticeable effect on the complex formation.

2.2.1.2. Tin(0) foil (BDH chemicals) was cut into 5mm discs using a paper punch and scratched with a scalpel to remove the surface oxide coating. This was etched in conc.HCl for one to two minutes until the surface appeared dulled then sequentially washed in absolute ethanol, 50% ethanol and distilled water. The discs were patted dry in paper towel and sealed under N\(_2\) in a 10ml reaction vial. Prepared in this fashion the tin remained chemically active for several days.

2.2.1.3. Sodium dithionite (Fluka chemicals) was prepared as a 46mM solution in 40mM sodium hydroxide at a pH>13. Degassed solvents were used and the solution was kept in an inert atmosphere, however, the reducing agent was always freshly prepared for immediate use as it was unstable in solution to oxidation/hydrolysis. In all cases, fresh reducing agent was made for the biodistribution studies.

2.2.2. Preparation of the complexes.
2.2.2.1. A 50mM ligand solution of the dithiadicarboxylic acid was prepared in tridistilled water. The pH of the ligand solution was typically about 5. One ml of this solution was added to a 10ml reaction vial with 1ml of generator eluant and 0.1ml of the tin(II) tartrate solution. When necessary, the pH was raised with 0.1molar
NaOH or lowered with 0.1molar HCl before the addition of the reductant. This reaction proceeded at room temperature.

2.2.2.2. A 50mM ligand solution of the S₄N₄-18 macrocycle was prepared in 1:1 ethanol:water. The method was similar to that above except that heating was required to ensure the reaction went to completion. A pressure cooker (Prestige) was used and the reaction vial heated for thirty minutes under pressure. This also had the desired effect of keeping the ligand in solution which, upon cooling, precipitated out, hence it was possible to remove most of the uncomplexed ligand before injection.

2.2.2.3. A 50mM solution of tiron (Sigma chemicals or Fisons) was prepared in tridistilled water at an ambient pH of 3.7. One ml of this solution and one ml of generator eluant were added to a vial containing activated tin foil discs (usually three were used) and the reaction was allowed to proceed at room temperature. The pH was raised to 7 by addition of 0.1molar NaOH to the ligand solution.

2.2.2.4. A 1% (w/v) solution of 'butylisonitrile (Fluka chemicals) was prepared in ethanol. 0.1ml of this solution was added to 0.4ml ethanol, 1.5ml water, 0.7ml generator eluate and 0.5ml sodium dithionite solution. This reaction mixture was heated for fifteen minutes in a water bath and cooled before assaying. The formulation is that developed by Loughborough University and Leicester Royal Infirmary for use in clinical studies (198).

Ligand and complex pH measurements were measured on a Pye Unicam PW 9409 pH meter fitted with a micro electrode. The electrode was calibrated at regular intervals using standard buffer solutions at
pH's 4.0, 7.4 and 9.0. Whatmann pH paper, narrow range, was only used as a guide.

Percentage complexation and stability were checked by paper chromatography at time intervals of 15, 30, 45, 60, 120, 180, 240 minutes and 24 hours. The results were analysed as described in 2.1.1. The effect of pH on complex formation was studied in a similar manner at pH's typically varying from 3 to 9, hence the optimum labelling conditions for each ligand were ascertained.

2.3. Ligand synthesis.

2.3.1. The dithiadicarboxylic acids.

These ligands were synthesised according to two literature preparations. The reaction of the parent dithiol with chloroacetic acid (212), or the reaction of a mercaptoalkyl acid with a dibromalkane (213).

2.3.1.1. Synthesis of 3,6 dithia 1,8 octandioic acid.

5ml. (0.069 moles) of mercaptoacetic acid (Aldrich chemicals) was added to 6.8g. (0.0357 moles) 1,2 dibromoethane in 300ml. ethanol. The mixture was stirred on a magnetic stirring plate and 0.2 moles KOH (12g./30ml.) was added dropwise. The solution was allowed to clear after each addition then the temperature raised to 40°C and maintained for three hours. The ethanol was removed on a rotary evaporator, a white precipitate was left which was acidified to pH 4 with concentrated HCl, washed with ether and filtered. The compound was recrystallised twice from hot water.

Lit. mpt.109°C, observed mpt. 108-109°C.
expected | found
---|---
C 34.3 | 34.44
H 4.8 | 4.9
N 0.00 | 0.01

2.3.1.2. Synthesis of 4,7 dithia 1,10 decanodioic acid

5ml. (0.057 moles) of 3 mercaptopropionic acid (Aldrich chemicals) was added to 5.45g. (0.0286 moles) 1,2 dibromocane in 300ml. ethanol. A similar method to that above was followed, heating for four hours at 40-50°C. The resultant white precipitate was recrystallised twice from hot water. Lit. mpt. 158°C, observed mpt. 158-159°C.

expected | found
---|---
C 40.3 | 40.29
H 5.88 | 6.05
N 0.00 | 0.07

2.3.1.3. Synthesis of 3,8 dithia 1,10 decanodioic acid

13.25g. (0.144 moles) of mercaptoacetic acid (Aldrich chemicals) were added to 8.68ml. (0.074 moles) of 1,4 dibromobutane (Fisons S.L.R.) in 700ml. deoxygenated ethanol. 0.2 moles KOH (12g/30ml) were added and the mixture refluxed gently for four hours. The ethanol was removed on a rotary evaporator and the remaining aqueous solution was acidified to pH 2. Recrystallisation from hot water gave a pink lustred white compound. Observed mpt. 107-108°C

expected (L.4H₂O) | found
---|---
C 30.96 | 31.75
H 5.8 | 4.82
N 0.00 | 0.01
S 20.6 | 21.62
2.3.1.4. Synthesis of 4,8 dithia 1,11 undecandioic acid

12.18g. (0.115 moles) of 3 mercaptopropionic acid (Aldrich chemicals) was added to 11.6g. (0.057 moles) of 1,3 dibromopropane (Fisons S.L.R.) and refluxed as above for three hours.

Recrystallisation gave a pink tinged powder. Observed mpt. 82-84°C expected (L.H₂O)
found
C 40.0 40.53
H 5.9 6.10
N 0.00 0.08
S 23.7 19.94

* The microanalyst reported the complex lost weight on the balance during the sulphur analysis, which he considered to be loss of H₂S.

2.3.1.5. Synthesis of 3,6 dithia 1,8 octandioic acid by the second method.

2ml. (2.38x10⁻² moles) 1,2 ethanedithiol was added to 250ml. of 50% ethanol in a round bottomed flask. Dropwise addition of 5.3g KOH (0.0952 moles), followed by 4.498g chloroacetic acid (4.76x10⁻² moles) resulted in a temporary formation of a precipitate which redissolved with stirring. The reaction was refluxed gently for three hours and the ethanol removed on a rotary evaporator. 50ml.ice/water was added and the solution acidified to pH2 with conc HCl. The precipitated compound was recrystallised from water.

The ligand syntheses outlined above were repeated several times over the course of this study and with the exception of 2.3.1.4. the yields were typically 65-75% based on the thiol. The yields obtained for 2.3.1.4. were never more than 30-40%. Infrared spectra were recorded for all these ligands but only the v(C=O) stretch at 1680-1720cm⁻¹ is
unequivocally assigned. The ν(C=S-C) stretch is not readily identified and its absorption may occur between 720 and 660 cm⁻¹.

2.3.2. The S₄N₈-18 macrocycles.

2.3.2.1. Synthesis of 4,7,13,16 tetrathia 1,10 diazacyclooctadecane.

The high dilution technique of Black and McClean (214) was employed in this preparation.

1,2 Ethanedithiol (Aldrich chemicals) as the disodium salt (0.06 moles) was added to 0.06 moles of 1,5 dibromo 3 azapentane (Aldrich chemicals) in a five litre round bottomed flask. Three litres of ethanol, distilled and stored over molecular sieves, were added and the mixture was refluxed for 48 hours. The volume was reduced on a rotary evaporator to approximately 500ml. The desired product was extracted with benzene (400ml.), washed with dilute NaOH, dried over Na₂SO₄ and concentrated to less than 50ml. The white precipitate was washed with diethyl ether to remove oily impurities and the compound recrystallised from ethanol to give fine white shiny needles. Lit. mpt. 124-125°C. Observed mpt. 124-125°C. Yield 4-10%, lit. yield. 4.6%.

No molecular ion was seen in the mass spectrum (cf. (215) 12% M, 326), with many fragmentation patterns apparent due to the large number of cleavage positions available on the ring. The proton nmr spectrum shows a singlet at δ=8.04ppm. (NH) and a multiplet at δ=7.1-7.3ppm. (CH₅), (cf. (214))]. The ¹³C spectrum shows a finally split doublet at δ=34ppm. (relative to TMS), a singlet at δ=46.8ppm. and a triplet at δ=75.4ppm.
2.3.2.2. A sulphonamide derivative of 4,7,13,16 tetrathia 1,10
diazacyclooctadecane.

165ml. of chlorosulphonic acid (Fisons A.R.) were freshly distilled
into a 3-neck, round bottom flask and stood in crushed ice. An
overhead mechanical stirrer was fitted and 67.5g. acetanilide (Fisons
S.L.R.) was slowly added over a period of an hour, during which time
the temperature of the reaction was maintained at 12°C, a reflux
condenser and drying tube were fitted and the solution was heated at
60°C for two hours, then left at room temperature overnight. Excess
chlorosulphonic acid was removed by decomposition with an ice/water
mixture and the desired product was separated by filtration, washed
with water and dried over P₂O₅(216). The yield was high (over 50g.)
but not recorded. Only 5g. were recrystallised for further use.
Recrystallisation of this para acetaminobenzenesulphonyl chloride(I)
was carried out in hot, dried benzene. 0.2g. (0.00062 moles) of the
macrocycle and 0.143g. (0.00062 moles) I were dissolved in 30ml.
dichloromethane with 0.12g. (0.0012 moles) N(Eth). The mixture was
left at room temperature, stirring for four days, after which TLC on
alumina plates showed no free I. The precipitate was filtered and
extracted on a soxhlet with ethanol for two days. The white, pink
lustred, flocculant compound was dried over silica.
A mpt. was not obtained since the complex did not melt below 220°C.

<table>
<thead>
<tr>
<th></th>
<th>expected</th>
<th>found</th>
</tr>
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<tbody>
<tr>
<td>C</td>
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<td>44.24</td>
</tr>
<tr>
<td>N</td>
<td>8.0</td>
<td>7.9</td>
</tr>
<tr>
<td>N</td>
<td>8.6</td>
<td>8.1</td>
</tr>
<tr>
<td>S</td>
<td>39.3</td>
<td>38.8</td>
</tr>
</tbody>
</table>
expected | found
---|---
C 44.1 | 44.24
H 8.0 | 7.9
N 8.6 | 8.1
S 39.3 | 38.8

No molecular ion was observed on the mass spectrum and again the fragmentation pattern was complicated.

2.3.2.3. A long chain fatty acid derivative of 4,7,13,16 tetrathia 1,10 diazacyclooctadecane.

The acid chloride of methyl hydrogen sebacate (I) (Aldrich chemicals) was prepared using thionyl chloride. 2g. (0.0093 moles) of (I) were refluxed in dichloromethane with a threefold excess of thionyl chloride for two hours then left at room temperature, in the dark, overnight. The solvent was removed by distillation and excess thionyl chloride removed by incubation in a boiling water bath (Bpt. SO_2Cl 79°C). A viscous yellow liquid remained. To verify the formation of the acid chloride (II), a derivative with aniline was prepared. Equimolar quantities of II and aniline reacted immediately to give a white precipitate in a strongly exothermic reaction. The precipitate was washed with water to remove any aniline hydrochloride and ether to remove excess aniline.

expected | found
---|---
C 70.8 | 70.22
H 8.8 | 8.97
N 4.6 | 4.95
O 15.8 | 15.86
II was used without further purification. 0.023 moles of II were reacted with 0.023 moles of macrocycle in dichloromethane. The reaction was instantaneous to form a milky slurry. After two hours of stirring and cooling in ice/water, the solvent was removed, leaving a waxy solid. This was washed with diethylether and methanol and dried over P₂O₅.

<table>
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<tr>
<td>H</td>
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<td>8.34</td>
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<tr>
<td>N</td>
<td>3.90</td>
<td>3.99</td>
</tr>
<tr>
<td>S</td>
<td>17.7</td>
<td>14.83</td>
</tr>
</tbody>
</table>

2.4. **Synthesis of the carrier added [⁹⁹Tc] complexes.**

2.4.1. Synthesis of [⁹⁹Tc],[Tc(SC(NH₂)₂)₅]Cl₂ (109).

25 ml. ethanol, 4 ml. conc. HCl and 0.88 g. (11.6x10⁻² moles) thiourea(tu) were mixed in a 50 ml. round bottomed flask, until the thiourea had dissolved. 2.5 ml. of an aqueous solution of NH₄[⁹⁹Tc]TcO₄ (2.02x10⁻⁴ moles) were added and the solution stirred until a red-orange precipitate was collected. This was washed with chilled ethanol and dried in vacuo over silica. The material was used without further purification.

2.4.2. Synthesis of [⁹⁹Tc]TcOCl₄⁻ (191).

10 ml. of the aqueous solution of NH₄[⁹⁹Tc]TcO₄ were placed in a round bottomed flask in an ice/water bath. 2.0 ml. 6M HCl were added and the mixture stirred at 0°C until the solution had turned a deep brown/yellow. Upon addition of excess aqueous NBu₄Cl, a green
precipitate was formed that was collected, washed with 6M HCl, ethanol and ether. The compound was recrystallised from CH₂Cl₂/hexane to give a final yellow/green complex that was used without further purification.

2.4.3. Synthesis of [²⁹⁰Tc]TcCl₅²⁻ (217).

5ml. of the aqueous solution of NH₄[TcCl₅] (4.04x10⁻⁴ moles) were placed in a round bottomed flask and boiled with 12M HCl until the solution turned bright yellow. 2ml. of a saturated KCl solution was added and the solution concentrated under vacuum, bright yellow crystals were precipitated and recrystallised from conc.HCl.

2.4.4. Synthesis of [²⁹⁰Tc]TcCl₅(PPh₃)₅ (83).

To 50mL of ethanol in a round bottomed flask were added 2ml.conc.HCl, 10ml.of the aqueous solution of NH₄[TcCl₅] (8.08x10⁻⁴ moles) and 1g PPh₃ (3.8x10⁻⁵ moles). The solution was stirred until a green precipitate was formed. This was collected, washed with ethanol and recrystallised from CHCl₃.

2.5. Synthesis of some copper complexes

The copper complexes of the dithiadicarboxylic acids were all prepared in a similar manner. 1g (5.5x10⁻⁵ moles) copper(II) acetate was dissolved in 10mL water. A twofold excess of an aqueous ligand solution was added, dropwise, with stirring. The reaction was instantaneous, giving an immediate precipitate that varied in colour from milky green to deep blue. The precipitates were collected, washed with water, ethanol and ether but not recrystallised as they
appeared to be insoluble in all common solvents. Analytical data is
given in chapter four.

An attempt was made to synthesise the copper complex of $\text{S}_6\text{N}_2\text{O}_6\text{-18}$. An ethanolic solution of the macrocycle was added to copper(II) acetate and refluxed gently. The solution turned pale green but did not precipitate any compound. When finally the solvent was removed, an oil was left that was not further purified.

2.6. Additional equipment and chemicals

Other chemicals were purchased as follows:
3,5 dithiao 1,7 heptadionic acid, Lancaster synthesis
4,6 dithiao 1,9 nonadionic acid, Lancaster synthesis.
HMPnAO, a gift from Amersham International plc.

All other reagents, unless stated, were of standard laboratory grade. Solvents were dried by standard methods and stored over molecular sieves or sodium wire. Where necessary in the organic syntheses, the oxygen free nitrogen (OFN) was also dried.

Melting points were measured using a Gallenkamp melting pt. apparatus and are reported uncorrected.

Infrared spectra were run on a Perkin-Elmer 257 spectrophotometer, calibrated with a polystyrene standard. The ligands were analysed as a nujol mull in NaCl discs, whilst the copper compounds were analysed as KBr discs.

NMR spectra were recorded on two different instruments. A Varian T-60 was used for proton nmr with TMS as standard and the carbon-13 spectrum of $\text{S}_6\text{N}_2\text{O}_6\text{-18}$ was recorded using a Bruker WD-80
Mass spectral data was collected using a Kratos MS80/DS-55 data system.

Ultraviolet spectra were recorded on a double beam Cecil CE5095 spectrophotometer, using 1cm² silica cells.

Magnetic moments of the copper complexes were calculated using standard techniques on a Gouy balance.

Microanalyses were provided by the University of Nottingham or the University of Manchester.
SYNTHETIC PATHWAYS

\[ \text{HS-}(\text{CH}_2)_n\text{-SH} + 2\text{Cl-CH}_2\text{-CO}_2\text{H} \rightarrow \text{HO}_2\text{C-CH}_2\text{-S-(CH}_2)_n\text{-S-CH}_2\text{-CO}_2\text{H} \]

\[ \text{HS-}(\text{CH}_2)_n\text{-CO}_2\text{H} + \text{Br-}(\text{CH}_2)_n\text{-Br} \rightarrow \text{HO}_2\text{C-}(\text{CH}_2)_n\text{-S-(CH}_2)_n\text{-S-(CH}_2)_n\text{-CO}_2\text{H} \]

\[ \text{Na}^+\text{-S-}(\text{CH}_2)_2\text{-S-Na}^+ + \text{Br-}(\text{CH}_2)_2\text{-NH-(CH}_2)_2\text{-Br} \rightarrow \]

\[ \text{C}_6\text{H}_5\text{-NHCOCH}_3 + \text{HOSO}_2\text{Cl} \rightarrow \text{C}_6\text{H}_6\text{-NHCOCH}_3 \]

\[ \text{MeO}_2\text{C-(CH}_2)_6\text{-CO}_2\text{H} + \text{SOCl}_2 \rightarrow \text{MeO}_2\text{C-(CH}_2)_6\text{-COCl} \]

\[ \text{MeO}_2\text{C-(CH}_2)_6\text{-COCl} + \text{H}_2\text{N-CH}_2\text{-NH}_3 \rightarrow \text{C}_6\text{H}_5\text{-CH}_2\text{-NHCO-(CH}_2)_6\text{-CO}_2\text{Me} \]
CHAPTER THREE

TECHNETIUM COMPLEXES OF TIRON

3.1. Introduction

Tiron, 1,2 dihydroxy 3,5 disulphonatobenzene(Figure 3.1.) is a derivative of catechol(1,2 dihydroxybenzene). The catecholate group has biological importance dopamine and adrenaline being examples of amino substitution although in this study the 1,2 dihydroxybenzene moiety is acting as a ligating group and thus the technetium complex would not be expected to mimic any binding to the specific receptors for such as the above. The sulphonato groups enhance both ligand and complex solubility in aqueous media and thus facilitate the formation of metal complexes in the latter medium. Tiron reacts readily with a host of first row transition elements(Z=21~30)(218) and has been used as a colorimetric test for iron. In the case of iron the nature of the complex formed is pH dependent. At pH1-3, a blue green complex is obtained while at pH3-5 a violet/blue solution is observed and above pH7 the reaction colour is deep red. These different colours are attributed to three different complexes, a 1:1 complex which is blue green, a 1:2(Fe:tiron) species which is violet/blue and a 1:3(Fe:tiron) deep red compound, although there must be some doubt about these rather simplistic assumptions(219). The pKₐ values for the ligand(220) given in Figure 3.1. indicate that at pH>0 the ligand exists as a dianion while at alkaline pH there is a competing irreversible autooxidation occurring, similar to that observed for the
Figure 3.1.: The ligand tiron
\[
\begin{align*}
\log K_1 &= 12.7 \\
\log K_2 &= 7.7 \\
\log K_3 &< 0 \\
\log K_4 &< 0
\end{align*}
\]
catecholamines, and so the 1:3 complex seems unlikely with the red complex being some product containing the ligand in an oxidised form.

### 3.2. Technetium catechol complexes

A suggestion that technetium and catechol might react together was made by Benson following early work on the tin(II) reduction of \[^{99m}Tc\text{ClTcO}_4\text{^-}\] in the presence of pyrogallol (1,2,3 trihydroxybenzene) and catechol (221). A subsequent work describes the formation of a \[^{99m}Tc\text{technetium(V) catecholate complex, from the reaction with TcOCl}_4\text{^-}\] in methanol. Their results appear to confirm the reaction stoichiometry as

\[
\text{TcOCl}_4\text{^-} + 2\text{LH}_2 \rightarrow \text{TcOL}_2\text{^-} + 4\text{HCl}
\]

The complex was isolated as golden crystals as the \(\text{Bu}_4\text{N}^+\) salt, which are soluble in methanol and acetone. The X-ray single crystal structure analysis confirms the square pyramidal symmetry with the benzene rings bent away from the oxo group. The \(\text{Tc}=\text{O}\) bond length, measured as 1.648\(\text{Å}\), is within the expected range (1.610-1.79\(\text{Å}\)) (186).

Other diolate complexes were synthesised in the same study, especially the ethyleneglycolate analogue. Interestingly, the reaction of the latter with \(\text{TcO}_4\text{^-}\) and sodium dithionite, does not yield a stable product, although a transient purple colour, the technetium(V) complex is purple, was observed. The reaction with catechol is not reported but it is reasonable to suppose that the reaction product with tiron is of the same \(\text{TcOL}_2\text{^-}\) structure.
3.3. Reaction of $^{99m}$TcTcO$_4^-$ with tiron.

3.3.1. Chemical studies.

Tiron was purchased from Fisons (A.R. grade) and used without further purification. A 50mM ligand solution in water was used at all times. The pH of this solution was 3.7. The percentage complexation, or labelling efficiency, was observed by standard chromatography methods, described elsewhere, over a pH range of 3.5-12 and is shown in Figure 3.2.

Tin foil was chosen as the reductant since it is a) a mild reducing agent very suitable for those ligands that will stabilise technetium(V) complexes and b) has the advantage over tin(II) reductants that the concentration of tin(II) and tin(IV) in solution is very low. Since tiron is a good complexing ligand the tin concentration should be kept as low as possible to prevent the formation of tin-tiron complexes. The precise mode of action of the tin foil as a reductant is unknown. However, its use has been patented (222) and it was incorporated in a freeze-dried radiopharmaceutical kit for the replacement of iodohippuran (223). It is likely that the etching process, described in chapter 2, removes the inert oxide coating and either the reduction is a surface one via a tin(0)/tin(II) couple or slow dissolution of tin(II) ions occurs into the solution and the reduction is then a tin(II)/tin(IV) one. In either case the presence of excess tin ions is minimised.

The effect of pH on complex formation indicated the unsuitability of this reduction at below pH6.5 and above 10.5 since there was evidence of TcO$_6^-$ and TcO$_4^-$ at these latter two pH values. However, between pH6.5 and 10.5 the reaction is essentially quantitative to form a $^{99m}$TcTciron complex. pH7 was thus chosen for the working solution since this would
Figure 3.2.: % Complexation of $^{99m}$TcIotiron as a function of pH(1)

Figure 3.3.: Complex stability as a function of time(2)
paper chromatography, Whatmann 3MM
MEK and 0.9% saline mobile phases
1. Complex $TcO_2$ to $TcO_4^-$

2. pH/0.5

% of each complex

Time/15 minutes
cause minimum antagonism in vivo. The pH of the ligand was therefore raised, with 0.1M NaOH, immediately prior to reaction.

The complex formed remained stable for up to 2h., with less than 5% disproportionation in this time, while after 24h. a chromatogram indicated >90% of the complex remained (Figure 3.3.). Stability in vitro was thus acceptable for animal studies. However, when electrophoresis was employed with this compound over a pH range it became apparent that the formulation does not give a single species at all pH's. Clearly the sulphonato groups of the ligand which will be uncoordinated may be protonated or deprotonated and due to the changes in ligand structure caused by coordination will have lower pKₐ's than the free ligand. However, since the pKₐ of the sulphonates is less than 1, in any technetium complex it may be assumed that they are fully deprotonated in the range of pH studied.

The pH4 formulation shows the presence of two complexes while that of the pH7 one shows merely a single species (Figure 3.4.). Thus the in vivo studies were carried out using only a single species and this is shown by the simplicity of the clearance and excretion curves. Nevertheless, at pH4 another anionic species is formed which has less mobility than the major complex and thus is probably of lower charge. It is a reasonable assumption that the major complex is similar in structure to that prepared by Davison and co-workers i.e. TcO₃⁻. Assuming deprotonation of the sulphonato groups and a technetium(V) oxidation state then the overall charge would be 5⁻. Of course in the generator eluant there is considerable Cl⁻ concentration, not present in the carrier added preparation. Loose coordination trans to the oxo ligand is well
Figure 3.4: Electrophoresis of the no carrier added \( ^{99m} \text{Tc} \) complex prepared at two pH values
1. prepared at pH4
2. prepared at pH7
3. TcO\(_4^-
\)
Tris buffer pH7, Whatmann no.1 paper
10V/cm. for 60 minutes
A=application point, R=cps
known and could possibly happen in this case to give a $\text{TcO}_\text{L}_\text{Cl}_6^-$ species. At the lower pH the catecholate groups of the free ligand will be essentially protonated. However, the chloride concentration will remain unchanged. Thus the competition which will exist between chloride ions and the deprotonated ligand on reduction of the $\text{TcO}_4^-$ may be disturbed such that at pH 4 not only is the 1:2 metal:ligand complex formed but also a 1:1 metal:ligand complex with the remainder of the coordination sites being occupied by chlorides. This complex, $\text{TcO}_\text{L}_\text{Cl}_4^-$, is of lower charge and thus will have the electrophoretic profile seen in Figure 3.4.

The competition of chloride ions in radiopharmaceutical formulations with ionisable ligands is usually neglected but the chloride ion is a good ligand in its own right and the use of isotonic saline in generator eluant means that the overall chloride concentration relative to other possible ligands may be high at low pH.

The octanol/saline partition coefficients confirmed that the $[^{99m}\text{Tc}]\text{Tc}^\text{tiron}$ complex is hydrophilic in nature (Table 3.1.).

<table>
<thead>
<tr>
<th>pH</th>
<th>Octanol/Saline (n=3)</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.036</td>
<td>-1.44</td>
</tr>
<tr>
<td>5.0</td>
<td>0.020</td>
<td>-1.69</td>
</tr>
<tr>
<td>7.0</td>
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<td>-1.63</td>
</tr>
<tr>
<td>9.0</td>
<td>0.010</td>
<td>-1.99</td>
</tr>
</tbody>
</table>

Table 3.1.

OCTANOL/SALINE PARTITION COEFFICIENTS
3.3.2. Animal biodistribution studies

The results above indicated that, at pH7, a single complex was formed in the formulation and buffering by the blood would maintain this complex. The degree of protein binding was measured by incubating the formulation with fresh human serum albumin for 30 minutes. The HSA-[\textsuperscript{\textsuperscript{99m}}Tc]Tc-tiron mixture was analysed by electrophoresis as described in section 2.1.4. Surprisingly for a hydrophilic species, there was 50% protein binding. However, the [\textsuperscript{\textsuperscript{99m}}Tc]Tc-DMSA complex is also a highly charged, hydrophilic and highly protein bound complex\textsuperscript{(224)}.

The biological distribution of the complex was examined using two species of rodent; mice and rats. An extensive human study involving a normal female volunteer was also carried out and will be discussed below.

The rat was studied, in duplicate, on a Baird \textsuperscript{\gamma} camera for two hours, with subsequent sacrifice as detailed in section 2.1.6. Mice were sacrificed, in triplicate, at timed intervals of 5, 15, 30, 60, 120 and 240 minutes to obtain dynamic clearance data from the blood, kidneys and other organs.

The clearance of this complex through a rat(Figure 3.5.) show rapid kidney uptake and retention with 14% of the injected dose present in the kidneys at sacrifice and 80% of the dose in the kidneys, bladder and urine(Figure 3.6.). There was virtually no other significant organ uptake although one of the rats showed slightly higher body background due probably to a slightly faulty injection. As such, this compound thus merits further investigation as a replacement for [\textsuperscript{\textsuperscript{99m}}Tc]Tc-DMSA used currently in nuclear medicine for static renal studies. However, if the mouse biodistribution data is considered, Figure 3.7., the complex passes
Figure 3.5.: Scintiphotos showing the biodistribution of the no carrier added $[^{99m}Tc]$ complex in rats

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes

Times are post injection
Figure 3.6.: Blood and renal clearance curves of the no carrier added $^{99m}$Tc complex in rats

1. blood clearance
2. kidney uptake
3. bladder accumulation
Figure 3.7: Biodistribution data of the no carrier added \( ^{99m} \text{Tc} \) complex in mice (n=3)

1. dissection data over 240 minutes
2. renal clearance
3. hepatobiliary clearance
4. blood clearance
through the kidneys rapidly into the bladder and as such could be useful for dynamic renal studies. In the mouse there is also some evidence of hepatobiliary clearance. This is another example of the common problem of inter-species variation of animal models, the most well established being that of \([^{99m}\text{Tc}][\text{TcCl}_3(\text{dmpe})_2]^+\) which gives excellent myocardial images in all animals except humans.

However, since this complex may have been of some use in nuclear medicine a human study was carried out which is described below.

3.3.3. Human studies

An original study was carried out at Northwick Park Hospital on a normal male volunteer. The \([^{99m}\text{Tc}]\text{TcTiron}\) complex was prepared under aseptic conditions and terminally sterilised by 0.22\(\mu\)m filtration. A renogram study, following the protocol for \([^{99m}\text{Tc}]\text{TcDTPA}\), was performed over a period of fifteen minutes with a urine sample collected at thirty minutes and blood samples taken at time intervals over four hours. The renal uptake was incomplete over this fifteen minute period and this complex obviously does not possess the properties of a renal function agent (Figure 3.5.). The plasma clearance curve showed considerable protein bound activity over the time of study which can be resolved into a triple exponential by plotting the semilogarithmic values (Figure 3.9.). The third exponential is poorly defined however due to a lack of data points.

It seemed appropriate to repeat this study, as the full potential of the complex had probably not been realised in fifteen minutes.
Figure 3.8.: Renal uptake in human volunteer
15 minute dynamic phase (NPH) (1)
A. right kidney
B. left kidney

Figure 3.9.: Plasma clearance curve (log/linear)
of human volunteer (NPH) (2)
A thirty one year old female volunteered for the study in which the renography was repeated at the Royal Liverpool Hospital. A dynamic study was carried out over thirty minutes followed by static imaging at 30 minute intervals. Blood samples were taken up to six hours post injection. Full details are reported below.

The complex was prepared under fully aseptic conditions with terminal sterilisation by 0.22um filtration. A 5ml dose(approximately 100MBq) was weighed and injected through an intravenous cannula into the right median cubital vein. The cannula was flushed with saline and retained for counting, the injection syringe was reweighed. Another cannula was inserted into the left median cubital vein and secured in place. With the aid of a three way valve, blood samples were taken through this cannula at 10, 20, 30, 40, 60, 90, 120, 180, 240 and 345 minutes post injection. The first two ml. withdrawn were discarded and the subsequent five ml. were placed into heparinised tubes for blood clearance studies and GFR calculations. At forty minutes post injection, the volunteer was asked to empty her bladder and all the urine was collected.

Immediately after injection of the complex, a dynamic study of 120 frames, in the posterior view, was undertaken to observe the kidney uptake and clearance behaviour over this initial period. The dynamic phase lasted 30 minutes (120x15second frames), at the end of which the first of the static pictures was acquired, for a time of 179 seconds. The length of study for a dynamic renogram (I23[Iodoschippuran] at the Royal Liverpool Hospital is generally sixteen minutes, sometimes extended to thirty minutes, with blood samples taken at twenty and forty minutes and urine collected after forty minutes. The study was carried out in
accord with this protocol. Further static pictures were acquired at 60, 
90, 120, 150, 180, 210, 240 and 345 minutes post injection. At 60 
minutes, views were taken of the head, chest and abdomen to observe the 
whole body biological distribution of the complex.

At the end of the study, the remaining uninjected complex was 
chromatographed, the octanol/saline partition coefficient calculated and 
the degree of protein binding assessed by paper electrophoresis and 
denatured protein precipitation. All showed the complex to be stable in 
vitro. Finally the urine sample was subjected to paper chromatography.

The absence of thyroid and stomach uptake in the volunteer 
confirmed the initial lack of TcO₄⁻ in the injected sample and that the 
complex did not hydrolyse in vivo. Analysis of the urine sample revealed 
that the complex is excreted, apparently unchanged, with no evidence of 
TcO₄⁻. There does appear to be another species, insoluble in saline 
which may be a function of the delay between collecting the urine and 
examining the specimen two hours later or it may be some protein bound 
species. Nevertheless, greater than 70% of the activity in the urine was 
due to the unchanged sample.

The plasma clearance curve and semilog clearance curve show a 
triphasic, probably three compartment system, with an initial period of 
rapid clearance (0-25 minutes), followed by a slower phase (30-180 minutes) 
and finally, a slow, almost indiscernible leaching of the complex from the 
blood (180-345 minutes) (Figure 3.10). Biological half-lives of the three 
phases in the plasma clearance curve were calculated by manually 
stripping the curves and calculating the individual gradients M₁, M₂ and
Figure 3.10.: Plasma clearance curve
(linear and ln/linear) of human volunteer (RLH)
1. linear clearance curve
2. ln/linear clearance curve
% per litre blood

Time/5 minutes

Ln(% per litre blood)

Time/5 minutes
The $t_{1/2}$ values were calculated from $0.693/X$, giving half lives of 9.9 minutes (20%), 77.1 minutes (20%) and 23.1 hours (60%).

The long biological half-life of the third phase effectively represents no excretion, as the radioactive half-life is a factor of three shorter and therefore the loss of tracer from the blood is indistinguishable between the two half-lives.

$^{99m}$TcTcDMSA, which is currently the radiopharmaceutical of choice for kidney morphological imaging, is also cleared from the blood in a triphasic mode, as determined by semi-logarithmic resolution into three exponential components (225). Biological half-lives of these three phases are quoted as 18 hours (12%), 50 minutes (44%) and 20 minutes (44%), with a total body retention of 72 hours for 80% of the dose. At six hours post injection, 75% of the plasma-associated activity is protein bound, rising to 90% after twenty-four hours. There is no diffusion into red cells, an observation also made for the $^{99m}$TcTciron complex. Biochemical assays performed on the $^{99m}$TcTcDMSA compound shows it penetrates the kidney cell and is localised in cytoplasmic protein and the mitochondria (226).

The initial clearance from the plasma is partly due to extraction through the kidneys, whether by glomerular filtration or tubular secretion. However, this does not wholly account for the drop in activity from the blood as only 6% of the injected dose was found in the urine after 40 minutes. The dynamic pictures (Figure 3.11.) and curves (Figure 3.12) indicate that this complex has low extraction efficiency and is therefore unsuitable for dynamic renal studies. This confirms the observations of Northwick Park Hospital. Figure 3.13. shows a normal $^{123}$Iodochippuran renogram where the peak renal uptake
Figure 3.16.: Regions of interest (R.O.I.) selected for human study (RLH) (1)

Figure 3.11.: Renal uptake in human volunteer
30 minute dynamic phase scintiphotographs (RLH)
2. 15X2 minute frames
Figure 3.12.: Renal uptake in human volunteer

30 minute dynamic phase linear curves (RLH)

1. left kidney

2. right kidney
Figure 3.13.: A normal renogram [123I]iodohippuran
(reproduced from reference 229)
Relative function

<table>
<thead>
<tr>
<th></th>
<th>2 min uptake</th>
<th>3 min uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney</td>
<td>43%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Right kidney</td>
<td>57%</td>
<td>10.3%</td>
</tr>
</tbody>
</table>

Percentage of dose vs. Time (min)
occurs between 3-5 minutes and rapid clearance over thirty minutes giving a classic renogram curve. The calculated GFR of 32ml./minute (normal range for women is 109±13ml./minute [227]) and the percentage dose in the urine after forty minutes (6.63%) also confirm that $^{99m}$TcTcTiron is unrepresentative of excretory renal function.

The final phase is probably due to the protein bound complex clearing slowly through the kidneys. Both protein binding assays indicated 48-53% protein binding, the higher value being that obtained by trichloroacetic acid precipitation. The precise value has considerable uncertainty due to the inherent inaccuracies in many of the standard methods of measurement [228]. The more rapid methods of ultrafiltration, trichloroacetic acid precipitation and paper or cellulose acetate electrophoresis are not as accurate as gel column chromatography and it is preferable to compare at least two methods than to rely on merely one. It is also important to point out that all the various methods may give different results for the same sample because they cause some disruption of the equilibrium.

The second phase in the plasma clearance curve is more difficult to interpret. Tests were performed, retrospectively, to examine the behaviour of the $^{99m}$TcTcTiron complex with whole blood. Standard procedures for red and white cell labelling were followed. Less than 2% of the added $^{99m}$Tc activity was associated with either cell type, indicating no significant cell labelling. The rationale for this second phase can be better understood if considered by the compartmental model of renal function [229]. If we consider a three compartment system, illustrated in Figure 3.14., compartment one represents the intravascular
Figure 3.14.: A three compartmental model of renal excretion. (reproduced from reference 229)
system where the tracer is exchangeable with the tubular cells of the kidney. Compartment two represents the urine, both in the kidney and bladder, and the clearance rate from compartments one to two is that which is to be calculated. This clearance is unidirectional (Fl→2). The third compartment represents the extravascular system and here diffusion of the tracer may occur in both directions (Fl→3; F3→1). The extravascular system includes such factors as red cell diffusion and extracellular fluid exchange. Therefore, when a radiopharmaceutical is injected into the blood stream, the value associated with compartment one (represented by blood plasma counts) begins to fall for two reasons. Firstly, uptake by the kidneys, represented by Fl→2, accompanied by diffusion into the extravascular system F1→3. The equilibrium attained by F1→3 and F3→1 is the rate determining step by which the injected radioisotope is cleared through the renal system into the urine.

A two component system, incorporating only the intravascular compartment and the urine would be represented by a single exponential rate of decrease.

When the semilogarithmic curves for both human and mouse studies are compared there is a significant difference between them (Figure 3.15). The plasma clearance in mice can be resolved into two exponential curves with an initial rapid loss of complex from the blood [$t_{1/2}=10.6$ minutes (60%)]. The second component has a half life of 287 minutes (40%). Both human plasma clearances show the same pattern of behaviour with poor extraction from the blood. The implication is that there is an additional component in human plasma that is more strongly binding to $[^{99m}Tc]Tctiron$ than was experienced in the animal studies.
Figure 3.15: Plasma clearance curves (log/linear) of mice and men

1. mouse
2. male human (NPH)
3. female human (RLH)
Quantification of the static renal pictures was necessary to determine the % uptake of the injected complex into the kidneys. A camera standard was prepared by taking 10% of the injected dose in a 100ml bottle of water and placing this in front of the camera inside a kidney phantom. A static image for 15 seconds was acquired and the sensitivity of the camera was computed for $^{99m}$Tc as 82 counts per second per MBq (cps/MBq). The data were obtained by drawing regions of interest (R.O.I.) around both kidneys and also selecting two background areas close to the organs (Figure 3.16.). In particular, this is important for the right kidney, where liver overlap often increases the counts in the right kidney R.O.I.. The area selected for background is normalised to the area of the kidney under consideration and the counts adjusted accordingly. The background count is then subtracted from the kidney count and it is this value that is plotted (Figure 3.17.). The graphs of counts in the kidney versus time represent accumulated counts over the 179 seconds that the frames were acquired for. Therefore, division by 179 gives cps and this then gives a renal uptake of one MBq approximately, or 1% of the injected dose. Such a low uptake of the complex is surprising. The original idea that a small, highly charged, hydrophilic complex would be cleared in some way through the kidneys is too simple an assumption. The lack of clearance from the blood is indicated in some degree by the one hour views of head, chest and abdomen (Figure 3.18.). The high counts observed in the heart and also the liver are both in agreement with this. The image of the head confirms the hydrophilic characteristics of the complex in vivo as there is no significant brain uptake. Also highly significant of the vascular
Figure 3.17.: Renal uptake in human volunteer over six hours post injection (RLH)

1. kidney accumulation
2. bladder accumulation
Figure 3.18.: Static images taken at 60 minutes p.i. of head, chest and abdomen (RLH)
ANTERIOR HEAD

ANTERIOR CHEST

ANTERIOR ABDOMEN

STATIC IMAGES
AT 60 MINUTES
activity is the body outline, clearly seen on all the static images up to and including the 345 minute study.

This must therefore imply that the renal image seen on all the static pictures is due mainly to the vascularity of the organ with very little renal cortex uptake (Figure 3.19).

The results in the human study were unexpected. The animal data, although showing species variation between rats and mice, did not suggest this tight protein binding that was experienced with the human plasma, nor did it imply such a low uptake into the renal cortex. It is assumed therefore that human plasma contains proteins that bind this highly charged \([^{99}\text{Tc}]\text{Tc-tiron}\) complex more strongly than the protein in mouse and rat plasma.

3.4. The carrier-added \([^{99}\text{Tc}]\text{Tc}\) reactions with tiron.

The reaction of \([\text{Bu}_4\text{N}]\text{TcOCl}_3\) and tiron in methanol under an anaerobic atmosphere gave a red solution with \(\lambda_{\text{max}}=310,435\ \text{nm}\). The catecholate analogue prepared by Davison (186) is also red and it may be assumed the complex is the 2:1 ligand:metal complex. After addition of excess \(\text{Bu}_4\text{NBr}\) no precipitation occurred and thus the solution was loaded onto an anionic (OH\(^-\)) exchange column and eluted with water. A deep red band was held on the column while a minor product an orange species eluted with water. The yield of this complex was too low for further study. The red band was impossible to remove from the column presumably due to the extremely high charge. Only 6M \(\text{NaCl}\), normally only used as a cleaning solvent, eventually leached off the remaining technetium from the
Figure 3.19: Static images of the kidneys taken at intervals (as marked) over the six hour study (RLH)
column. However, the leaching process appeared to decompose the complex and further identification was not possible.
CHAPTER FOUR
TECHNETIUM COMPLEXES OF DITHIADICARBOXYLIC ACIDS

4.1. The Ligands

4.1.1. Introduction

The dithiadicarboxylic acids, of general formula

\[ \text{HO}_2\text{C(CH}_2)_n\text{S(CH}_2)_m\text{S(CH}_2)_n\text{CO}_2\text{H} \]

were synthesised by either literature preparations or purchased as denoted in table 4.1.

<table>
<thead>
<tr>
<th>Ligand Name</th>
<th>Structure</th>
<th>Chelate Rings</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5 dithia 1,7 hepta dioic acid</td>
<td>HO_2CCH_2SCH_2SCH_2CO_2H</td>
<td>545</td>
<td>Aldrich</td>
</tr>
<tr>
<td>3,6 dithia 1,8 octan dioic acid</td>
<td>HO_2CCH_2S(CH_2)_2SCH_2CO_2H</td>
<td>555</td>
<td>Ref. 212, 213</td>
</tr>
<tr>
<td>4,6 dithia 1,9 nona dioic acid</td>
<td>HO_2C(CH_2)_3SCH_2S(CH_2)_3CO_2H</td>
<td>646</td>
<td>Aldrich</td>
</tr>
<tr>
<td>4,7 dithia 1,10 decan dioic acid</td>
<td>HO_2C(CH_2)_4S(CH_2)_4S(CH_2)_4CO_2H</td>
<td>656</td>
<td>Ref. 213</td>
</tr>
<tr>
<td>4,8 dithia 1,11 undec andioic acid</td>
<td>HO_2C(CH_2)_4S(CH_2)_4S(CH_2)_4CO_2H</td>
<td>666</td>
<td>Ref. 213</td>
</tr>
<tr>
<td>3,8 dithia 1,10 decan dioic acid</td>
<td>HO_2CCH_2S(CH_2)_4SCH_2CO_2H</td>
<td>575</td>
<td>Ref. 213</td>
</tr>
</tbody>
</table>

Table 4.1.

LIGAND STRUCTURES AND ABBREVIATIONS

These ligands are potentially quadridentate forming a 1:1 complex with a metal atom. The structure of the complex thus comprises three "n"

membered rings. Alternatively the ligands may act in a bi or monodentate
fashion. The likelihood of which type of complexation can be qualitatively discussed with reference to the value of "n". Traditionally, a five membered ring is sterically the most stable with minimum angular strain imposed on the bonds. Using a ball and stick model, and estimated bond lengths it may be shown that the most favoured technetium complex (i.e. least strained) would be formed with 3,6 dithia 1,8 octandioic acid, giving three 5 membered rings(230). Similarly, a 545 configuration as postulated for 4,6 dithia 1,9 nonadioic acid would be unlikely due to the close proximity of the two sulphur atoms. Thus a 2:1 ligand:technetium complex would be expected. The ligand 4,8 dithia 1,11 undecandioic acid, if reacting in 1:1 stoichiometry would give rise to a 666 configuration which is generally considered to be of good stability. Surprisingly, this was found not to be the case.

Similarly a technetium complex containing N,N' propylenebis(benzoyl 3 mercaptopropionamide) could not be isolated from the reaction of pertechnetate in the presence of the ligand, the product being TcO$_2$.xH$_2$O(231). In the same study, quantitative yields of a complex using the dimercaptodiamide, N,N' ethylenebis(2 mercaptoacetamide) which has a 555 configuration were obtained.

4.1.2. The coordination chemistry of the dithiadicarboxylic acids

Bellaart and Verbeek(232) synthesised the copper(I) and silver(I) complexes of 4,7 dithia 1,10 decan dioic acid (called in their paper 3,6 dithiooctane 1,8 dioic acid) which they assigned through elemental analysis and spectral data to a 2:1 complex with coordination through the sulphur atoms(Figure 4.1.). The reaction of copper(I) oxide with the
Figure 4.1.: 2:1 Copper:ligand complex with 4,7 dithia 1,10 decandioic acid (656)
ligand gave two complexes, one colourless and identified as \([\text{Cu}(\text{LH})_2]^-\) and an unidentified turquoise species, probably the copper(II) complex.

Van der Meer confirmed the structure of the copper(I) species with sulphur bonding and a tetrahedral arrangement around the central atom\(^{(233)}\). The \(-\text{SCH}_2\text{COH}\) residues of the ligands are orientated according to hydrogen bonding.

Further studies on the coordination of 3,6 dithia 1,8 octandioic acid with a number of different first row transition metals have been carried out\(^{(212,234)}\). Ford, Pettit and Sherrington concluded that the S atoms bonded to a \(-\text{CH}_2\text{COH}\) group are better ligating moieties due to the electron donating properties of the carboxyl moiety and that, with respect to copper(II), nickel(II), cadmium(II) and zinc(II), coordination through at least one sulphur atom noticeably contributes to the overall complex stability. Podlaha and Podlahova mixed metal sulphates with the sodium salt of 3,6 dithia 1,8 octandioic acid and isolated the barely soluble complexes. They concluded that \(\text{Ni}^{2+}\) and \(\text{Cu}^{2+}\) have an unusually elevated affinity for sulphur donors and that a complex of stoichiometry 1:1 was formed. A preliminary single crystal X-ray determination on the \(\text{NiL}_2\text{H}_2\text{O}\) complex confirms the correct assignment of their other physical data\(^{(234)}\). Both the above thus illustrate the ability of some of these ligands to coordinate in a quadridentate fashion the determining factor being the affinity of the thioether sulphur for the metal centre.
4.1.3. Technetium complexes containing thioether or carboxylate ligands.

The chemistry of thiolato complexes is well established, in particular, \([\text{TcO}(S\text{CH}_2\text{CH}_2\text{S})_2]^-\), \([\text{TcO}(\text{ema})]^-\) and derivatives thereof \(109,135,153,185\). However, the affinity of the thioether moiety for technetium in any oxidation state is only poorly documented. Jurisson reported that the reaction between \(\text{TcCl}_6^{2-}\) and 2,5 dithiahexane yielded, after precipitation with \(\text{NaBPh}_4\), a product which was tentatively characterised as \(\left[^{99}\text{Tc}\left(\text{Tc}(\text{CH}_2\text{S}\text{CH}_2\text{S})_2\text{Cl}\right)\right]^{2-}\). In the same work, it was suggested that the reaction between 1,5,8,12 tetrathiacyclodecane and \(\text{Bu}_4\text{NTcOCl}_4\) did not give a discrete complex\(235\). Also, as described in chapter five, the reaction has been reported of a \(\text{S}_8\text{N}_2\text{O}_{14}\) macrocycle with \(\left[^{99}\text{Tc}\text{OBr}_2\right]^{-}\) giving a 1:1 complex of technetium(V) with a dioxo core\(236\).

By contrast, the carboxylates have been reported to react readily with various high oxidation state technetium precursors. The aminocarboxylates, particularly DTPAH\(_5\), EDTAH\(_4\) and IDAH\(_2\) are described in section 1.3.2.3. In all these cases, there is coordination through the nitrogen atom as well as through the carboxylate groups, whilst those ligands that do not have other donors, such as acetate and formate, do not form readily identifiable technetium oxo species\(237\). The hydroxycarboxylates, such as gluconate and glucoheptanoate were used in early radiopharmaceuticals and do form discrete complexes in quantitative yield but only in the presence of a large excess of ligand\(238\).

Although there has been much conjecture on the structure of these complexes formed by the simple reduction of \(\text{TcO}_4^-\) in solutions containing
these ligands, no single crystal X-ray structure of such a complex has yet been published. These results indicate that the complete identification of technetium complexes with the dithiadicarboxylates investigated here would be unlikely, although such complexes may be stable at the no carrier added [$^{99m}$Tc] concentration with very high ligand:metal ratios. The results reported below confirm this theory.

4.2. No carrier added [$^{99m}$Tc] reactions of the dithiadicarboxylic acids

4.2.1. The reactions of 3,6 dithia 1,8 octandioic acid(555) with technetium.

The most commonly used reductant in radiopharmaceuticals is the tin(II) ion and this simple method of reducing the TcO$_4^-$ was employed in this work. The details of the formulation procedure are given elsewhere. The $K_a$ values for this ligand are 4.15X10$^{-4}$ and 4.45X10$^{-5}$ (239) and thus at the pH of isotonic saline both carboxylates are deprotonated. The formulation was carried out over the range of pH 3 to pH 10 and the effect on the labelling efficiency monitored. The lower limit of pH was chosen as the minimum for intravenous injection. At this pH the carboxylates will be completely protonated. Above this pH the carboxylates will be deprotonated. The effect of pH on complex formation (or labelling efficiency in the current terminology of radiopharmacy) is shown in Figure 4.2. In the range pH 4 to pH 9, there is no noticeable deterioration in the labelling efficiency with only significant TcO$_2^-$ and residual TcO$_4^-$ at the extreme pH's. The stability of this formulation in the intermediate pH range was studied over a 24h period and negligible hydrolysis and disproportionation was observed for
Figure 4.2.: % Complexation as a function of pH of the no carrier added $^{99m}$Tc reaction with 3,6 dithia 1,8 octandioic acid. Paper chromatography, Whatmann 3MM paper MEK and 0.9% saline mobile phases. Subsequent paper chromatography was carried out under the same conditions.
of each complex

% of each complex

TrO2
TrO4–
Complex

pH/0.5
at least 8h post preparation for the pH5-7 formulations. However some degradation of the formulation occurred after 4h for the reaction carried out at pH9. This probably reflects either the decomposition of the residual tin(II) reagent which prevents oxidation of the complex or extended base attack on the complex.

The octanol/saline partition coefficients show that the formulation yields hydrophilic species (Table 4.2.) while electrophoresis showed the species to be all negatively charged.

<table>
<thead>
<tr>
<th>Ligand in complex</th>
<th>Octanol/Saline Value</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>0.035</td>
<td>-1.45</td>
</tr>
<tr>
<td>545</td>
<td>0.110</td>
<td>-0.96</td>
</tr>
<tr>
<td>646</td>
<td>0.087</td>
<td>-1.06</td>
</tr>
<tr>
<td>656</td>
<td>0.069</td>
<td>-1.16</td>
</tr>
<tr>
<td>575</td>
<td>0.110</td>
<td>-0.96</td>
</tr>
</tbody>
</table>

Table 4.2.

PARTITION COEFFICIENTS FOR NO CARRIER ADDED [\(^{99m}\)Tc] REACTIONS

Since the simple paper chromatography system serves to merely separate TcO\(_2\), TcO\(_4\)\(^-\) and any technetium-ligand complexes but not the number of complexes in the formulation an alternative analytical tool HPLC, high performance liquid chromatography, was employed to try to separate discrete technetium-ligand complexes.

The mobile phase of the HPLC system which employed a reverse phase column was chosen such that the aqueous component contained ligands which were not likely to compete with the ligand to complex the technetium. Since SO\(_4\)\(^2-\) and CH\(_3\)CO\(_2\)\(^-\) had previously been shown to
effectively separate anionic technetium complexes an acetic acid/acetate/acetonitrile(90:10) was employed in this study. Although this medium clearly may have the capability to encourage ligand exchange essentially identical results were obtained using a sulphate based mobile phase indicating that the chosen system was innocent in changing the nature of the complexes in the formulation.

The formulation was carried out at pH5, the labelling efficiency checked by paper chromatography and 20μl. injected onto a Spherisorb PRP-DVB column immediately. The eluate was monitored using a NaI/Tl crystal well detector. Altering the pH of the buffer employed in the mobile phase resulted in a number of chromatograms for the range 3.02-6.48. These are shown in Figure 4.3. and tabulated in Table 4.3.

<table>
<thead>
<tr>
<th>pH</th>
<th>3.02</th>
<th>4.25</th>
<th>5.04</th>
<th>6.48</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>4.3</td>
<td>3.0</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td>4.5</td>
<td>3.5</td>
<td>3.2</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>5.0</td>
<td>3.8</td>
<td>6.8</td>
<td>7.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3.

HPLC BEHAVIOUR OF NO CARRIER ADDED [99mTc] REACTIONS

Although the exact definition of pH in mixed aqueous-organic solutions is a matter of some discussion the results below are reported relative to the pH of the original buffer which, since the mobile phase is 90% aqueous, is an acceptable approximation although the values are not meant to represent true pH(147). In assigning the peaks in the chromatograms
Figure 4.3.: HPLC behaviour at different buffer pH's of the no carrier added \( ^{99m} \text{Tc} \) reaction with 555

a. pH 6.48
1. \( R_v = 2.8 \) ml.
2. \( R_v = 3.7 \) ml.
3. \( R_v = 7.0 \) ml.

b. pH 4.25
1. \( R_v = 2.9 \) ml.
2. \( R_v = 3.5 \) ml.
3. \( R_v = 3.8 \) ml.

c. pH 3.02
1. \( R_v = 3.5 \) ml.
2. \( R_v = 4.3 \) ml.
3. \( R_v = 4.5 \) ml.
4. \( R_v = 5.0 \) ml.
5. \( R_v = 7.0 \) ml.

Spherisorb ODS column, 20cm. long,
eluant 90% 0.1MNaOAc/ HoAc 10% CH\(_3\)CN
Flow rate 1 ml./minute
T=time, R=cps
an added complication is that the $pK_a$ values of a ligand may be radically altered on coordination to a metal centre generally being lowered.

Below the $pK_a$ of the ligand, five peaks are observed. At pH4.25 the chromatogram alters and above pH5, only two major species are observed with a small amount of a third well-retained species.

That the number of species decreases as pH increases may be attributed simply to deprotonation of the free carboxylate groups on the coordinated ligands. However, at pH values close to the coordinated ligand $pK_a$, the protonated and deprotonated species will be in equilibrium, giving rise to a broadening of the peak which will deviate from classical HPLC shape.

If a diprotic complex, $MLH_2$, is considered there are three possible ionisable states $ML^{2-}$, $MLH-$ and $MLH_2$ which will, in part, be related to the complexes observed at pH3.02 and pH6.48. The complex shown in Figure 4.4. is the $ML^{2-}$ species and there must be some doubt that $MLH-$ would possess a sufficiently different capacity factor from it to ensure complete separation of these two complexes on a reverse-phase column, and the two complexes would give a single broad rather than two distinct peaks (240).

The implication is that the reaction solution contains more than one complex type, all with ionisable protons, with probable coordination through a SO donor set, although alternative, though less likely, structures are those involving coordination through the sulphur atoms alone or a polynuclear species with a Tc-O-Tc bridging core. The latter has precedence in carrier-added preparations but is unlikely in solutions of very high ligand:metal ratios.
Figure 4.4: A metal complex containing the fully deprotonated 555 ligand
On a reverse phase column, the more lipophilic the species the greater the capacity factor. As the buffer pH is decreased, the free carboxylate groups are protonated and the complex lipophilicity increases. This is indicated by a longer retention time on the column. If the data for pH values 3.02 and 4.25 are compared, this is occurring in this system as the R_v factors noticeably alter. This suggests that at least one of the carboxylate groups is non coordinating and therefore a 2:1 coordination is preferred. Geometric isomerism of the cis and trans complexes is probable and it is likely that these are the two complexes separated at pH 6.48. If a 1:1 complex were formed, then both carboxylate groups would be coordinating and a change in buffer pH would have no effect. This appears to be the case with the highly retained species, R_v = 7 ml, which is only formed in low quantity and is assigned the 1:1 complex. The additional complexes separated at the intermediate pH's are not sufficiently described to be able to interpret them with any certainty. However, if a tentative structure may be assigned of \([^{99m} \text{Tc}{(\text{OH})_2}]^2-\), then at low pH's the hydroxyl group may protonate and be less strongly coordinated. This would affect the complex charge and may account for the more retained species seen at R_v 4.5 and 5.0. On steric grounds, the trans isomer is the more stable of the two and is therefore expected to form preferentially, this is assigned R_v = 2.9 ml at pH 6.48, changing to R_v = 3.5 ml at pH 3.02. The cis isomer is assigned to R_v = 3.8 ml at pH 6.4, changing to R_v = 4.3 ml at pH 3.02. The relative proportions of each isomer formed alters at the lower pH. The trans orientation being more stable, this is possibly due to weak intra
molecular bonding between the free carboxylate and the core hydroxyl group which may be better oriented in the trans than the cis isomer.

4.2.2. The question of the double peaks.

There are technical reasons for double peaking which are leaching of the sample off the column or the result of the top of column bed being pitted. Neither of these account for the two peaks observed in this system. Multiple speciation in a radiopharmaceutical has been reported previously, the most established of which being the seven radiochemical species separated by HPLC in a preparation of \( ^{99m} \text{Tc} \) \( \text{Tc(HEDP)} \) \( (241) \).

The most relevant to this work is that reported by Nowotnik et al. on the \( ^{99m} \text{Tc} \) studies of thiodiglycollic acid (41a). This ligand is similar to those being studied here (Figure 4.5). \( ^{99m} \text{Tc} \) \( \text{Tc(DG)} \) gives two distinct radiochemical species which may be separated on an anion exchange HPLC column. The relative proportions of these species altered with age or, more quickly, by heating. The nature of the two species was not discussed. Ikeda et al. found the reaction between DMSAH\( _4 \) and \( \text{TcO}_4^- \) yielded two complexes which are suggested as the technetium(IV) and technetium(III) compounds (101,102). The latter is purportedly formed in the presence of excess tin(II) at the carrier added\( ^{99m} \text{Tc} \) level and to the exclusion of the former complex at the no carrier added\( ^{99m} \text{Tc} \) level.

Sundrehagan and Nakken (242) reacted pertechnetate with 2 thiocarboxylic acid derivatives and determined two species by electrophoresis and thin-layer chromatography which he argues as the 1:1 and 2:1 ligand:metal complexes. These assignments are however based almost entirely on
Figure 4.5: The ligand TDGH₂
charge determination without any knowledge of the oxidation state of the technetium ion.

Thus there is precedence for this type of ligand to form complexes which resolve into two radiochemical species. This appears to be the case with the dithiadicarboxylic acids. Determination of the metal oxidation state in radiopharmaceuticals always presents a major problem for analysis. Hwang et al. reacted $^{99m}$Tc$\text{gluconate}$ with 8-hydroxyquinoline which resulted in a neutral complex that was extracted into chloroform (243). As technetium(V) supposedly gives neutral complexes with 8-hydroxyquinoline, the $^{99m}$Tc$\text{gluconate}$ is assigned Tc(V). By a similar reasoning the technetium complexes of citric acid and mannitol are also assigned technetium(V). Such a system, however, is limited to those complexes that are labile and relies on the assumption that no oxidation/reduction takes place during ligand exchange and finally these assignments do not concur with those of some other workers, notably Russell and Cash, who studied these systems by electrolytic methods (244).

4.2.3. The no carrier added $^{99m}$Tc reactions of pertechnetate with the 656 and 646 ligands.

Both these ligands contain the HO$_2$C-CH$_2$-CH$_2$-S- group and their complexes are less facile to prepare if compared with the 555 ligand. Complexation occurs under similar conditions to those employed for 555, but the ligand concentration is a more important factor. Below concentrations of 50mM the paper chromatogram shows a second labelled species with an $R_f$ of 0.25 in saline. At ligand concentration of 5mM,
very little of the desired complex is formed and this second species is preferred. The 646 ligand could not be prepared by a similar method as the yield of the desired complex was very low. A typical paper chromatogram trace is shown in Figure 4.6 and indicates a mixture of $\text{TcO}_2\cdot\text{xH}_2\text{O}$, desired complex and species $R_f 0.25$. Millipore filtration gave a preparation that was free from colloidal material but still showed evidence of the two compounds. Sodium dithionite was used as an alternative reducing agent at pH 8.5 and resulted in a reaction mixture of $\text{TcO}_2\cdot\text{xH}_2\text{O}$ and desired complex ($R_f 0.95$), however, the % yield was low and not particularly reproducible, varying from 10-45%.

The HPLC of these no carrier added preparations was carried out to obtain better information on the species formed.

In the latter case, the elution profile for this preparation with a buffer pH 6.6 was very different to that observed with the 556 ligand and appears to be leaching slowly off the column. When the $[^{99m}\text{Tc}]{\text{TcO}}_2\text{tartrate}$ complex was prepared and chromatographed under the same conditions a similar elution profile was observed (Figure 4.7). Additionally, the paper chromatogram trace for the tartrate complex shows a species with $R_f 0.25$. It would appear that the reaction between $[^{99m}\text{Tc}]{\text{TcO}}_2$ and 646 preferentially forms the technetium-tartrate complex. Some complex is formed however, as indicated when using $\text{Na}_2\text{S}_2\text{O}_4$ as reductant, although it appears not to be very stable. An empirical comparison may be drawn between 656 and 646. The former will react to give the required product in a stable form and with reasonable yield, if the ligand is well in excess of tartrate concentration. The latter produces a far less stable species in much lower yield.
Figure 4.6.: A typical paper chromatogram trace in 0.9% saline for the no carrier added $[^{99m}Tc]$ complexes formed with the 646 ligand

1. $R_s = 0$
2. $R_s = 0.25$
3. $R_s = 0.85$

A=application point, S=solvent front
R=cps

Figure 4.8.: Typical paper chromatogram traces in 0.9% saline and MEK for the no carrier added $[^{99m}Tc]$ complexes formed with 545 ligand

1. saline
2. MEK
R=cps
Figure 4.7.: A qualitative comparison of the HPLC behaviour of the no carrier added [$^{305}$Tc] complexes formed with tin(II) tartrate and 646 ligand
1. 646
2. tin(II) tartrate
R,T as 4.3
irrespective of ligand concentration. This implies the instability is due to the close proximity of the two sulphur atoms in the S-CH₂-S backbone of the ligand. The HPLC elution profile for the 656 ligand was similar to that observed for the 555 ligand, with two major species eluting with similar retention times.

4.2.4. The no carrier added [⁹⁹ᵐTc] reactions of the 545, 666 and 575 ligands.

These are considered together and were not studied in as great detail as the 555 or 656 ligands. The technetium complexes containing the 545 ligand were never satisfactorily isolated free from [⁹⁹ᵐTc]TcO₂·xH₂O and yields were very low. When sodium dithionite, sodium borohydride or tin(0) foil were used as alternative reducing agents, the yield was not improved and in particular for sodium borohydride, the proportion of TcO₂·xH₂O was increased considerably. The optimum yield of the complex was given at pH 4.6 and for animal biodistribution studies, the complex was formed at this pH and filtered twice through a 0.22μm millipore filter. The quality of the paper chromatogram trace makes it difficult to assess whether or not there is a species present with Rₜ 0.25 (probably the tartrate complex) because there is considerable smearing from the application point over the entire elution path (Figure 4.8.). This is generally due to breakdown of the compound being chromatographed but such instability in the presence of mild eluants like saline is unlikely and its in vivo stability, as shown by the biodistribution study, also makes this an improbable explanation. The 575 ligand reacted with pertechnetate in a predictable fashion and
was stable over a range of pH's for several hours. It seems as though in general, the \( \text{NO}_2\text{C-CH}_2\text{-S-} \) group complexes more efficiently to technetium than does the \( \text{NO}_2\text{C-CH}_2\text{CH}_2\text{-S-} \) group. Such an observation has been made before with respect to copper complexes(212) and these results appear to uphold their findings. The 666 ligand was unusual, as mentioned in section 4.1.1. It did not form a complex that resembled the other complexes reported here, being more lipophilic, the ligand itself was more difficult to prepare and less stable in storage. The lipophilicity of the complex is demonstrated in its biological behaviour, having high muscle uptake and slow blood clearance.

A summary of the details of all the no carrier added \( ^{99m}\text{Tc}\text{Tc} \) complexes with these ligands are given in Tables 4.4. and 4.5.

<table>
<thead>
<tr>
<th>Ligand in complex</th>
<th>MEK</th>
<th>Saline</th>
<th>( pK_a ) when available*</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>0.0</td>
<td>0.85-0.95</td>
<td>4.15x10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.45x10^{-5}</td>
</tr>
<tr>
<td>656</td>
<td>0.0</td>
<td>0.80-0.90</td>
<td>3.44x10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.78x10^{-5}</td>
</tr>
<tr>
<td>575</td>
<td>0.0</td>
<td>0.84</td>
<td>4.9x10^{-9}</td>
</tr>
<tr>
<td>646</td>
<td>0.0</td>
<td>0.25-0.85</td>
<td>4.52x10^{-8}</td>
</tr>
<tr>
<td>545</td>
<td>0.0</td>
<td>0.0-0.9</td>
<td></td>
</tr>
<tr>
<td>666</td>
<td>0.86</td>
<td>0.65</td>
<td></td>
</tr>
</tbody>
</table>

* 18°C, 0.1M Ionic Strength (239)

Table 4.4.

PAPER CHROMATOGRAPHIC BEHAVIOUR OF NO CARRIER ADDED \( ^{99m}\text{Tc} \) REACTIONS
The interpretation of these results is difficult when only tracer level reactions are known. Because of the variety of complexes formed and stabilities observed it was decided to limit the carrier added \(^{99m}\text{Tc}\) investigations to three of the ligands only.

<table>
<thead>
<tr>
<th>Ligand in complex</th>
<th>Electrophoretic Movement (value relative to TcCl\textsuperscript{-})</th>
<th>pH of a 50mM solution in H\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>Anionic; 0.9</td>
<td>4.53</td>
</tr>
<tr>
<td>656</td>
<td>Anionic; 0.9</td>
<td>4.96</td>
</tr>
<tr>
<td>575</td>
<td>Anionic; 0.9</td>
<td>4.30</td>
</tr>
<tr>
<td>646</td>
<td>Anionic; 0.4</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>545</td>
<td>Anionic; 0.4</td>
<td>4.20</td>
</tr>
<tr>
<td>666</td>
<td>Anionic; 0</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5.

ELECTROPHORETIC MOBILITY OF NO CARRIER ADDED \(^{99m}\text{Tc}\) REACTIONS

The 555 and 656 were both chosen as they had reacted at the no-carrier added level with a good yield giving a stable product. It was also felt that these two were the most likely to form stable 1:1 complexes, coordinating through both thioether and carboxylate functional groups if the correct reaction conditions were found. For that reason, the 545 was considered, as the ligand most likely to form a 2:1 (ligand:metal) complex, probably coordinating through a SO donor set or, less likely, through an OD donor set.
4.3. Carriers added \(^{99}\)Tc reactions of the dithiadicarboxylic acid ligands.

4.3.1. Introduction

The reactions were designed to imitate the radiopharmaceutical preparation, hence pertechnetate was the starting material of choice. Several reducing agents were considered, tin(II) tartrate, sodium dithionite, sodium borohydride and sodium bisulphite. This last was the only one suitable for the system. Sodium bisulphite reacted slowly over a period of twenty-four hours and did not result in the formation of \(^{99}\)TcO\(_2\cdot xH_2O\) as did the others, which in the case of sodium borohydride was instant and quantitative. This choice of reductant reflects the weakness of the ligand strength for technetium and affords a comparison between thiol sulphur and thioether sulphur as the former complexes readily with technetium with any of the above reductants\(^{(45)}\). Details of the reactions are tabulated in Table 4.6.

4.3.2. Solution Studies

As the reaction proceeds, the solution becomes coloured from yellow, through orange to deep-red. Column chromatography of this red solution separated two species on cellulose, one pink (\(\lambda_{max} \approx 490,300\)nm.) the other brown/yellow (no strong absorption between 230 and 800nm.). The pink solution, eluted with a methanol/water (60:40) solution slowly turned brown/yellow on standing. To the red solution was added a saturated solution of Ph\(_4\)AsCl, followed by ethanol to dissolve the Ph\(_4\)As\(^+\) salt of the ligand. A pink coloured precipitate remained. \(^{99}\)Tc\(^{\text{III}}\) analysis, by liquid scintillation counting, gave a technetium analysis of 9%. 

Recrystallisation of the compound resulted in an oil which could not be further purified without decomposition. The technetium analysis implies either coprecipitation of the technetium containing complex with another non technetium compound or a technetium complex of molecular weight 1100 daltons approximately. If the former, then purification proved too rigorous a procedure for the complex, if the latter, then the stoichiometry is 5:1 ligand:metal, which is considered unlikely.

**Reaction Conditions**

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{TcO}_4^{-}+545+S_2O_4^{2-}$</td>
<td>$\text{N}_2,\text{room temp}$ Colour change: yellow $\rightarrow$ red brown $\rightarrow$ brown $\text{+Bu}_4\text{NBF}_4$ in $\text{iPrOH}$ $\rightarrow$ No. ppt. $\rightarrow$ removed $\text{iPrOH}$ $\rightarrow$ $\text{TcO}_2$ $\rightarrow$ $\text{R}_r=0$ (MEK), $0.8$ (Saline) red soln.</td>
</tr>
<tr>
<td>$\text{TcO}_4^{-}+556+S_2O_4^{2-}$</td>
<td>$\text{N}_2,\text{room temp}$ Immediate $\text{TcO}_2$ ppt.</td>
</tr>
<tr>
<td>$\text{TcO}_4^{-}+555+\text{HS}_2\text{O}_6^{-}$</td>
<td>$\text{N}_2,4^\circ\text{C}$ Colour change: red brown $\rightarrow$ red ppt. $\rightarrow$ $\text{Bu}_4\text{NCl}$ $4^\circ\text{C}$/$\text{12h}$ $\rightarrow$ red ppt. $\rightarrow$ Recryst. $\text{H}_2\text{O}/\text{iPrOH}$ $\rightarrow$ $\text{TcO}_2$ $\rightarrow$ $\text{R}_r=0$ (MEK), $1$ (Saline), $0.9$ (40% MeOH) red solution</td>
</tr>
<tr>
<td>$\text{TcOCl}_4^{-}+545+\text{NaOH}$</td>
<td>$\text{N}_2,\text{room temp}$ After $\text{24h}$ $\rightarrow$ orange $\rightarrow$ purple ppt. $\rightarrow$ Recryst. $\text{H}_2\text{O}$ $\rightarrow$ EtOH $\rightarrow$ brown yellow $\rightarrow$ no further isolation possible</td>
</tr>
<tr>
<td>$\text{TcOCl}_4^{-}+545+\text{NaOEt}$</td>
<td>$\text{N}_2,\text{room temp}$ Decomposed slowly to $\text{TcO}_2$</td>
</tr>
<tr>
<td>$\text{TcCl}_6^{2-}+545+\text{NaH}$</td>
<td>$\text{N}_2,\text{room temp}$ Colour change: red/brown $\rightarrow$ dissolve MeOH $\rightarrow$ add C$_2$H$_5$, grey ppt (only 3% Tc) $\rightarrow$ residual solution $\text{TcO}_4^{-}$ $\rightarrow$ $\text{R}_r=0$ (MEK), $0.9$ (Saline) red solution</td>
</tr>
</tbody>
</table>

Table 4.6.

 SOME CARRIER ADDED [\(^{99m}\text{Tc}\) REACTIONS WITH DITHIADICARBOXYLIC ACIDS
When Bu₄N⁺ was used to precipitate the red complex only an oil was formed. These observations were similar for all three ligands studied and in no case was a stable purified product obtained for analysis, frequently the coloured solutions slowly decomposed during work up procedures. The inability to crystallise and purify these complexes is a similar phenomenon to that experienced with other classes of ligand, particularly the aliphatic hydroxy and carboxylic acids (23?a).

Unsubstituted carboxylic acids give quantitative yields of \([^{99}\text{Tc}]\text{TcO}_2\cdot x\text{H}_2\text{O},\) whilst the hydroxy carboxylic acids give rise to two coloured species, one red/violet \(\lambda_{\text{max}} 510-560\text{nm}, 280-300\text{nm}.,\) the other brown/yellow (no \(\lambda_{\text{max}}\) quoted) (23?b). This latter is attributed to a polymeric soluble species. It is feasible that these complexes do not readily crystallise because their coordinating positions are partially filled with solvent molecules, which dissociate and cause the degradation of the complex. It is also possible that the colour change from red-brown/yellow, seen after column separation on cellulose was a hydrolysis effect as the complex was no longer in the presence of excess ligand, it having been removed on the column. A presumption therefore could be made that the red solution contains \([^{99}\text{Tc}]\text{Tc}^{-}\text{L}_{2}\) and the brown/yellow solution contains \([^{99}\text{Tc}]\text{Tc}^{-}\text{L}^{-}(\text{H}_2\text{O})_{\infty}.\)

4.3.3. The effect of total technetium concentration on the reaction products by serially diluting a solution of \(\text{NH}_4[^{99}\text{Tc}]\text{TcO}_4.\)

The inability to transfer chemical reactions from the no carrier added to the carrier added concentration levels has been highlighted several times by other workers (116,245). In particular, for this work,
Garcia et al. expanded the work of Ikeda to study the various
[\textsuperscript{99m}Tc]TcDMSA complexes formed but failed to identify the one formed under
radiopharmaceutical conditions\cite{103}, whilst with the
polyaminocarboxylates, the millimolar level of reactions lead to formation
of \( \text{O}_2 \) bridged dimers of technetium(III) or technetium(IV) compounds, when
the macromolar level is understood to give monomeric technetium(V)
species\cite{116}. Two theories have been developed which explain this
behaviour\cite{101,245}. Firstly, that the large excess of reductant present
in the no carrier added formulation will encourage a different redox
reaction and the final oxidation state in this case will be lower than in
the carrier added formulation. Hence, the reduction potential from
technetium(VII) to technetium(IV) is achieved with tin(II)/tin(IV)
oxidation, but the further reduction of technetium(IV) to technetium(III)
requires a large excess of the reductant. Secondly, that the total
technetium concentration may be part of the rate determining step for the
reaction. The total concentration between carrier added and no carrier
added technetium may vary by up to \( \times 10^4 \), hence if a reaction path depends
upon a reaction of two technetium containing intermediates, such a
reaction is less likely to occur in the no carrier added preparation. To
ascertain whether or not a reaction does transfer from carrier to no
carrier added levels, the technetium concentration is gradually reduced
and a standard chromatography technique is used to monitor the changes.
Ideally HPLC is the method of choice, this was not possible in this case.
For this reason, serial dilutions were performed on two systems to
observe the variation in behaviour for these complexes. The
concentration of \( \textsuperscript{99m}Tc \) was changed from \( 8.08 \times 10^{-5} \) to \( 8.08 \times 10^{-9} \) moles by
simple dilution factors. The ligand(545) concentration was initially
twice that of the technetium, as this was thought most likely to form a
2:1 complex and the concentration of the reductant tin(II)tertrate was at
the same initial concentration as the technetium. Therefore, the ligand
and reductant are increasingly in excess relative to technetium, which
emulates the conditions for the no carrier added preparation. The
reactions were monitored by paper chromatography as described previously.

At \([Tc]=10^{-5}\) moles, about 50\% of the activity is associated with a
compound that has an \(R_f=0\) in butan-2-one and the remaining 50\% moves to
\(R_f=0.5\). In saline, 60\% of the activity is associated with \(R_f=0\), smearing
to \(R_f=0.35\) and the remaining 40\% is at \(R_f=0.85\). Since no pertechnetate
is observed on these simple chromatograms, it is assumed that complete
reduction has occurred but the reaction products are different to those
obtained at the no carrier added level. This second species, more
lipophilic, could possibly be similar to the \(O_2\) bridged dimers reported
in the polyaminocarboxylate studies and of the form (LTc-O-TcL)\(^n\) or
(LTc-O\(_2\)-TcL)\(^m\)-(116). There is some evidence of a species that has the
same \(R_f\) values as the no carrier added product.

At \([Tc]=10^{-6}\) moles, 100\% of the measured activity is associated with
a compound that has \(R_f=0\) in butan-2-one and a broad band is observed on
the saline chromatogram, \(R_f=0-0.4\). About 70\% of the activity is
associated with this compound, there is no clear band at \(R_f=0.85\). This
is radically different to that above, indicating a different reaction
product when the reactants are no longer in equimolar or stoichiometric
quantities. At \([Tc]=10^{-7} -> 10^{-8}\) moles, essentially the no carrier
added(Tc) preparation is achieved.
It was noticed that when the technetium concentration was \(10^{-5}\) moles, the solution turned brown, with a red reflex. This decomposed over a period of twenty-four hours, to \(^{99m}Tc\)TcO\(_4^-\) and TcO\(_2\).xH\(_2\)O. However, when the total technetium concentration was diluted to \(10^{-5}\) moles, the solution was pink and remained stable to decomposition. Since in this latter case, the ligand concentration was now twenty times that of the technetium, this is a probable reason for its additional stability. The nature of the product formed is unknown. The absence of any species with the same \(R_f\) values as the radiopharmaceutical implies no simple monomeric complex is formed but possible structures are discussed in 4.5.2..

The second system investigated was that of 545 and sodium dithionite. A different reducing agent was used since the tartrate moiety seemed to be influencing the final reaction product and needed therefore to be removed. Under these conditions the radiopharmaceutical preparation gives a very low yield of the desired complex, the majority of the product being \(^{99m}Tc\)TcO\(_4^-\).xH\(_2\)O. The same concentrations as previously described, in the same ratios, were used where the pH of the reductant was initially 12 and the final pH of the reaction solution was 8-9. Chromatograms were developed at thirty minutes post formulation and electrophoresis strips were also run for each solution in a phosphate buffer pH=7.4, for one hour with pertechnetate as a standard.

The reaction was slower than previously observed with tin(II) tartrate, since after thirty minutes there was free pertechnetate at both \([Tc]=10^{-5}\) and \(10^{-6}\) moles, (80% and 20% respectively). Additional chromatograms were developed in 40% methanol \((R_f, \text{TcO}_4^-=0.5)\) and
confirmed the presence of pertechnetate. There was, however, in the latter case, evidence of a small proportion of a complex having an $R_v$ in methanol of 0.0-0.3 and moving on the electrophoresis strip with an $R_v(TcO_4^-)$ of 0.66. When $[^{99}\text{Tc}]=10^{-7}$ moles, the chromatograms indicate 85-90% $[^{99}\text{Tc}]\text{TcO}_4^-$ and $\text{TcO}_4^-$ with 10-15% of this complex. Further dilutions of the pertechnetate concentration resulted in only colloidal formation.

That a reaction involving the ligand has occurred is confirmed by the disproportionation products. If $\text{Tc(V)}$ is assumed to be the oxidation state, then $3\text{Tc(V)} \rightarrow \text{Tc(VII)} + 2\text{Tc(IV)}$. In the absence of any complex formation, the reaction would proceed through reduction of pertechnetate to $\text{TcO}_4^-$ with $\text{TCO}_2.x\text{H}_2\text{O}$. This indeed was the case for the more dilute solutions and concurs with the results found at no carrier added $[^{99}\text{Tc}]$ concentrations.

Sodium dithionite is a poor reductant for this system. It was chosen because there is no known evidence of dithionite complexing with a technetium complex or in any way coordinating to the reaction products. Tin(II) tartrate appears to stabilise these reactions at the no carrier added level and tin(II) salts have been shown to be incorporated into technetium complexes at the carrier added $[^{99}\text{Tc}]$ level (130). This particular ligand was chosen because it demonstrated most clearly the tartrate effect of all the systems studied and therefore despite it not being the most easy to interpret was considered to be the best example.
4.3.4. The carrier added $[^{99}\text{Tc}]$ reactions of thiodiglycollic acid (TDGH$_2$).

To help characterise the dithiadicarboxylic acid reactions with $[^{99}\text{Tc}]\text{TCO}_4^-$, the synthesis of a $[^{99}\text{Tc}]\text{TDG}$ complex was undertaken. The no carrier added reactions for this ligand had been fully investigated ([41a]), reported and the complex was the subject of clinical trials as a potential replacement for $^{123}\text{Iodohippuran}$ ([223]). The radiopharmaceutical formulation was known to yield two complexes which could be separated by anion exchange. Thermal conversion from one to the other was effected at high temperatures (100°C) although it slowly converted at room temperature and complex II (100°C) was shown to have better biological clearance rates than complex I. At low temperatures (0-2°C), with the no carrier added reaction, only complex I was isolated. When the carrier added reaction was attempted at low temperatures, only $[^{99}\text{Tc}]{\text{TCO}}_{4-x}{\text{H}}_2{\text{O}}$ was formed. A list of the reactions attempted and results or observations noted is given in Table 4.7. In summary it can be said that the carrier added reaction with TDGH$_2$ produces a mixture of products which do not separate on electrophoresis, except by colour, nor do they separate on a cellulose chromatography column. The similar behaviour of TDGH$_2$ compared to the dithiadicarboxylic acids again confirms the unsuitable nature of these ligands if starting from pertechnetate. Other starting materials were prepared, with technetium(V) and technetium(IV) oxidation states, $\text{TcOCl}_4^-$, $\text{TcCl}_6^{2-}$ and $\text{TcCl}_4(\text{PPh}_3)_2$. It seemed likely that a pre-reduced technetium centre might be a more accessible route. A reaction between TDGH$_2$ and $[^{99}\text{Tc}]{\text{TcCl}}_4(\text{PPh}_3)_2$ in an ethanol/water (50/50) mixture, did not occur.
When stoichiometric quantities of $[^{99}\text{Tc}]\text{TcOCl}_4^-$ and TDG$_2$ (1:2) were reacted together in methanol, nothing happened until a base was added to deprotonate the carboxylic acid, then $[^{99}\text{Tc}]\text{TcO}_{2-x}\text{H}_2\text{O}$ was precipitated. When $[^{99}\text{Tc}]\text{TcCl}_2^{2-}$ in ethanol was reacted with TDG(Na salt) in ethanol/NaH, a red/brown solution ($\lambda_{\text{max}}$ 476, 356nm) formed which yielded a brown precipitate. Recrystallisation from methanol/toluene gave a grey/brown compound that gave a $^{99}\text{Tc}$ analysis of 5% by scintillation counting. The supernatant was highly radioactive, colourless and chromatography showed it to be pertechnetate. It seems likely that the 5% $^{99}\text{Tc}$ content is contamination of the precipitate.

The methods required to prepare stable complexes of these ligands with technetium have not yet been ascertained. It seems likely that the sixth coordinating position must be stabilised by a strong ligand that
will hold the complex together. Possible routes might be the CO, NO⁺ or N≡N derivatives, by forming Tc(O)L₂(H₂O) and replacing H₂O with one of the above, or indeed, starting with the [⁹⁹ᵐTc][Tc(NO)Cl₄]⁻ precursor. The more common reaction precursors, with their oxo type cores or labile halide ions are clearly not suited for these ligands.


4.4.1. Introduction.

The complexes were synthesised using freshly prepared solutions and checked for radiochemical purity by paper chromatography. Three different studies were performed, dynamic γ camera pictures with subsequent dissection after two hours for the 555, 656, 666 and 575 [⁹⁹ᵐTc] complexes, dynamic γ camera pictures with regions of interest selected for blood clearance and kidney uptake for the 646, 656 (Na₂S₂O₃) and [⁹⁹ᵐTc]TcSntartrate complexes, finally periodic dissections of mice, in triplicate, at six time intervals up to four hours, described in chapter two, for 545, 656 and [⁹⁹ᵐTc]TcSntartrate complexes. The percentages given represent the measured uptake per organ relative to the injected dose. For blood and muscle, correction factors of body composition were used (5.8% and 43% respectively for the rat and 7% blood for the mouse). Frequently, when discussing a number of similar ligands, a structure-distribution relationship (SDR) can be argued and patterns of behaviour based on molecular weight, determined(120,246). One of the features of these complexes is that such a relationship is not obvious. This is partly due to the uncertainty of the actual radiochemical species.
involved such that simple molecular weight calculations based on the ligand may not be sufficient.

The term 'complex' in the following discussion does not necessarily imply a discrete radiochemical species, rather it is used to describe the reaction solution that may well contain at least two identifiable radiochemical components. 'Complex' is used for simplicity.

4.4.2. Discussion.

There is a degree of protein binding with these complexes which accounts for the presence of radioactivity in the blood and muscle after two hours. This is especially true for 656 where 30% of the injected dose is present in the blood and muscle at dissection and there appears little difference between the hepatobiliary clearance and the renal clearance (29% and 25% respectively). (Figure 4.9.) When this complex was prepared at pH1, ensuring complete protonation of the carboxylate ligands, this biodistribution changes to 8% residual dose in the blood and muscle, with 49% handled by the kidneys and 23% by the hepatobiliary system. (Figure 4.10.) The stomach is clearly outlined, although absence of thyroid uptake confirms the integrity of the injected complex as pertechnetate is accumulated in stomach and thyroid as shown in Figure 4.11. It is probable that this low pH has a pharmacological effect, although blood can buffer small volumes of material and will buffer acidic formulations better than basic ones (247), therefore this preparation, although apparently stable in vivo, was not used in subsequent studies. Where a compound is known to be protein-bound, the apparent organ uptake is falsely elevated due to the blood supply. It is
Figure 4.9: 120 minutes p.i. biodistribution data of rats and static scintiphotos of the no carrier added $^{99m}$Tc complexes formed with 656 ligand

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes
* = Bladder and urine
Figure 4.10.: 120 minutes biodistribution data of rats and static scintiphotos of the no carrier added $^{99m}$Tc complexes formed at pH1 with 656 ligand

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes
Liver 4.20
S.I./L.I. 18.52
Carcass 8.92

B/U 33.31
15.08 Kidneys
8.45 Blood
8.84 Muscle
14.83 Injection

* = Bladder and urine
Figure 4.11.: Scintiphotographs showing the biodistribution in rats, of $^{99m}$Tc TeO$_4^-$

1. 0 minutes
2. 60 minutes
therefore interesting to consider the blood and kidney clearance curves which were constructed from 656 mouse dissection data and show a very similar trend. (Figure 4.12.) It could be inferred from this that the % dose in the kidneys is merely a reflection of the blood supply to those organs and does not arise from any extraction, however, the high accumulation at an early stage, of activity in the urine shows very efficient filtration of this compound is taking place through the kidney excretory functions. The % hepatobiliary clearance remains fairly constant at 18-20% although it can be seen to clear slowly from the liver into the small intestine (Figure 4.13.). The complexes formed with 656 were studied in both rats and mice as the tin(II) formulation and also in rats as the dithionite formulation. Comparing the two animal types, this complex is cleared more rapidly from the blood in mice than in rats.

Different biological handling between animal species is, of course, a well-known phenomenon, indeed, intra-species variation is also recognised, particularly between male and female. When choosing an animal model therefore, it is important to remember that the observations are neither absolute nor unequivocal. When the 656/dithionite complex was prepared, it was filtered through a 0.22μm filter to remove all trace of colloid before injection. HPLC of the injected material showed two distinct species in approximately equal proportions and the subsequent biodistribution showed clean, renal clearance with a low body blood background and very little hepatobiliary activity (Figure 4.14.). From this experience with the 656 complex, it appears that the choice of reductant does influence the biodistribution pattern of the material and this is an observation that has also been made with respect to
Figure 4.12.: Blood and kidney clearance curves in mice of the no carrier added $[^{99m}Tc]$ complexes formed with 656 ligand

1. kidney clearance
2. blood clearance
% Injected dose

Time/5 minutes

% Injected dose

Time/5 minutes
Figure 4.13.: Biodistribution data and hepatobiliary clearance in mice over 240 minutes of the no carrier added [$^{99m}$Tc] complexes formed with 656 ligand

1. dissection data

2. hepatobiliary clearance
Figure 4.14.: Scintiphotographs of the biodistribution of the no carrier added $^{99m}$Tc complexes formed with 656 ligand using sodium dithionite as reductant

1. 1-4 minutes
2. 5 minutes
3. 10 minutes
4. 20 minutes
5. 30 minutes
6. 60 minutes
7. 120 minutes
formamidine sulphinic acid (47b). The comparison of $^{99m}$TcTcHIDA (SnCl₂) and $^{99m}$TcTcHIDA (PSA) showed considerably more renal clearance in the latter case.

The $^{99m}$Tc complex showed an unusual biodistribution (Figure 4.15.). Studied in mice only, there was a constant activity of 17±18% present in the liver over the span of four hours. This implies colloid in the liver which was not detected by paper chromatography either before the study or at its conclusion, some six hours later. The complex appears to be stable in vitro, but not very stable in vivo, or more readily metabolised by the body. If a correction is made to the % hepatobiliary excretion to account for colloid in the liver, then this compound is overwhelmingly cleared by the kidneys.

The $^{99m}$Tc complex, again, showed 20% liver uptake and retention at dissection and as before with the $^{99m}$Tc, no colloid had been present in the solution prior to injection (Figure 4.16.) However, in this case, it seems the liver activity may be due to a slow clearing component. HPLC of the injected material again separated two radiochemical species and although their chromatographic properties are similar, it is probable that the two components are cleared by different means through the body. The logical step to follow is to separate these two components on the HPLC and study them independently of each other, also, it would be an interesting hypothesis that if this complex were formulated with dithionite, the biological handling of the resultant solution would be different, clearing more through the kidneys.

The biodistribution of the $^{99m}$Tc complex (Figure 4.17.) is not discussed in detail. There is no obvious trend of increased hepatobiliary
Figure 4.15.: Biodistribution data and clearance curves in mice over a four hour study of the no carrier added \[^{99m}\text{Tc}\] complexes formed with 545 ligand

1. dissection data
2. renal clearance
3. hepatobiliary clearance
Figure 4.16.: Biodistribution data of rats and static scintiphotographs (up to 60 minutes) of the no carrier added \(^{99m}\text{Tc}\) complexes formed with 555 ligand

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 60 minutes
Liver 20.91
S.I./L.I. 7.82
Carcass 10.23
Injection 19.93
10.29 Blood
12.48 Muscle
10.68 Kidneys
25.70 B/U

* = Bladder and urine
Figure 4.17: 120 minutes biodistribution data of rats and static scintiphotosgraphs of the no carrier added $[^{99m}Tc]$ complexes formed with 575 ligand

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes
B/U 29.64
Liver 13.22
S.I./L.I. 8.59
23.24 Kidneys
6.66 Blood
7.24 Muscle
1.66 Injection
6.65 Carcass

* = Bladder and urine
clearance from 555 to 575, nor any similarity between 656 and 575 which are the same molecular weight. The data shows more renal clearance than has been previously observed with the exception of the pH1 formulation of the 656 and no comparison is drawn between these two. As was said in the introduction, the 656 ligand complexed with technetium in a different manner to the rest of the ligands and therefore its biodistribution must be viewed independantly, although the urinary and hepatobiliary clearances are not particularly interesting (Figure 4.18.). Finally, the 646 complex shows a clearance pattern in rats that is similar to \(^{99m}\text{Tc} \text{TcSntartrate}\) (Figures 4.19. and 4.20.). The biodistribution data in mice (Figure 4.21.) shows rapid clearance from the blood, early passage through the kidney into the bladder (14% of the dose after 15 minutes) and about 20% excreted via the hepatobiliary system. The absence of any sustained activity in the liver and any thyroid or stomach uptake confirm the rather surprising finding that the \(^{99m}\text{Tc} \text{TcSntartrate}\) complex is a stable radiochemical species that does not break down in vivo, as might have been expected. This is taken as further evidence that the reductant is actively stabilising some of the \(^{99m}\text{Tc} \text{Tcdithiodicarboxylate}\) complexes that are not sterically favourable to form strong unique complexes.

4.4.3. Some final observations.

The effect of the tartrate presence has been emphatically stated, however, mention must also be made of the effect of tin to a radiopharmaceutical. Despite the fact that most 'cold' kits available for radiopharmaceuticals contain a tin(II) reducing agent, it is known that
Figure 4.18: 120 minutes biodistribution data of rats and static scintiphotos of the no carrier added $^{99m}$Tc complexes formed with 866 ligand
1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes
* = Bladder and urine
Figure 4.19.: Scintiphotographs of the biodistribution of the no carrier added [99mTc] complexes formed 646 ligand

1. 15s, 30s, 45s, 60s
2. 2-5 minutes
3. 10 minutes
4. 15 minutes
5. 30 minutes
6. 60 minutes
7. 120 minutes
Figure 4.20.: Scintiphotos of the biodistribution of the no carrier added $[^{99m}\text{Tc}]$ complexes formed with tin(II) tartrate
1. 1-4 minutes
2. 10 minutes
3. 30 minutes
Figure 4.21: Biodistribution data and clearance curves in mice over a four hour study of the no carrier added $^{99m}$Tc complexes formed with tin(II) tartrate
1. dissection data
2. hepatobiliary clearance
3. renal clearance
Sn²⁺ has an affinity for red blood cells and is used as an 'in vivo' method for red blood cell labelling (247). Therefore, when an excess of Sn²⁺ is administered in the injection vehicle, there is an increased possibility of persistant blood levels, soft tissue accumulation and biliary excretion (248). The use of tin(0) foil or wire is a better method as this reduces the amount of free Sn²⁺ in solution. Unfortunately, although tin(0) foil was suitable for TDGH₂, it was not effective for the dithiadicarboxylic acids.

Finally, the 656 formulation at pH 1 may well represent a different oxidation state, as it is well known that oxidation states, particularly III, IV and V, change at low pH's (54).

4.5. Conclusions

4.5.1. Possible structures of the no carrier added [⁹⁵mTc] complexes.

Figure 4.22. shows the 1:1 and 2:1 coordination of the 555 ligand with technetium. In both these configurations, the coordination number is four and it is probable that solvent or oxo molecules will increase this to six. The nature of the technetium core is influenced by the oxidation state and it is recognised that most technetium(V) compounds, with very few exceptions, form Tc-oxo cores, as explained in chapter one. This preponderance of Tc-oxo though, merely reflects the reaction routes employed, as reduction from pertechnetate to technetium(V) is facile and occurs with many different reducing agents. The reaction precursors for technetium(V) themselves have Tc-oxo cores, for example the [⁹⁵mTc]TcOX₄⁻ ligands, where X=halide. The nature of, or absence of, any group in the sixth coordination position, trans to Tc=O, has a marked dependance on
Figure 4.22.: Configurations of the 1:1 and 2:1
555 ligand:technetium complexes
the electronic nature of the cis ligands. π-acid ligands, in particular, render this position substitutionally labile and encourage hydrolysis or solvolysis to produce \([\text{Tc}(\text{O})\langle\text{OR}\rangle]^2^+\) or \([\text{Tc}(\text{O})_2]^+\) species readily. The reaction of KCN in methanol with \(\text{TcOCl}_4\) gives \((\text{TcO}(\text{OMe})(\text{CN})_4)^2^+\) (28), whilst the reaction of 2,2'-bipyridine (bipy), in ethanol gives \(\text{TcO}(\text{OEt})\text{Cl}_2\text{(bipy)}\) (249). In this latter reaction, if the ethanol is acidified with HCl, then the reaction product is \(\text{TcOCl}_2\text{(bipy)}\). The electronic nature of the dithiadicarboxylic acids is not well documented. The π-acceptor capacity of sulphur as a thioether is greater than that of a thiol (250) and less than the tertiary phosphines or arsines. This places the ligands in an intermediate position between an oxo and dioxo core. Technetium(IV) complexes are not so clearly defined. Many of these are characterised with halogeno ligands in the fifth and sixth coordinating positions, particularly true of the phosphino and schiff base complexes, whilst some carboxylate complexes are assigned \(\text{Tc}^{\text{IV}}\) with an oxo/hydroxy core. From current knowledge of technetium chemistry, either of these oxidation states is possible for these ligands, with oxo, dioxo and hydroxy cores all likely to be involved. It cannot be ruled out that the Cl⁻ ion, present in high quantities in the generator eluate, is also a probable coordinating moiety as explained in chapter three and indeed this may also be a possible explanation of the variation seen in the biodistribution between 656 and 656 prepared at pH1, the pH being lowered with conc. HCl which would obviously increase Cl⁻ concentration quite markedly. As yet however, no carboxylate or thioether complex has been fully characterised with a dioxo core, the only one currently reported has yet to be confirmed. For this reason, the 2:1 structure is suggested for
the 555 ligand shown in Figure 4.22. The 1:1 structure is ruled out on the grounds that at no carrier added concentrations, the ligand is in excess and therefore the likelihood of two molecules of ligand reacting with one molecule of technetium is high, also, the affinity of this ligand type for technetium does not appear to be sufficiently strong that the extra stability conferred by the chelate effect is not a contributing factor.

The lesser tendency for the 656 to complex is explained by the weaker bonding capabilities of the HO₂C-CH₂-CH₂-S- backbone. Finally, the difficulty observed in the 545 and 646 ligands must be the steric interference of the two bulky sulphur groups, separated only by a -CH₂- group, which causes destabilisation of the complex. It is an interesting possibility that the 666 ligand which reacts to form a complex with different characteristics to the other ligands does in fact form a 1:1 complex.

If these assumptions are made, then the two components present in the radiopharmaceutical preparation are the cis and trans isomers (Figure 4.23.). Their similarities in chromatographic behaviour would therefore be expected.

The possibility of a multi-centred complex of the type found in the aminocarboxylate complexes is not considered as this is unlikely in the high dilution environment of the radiopharmaceutical preparations.

4.5.2. Possible structures of the carrier added[⁹⁹Tc] complexes.

To assign chemical structures to the carrier added complexes is rendered difficult by the absence of any inorganic analysis on these
Figure 4.23.: The geometric isomers, cis and trans, of the 2:1 555 ligand:technetium complex
1. cis
2. trans
preparations. However, by comparing them to the polyaminocarboxylates and the hydroxycarboxylates, some structures may be suggested. In the former studies, the no carrier added [$^{99m}$Tc] preparations are believed to be technetium(V) whilst the carrier added [$^{99}$Tc] preparations are mixed technetium(III) and technetium(IV). However, with the polyaminocarboxylates, the ligands are coordinating through a tetradeutate set, allowing the formation of ($\mu$-O)$_2$ bridged dimers with one ligand coordinating to each technetium. The equivalent reaction in the dithiadicarboxylate system would require two ligands per technetium and the constraints placed upon a resulting L$_2$Tc($\mu$-O)$_2$TcL$_2$ structure would not make it a particularly stable species. Both ligands on each technetium centre possess a -CH$_3$SCH$_2$CO$_2$H tail that are restrained by the ($\mu$-O)$_2$ bridge. A single oxo bridge would be more acceptable as this would permit rotation around the technetium-technetium bond to separate the ligand tails. It is possible that this is the species observed in the serial dilutions at 10$^{-5}$ moles technetium concentration, that was hydrolysed slowly in the absence of excess ligand to [Tc(O)L$_2$(solvent)]$^{3-}$ and finally to TcO$_{2-x}$H$_2$O. An alternative is the Tc$_2$O$_{3-x}$ core, which would give rise to L$_2$TcO($\mu$O)-OTcL$_2$. A technetium(V)/technetium(V) centre would result in a 4- charge on the complex, while a mixture of technetium(IV)/technetium(V) would result in lower overall charge. The hydrolysis of this $\mu$-oxo bridge would occur in acid solution and it is unlikely that the products would be stable.
4.6. Copper complexes of the dithiadicarboxylic acids.

The copper complexes of these ligands were synthesised as detailed in chapter two to assess the potential complexing of the ligands in a quadridentate fashion. The work of Podlaha and Podlahova had confirmed the affinity of this ligand type for copper(II) and their preliminary work on the 555 ligand showed 1:1 metal:ligand complexation as the dihydrate (234). This is similar to the findings of Ford et al. except that they analyse the complex as the monohydrate (212). The neutral complexes readily precipitated from water and no suitable solvent was found to recrystallise them, due to their low solubility, consequently they were washed with water, ethanol and ether and allowed to air dry. The elemental analyses of these compounds is given in Table 4.8, and clearly indicate that 1:1 complexes are formed as either the mono or dihydrate.

<table>
<thead>
<tr>
<th>LIGAND</th>
<th>FOUND</th>
<th>EXPECTED CuL(H₂O)</th>
<th>EXPECTED CuL(H₂O)₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>S</td>
</tr>
<tr>
<td>555</td>
<td>23.9</td>
<td>3.3</td>
<td>22.6</td>
</tr>
<tr>
<td>545</td>
<td>21.7</td>
<td>3.0</td>
<td>22.8</td>
</tr>
<tr>
<td>575</td>
<td>23.6</td>
<td>5.0</td>
<td>19.0</td>
</tr>
<tr>
<td>656</td>
<td>30.5</td>
<td>3.9</td>
<td>21.0</td>
</tr>
<tr>
<td>646</td>
<td>28.2</td>
<td>3.9</td>
<td>21.0</td>
</tr>
<tr>
<td>666</td>
<td>35.0</td>
<td>5.1</td>
<td>18.9</td>
</tr>
</tbody>
</table>

* = C: H: S ratio is C₁₀H₁₇S₂
Ligand requires C₀H₁₄S₂

Table 4.8.

ELEMENTAL ANALYSIS OF THE COPPER COMPLEXES.
Only the 666 ligand is not easily resolved, this is consistent with the difficulties experienced with this ligand throughout this work and is not considered further. The other complexes resolve readily into mono or dihydrate apart from 555 which has equivocal analysis.

If the infrared spectra are considered, Table 4.9, then it is clear that coordination has occurred consistently through the carboxylic acids. This is to be expected of a copper(II) complex which will have greater affinity for carboxylate donors than copper(I). Hence when this ligand was complexed with copper(I), only coordination through the sulphur was reported(232,233). The absorption shifts are not so clearly defined however, for the thioether donors. There is a significant shift to a lower wavenumber for the 555 and 545 ligands with a lesser shift occurring for the 575 ligand. The spectra obtained for 656 and 646 imply little, if any, coordination through the sulphur atoms.

<table>
<thead>
<tr>
<th>Species</th>
<th>I.R.Absorptions(cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;C=O</td>
</tr>
<tr>
<td>555</td>
<td>1685,1720</td>
</tr>
<tr>
<td>Ligand</td>
<td>1602</td>
</tr>
<tr>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td>545</td>
<td>1690,1710</td>
</tr>
<tr>
<td>Ligand</td>
<td>1590</td>
</tr>
<tr>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td>656</td>
<td>1680,1710</td>
</tr>
<tr>
<td>Ligand</td>
<td>1600</td>
</tr>
<tr>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td>646</td>
<td>1685</td>
</tr>
<tr>
<td>Ligand</td>
<td>1590</td>
</tr>
<tr>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td>575</td>
<td>1700</td>
</tr>
<tr>
<td>Ligand</td>
<td>1605</td>
</tr>
</tbody>
</table>

Table 4.9.

SELECTED I.R.ABSORPTIONS
Since copper(II) has d⁹ electronic configuration, it is paramagnetic and the magnetic properties is a feature of all copper(II) compounds, as is the colour—they are virtually all blue or green.

The magnetic properties of these complexes were measured on a Gouy balance and are listed in Table 4.10.

<table>
<thead>
<tr>
<th>LIGAND</th>
<th>MAGNETIC MOMENT (B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>1.67 ( \neq n\leq 1 )</td>
</tr>
<tr>
<td>545</td>
<td>1.61 ( \neq n\leq 1 )</td>
</tr>
<tr>
<td>575</td>
<td>diamagnetic</td>
</tr>
<tr>
<td>656</td>
<td>diamagnetic</td>
</tr>
<tr>
<td>646</td>
<td>1.35 ( \neq n\leq 1 )</td>
</tr>
<tr>
<td>666</td>
<td>0.61 ( \neq n\geq 0 )</td>
</tr>
</tbody>
</table>

Table 4.10.

MAGNETIC MOMENTS OF THE COPPER COMPLEXES

For a simple monomeric copper(II) complex the magnetic moment (\( \mu_{eff} \)) lies within the range 1.75-2.20 BM. However, the low symmetry of the environment that is characteristic of a Cu²⁺ ion often results in a lower value than anticipated. If a binuclear complex is formed, which is less common in copper(II) than in copper(I), then the paramagnetic effect can be virtually nil. There is an intermediate state where binuclear complexes are formed without a formal Cu-Cu bond and weak coupling of the unpaired electrons result in a \( \mu_{eff} \) in the range of 1.3-1.7 BM. The magnetic moments for these complexes indicate that the 555 and 545 ligands both form monomeric species although the values of \( \mu_{eff} \) do imply a low symmetry of the copper(II) ion. The 646 ligand has a magnetic
moment that suggests a binuclear species with weak electron coupling, the value of \( \mu_{\text{eff}} \) lying well within the range. The 656 and 575 ligands form complexes that are diamagnetic and therefore are dimers exhibiting strong coupling between the unpaired electrons. It is possible that in the 656 complex, dimerisation has occurred through a Cu-O bridge similar to copper(II) acetate whilst with the 575 ligand, where there is evidence of coordination through the sulphur donors, the methylene backbone (CH₂)₄ is sufficiently long to promote coordination between two copper centres. The infra red absorption assigned to this methylene chain is noted to shift very slightly from 800 to 790 cm⁻¹. It appears that the coordination occurs primarily through the carboxylate group with secondary coordination through the thioether groups if the ligand possesses the HO₂C-CH₂-S- moiety.
A hexadentate ligand which was known to react with first row transition metals to give 1:1 metal:ligand complexes was synthesised, initially by the method of Black and McClean and later supplied by Lancaster Synthesis plc. This ligand was chosen as the methylene framework was large enough to allow encapsulation of the technetium metal ion, occupying six coordination sites. Assuming that the resulting complex would have an octahedral arrangement of donor atoms then since the macrocycle confers no charge the overall charge of the complex will reflect the technetium oxidation state. However, a seven coordinated metal ion c.f. \([\text{TcO(EDTA)}]^-\) would allow the formation of the typical technetium(V)oxo core. The thioether group lies between amino nitrogens and tertiary phosphines or arsines in its \(\pi\) acceptor capacity. Since it is known that the technetium cations, of the general formula \(\text{TcO}_L\text{L}^+\) (where \(L=\text{amine or PR}_3\)) containing the former do not show even transient myocardial uptake whilst the latter containing complexes do even if only in some species the study of analogous thioether containing complexes is of interest.

The reaction of nickel(II) picrate and cobalt(II) picrate in ethanol with 1,4,10,13 tetrathia 7,16 diazacyclooctadecane (S₄N₁₀₋₁₈) gave \((\text{CoL})(\text{picrate})_2\cdot\text{H}_2\text{O}\) and \((\text{NiL})(\text{picrate})_2\) with the metal centre having...
Figure 5.1.: The $\text{S}_4\text{M}_2$-18 macrocycle
octahedral geometry with the ligand taking all six coordination sites.

Two configurations are possible (Figure 5.2).

In one the four sulphur atoms occupy the equatorial plane with the secondary amine groups mutually trans. In the other the SNS donor atom sequences lie in the same plane of the coordination sphere. The two amino nitrogens are still mutually trans but in the former the macrocycle is less strained and is thus the one favourably produced.

The reactions of macrocyclic ligands have principally been investigated with first row transition elements and while these do not readily translate to the larger second row elements there is sufficient evidence that heterocyclic eighteen membered ring systems will stabilise the first row transition elements to warrant further study (251).

It has been reported that the reaction of 1,4,7,10,13,16 hexathiacyclooctadecane ($S_6-18$) with either $MoO_2Cl_2$, $MoCl_5$ or $MoCl_6$ yielded a 1:2 ligand:metal complex in which the $S_6-18$ moiety acts as a bridging ligand bridging two metal centres (252).

In the one previous study of a $S_4-14$ macrocycle, 1,4,8,11 tetrathiacyclotetradecane, with $TcO_4^-$, no discrete complex was characterised or indeed even isolated in a pure form (235). As it is well established that amino nitrogens form stable complexes with technetium in a variety of complexes in different oxidation states a mixed amino thioether macrocycle, $S_4N_2-18$, was chosen for this study.

Recently, the reaction of 1,4 dithia 8,11 diazacyclotetradecane ($S_2N_2-14$) with $TcOBr_4^-$ has been reported giving a proposed 1:1 metal:ligand complex of the form $TcO_2L^+$. From the data reported absolute identification is not possible but electrophoresis indicates a cationic
**Figure 5.2.** Possible configurations of the O₆ complex with the S₄N₄-18 macrocycle
species and I.R. spectroscopy indicates a $v(Tc=O)$ vibration. Both results in this thesis and those discussed earlier suggest the complex may be the $TcO_2L_2^+$ species with bonding through the nitrogens only. This will only be resolved when an X-ray crystal structure of the complex is available (236).

5.2. The reactions of $S_{4N}-18$ at the no-carrier added [$^{99m}Tc]$ level.

5.2.1. Reactions using tin(0) and tin(II) as reductants.

The reactions were carried out by methods previously described with the ligand dissolved in a 1:1 ethanol:water mixture. When tin(II) tartrate was used as the reductant the yield of complexes containing the ligand was small, with varying amounts of $TcO_2^-$ (30-50%) being formed in the reaction mixture. This implied that the ligand was a rather weak one and possibly the kinetics of complex formation were slower than those of $TcO_4^-$. Tin foil was thus substituted for the tin(II) tartrate since the low concentration of tin(II) ions in these solutions may inhibit $TcO_2^-$ formation. Surprisingly, the chromatographic profiles of these two reactions are remarkably different. Figure 5.3. illustrates a typical paper chromatogram plot for tin(II) tartrate reduction with no free pertechnetate and 35% $TcO_2^-$. Figure 5.4. is the same using tin foil as the reductant. The paper chromatogram resembles that of $TcO_2^-$: (butan-2-one $R_f$ 0.9, saline $R_f$ 0.75, Whatmann 3MM paper). Electrophoresis, however, indicates the species is not pertechnetate, and this is borne out by animal biodistribution studies. Clearly, the complex (or complexes) formed in the latter reaction is unexpectedly different to that prepared in the former reaction. Attempts to improve the percentage of complex formation
Figure 5.3.: A typical paper chromatogram trace in MEK and saline for the no carrier added $^{99m}$Tc complexes formed with $S_{4}N_{2}-18$ using tin(II) tartrate and $S_{2}O_{4}^{2-}$ as reductants

a. tin(II) tartrate reaction

b. $S_{2}O_{4}^{2-}$

1. MEK

2. 0.9% saline
Figure 5.4.: A typical paper chromatogram trace in MEK and saline for the no carrier added $^{99m}$Tc complexes formed with $S_4N_2$-18 using tin(0) foil as reductant.

1. MEK
2. 0.9% saline
with tin(II) tartrate proved fruitless and the different behaviour between the two reductants may be due to competition between the ligand, chloride and tartrate.

To overcome these problems an alternative reductant, sodium dithionite was employed.

5.2.2. Reaction using sodium dithionite as the reductant.

A stock solution of sodium dithionite (46mM) in sodium hydroxide (40mM) was used throughout the experiments. The ligand:reductant ratio was varied by maintaining a ligand concentration of 50mM and varying the reductant concentration to ascertain optimal conditions to minimise TcO$_4^-$/$TcO_2$ formation. Figure 5.5. shows that 28mM reductant concentration is the best studied. The amount of colloidal TcO$_2$ noted in this formulation was considerably less than the tin(II) tartrate reaction. Filtering the reaction solution through a 0.22µm filter reduced even this to ~3% of the technetium present. The paper chromatogram otherwise resembles that of the tin(II) tartrate reaction. Electrophoresis of the filtered reaction solution indicates that no TcO$_4^-$ is present and the complex(es) does not appear to be cationic, remaining on the application point, spreading in the direction of the anode. Figure 5.6. is a representation of this. A sample of TcO$_2^-$ was subjected to the same experimental conditions and this is marked, as is the slight anodic shift. A pH9.2 $CO_3^{2-}/HCO_3^-$ buffer was employed at a constant voltage of 10V/cm.

Cationic complexes, which are lipophilic, sometimes do not move under electrophoresis, while the anodic drift is not unusual, and thus
Figure 5.5.: % Complexation as a function of reductant concentration for the no carrier added [\$^{99}\text{Tc}\$] complexes formed with S$_4$N$_2$-18 using sodium dithionite as reductant.
% of each complex

mM/4 dithionite

* TcO4-
* TcO2
* Complex
Figure 5.6: Electrophoretic behaviour of the no carrier added $^{99m}$Tc complexes formed with S$_4$N$_2$-18 and sodium dithionite

1. TcO$_4^-$
2. S$_4$N$_2$-18 reaction
electrophoresis was not considered to be useful in this study except to quantify TcO$_4^-$ contamination.

To establish the charge on the complex(es) cation and anion exchange resins were used. The reaction was monitored prior to ion exchange to confirm complexation was complete. Recharged anion exchange resin (Cl$^-$) and cation exchange resin (Na$^+$) were placed into the reaction vials, subsequently resealed and gently agitated for five minutes to ensure maximum surface contact of the reaction mixture with the resin. The supernatant was removed and the resin washed twice with ethanol to remove superficial radioactivity adhering to the beads. The supernatant was rechromatographed and the radioactivity content in the resins counted. With the sodium dithionite formulation, there was 65% exchange onto the cation resin beads (based upon calibration of the reaction vial before exchange and of the resin beads and total washings + supernatant), this therefore implies 35% of the reaction product that is either neutral, anionic or cationic but does not exchange. The possibility of an anionic component is ruled out as the electrophoresis did not indicate this and while it has been observed that cationic compounds do not always move under electrophoresis, this has not been shown to occur with anionic complexes. If the exchange resin were saturated, then addition of fresh resin would remove the complex in the supernatant, this did not happen, the evidence appears to indicate a neutral complex. The formulation with tin(0) foil could not be assessed in a similar fashion as the complex adhered to the resin beads, both anionic and cationic. Different resin types were tried but none seemed suitable.
5.3. High performance liquid chromatographic separation of the S=H=18 containing complexes.

A reverse phase-ODS column was chosen for this study. Using a mixed aqueous/acetonitrile eluant, the nature of the complex(es) formed with sodium dithionite, at different pH and upon aging were considered. Once again, the paper chromatography proved to be inadequate in identifying the number of species obtained in the reactions. A complicated mixture of species was eluted from the column, comprising at least three major components. Table 5.1. gives the change in $R_v$ with pH for these major species. The only significant change throughout the study was the retention of the third major species, which becomes less retained as pH decreases. There are several minor species at 3.6, 4.0, 4.5 and 6.6. Only the latter alters significantly, being eluted with an $R_v$ of 5.6 at pH 3.67.

<table>
<thead>
<tr>
<th>PEAK</th>
<th>$R_v$ at three pH values</th>
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<tr>
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<td>7.35</td>
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<td>1</td>
<td>2.6</td>
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<td>5.4</td>
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<td>'4'</td>
<td>6.6</td>
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Table 5.1.

CHANGES IN $R_v$ AS A FUNCTION OF BUFFER pH
Aging has no significant effect on the reaction solution with the relative proportions of each of the major components remaining effectively the same.

That the ligand $S_4N_4$ forms stable complexes at the no carrier added [$^{99m}$Tc] concentration level is borne out by the biological behaviour as discussed in section 5.6. It is feasible that one of the major products is the 1:1 metal:ligand complex initially desired. It is also probable that coordination through an $S_2N_2$ donor set has occurred resulting in $TcOClCl^+$, $(TcOL(OH))^+$ or $TcOCL^+$ species or even coordination through only the $N_2$ donor set giving $TcOCL^+$. This latter complex would be a large, bulky and fairly lipophilic species. The possibility of polymeric complexes is usually discounted in no carrier added formulations due to the very large metal:ligand ratios used, and can probably be ignored.

Chromatographic analysis of the tin foil preparation was not possible as it adhered to the top of the RP-ODS column and could only be removed using a weakly oxidising solution of $H_2O_2$ which decomposed the complex. Since $TcO_4^-$ is eluted from the column close to the void volume using the mobile phase employed in these experiments, it is clear that the tin foil formulation does yield discrete species.

The complexes formed with sodium dithionite are hydrophilic with octanol/saline partition coefficients of 1-9, while those formed with tin foil are more lipophilic with considerable solubility in butan-2-one. It is not known whether this difference is due to direct incorporation of tin into the technetium complex, $(Tc\{DMG\}_2SnCl_3(OH))$ or due to a different oxidation state of the technetium. Many neutral complexes of
technetium are known to label blood cells and the $S_{2}N_{2}-18$ complexes formed with tin(0) are slow to clear from the blood as is detailed in 5.6.2.

5.4. The carrier added $^{99m}$Tc reactions of $S_{2}N_{2}-18$

5.4.1. Solution studies.

Initially, the reaction conditions were chosen to reflect the no carrier added preparation. However, this was unsuccessful and more stringent conditions were considered. To minimise hydrolysis of the product, dry ethanol and sodium borohydride were used. Ammonium pertechnetate (supplied in an aqueous solution) was stripped to dryness and redissolved in ethanol. All solvents were degassed and the reaction proceeded in an inert atmosphere. Despite these precautions, after an initial colouration of yellow/brown, the mixture decomposed to $^{99m}$TcTcO$_2$·$\Delta$H$_2$O. Attempts to trap the reaction by rapid cooling were unsuccessful. Sodium borohydride is too powerful a reducing agent for this system and the handling of any reaction products arising from the technetium(VII) / reduction route is apparently too difficult under the conditions explored here. Several reaction precursors were synthesised in order to study ligand exchange behaviour. $[^{99m}$TcTc(tu)$_2$]$Cl_3$; $^{1}{\text{Bu}}_4$NTcOCl$_4$; $K_2$TcCl$_6$ and TcCl$_4$(PPh$_3$)$_2$; (technetium(III), (V), (IV) and (IV) respectively,) were all prepared by standard literature methods. A number of reactions were carried out using different solvents, CH$_2$Cl$_2$, THF, ethanol and chlorobutane. It was noticed that a solution of $^{99m}$Tc TcCl$_4$(PPh$_3$)$_2$ in THF gradually changed, on standing at room temperature, from green to colourless, presumably forming a TcX$_{6-\eta}$(THF)$_\eta$ intermediate
of unknown oxidation state and composition, \(X=\text{Cl}/\text{PPh}_3\). To minimise the loss of \(\text{PPh}_3\) from the complex and encourage ligand exchange with the chloride ligands, excess \(\text{PPh}_3\) was added to the reaction solution. Immediately upon addition of the macrocycle, a purple colour is observed, which intensifies very quickly (\(\lambda_{\text{max}}: 475\text{nm}\)). This reaction is immediate, even at 0°C. Filtration of this purple solution gave a tarry precipitate that decomposed on the filter frit and a purple supernatant that rapidly changed colour to brown. No reaction product was isolated. This purple complex was frequently seen irrespective of the reaction precursor and it is assumed therefore that it is caused directly by a reaction with \(\text{S}_4\text{N}_4^-\text{-18}\), although the technetium intermediate responsible was never identified.

Qualitatively, to assess potential colour changes in these systems, a solution of \(^{99}\text{Tc}\text{[TcCl}_4(\text{PPh}_3)_2}\) in THF was added, separately, to butylsulphide and heptylamine. In the first instance, the solution turned green with a clear red reflex, in the latter case, the solution turned purple. Although no absolute conclusions could be drawn from such observations, it suggested that the main coordination power of the \(\text{S}_4\text{N}_4^-\text{-18}\) macrocycle appears to be through the amino groups.

When the hexachlorotechnetate(IV) and oxotetrachlorotechnetium(V) were the precursors for the reaction, the solution turned a deep brown, but when diluted, appeared to have a strong red reflex. No stable products were isolatable however and the reaction did not remain stable in solution. \(^{99}\text{Tc}\text{[Tc(tu)_6Cl}_3\) showed no visible signs of a reaction which may indicate that technetium(III) is too low an oxidation state for this ligand.
That the S₄N₃⁻₁₈ macrocycle will coordinate with technetium has been demonstrated at different oxidation states, technetium(IV), (V) and (VII). However, the reaction is unlikely to be the formation of a 1:1 complex as had been hoped, due to extreme difficulty in isolating any stable products. The correct conditions for this reaction have not been achieved and difficulty will arise from the fact that the ligand may act as a uni- to hexa- dentate ligand, which may give rise to a number of different reaction products. What seems most probable is that the coordination is largely occurring through an NN set, similar to ethylenediamine, but the bulk of the ligand makes this a difficult complex to crystallise.

5.5. The no carrier added [⁷⁹mTc] reactions of the derivatised S₄N₃⁻₁₈ ligand.

5.5.1. Introduction.

The purpose of derivatisation was two-fold. Firstly, to alter the biological behaviour of the technetium complex and to attempt to target the molecule in the body by judicious choice of derivative, secondly, to assess the strength of the thioether bond if the amino groups are no longer contributing to the bonding in the complex. It was not the intention to doubly derivatisate at both amine groups, the reaction conditions were not found where single derivatisation preferentially occurred. A technetium complex incorporating the sulphonamide group has been reported as a red cell labelling agent(253) whilst the long chain fatty acid is well known for its myocardium uptake(254).
5.5.2. Experimental methods and results.

A number of different methods were tried which have not been discussed in chapter two. Therefore, these will be detailed here. The inherent insolubility of the ligands, particularly the sulphonamide derivative, made standard reaction conditions unsuitable for these ligands. The normal ligand concentration (50mM) was considered too strong and therefore 5mM was the target. Several different solvents were tried, methanol, ethanol and isopropanol; DMSO and DMF. In all cases the ligand was heated in the solvent and placed for an hour in an ultrasound bath. The resulting suspension was then used, on the assumption that some of the ligand was indeed in solution. Standard reaction techniques were then employed, using tin(II) tartrate, sodium dithionite and tin(0) foil as reductants, with paper chromatography for analysis of any complex formed. None was observed.

A dual phase labelling was attempted using a screw-top Soveril tube. The ligand was suspended in CH₂Cl₂, having some solubility in this solvent, while aqueous pertechnetate was layered above. In this case a reductant was used that would have contact with both phases. Tin wire, cut to a suitable length for the tube, was prepared in a manner similar to the foil discs. The tube was gently agitated for 30 minutes and both layers chromatographed. Only [⁹⁹m⁸⁻Tc]TcO₂⁻ and TcO₂.xH₂O were found.

The aqueous pertechnetate was stripped to dryness and a solution of the ligand in CH₂Cl₂ was added. Sodium borohydride was used as the reductant but only [⁹⁹m⁸⁻Tc]TcO₂.xH₂O was formed.

Finally, Tween 80 (surfactant) was used as a solubilising aid. 1ml of Tween and 10mg ligand were warmed together and diluted with 5ml.
ethanol. Both sodium dithionite and tin(II) tartrate were used as reductants. The use of Tween 80 has been documented before (246a) as an effective method of solubilising certain ligands, however, in this case, there was none of the desired reaction product formed.

The inability to prepare a technetium complex of these derivatives is taken as an indication of the important role played by the amino groups in the complexation. Had a single derivative been prepared, then it is possible that some complexation would have occurred.

5.6. Biodistribution studies of the no carrier added \[^{99m}Tc\] complexes of S4N3-18

5.6.1. Complexes of S4N3-18 formed with sodium dithionite.

The complexes were prepared as previously described and the radiochemical purity of the solution checked by paper chromatography. Protein binding of this preparation to human serum albumin was assessed by the protocol devised at Amersham International plc. using HPLC and a UV detector. The protein binding, by this method was 5%.

This study was performed in two parts. In the first instance, a 2 minute heart/brain dissection was performed, where the partial dissection data, given in Figure 5.7, shows a heart/blood ratio of 0.17 and a brain/blood ratio of 0.07. The absence of any significant brain uptake is an indication that the charge on these complexes is not neutral. The two hour dissection pictures, Figure 5.8, show mixed hepatobiliary and renal clearance with about 25% of the dose present in the bladder at the end of the study. The blood clearance confirms the protein bound findings with only 2% remaining in the blood at the end of two hours. There is nothing
Figure 5.7.: Heart/brain 2 minute dissection data on rats of the no carrier added $^{99m}$Tc complexes formed with S$_4$N$_2$-18 and sodium dithionite.
Brain 0.26

15.87 Blood

0.24 Heart

10.23 Liver

1.14 Lung

2.18 Tail

Carcass 71.94
Figure 5.8: 120 minutes biodistribution data on rats and static scintiphotos of the no carrier added [\(^{99m}\)Tc] complexes formed with S\(_2\)N\(_2\)-18 and sodium dithionite

1. 0 minutes
2. 10 minutes
3. 20 minutes
4. 30 minutes
5. 40 minutes
6. 60 minutes
7. 120 minutes
significant about the biological handling of this preparation as it is known that the solution does contain a number of complexes of unknown composition and charge and hence a mixed clearance pattern would be expected.

5.6.2. Complexes of $S_{4}N_{2}$-18 formed with tin(0) foil.

Protein binding assessment for this preparation could not be done by the same method as in 5.6.1., as it was not possible to elute the products from a column. Hence, trichloroacetic acid precipitation was substituted and it was found to be 30%. The rats were viewed on the γ camera for one hour and then sacrificed. At the end of this hour, there was considerable activity associated with the carcass (>25%), high muscle uptake (>13%) and 10% still present in the blood. The main excretory route from the body is through the hepatobiliary system, with considerably less renal clearance than was observed with the sodium dithionite preparation (Figure 5.9.). A two minute heart/brain dissection was not performed as the blood clearance was considered too slow to make it meaningful. As the subsequent biodistribution did not indicate any interesting organ uptake this decision seemed reasonable.

5.6.3. Conclusions.

The biological behaviour of the $S_{4}N_{2}$-18 complexes show no evidence of either cationic or neutral compounds. This, however, confirms that not all cationic species are taken up into the myocardium tissue nor every neutral complex will cross the blood brain barrier and be retained. The assorted complexes formed by $S_{4}N_{2}$-18 with technetium will be cationic or
Figure 5.2: 60 minutes biodistribution data on rats and static scintiphotos of the no carrier added \(^{99m}\text{Tc}\) complexes formed with \(S_2\text{N}_2\text{-18}\) and tin(0) foil

1. 0 minutes
2. 15 minutes
3. 30 minutes
4. 60 minutes
15.91 Liver
B/U 24.33
6.07 Kidneys
9.79 Blood
12.85 Muscle
S.I./L.I. 16.48
25.00 Carcass
neutral in charge, but share with $[^{99m}Tc]HTcO_2(\text{en})_2^+$, a cationic complex, no myocardium imaging (39) and the tris acac complex, $[^{99m}Tc]Tc(acac)_3$, a neutral complex (30) which has not been reported for brain imaging. The biodistributions observed here are representative of solutions that contain more than one radiochemical entity with different modes of excretion.
CHAPTER SIX
TECHNETIUM NITROSYL ISONITRILE COMPLEXES

6.1. Introduction

As noted in Chapter 1 there is considerable interest in the chemistry of low valent technetium complexes as potential myocardial imaging agents. Probably the most advanced clinically of these is the series of complexes of the general formula \([\text{Tc}(\text{RNC})_6]^{+}\) developed by Davison, Jones and co-workers of which the parent compound was that with \(R=\text{tertiary butyl}(198)\). The second and third generation of reagents have overcome many of the high lung and liver uptake problems associated with this reagent(255, 256) although it is doubtful if even the most probable of the complexes, \([\text{Tc}(\text{MIBI})_6]^{+}\) (MIBI=methoxyisobutyl isonitrile), which will go into routine clinical use is the ideal reagent. All these derivatives have relied upon altering all six of the ligands together to affect the biological distribution. In this study a different route was taken by seeking to exchange one or more of the isonitrile ligands by other \(\pi\) acceptor ligands, in particular the NO\(^+\) ligand. Other workers have considered the mixed isonitrile-tertiary phosphinetechnetium(I) complexes, \(\left[\text{Tc}^{3+}\text{Tc}(\text{CNR})_2(\text{P-P})_2\right]^{+}\) (P-P=ditertiaryphosphine), and found them to be good myocardial imaging agents in animal models but again extensive protein binding in human results in a blood pool imaging agent(94). This is another marked example of human plasma acting differently from that of other species.
The NO ligand was chosen as a suitable ligand since, as noted in chapter 1 it readily forms technetium complexes and is additionally of interest since it confers an extra positive charge on the complex.

6.2. Chemical studies of the reactions of NO with $^{99m}\text{Tc}[\text{Tc(RNC)}_6]^+$

The synthesis of complexes of the general formula $^{99m}\text{Tc}[\text{Tc(RNC)}_6]^+$ is well established and possible by a number of routes. The simplest method was found to be the direct reduction of TcO$_4^-$ by sodium dithionite in the presence of the free ligand. Heating for 15 minutes in a boiling water bath gave an essentially quantitative yield of the desired complex. The reaction course was routinely monitored using the paper chromatography system described in chapter 2. This proved more convenient than the HPLC system for quick analysis of the reaction course. The reactions were carried out by either bubbling through NO gas, generated from the reaction of copper and nitric acid, from a purchased NO gas cylinder, or indeed by adding nitric acid to the solution. Although the latter gives the same reactions the NO gas route was found to be the easier to control to give reproducible results.

The reaction was found to give different products depending upon the temperature of the reaction and the reaction time. Three basic reaction temperatures 0°C (or room temperature=18°C), 50-60°C and 100°C. Each will be considered in turn.

Bubbling NO through a solution of $[\text{Tc(BuNC)}_6]^+$ at room temperature resulted in a slow reaction during which the paper chromatography profile changed from that typical of the starting material $R_r=0.9$ (MEK), $R_r=0$ (saline) until all the $[\text{Tc(BuNC)}_6]^+$ had reacted. The lack of any
activity with an $R_v = 0.9$ (MEK) reflects this. To reach this stage took between 25 and 50 minutes and the chromatogram had a radioactivity profile as shown in Figure 6.1. However, the paper chromatogram is clearly a mixture of a number of species and thus reverse phase HPLC studies using a Hamilton PRP-1 column and 50% CH$_3$CN/50% 0.1 M NH$_4$O$_2$CCH$_3$ as the mobile phase were undertaken on the reaction solution. Surprisingly, from this (Figure 6.2.) it appears that there is mainly only one complex formed, that with a $R_v = 2.64$ ml., with smaller amounts of a less lipophilic species with an $R_v = 1.52$ ml. also present. This reaction solution is not, however, stable over long time periods and the paper chromatographic profile changes to that in Figure 6.3. with a MEK soluble species being formed. The HPLC showed that the major species was now a complex with an $R_v = 4.32$ ml. with smaller amounts of a complex with $R_v = 6.0$ ml. (Figure 6.4.). When the intermediate reaction solution with the $R_v = 2.64$ ml. species as the major component was heated to 100°C for 15 minutes a drastic change in the composition of the solution occurred. The paper chromatogram is not noticeably different to the aged room temperature sample (Figure 6.5.) but HPLC shows that the solution has an approximately equal mixture of two complexes, the initial $R_v = 2.64$ ml. species and the $R_v = 1.52$ ml. (Figure 6.6.).

Secondly, since the reaction of $[^{99m}\text{Tc}][\text{Tc}(-\text{BuNC})_2]^+$ with NO appeared to be complicated and seemed to be preparing three different species the reaction was carried out by passing NO through a solution of the starting material heated at 100°C. Within 10 minutes the reaction seems to have progressed so that all the $[\text{Tc}(-\text{BuNC})_2]^+$ has reacted but the paper chromatogram resembled that of $\text{TcO}_4^-$ (Figure 6.7.).
Figure 6.2.: HPLC of the reaction solution produced after 25-50 minute bubbling of NO at room temperature
Hamilton PRP-1 column
Eluant 50%CH₃CN/50%NH₄OAc, Flow 2.2 ml./minute
1. Rₜ=1.52 ml.
2. Rₜ=2.64 ml.

Figure 6.1.: Paper chromatographs of the reaction solution produced as above
1. MEK
2. 0.9% saline
Figure 6.3: Paper chromatographs of the aged room temperature reaction solution
1. MEK
2. 0.9% saline

Figure 6.4: HPLC of the aged room temperature reaction solution
Conditions as 6.2. except
flow 1.1 ml./minute
1. Rv=1.54 ml.
2. Rv=2.64 ml.
3. Rv=4.32 ml.
4. Rv=6.00 ml.
Figure 6.5.: Paper chromatographs of the reaction solution from 6.1. heated at 100°C for 15 minutes
1. MEK
2. 0.9% saline

Figure 6.6.: HPLC of reaction solution described in 6.5.
Conditions as 6.2. except flow 2.0 ml./minute
1. Rv=1.52 ml.
2. Rv=2.64 ml.
Figure 6.7.: Paper chromatographs of reaction solution produced by passing NO through the reaction 100°C for 10 minutes.

1. MEK

2. 0.9% saline
Electrophoresis showed that there was no TcO$_4^-$ present and only cationic species were present (Figure 6.8.). Indeed, all the reaction solutions contained only cationic complexes, although it was extremely difficult to determine the maximum of the electrophoretic movement since the profiles were broad. HPLC, however, showed that there were two main complexes with the major one being the $R_v=1.52$ ml. species and the minor being the $R_v=2.64$ ml. complex (Figure 6.9.). It was observed that, although this was the typical product distribution there was sometimes observed a small amount of the $R_v=4.32$ ml. species.

The retention volume of the starting material with this mobile phase was considerably longer than that of these species being ca. 20 ml. TcO$_4^-$ is not retained and is eluted at the void volume of the column ($R_v=1.1$ ml.) and thus the complexes formed in the reactions were cationic but clearly of considerably lower lipophilicity than [Tc(BuNC)$_4$]$^+$. At the two reaction temperatures above, the yield of the most lipophilic complex ($R_v=4.32$ ml.) was very low, experiments were carried out to maximise the yield of this species.

It was found that if the reaction of [Tc(BuNC)$_4$]$^+$ with NO was carried out at 50-60°C a change in the paper chromatogram occurred. HPLC separated the reaction mixture into three components, all those itemised above but by far the major species is the $R_v=4.32$ ml. complex (Figure 6.10.). Heating this reaction mixture at 100°C converted all the lipophilic complex to the $R_v=1.52$ ml. complex and only a small yield of the 2.64 ml. compound (Figure 6.11.).

Thus, it was possible, by altering the reaction conditions to prepare solutions which contained almost entirely one of the three
Figure 6.8.: Electropherogram of the solution described in 6.7.

Whatmann no. 1 paper

12V/cm., PO₄³⁻ buffer, pH 7.5

2 hours

1. [Tc(BuNC)₆]⁺

2. TcO₄⁻

3. reaction solution
Figure 6.9.: HPLC of the reaction solution described in 6.7.
Conditions as 6.2. except
flow 0.8 ml./minute
1. Rv=1.52 ml.
2. Rv=2.64 ml.

Figure 6.10.: HPLC of the reaction solution produced when NO is passed through at 50°C
Conditions as 6.2.
1. Rv=1.52 ml.
2. Rv=2.64 ml.
3. Rv=4.32 ml.
Figure 6.11.: HPLC of the reaction solution
produced by heating 6.10 at 100°C for 15 minutes
Conditions as 6.2. except
flow 1.9 ml./minute
1. RV=1.52 ml.
2. RV=2.64 ml.
complexes. The individual components were isolated from the HPLC column and then re-injected onto the HPLC column. These experiments confirmed that reaction had not occurred on the column and that the products obtained from the HPLC were indeed those injected.

Additionally, by collecting the HPLC effluent and using C-18 Seppak cartridges it may be possible to inject discrete single complexes into laboratory animals.

Only in the case of the most lipophilic complex was it possible to obtain, from the HPLC fraction a sample which was suitable for intravenous injection. Adsorbing this component onto a C-18 Seppak cartridge and subsequent elution using ethanol gave a salt free solution. Evaporation of most of the ethanol and the addition of sterile isotonic saline yielded the required solution.

Although the system studied in detail was the $[^{99m}Tc]Tc\left(\text{tBuNC}\right)_6^{+}$ similar results were obtained using cyclohexyl and benzylisonitrile. These are reported where appropriate.

The no carrier added$[^{99m}Tc]$ reactions produced complexes which were all cationic and less lipophilic than the starting material. It is clear that the $R_v=1.52$ mI. is the most stable species while the $R_v=2.64$ mI. species formed at room temperature is relatively short lived and the $R_v=4.62$ mI. complex may also be converted to the least lipophilic compound.

The recent work of Linder and Davison has shown that two complexes are formed on a carrier added$[^{99m}Tc]$ scale, $\left(\text{TcNO(\text{tBuNC})}_6\right)^{2+}$ and $\left(\text{Tc(\text{tBuNC})}_6\right)^{2+}$, and it is probable that at the $^{99m}Tc$ scale these complexes are also formed. However, other studies of the reaction of
TcNOCl\(_4\) with \(\text{BuNC}\) only resulted in \([\text{TcNO}(\text{BuNC})_4\text{Cl}]^+\), even with a large excess of the isonitrile and attempts to isolate the two complexes described by Linder have proved impossible. Indeed, the synthetic details of the \([\text{Tc}(\text{BuNC})_6]^2+\) complex are not readily available. Since the reaction at room temperature is relatively mild, and the complex \([\text{Tc}(\text{BuNC})_6]^+\) is rather inert to ligand substitution the initial product is probably \([\text{Tc}(\text{BuNC})_6]^2+\). As a technetium(II) complex this is a more labile species and ready ligand replacement may occur. Since the final product under forcing conditions at the carrier added concentration is \([\text{TcNO}(\text{BuNC})_6]^2+\), the species with \(R_v=1.52\) ml. may be this compound or a complex of the form \([\text{TcNO}(\text{BuNC})_6X]^+\), where \(X\) is either NO\(_3^-\) or NO\(_2^-\). The more lipophilic complex is probably formed by ligand substitution of \([\text{Tc}(\text{BuNC})_6]^2+\). Since the complex is more lipophilic than the other complexes formed and it is readily converted at higher temperatures this may be \([\text{Tc}(\text{BuNC})_6X]^+\). A complex of this form would be more lipophilic than the other two, being of lower charge but lacking only one isonitrile ligand but this would make it less lipophilic than \([\text{Tc}(\text{BuNC})_6]^+\). The very low yield complex with \(R_v=6.0\) ml. may have a different \(X\) ligand, which changes the retention time slightly. Equally, comparing the \([\text{Tc}(\text{BuNC})_6]^2+\) and \([\text{TcNO}(\text{BuNC})_6]^2+\) complexes it may be supposed that the latter will be more lipophilic than the former. If one of the isonitriles of the latter is replaced by a NO\(_3^-\) or NO\(_2^-\) ligand the loss of the extra isonitrile may more than counteract the extra charge.
6.3. **Biological distribution studies.**

The chemical studies described above showed that three new technetium-tertiary butylisonitrile containing complexes were formed in the reactions with NO, or HNO₃ and thus biological studies were carried out at Northwick Park Hospital to determine the biodistribution of these complexes.

Initially, since the reaction could be made to proceed such that predominantly one complex was formed, studies on rats were carried out using the three reaction mixtures 0°C, 50°C and 100°C.

The crude 50°C product was injected into the tail vein and γ camera pictures taken over the first 30 minutes post injection. These images obtained after 2, 5, 8, 11, 15, 20 and 28 minutes are shown in Figure 6.12. After 30 minutes further static pictures (Figure 6.13.) were obtained, analysis of the pictures reveals that the complex mixture passes rapidly from the blood and accumulates in the kidneys and bladder. The enhanced static picture shows some residual activity in the myocardial region, more than merely blood pool.

The reaction mixture from the 100°C reaction containing predominantly the Rᵥ=1.52 ml complex has a radically different biological distribution. The dynamic pictures up to 20 minutes only, were collected (Figure 6.14.). As might be expected this complex appears to be highly retained in the liver, with some renal clearance, but little hepatobiliary clearance.

The 0°C reaction mixture, the 30 minute static of which is shown in Figure 6.15. shows no liver activity and considerable hepatobiliary clearance.
Figure 6.12.: Scintiphotographs of the crude 50°C reaction product in a rat Images acquired at 2, 5, 8, 11, 15, 20 and 28 minutes as shown.
8. static 30 minutes p.i.
Figure 6.13.: Reframe of 8 in 6.12
Figure 6.14.: Scintiphotographs of the crude 100°C reaction product in a rat. Images acquired at 1, 5, 10 and 20 minutes as shown.
Figure 6.15.: Scintiphotos of the crude 0°C reaction product in a rat

Image acquired at 30 minutes p.i.
However, since the 50°C reaction appeared to produce the most lipophilic compound and also because this was the only complex which could be satisfactorily separated and synthesised in a sterile form, it was decided to concentrate on these reaction conditions. A sterile formulation of the pure R_v=4.62 ml. complex was prepared and injected into a normal human volunteer. The result of this study is shown in Figure 6.16. The dynamic pictures were again obtained over a 30 minute period post injection with images obtained at 1, 3, 6, 11, 18 and 30 minutes post injection. A static image with enhancement was taken approximately 10 minutes later. The results show initial blood pool within the heart with exceedingly rapid clearance through the liver into the gall bladder of the subject, where it remained for a prolonged period. Rats do not, of course, possess gall bladders so this could not have been predicted from the animal study. Additionally, the complex gives a very good gall bladder image, with virtually no liver retention. If this complex could be formulated in a better manner then it may be useful clinically.

However, the complex did not show myocardial uptake and so two other isonitrile complexes were formulated, the cyclohexylisonitrile containing complex, the R_v of which was 6.1 ml. and the more lipophilic benzylisonitrile containing species (R_v=7.5 ml.). The cyclohexylisonitrile complex was formulated using NO, while the benzylisonitrile was formulated by the alternative reaction using HNO_3. In both cases the complexes were purified using the Seppak technique. The cyclohexylisonitrile complex whose dynamic image is shown in Figure 6.17, shows wholly rapid hepatobiliary clearance but no myocardial uptake.
Figure 6.16.: Scintiphotos of pure $R_\omega=4.52$ ml.
complex in a human volunteer (NPH)
Images acquired at 1, 2, 3, 6, 11, 18 and 30 minutes p.i.
8. static acquired at 40 minutes p.i.
Figure 6.17: Scintiphotographs of the pure lipophilic complex formed from the cyclohexylisonitrile analogue reaction at 50°C(HNO₃) in a rat. Images acquired at 6, 20 and 30 minutes p.i. 4. static acquired at 45 minutes p.i.
However the benzylisonitrile complex (Figure 6.18.) was rather more interesting with some evidence of hepatobiliary clearance and myocardial uptake, although the latter does appear to wash out. Thus the latter was injected into the same human volunteer (Figure 6.19.). A dynamic view of the myocardial area reveals only a blood pool image which when a whole body image was taken 75 minutes post injection showed that the complex is retained in the blood and is clearing slowly through the hepatobiliary system, with no kidney uptake.

6.4. Conclusions

The complex \[^{99m}Tc(Tc('BuNC)_6]\] has a biological distribution which consists of rapid liver, lung and myocardium uptake with the myocardium being visualised only after the lungs have cleared. The reaction of this species with NO radically changes the biological distribution and all three products show little or no myocardial uptake. Since the products are all much less lipophilic than the starting material this may not be too surprising. Attempts to produce more lipophilic complexes were successful but possibly they were still not lipophilic enough for myocardial uptake. The gall bladder imaging complex may be useful for jaundiced patients or those with gall stones but the preparation needs to be modified dramatically before routine use would be possible.
Figure 6.18.: Scintiphotographs of the pure lipophilic complex formed from the benzylisonitrile analogue reaction at 50°C in a rat. Images acquired at 1, 10 and 28 minutes. 4. liver and heart clearance curves.
Figure 6.19.: Scintiphotographs of the complex described in 6.18. in a human volunteer (NPH) Images acquired at 10, 15 and 25 minutes.
4. static acquired at 30 minutes p.i.
5. whole body distribution
CHAPTER SEVEN

TECHNETIUM COMPLEXES OF HM-PnAO [Pn26]

7.1. Introduction

The technetium complex of HM-PnAO is currently marketed as a brain imaging agent under the Amersham trademark 'Ceretec'. In this chapter it will be referred to by its Amersham code of Pn26, being the twenty-sixth derivative of PnAO synthesised and studied. Some of the results related in this chapter have been taken from project reports supplied by Amersham, which originate from both Amersham and the University of Missouri, which is acknowledged in the text.

[^59mTc]TcPn26 was shown to be a mixture of complexes, only one of which is the brain imaging agent(143). This study was intended to contribute to the information being gathered on the identity of these complexes. Since this chapter contains a comparison of results drawn from internal Amersham International reports it is treated in a different manner from the remainder of the thesis and the experimental procedures are included within the chapter rather than chapter two.

7.2. Experimental methods.


Pn26 itself is known to have two diastereoisomeric structures, meso and d,l(Figure 7.1.). At all times in this study, the d,l form was used.

The reaction of [^59mTc]TcO₄⁻ with Pn26 was carried out as a modification of the method described by Jurrison et al.(Missouri).
Figure 7.1: The diastereoisomers of $d,l$ and meso HXPAQ.
(reproduced from reference 143)
2.2mg (8x10^{-6} moles) Pn26 was dissolved in 1ml saline, with the addition of sufficient 5M HCl to ensure complete dissolution. The pH of this solution was ca.4. To this solution was added 1.4mg (8x10^{-6} moles) [\textsuperscript{99m}Tc]NH4TcO\textsubscript{4} in saline (1ml), spiked with 94 MBq [\textsuperscript{99m}Tc]TcO\textsubscript{4}\textsuperscript{-}, with stirring and the solution was made alkaline to pH9 with 1M NaHCO\textsubscript{3}. Tin (II) tartrate was suspended in saline (1.5ml) and then added slowly, continually stirring. The reaction solution was then left at room temperature for twenty minutes. At the end of this time, the solution had turned a brown-yellow colour, diethylether (25ml) was added to the reaction mixture and the organic phase turned bright yellow. This layer was separated from the aqueous phase, now a red-brown colour, which was further extracted with two 15ml aliquots of ether, yielding paler yellow solutions, the three extracts were combined and evaporated under a stream of N\textsubscript{2}. A red crystalline compound formed around the edge of the vessel, with yellow crystals at the base of the flask. Both type of crystals, on dissolution in methanol, gave an orange-red solution of similar appearance. The aqueous layer was further extracted, with CH\textsubscript{2}Cl\textsubscript{2} (2x10ml aliquots) and the two phases separated. The organic phase was yellow, the aqueous phase was red-pink. If an ether layer is washed with dilute HCl, then the colour is discharged and a pink-red aqueous layer is seen. Neutralisation with NaHCO\textsubscript{3} to pH8.5 did not restore the yellow colour to the organic layer.

7.2.2. No carrier added [\textsuperscript{99m}Tc] experiments of Pn26.

Two different formulations were carried out, based on the Amersham and Missouri reports.
7.2.2.1. 0.5ml. of a solution of Pn26 (1mg/ml) in water was mixed with 0.2ml. of a tin(II) solution, prepared by dissolving tin(II) chloride hydrate (38mg.) in one litre of N₂ purged water. 1ml. of generator eluate was added and the final pH of this solution was generally 8.5-9.0. The reaction mixture was left at room temperature for thirty minutes.

7.2.2.2. 1ml. of a solution of Pn26 (1x10⁻⁷M in saline) was added to 1ml. 0.1M NaHCO₃, 3ml. of saline and 1ml. of generator eluate. To this mixture was added 0.2ml. of a saturated solution of tin(II) tartrate in saline. The solution was left to stand for fifteen minutes at room temperature.

7.2.3. Analysis of the complexes formed at carrier and no carrier added level.

HPLC analysis was done as described in chapter two using a Hamilton PRP-1 15cm. reverse phase column with an isocratic mobile phase of 25%THF/75% 2%NH₄ coupled to a Radiomatic β-flow detector. ITLC-SG and paper chromatograms were run in the normal fashion, using butan-2-one and saline(ITLC-SG) or butan-2-one,saline,40% MeOH and 50% acetonitrile/water(Whatmann no.1). The Rₚ values of the no carrier added preparations are given in Table 7.1. The most noticeable difference is the absence of butan-2-one soluble species in the second preparation. Since this is the Missouri preparation and corroborates their results, it is probable that this difference is due to the high chloride concentration in the formulation. Therefore, it is suggested that the complex prepared by 7.2.2.2. is a chloro derivative or oxochloro derivative of [³⁹TcI]Tc(Pn26).
Table 7.1.

R_v VALUES OF NO CARRIER ADDED \([^{99m}Tc]\) PREPARATIONS

The R_v values for the carrier added preparations are given in Table 7.2. There is no distinct correlation between these and the no carrier added R_v values, therefore it appears that the lipophilic species extracted into ether is not the same as the lipophilic radiopharmaceutical. It is reasonable to assume that the yellow complex extracted into ether and dichloromethane is the \([^{99m}Tc]technetium(V)\) complex \(TcO(Pn26)\), similar to the technetium complex reported by the Missouri group\(^{146}\) of \(PnAO-3H\). The aqueous phase shows some evidence of pertechnetate, this had been noted during the course of the reaction and, in fact, under the conditions of Missouri, the reaction does not go to completion. Consequently, additional ligand, reductant and \(NaHCO_3\) were added to increase the yield of desired product. The aqueous phase also shows evidence of a mixture of components which include \([^{99m}Tc]TcO_2.xH_2O\), however, that may be filtered to leave a clear pink-red solution that probably includes the chloro species, \(Tc(Pn26)Cl_2\)^+ and other hydrophilic complexes. The activity that remains on the base-line in all three solvents is not
necessarily colloid as the filtered solution also shows the same activity.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>TcO₄⁻</th>
<th>Ether</th>
<th>CH₂Cl₂</th>
<th>Aqueous</th>
<th>Ether/MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEK</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0;80%</td>
<td>0;85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1;20%</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5;50%</td>
<td>0;70%</td>
<td>0;50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0;27%</td>
<td>1;9%</td>
<td>1;30%</td>
</tr>
<tr>
<td>50%CH₂CN</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0;45%</td>
<td>0.9;65%</td>
</tr>
<tr>
<td>/H₂O(Whatmann no.1)</td>
<td></td>
<td></td>
<td></td>
<td>1;45%</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2.

Rₜ VALUES OF THE CARRIER ADDED [⁹⁹Tc] PREPARATION

These Rₜ's are in fact similar to those reported by Missouri in the no carrier added [⁹⁹Tc] formulations. The dissolution of the ether extract into methanol gives a third dimension to this problem. Although the profile in saline is not clean, there is no apparent decomposition to pertechnetate or [⁹⁹Tc]TcO₂ₓ.nH₂O and a more hydrophilic species is in evidence here. The HPLC results are shown in Figure 7.2. The yellow ether extract, assumed to be TcO(Pn26), has a relatively short retention time on the column, eluting in a volume of 5.6ml. For a lipophilic species, on a reverse phase column, this was surprising and again confirmed the difference between the carrier and no carrier added preparations, since in the latter case, the lipophilic complex is reportedly eluted off a similar column, but with a different buffer, in 24ml. Obviously, no direct comparison can be made between the two systems but the retention volume of 5.6ml is low, particularly as pertechnetate elutes in 2.6ml and the aqueous extract shows poorly retained and resolved species eluting in a volume of 2.4-4.0ml. It was
Figure 7.2: HPLC behaviour of the carrier added $^{99m}$Tc complexes of HMPnAO

Hamilton PRP-1 column

Eluant 25%THF/75% 2%$\text{NH}_3$ solution

Flow 1 ml./minute

1. aqueous extract $R_v=2.6$ ml.
2. ether extract $R_v=5.6$ ml.
found however that the aged complex altered in elution characteristics although not in a visible way, giving two compounds, one more hydrophilic, $R_v=3.4\text{ml}$. the other more lipophilic, $R_v=17\text{ml}$. A similar elution profile is also seen in the methanolic solution of the ether extract. If the ether extract is allowed to evaporate in air and is redissolved in dichloromethane, then the ensuing red-brown solution has a similar ITLC profile to the Missouri no carrier added $^{99m}\text{Tc}$ formulation. It is possible that the $\text{TcO(Pn26)}$ complex has coordinated a Cl$^-$ from the solvent.

Unfortunately, the $^{99m}\text{Tc}$ complexes were not subjected to HPLC analysis under identical conditions to the carrier added $^{99m}\text{Tc}$ solutions. This means that direct comparison between the eluted compounds is not possible. However, some additional work on this was done by Amersham and these results are now summarised. It was felt that the mobile phase might be in some way affecting the nature and resolution of the complex. Therefore, $2\text{ml. of }2\% \text{ NH}_3$ were added to a preparation of $^{99m}\text{TcPn26}$ and incubated for 15 minutes. HPLC analysis was done using a Hamilton PRP-1 column with a gradient buffer system of THF/50% phosphate. The ammoniacal solution was then sampled at 15 and 90 minutes and the results are shown in Table 7.3.

Although it can be seen that the % of the lipophilic species is reduced with time, converting to other hydrophilic species, this is also the case of the normal Pn26 preparation which shows signs of degradation as early as thirty minutes post formulation. Indeed, the instructions accompanying 'Ceretec' provided to radiopharmacies stipulate the radiopharmaceutical must be used within a maximum of thirty minutes post reconstitution.
There does not appear to be any evidence that the 2% NH₃ radically affects the [⁹⁹ᵐTc]TcPn26 complex. Even when 2ml conc. NH₃ was added to the preparation and incubated for thirty minutes, which represent considerably more stringent conditions than those experienced on the HPLC column, there is still 29% of the lipophilic species remaining.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Retention Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pn 26</td>
<td>1.83 4.92 10.29</td>
</tr>
<tr>
<td>15 minutes after addition of NH₃ (2%)</td>
<td>24% 11.8% 51%</td>
</tr>
<tr>
<td>90 minutes after addition of NH₃ (2%)</td>
<td>39% 12.3% 35.9%</td>
</tr>
<tr>
<td>60 minutes after addition of c. NH₃ (2ml.)</td>
<td>39% 13% 28%</td>
</tr>
</tbody>
</table>

Table 7.3.

RETENTION TIMES OF [⁹⁹ᵐTc]TcHMPnAO INCUBATED WITH NH₃ SOLUTION

<table>
<thead>
<tr>
<th>Retention Times (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.71 4.85 10.13</td>
</tr>
<tr>
<td>Gradient Elution</td>
</tr>
<tr>
<td>22% 10% 57%</td>
</tr>
<tr>
<td>0.78 1.20 5.39</td>
</tr>
<tr>
<td>Isocratic Elution</td>
</tr>
<tr>
<td>22% 21% 56%</td>
</tr>
</tbody>
</table>

Table 7.4.

RETENTION TIMES OF [⁹⁹ᵐTc]TcHMPnAO WITH DIFFERENT ELUTION SYSTEMS

The final study involved a comparison of the Pn26 preparation under both type of HPLC conditions, i.e. the gradient and isocratic
Unfortunately, there is no data recording the length of time, if any, for equilibration of the column after the change in mobile phase. The first system used was the gradient mobile phase of THF/50% phosphate, which was then altered to the isocratic system of THF/2% NH₃. Assuming a reasonable length of time was allowed for equilibration, the nature of the radiopharmaceutical would have altered naturally through aging, if equilibration time was not given, then the mobile phase would be an unknown concentration of THF/phosphate/2% NH₃. It is also true that a lipophilic compound will elute quicker from a reverse phase column with an isocratic system of constant non-aqueous content than from a gradient system where the % organic phase is introduced over a gradient. The separated species eluted at the void volume (43%) and 57% at $R_v = 6.2\text{ml}$.

7.3. Discussion of results.

The irreversible reaction that occurs when dilute HCI is washed through the ether layer could be a protonating rearrangement of the complex or simple coordination of a chloride ion in the sixth coordinating position trans to the oxo to give the $[^{99}\text{Tc}]\text{TcOPn26Cl}^+$ ion. The $R_v$ values obtained from ITLC-SG analysis show similarities to those observed in the aqueous extract from the reaction mixture. There remains a species, obviously water soluble, that has $R_v$ values of 0.00 in saline and butan-2-one. It is interesting that the ITLC-SG data given in the 'Ceretec' insert for the secondary, degraded Pn26 complex also has $R_v$ values of 0.00 in both these solvents as does the Missouri no carrier added $[^{99}\text{Tc}]$ preparation.
The retention volume of 3.4 ml. that was observed on a few occasions is not interpreted as being the same radiochemical entity. It is likely that this merely reflects the elution of hydrophilic species from a reverse phase column.

When the carrier added \(^{99m}\text{Tc})\text{TcPn26} complex degrades, whether by aging or by chemical means, it appears to form two major species, one very lipophilic and the other very hydrophilic. The ease of decomposition of the complex suggests that there is an ether molecule loosely coordinated in the sixth coordination position, which may be replaced by stronger coordinating solvents in a suitable medium, whether \(\text{Cl}^-, \text{MeO}^-\) or \(\text{OH}^-\). However this is not a complete explanation as this degradation occurs in ether solution over a long period of time (24h). The formation of dimeric or polymeric species cannot be ruled out, particularly a \(\text{Tc}_2\text{O}_3\) configuration. To summarise, the carrier added \(^{99m}\text{Tc}\) reaction of \text{Pn26} formed a neutral, lipophilic complex which has different ITLC-SG characteristics to the no carrier added \(^{99m}\text{Tc}\) preparation. This lipophilic complex is believed to be \(^{99m}\text{Tc})\text{TcOPn26} which readily undergoes aerial or chemical degradation to a mixture of lipophilic and hydrophilic complexes. The chemical nature of the former is of great interest as it may well be similar to the lipophilic species observed at no carrier added\(^{99m}\text{Tc}\) levels which is suitable for cerebral imaging, the identity of the latter is tentatively suggested as \(^{99m}\text{Tc})\text{TcOPn26(solvent)}^+\).

7.4. The alternative Amersham views.

Since this project was completed further work has been undertaken at both the Amersham laboratories and Missouri University. The results
of these studies are confidential and therefore not accessible being of a commercially sensitive nature.

To date, there is no definite knowledge of the nature of this degraded species. Current Amersham philosophy is that the radiopharmaceutical preparation is indeed the same as the carrier added $[^{99}\text{Tc}]\text{TcOFn26}$ complex and that the two components, the hydrophilic and lipophilic species are two isomers of the same complex. Studies were done on the EE,EZ and ZZ configurations of the oxime groups in the belief that these oxime groups reorientated themselves from the EE configuration required of the complex to EZ or ZZ. The complex requires EE orientation to ensure the ring closure through hydrogen bonding. However, synthesis, separation and labelling with $[^{99}\text{Tc}]$ of the EZ configuration showed slow isomerisation to the EE complex. This indicates that the reverse reaction is unlikely to spontaneously occur (150).
CHAPTER EIGHT

FURTHER WORK

The results contained in this thesis lead to the recommendation that thioether donor ligands, despite the experiences of TDG\textsubscript{X}, are not suitable for exhaustive studies as potential new radiopharmaceuticals. The problems inherent in their carrier added \textsuperscript{99m}Tc chemistry makes the characterisation of any potential complex a difficult procedure. Since the progress of technetium chemistry and radiopharmaceutical chemistry relies upon the ability to achieve this characterisation, it leads back to a serendipity method that does not help the technetium inorganic/radiopharmaceutical chemist.

Complexes of technetium containing macrocyclic ligands, however, still have potential although the evidence to date is unfavourable for both crown ethers and thioethers and only positive for nitrogen donor cyclics such as cyclam. A suitable mixed donor heterocyclic ligand may succeed where most others have so far failed and it is with great interest in this possibility that the results of Iovanici et al. with the mixed \textsubscript{S,N} ligand are awaited.

The water-soluble complexes formed with tiron and other catecholate derivatives are a possible route for further study. The simplicity of complexation and affinity of this ligand type for technetium makes the 1,2 dihydroxybenzene moiety a useful precursor. Derivatisation of the benzene ring would lead to a number of different classes of complex limited only by the constraints of organic chemistry. In particular, it would be useful to study the
monosulphonato derivative of catechol at both carrier $^{99m}$Tc and no carrier $^{99m}$Tc added levels as the reduction in overall charge on the complex formed, assuming a TcOL₂ configuration, would make the $^{99m}$Tc chemistry more accessible. There is possibly scope here, based on the experience of $^{99m}$TcTetron, to seek the technetium radiopharmaceutical replacement for $^{123}$Iodochiippuran and supercede $^{99m}$TcToDTPA.

The isonitriles are probably the most exciting route forward. Replacement of one or more of the isonitrile ligands by other $\pi$ acceptors have been shown to radically alter the biological handling of the complex and a study to rationalise this effect with the concomitant change in the chemistry of the complex would be fascinating and of great value in the field of low oxidation state technetium complexes. The number of $\pi$ acceptors available for substitution, or indeed addition reactions if a seven coordinate complex is envisaged, is large and the technetium isonitrile compound is apparently reactive to these ligands.

Finally, in the field of radiopharmacy, all current technetium radiopharmaceuticals are prepared from simple addition of pertechnetate to a freeze-dried solution of ligand with reducing agent. It is near the time when radiopharmaceuticals will become more sophisticated in their preparation and radiopharmacists will handle more than one step reactions. This will open up the field of second generation radiopharmaceuticals where the technetium-oxo core is replaced by nitrido, sulphido, nitrosyl or even carbonyl cores and all the biological findings that accompany this.
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