

THE CHEMICAL STATE OF ^{99m}Tc IN BIOMEDICAL PRODUCTS. II. THE CHELATION OF REDUCED TECHNETIUM WITH DTPA

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Many reviews of ^{99}Tc chemistry (1) show that the IV and the VII oxidation states of technetium are the most stable in aqueous solution and that various hydrolyzed forms of the reduced state are possible, as is the case for most transition elements. Most of these data, however, have not been related to the ^{99m}Tc biomedical products because of the lack of analytical techniques suitable for measuring the oxidation state of carrier-free ^{99m}Tc . Attempts to use analytical methods which allow extrapolation from ^{99}Tc chemistry, which involves millimolar amounts, to carrier-free ^{99m}Tc , which is present in much smaller amounts, have been limited. Physical measurements involving spectroscopy and resonance techniques are difficult with carrier-free amounts. Chromatography is useful at the carrier-free level but care must be taken to exclude interference by trace impurities and air oxidation. The large number of mixed aquo-chloro species also makes it difficult to obtain straightforward data (2,3).

Earlier attempts at this laboratory (4) to elucidate the chemical state of ^{99m}Tc in biomedical products showed that technetium is indeed reduced and not present as a pertechnetate metal complex, but the precise oxidation state was not determined. This paper describes attempts to determine the precise oxidation state of ^{99m}Tc produced by various reducing agents. DTPA was used to chelate the reduced species, and chelation efficiencies were determined for various concentrations of ^{99m}Tc down to the carrier-free level. Assuming that the oxidation state of the carrier ^{99}Tc is known for a certain reducing medium, the oxidation state of the carrier-free ^{99m}Tc can be determined by extrapolation if indeed the behavior toward DTPA is identical over the complete concentration range. This requires, however, that the reactivity of DTPA be specific for a single oxida-

tion state, an assumption that we show is valid for the IV oxidation state.

METHODS AND MATERIALS

Preparation of $^{99m}\text{TcO}_4^-$. $^{99m}\text{TcO}_4^-$ was obtained by a MEK extraction process from $^{99}\text{MO}^\dagger$. The ^{99}Mo contamination was less than $0.01 \mu\text{Ci}/\text{mCi}$, and the aluminum contamination was below the detectable limit of $1 \mu\text{g}/\text{ml}$. $^{99}\text{TcO}_4^-$ was obtained from Oak Ridge National Laboratory as a solution of NH_4TcO_4 .

Technetium VII reduced by stannous ion. To 0.1–0.3 ml of TcO_4^- in saline is added an appropriate weight of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 0.1–0.5 ml of concentrated HCl with heat. The solution is stirred under nitrogen for the times stated in Table 1. An appropriate volume of DTPA is added and the solution is mixed for 5 min. The pH is raised to 5 with 3 N NaOH, 2 ml of 0.1 M KH_2PO_4 is added, and the pH is brought to 7 with additional 1 N NaOH.

Technetium VII reduced by concentrated HCl. To 0.1–0.3 ml of TcO_4^- in saline is added 10–20 ml of concentrated HCl. The solution is mixed under nitrogen at the time and temperature given in Table 2. To 0.1 ml of this solution is added 1 ml of appropriate concentration of DTPA. The pH is raised to 5 with 3 N NaOH, 2 ml of 0.1 M KH_2PO_4 is added, and the pH is brought to 7 with additional 1 N NaOH.

Technetium VII reduced by HCl-HI-KI. To 0.1–0.3 ml of TcO_4^- is added 2 ml of concentrated HCl,

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† Purchased from Union Carbide, Tuxedo, N.Y., in 3 N NaOH solution.

TABLE 1. Tc VII REDUCED BY STANNOUS ION

Final concentration in solution before analysis			(HCl)	Time* (min)	Chelate (%)	TcO ₄ ⁻ (%)	Absorbed† (%)
(Sn)	(Tc)	(DTPA)					
1 × 10 ⁻⁴	2 × 10 ⁻⁵	4 × 10 ⁻²	9	0	97	0	3
2 × 10 ⁻⁵	3 × 10 ⁻⁶	1 × 10 ⁻¹	0.8	0	95	0	5
5 × 10 ⁻⁶	CF	2 × 10 ⁻⁴	12	0	97	1	2
5 × 10 ⁻⁶	CF	4 × 10 ⁻²	12	0	98	1	1
5 × 10 ⁻⁶	CF	4 × 10 ⁻²	12	90	100	0	0
2 × 10 ⁻⁵	3 × 10 ⁻⁶	1 × 10 ⁻¹	0.8	0	95	0	5
2 × 10 ⁻⁵	3 × 10 ⁻⁶	1 × 10 ⁻¹	0.8	30	85	1	14
2 × 10 ⁻⁵	3 × 10 ⁻⁶	1 × 10 ⁻¹	0.8	120	74	3	23
8 × 10 ⁻⁶	1.7 × 10 ⁻⁶	4 × 10 ⁻²	0.1	10	71	0	29
8 × 10 ⁻⁶	1.7 × 10 ⁻⁶	4 × 10 ⁻²	0.1	60	69	0	31
8 × 10 ⁻⁶	CF	4 × 10 ⁻²	0.1	5	81	0	19
8 × 10 ⁻⁶	CF	4 × 10 ⁻²	0.1	60	77	0	23
8 × 10 ⁻⁴	1.7 × 10 ⁻⁶	4 × 10 ⁻²	0.03	10	67	1	33
8 × 10 ⁻⁴	1.7 × 10 ⁻⁶	4 × 10 ⁻²	0.03	60	64	1	36

* Time of mixing at HCl concentration shown.
† Percent of ^{99m}Tc activity not eluted from gel chromatography column with 80 ml of N₂ purged eluant.

1 ml of concentrated HI (47%), and 10 mg of solid KI. The solution is heated at 50°C for 30 min. To 0.1 ml of this solution is added 1 ml of appropriate concentration of DTPA. After mixing for 5 min the pH is raised to 7 with 1 N NaOH.

Technetium VII reduced by HCl-H₃PO₂. To 0.1–0.3 of TcO₄⁻ in saline is added 1 ml of concentrated HCl and 0.1–0.5 ml of H₃PO₂ (50%). The solution is heated under nitrogen for 1 hr at 80°C. To 0.1 ml of this solution is added 1 ml of DTPA of appropriate concentration. The pH is raised to 5 with 1 N NaOH, 2 ml of 0.1 M NaH₂PO₄ is added, and the pH is raised to 7 with 1 N NaOH.

Technetium VII reduced by ascorbic acid. To 0.1–0.3 ml of TcO₄⁻ in saline is added 1–2 ml of water followed by 0.35 ml of concentrated H₂SO₄. Then 50 mg of solid ascorbic acid is added and mixed in under nitrogen for 5 min. To this solution is added 1 ml of appropriate concentration of DTPA solution. After mixing for 5 min the pH is raised to 7 with 1 N NaOH.

Technetium VII reduced by ferrous ion. To 0.1 ml of TcO₄⁻ in saline is added 1–2 ml of water, 10 mg FeSO₄·7H₂O, and 0.35 ml concentrated H₂SO₄. After mixing for 5 min, 1 ml of 0.4 M DTPA is added. After mixing for 5 min, the pH is raised

TABLE 2. Tc VII REDUCED BY CONCENTRATED HCl

Final concentration in solution before analysis			Temperature (°C)	Time* (hr)	Chelate (%)	TcO ₄ ⁻ (%)	Absorbed† (%)
(Tc)	(DTPA)	(HCl)					
2 × 10 ⁻⁷	4 × 10 ⁻²	11.8	25	1/6	6	11	83
				1/2	4	20	76
				2	6	28	66
				3	8	30	62
				23	9	37	54
2 × 10 ⁻⁷	4 × 10 ⁻²	11.8	70	1/6	5	45	50
				1	12	60	28
				3	10	76	14
CF	4 × 10 ⁻²	11.4	25	1/6	41	8	51
				1/2	42	10	48
				2	43	22	35
				3-2/3	30	34	36
CF	4 × 10 ⁻²	12.0	95	1/6	13	63	24
				1/2	15	70	15
				2/3	14	66	20
				1-1/2	11	66	23

* Time of mixing at HCl concentration shown.
† Percent of ^{99m}Tc activity not eluted from gel chromatography column with 80 ml of N₂ purged eluant.

to 5 with 9 *N* NaOH, 2 ml of 0.1 *M* KH_2PO_4 is added, and the pH is raised to 7 with 1 *N* NaOH.

Technetium VII reduced by ferric ion and ascorbic acid. To 0.1 ml of TcO_4^- in saline is added 1–2 ml of water followed by 0.35 ml of concentrated H_2SO_4 , 9.7 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 15 mg ascorbic acid. The solution is stirred for 2 min and 1 ml of 0.4 *M* DTPA is added. The pH is raised to 4 with 9 *N* NaOH, 2 ml of 0.1 *M* KH_2PO_4 is added, and the pH is raised to 7 with 1 *N* NaOH.

Technetium VII reduced by thiocyanate. To 0.1 ml of TcO_4^- is added 4 ml of 4 *M* H_2SO_4 and 0.5 ml of 4 *M* NH_4SCN . The solution is mixed for 35–40 sec and 6 ml of acetone is added. The solution is mixed well and to 0.1 ml is added 1 ml of 0.4 *M* DTPA. The pH is raised to 5 with 9 *N* NaOH, 2 ml of 0.1 *M* KH_2PO_4 is added, and the pH is raised to 7 with 1 *N* NaOH.

Gel chromatography (5). The analytical chromatography was performed on a 0.9×35 -cm gel chromatographic column (Sephadex G25-M). The column was eluted either with nitrogen-purged saline or with 0.1 *M* KH_2PO_4 as stated in the appropriate tables. With a gel chromatographic column of this size the chelate fraction is eluted in 12–20 ml and pertechnetate is eluted in 38–46 ml. The eluted chelate fraction was recycled over an identical column with quantitative recovery in the chelate fraction. The quantitative reelution does not necessarily occur with weak chelate. The technetium adsorbed on the column represents reduced hydrolyzed technetium as shown in previous work (4). The adsorbed technetium was removed from the Sephadex G25 as pertechnetate by elution with 2 ml of 0.1% H_2O_2 solution followed by the appropriate eluant. A standard was taken to assure activity balance.

RESULTS

Table 3 lists the oxidative states indicated by ultraviolet spectroscopy for carrier technetium reduced by various agents. Our data in agreement with these published data indicate that at the carrier level technetium is reduced to the IV state to varying extents by stannous ion, HCl plus H_3PO_2 , and HCl-HI-KI. Concentrated HCl seems to produce the V state as does the thiocyanate system.

Table 2 presents data for the reduction of carrier-free and carrier technetium by stannous ion. The DTPA seems to chelate the reduced species effectively at all the technetium concentrations shown when the reactants are mixed in strong acid.

When reduction is by concentrated HCl with or without heat (Table 3) the chelation efficiency seems to be lower for all concentrations of technetium including the carrier-free level. The UV data indicate

TABLE 3. CARRIER ^{99m}Tc VII REDUCED BY VARIOUS AGENTS

Reducing agent	Literature data	Oxidation state
Stannous ion	UV spectroscopy (Ref. 11) Potentiometric titrations (Ref. 12)	IV
Concentrated HCl	UV spectroscopy (Ref. 12) Potentiometric titrations (Ref. 12)	V
Concentrated HCl and heat	As above	V
Ascorbic acid	UV spectroscopy (Ref. 13)	IV
Thiocyanate	UV spectroscopy (Ref. 8)	V
Stannous ion in 1 <i>N</i> HCl	UV spectroscopy (Ref. 12)	IV
HCl HI KI	{ UV spectroscopy (Ref. 11) Paramagnetism (Ref. 11) X-ray diffraction (Ref. 11)	IV
HClH_3PO_2		IV

the presence of the TcCl_6^{2-} species, but because of differences in molar absorptivity the major species is not necessarily the one with the highest absorption. Pertechnetate also absorbs strongly in this region. Reduction of technetium by HCl plus H_3PO_2 , a method less commonly used with radiopharmaceutical preparations, produces a chelation efficiency intermediate between that found with concentrated HCl and with stannous ion reducing agents.

Iodide has been used to reduce technetium (6). The UV spectra of the carrier reduced species could not be obtained because of the strong absorption of the iodide, but we studied the chelation efficiency of technetium reduced by the HCl-HI-KI solution. Our data indicate that DTPA can efficiently chelate the reduced species. About 13% of the technetium appears to be in a nonreactive form. The use of a phosphate buffer to raise the pH more uniformly resulted in a lower chelation efficiency, apparently because of competition for the technetium by the phosphate buffer.

The ferric ascorbate system (7) (Table 4) was one of the early ones used with radiopharmaceutical preparations. The technetium is reduced and chelated efficiently in this system and in the similar ferrous ion system. The ferric ascorbate system seems to be essentially a ferrous ion reduction system, since ascorbic acid alone does not efficiently reduce technetium and therefore its role must be to reduce the ferric ion.

The thiocyanate system (8) (Table 4) is thought to give the V state, at least at the carrier level. Early work has indicated that the organic solvent added stabilizes the technetium in the V oxidation state. The iron ascorbate thiocyanate system proposed by

Weber and Howard (9) also produces the V oxidation state if an organic solvent is added.

Control experiments to indicate possible complications due to hydrolysis of technetium were run as time studies at various pH values with stannous ion as the reductant. The results (Table 2) show that at relatively acidic conditions the technetium can be converted to a nonchelated form. This does not contradict previously reported studies since the DTPA was added only to strong acid solutions, but it does indicate the conditions necessary for high-yield chelate formation. These data are in general agreement with the recently derived hydrolysis constant for technetium reduced with hydrazine (10).

Further controls were run to indicate the chromatographic distribution of the activity when chelate is not present and the results show that an insignificant amount of activity (<5%) can appear in the chelate elution volume.

CONCLUSIONS

The data obtained on the oxidation state of carrier ^{99}Tc reduced by stannous ion and concentrated HCl seem to indicate that DTPA is an effective chelating agent for the IV oxidation state but not for the V state.

Obviously the chemical form of the technetium being chelated must be considered. It would be naive to expect results based on data from only two reduc-

ing agents in specific solvents to hold for all chemical forms of the IV and V oxidation states. The most likely form of the IV oxidation state for carrier technetium in the strong HCl solutions used (11) is TcCl_6^{2-} . Since this anion certainly would not chelate with DTPA, a cationic species must be formed as the pH is raised. This is postulated by Gorski et al (10), who present electrophoresis data for the existence of TcO^{2+} and TcOOH^+ and at higher pH the well-known TcO_2 . Our hydrolysis experiments in the stannous ion system indicate that chelation takes place in acidic solutions but decreases with increasing pH and the data are in general agreement with the hydrolysis constants obtained by Gorski et al (10). We therefore think that the form of the IV state present when chelation occurs is the dipositive ion and that further hydrolysis results in the weakly chelating singly charged cation and finally the neutral oxide, TcO_2 .

The chemical form of the V state for carrier technetium is less well defined. Busey (12) claims a TcOCl_4^- species in the concentrated HCl system but no species has been isolated. Ossicini et al (2) claim a neutral species, TcOCl_3 , which forms an anionic complex in increasingly acid solutions. No cationic character has been reported for the V state. We think that the assumption of nonbinding of the V state should be limited to species of the chemical form proposed by Busey and by Ossicini et al and apparently present in our systems, but the possibility

TABLE 4. Tc VII REDUCED BY FERRIC ASCORBATE AND FERROUS ION

Reducing agent	Final concentration in solution before analysis				Chelate (%)	TcO_4^- (%)	Adsorbed* (%)
	(Fe)	(ASA)	(Tc)	(DTPA)			
Fe^{2+} ASA	3.6×10^{-3}	8.5×10^{-3}	CF	4×10^{-2}	95	1	4
Fe^{2+} ASA	3.6×10^{-3}	8.5×10^{-3}	2.2×10^{-5}	4×10^{-2}	81	2	17
Fe^{2+} ASA	3.6×10^{-3}	8.5×10^{-3}	CF	4×10^{-2}	73	20	7
Fe^{2+} DHA	3.6×10^{-3}	8.5×10^{-3}	CF	4×10^{-2}	87	4	9
Fe^{2+} ASA	1.8×10^{-3}	8.5×10^{-3}	2.2×10^{-5}	4×10^{-2}	77	12	11
Fe^{2+}	3.6×10^{-3}	—	CF	4×10^{-2}	92	2	6
Fe^{2+}	3.6×10^{-3}	—	2.2×10^{-5}	4×10^{-2}	73	3	24
ASA	—	8.5×10^{-3}	CF	4×10^{-2}	1	96	3

Tc VII Reduced with NH_4SCN						
	Final concentration in solution before analysis			Chelate (%)	TcO_4^- (%)	Adsorbed* (%)
	(Tc)	(SCN)	(DTPA)			
CF		2×10^{-3}	4×10^{-2}	1	95	4
2.2×10^{-7}				4	73	23
$2.2 \times 10^{-7}\dagger$				38	8	54
$2.2 \times 10^{-7}\ddagger$				58	4	62

* Percent of ^{99m}Tc activity not eluted from gel chromatography column with 80 ml of N_2 purged eluant.

† With stannous ion.

‡ With stannous ion, no acetone.

ASA = ascorbic acid.

DHA = dehydroascorbate.

of a cationic form of the V state in aqueous solution does seem remote.

We therefore propose that the amount of chelate formed with ^{99}Tc reduced by various agents indicates the proportion of the IV oxidation state present. If the chelation efficiency for the carrier-free technetium is similar, then it can be postulated by extrapolation that the percent of the IV oxidation state of ^{99m}Tc is the same for the same reducing system.

In the stannous ion experiments the ^{99}Tc is chelated efficiently by the DTPA, as is the ^{99m}Tc (Table 1), and potentiometric titration shows the carrier technetium to be in the IV oxidation state. By extrapolation from these findings it appears that the oxidation state of ^{99m}Tc in chelate used in biomedical products is the IV state.

Similar arguments for the systems HCl-HI-KI , ferrous ion, ferric ascorbate, and $\text{HCl-H}_3\text{PO}_2$ show that the majority of the technetium is in the IV state for the first three systems, ~45% for the $\text{HCl-H}_3\text{PO}_2$ system.

Appreciable amounts of the IV oxidation state are also obtained in the ferrous ion and ferric ascorbate systems. In the latter, the technetium has been shown to be in the V oxidation state by the work of Crouthamel (8) and Howard et al (9). However, when this system was used in radiopharmaceutical applications (7) the organic solvent was not added to stabilize the technetium in the V state. Our data (Table 4) show that absence of the organic compound allows high yields of the IV oxidation state.

In the concentrated HCl system at the carrier level the IV state does not seem to be present in amounts above 20%. At the carrier-free level, without heat the yield is much higher, but with heat it decreases to that at the carrier level most likely due to the distillation of the HCl and a resulting less acidic reducing media. The existence of the IV state at the carrier-free level with concentrated HCl is postulated by Williams et al (3) on the basis of paper chromatography.

When the V state is prevalent, a large fraction of the activity is adsorbed to the gel column. This behavior is similar to that found in paper chromatography (2), which is not surprising in view of the similarity between the structures of cellulose and of the polysaccharide in the gel. In paper chromatography the V state is postulated as being in the form of TcOCl_3 . This does not preclude the adsorption of the hydrolyzed form of the IV state in the present work, especially in the presence of easily hydrolyzed substances such as tin and iron.

The practical implications are that the technetium should be reduced with a strong reducing agent, e.g.,

stannous ion or ferrous ion, and that the technetium must be kept in an acid condition to prevent hydrolysis of the IV state. As previously shown, all work should also be performed in a nitrogen atmosphere to prevent oxidation of the technetium.

SUMMARY

It has been demonstrated that the probable oxidation state of ^{99m}Tc in chelates used in biomedical products is in the IV state. It has been shown that this state can be made nonreactive by allowing hydrolysis of the species.

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