

# CHEMICAL STATE OF $^{99m}\text{Tc}$ IN BIOMEDICAL PRODUCTS

W. Eckelman, G. Meinken, and P. Richards

*Brookhaven National Laboratory, Upton, New York*

The field of nuclear medicine employs a wide variety of products containing  $^{99m}\text{Tc}$  because of its superior physical properties. However, the chemical state of technetium in the various biomedical products in use is not well defined. It is generally conjectured (1) that a reducing agent is required to produce a cationic technetium species, which is then chelated in such products as  $^{99m}\text{Tc}$ -human serum albumin (HSA) and  $^{99m}\text{Tc}$ -diethylenetriaminepentaacetic acid (DTPA).

However, a series of papers by Benjamin (2) postulates that although sufficient potential was applied to reduce technetium in zirconium crucible electrolysis experiments, this potential is not necessarily used in forming the technetium-labeled product; that is, the technetium reacts as an anion. Benjamin claims "that anodic dissolution of zirconium in the presence of, or followed by, the addition of  $^{99m}\text{TcO}_4^-$  and HSA results in nearly quantitative binding of the technetium with the protein. The mechanism of the reaction of technetium and zirconium does not seem to involve the formation of hydrous zirconia with  $\text{TcO}_4^-$  adsorbed but rather the ionic addition of  $\text{TcO}_4^-$  to  $\text{Zr}^{4+}$  followed by molecular addition of albumin as a ligand."

We have attempted to resolve this dichotomy by employing various analytical tools available to us, including ultraviolet spectroscopy, solvent extraction, and gel chromatography to characterize the chemical state of the technetium in chelates, including HSA, containing zirconium or iron.

## MATERIALS

Benjamin's electrolytic cell consisted of a 35-ml zirconium crucible as the anode and a rotating micro platinum electrode (SARGENT Synchronous Rotator, constant speed 600 rpm) as the cathode. The current (d-c) was supplied from a voltage-regulated power supply.

The electrolytic solution normally consisted of different combinations of the following:

1. 0.05–0.1 ml of 25% human serum albumin.
2. 0.01–0.5 ml of 1 N HCl.
3. 3–5 ml of  $\text{TcO}_4^-$  in 0.9% saline.
4. 1 N HCl and 1 N NaOH to adjust pH to 7–8.

We have refined the zirconium crucible (Oremet Co., Albany, Ore.) electrolysis equipment of Benjamin by isolating the platinum electrode to prevent hydrogen production and subsequent reduction of technetium.

The product analysis was performed on a  $0.9 \times 35$ -cm gel chromatography column (Sephadex G25) eluted with  $\text{N}_2$  purged saline. The percentage adsorbed to the column represents the hydrolyzed unchelated fraction of technetium. A standard was counted to assure activity balance of  $^{99m}\text{Tc}$ .

## METHODS

**Electrolysis.** In the modified electrolysis apparatus chelates of hydroxyethylethylenediaminetriacetic acid (HEDTA) have been prepared by adding the pertechnetate after electrolysis. In this manner, the technetium could not have been reduced at the cathode. This reaction is not inconsistent with Benjamin's postulate that the technetium is present as pertechnetate in the complex. Furthermore, filtration of the electrolysis solution through a 25-m $\mu$  Millipore filter before pertechnetate addition to exclude the possibility of large reactive zirconium metal particles did not decrease the yield. Reaction of  $\text{TcO}_4^-$  in acid solution in the presence of reactor-grade 200-mesh zirconium particles (ROC/RIC, Sun Valley, Calif.) produced no  $^{99m}\text{Tc}$ -HEDTA chelate, further substantiating our feeling that zirconium particles are not reducing the technetium. However, when the zirconium crucible and the platinum electrode were separated by dialysis tubing (Union Carbide, Chicago, Ill.) with an average pore size of

Received Nov. 9, 1970; revision accepted Feb. 12, 1971.

For reprints contact: William C. Eckelman, Medical Radionuclide Group, Dept. of Applied Science, Brookhaven National Laboratory, Upton, N.Y. 11973

~2.5 m $\mu$ , and the pertechnetate was again added after electrolysis, the  $^{99m}\text{Tc}$ -chelate yield using the cathode solution dropped precipitously. This appears to indicate that very fine particles, possibly of zirconium metal, are reducing the technetium. The solution was analyzed for zirconium content to assure that the zirconium ions had crossed the membrane. Sufficient zirconium had crossed the membrane to give high-yield compounds in non-membrane experiments.

Variation of the chelating group in the same system gave an indication of the nature of the ligands required to bind the reduced technetium. These data and those of the other electrolysis experiments are given in Table 1. It is interesting to note that the high incorporation of technetium and zirconium was achieved with EDTA-type ligands. With simple diacid types, e.g. citric, malic, and aspartic, soluble zirconium complexes were formed at neutral pH but

technetium was not incorporated to any extent. In a third group of monoacids, e.g. acetic, glycine, lysine, precipitation occurred upon raising the pH to 7.

**Ultraviolet spectroscopy.** Ultraviolet spectra using  $^{99}\text{Tc}$  carrier were not informative in helping us decide between the presence of reduced technetium or pertechnetate in either zirconium or ferrous compounds. On the basis of the early zirconium crucible work we had hoped to look at the characteristic pertechnetate spectra in chelate solutions. However, we have discovered that a high metal-to-technetium molar ratio (1,250:1 for zirconium and 1,200:1 for iron) is necessary to produce high-yield chelates with EDTA-type analogs and that both the reducing metal and the chelate absorb too strongly in the pertechnetate region. Therefore the spectral work gave us no indication of the chemical state of technetium.

**Extraction of  $^{99m}\text{Tc}$ .** Pertechnetate is efficiently

TABLE 1. YIELDS OF BIOMEDICAL PRODUCTS LABELED WITH  $^{99m}\text{Tc}$

Reducing agent ( $\mu\text{M}$ )	Chelate ( $\mu\text{M}$ )	Conditions	Percent $^{99m}\text{Tc}$ appearing in chelate fraction	Percent $^{99m}\text{Tc}$ appearing in $\text{TcO}_4^-$ fraction	Percent $^{99m}\text{Tc}$ adsorbed on Sephadex*
1.1 $\text{Zr}^{4+}$	120 HEDTA†	Std. proc. of Benjamin (see text), Zr crucible	35	16	49
3.3 $\text{Zr}^{4+}$	120 HEDTA	Std. proc. of Benjamin 25-m $\mu$ filter	38	22	40
$\text{Zr}^{4+}$	120 HEDTA	200-mesh Zr powder	1	99	—
1.1 $\text{Zr}^{4+}$	120 HEDTA	Zr crucible, dialysis tubing	2	96	2
1.1 $\text{Zr}^{4+}$	120 NTA†	Std. proc. of Benjamin. Product	7	93	0
1.1 $\text{Zr}^{4+}$	120 DCTA†	heated at 100°C for 20 min	78	21	1
1.1 $\text{Zr}^{4+}$	120 citric acid		—	98	2
1.1 $\text{Zr}^{4+}$	120 malic acid	↓	1	99	—
1.1 $\text{Zr}^{4+}$	120 aspartic acid		7	70	23

\* Percent adsorbed is the fraction of the  $^{99m}\text{Tc}$  activity which was not eluted from a 35-cm Sephadex G25 column in 80 ml nitrogen purged isotonic saline.

† HEDTA—N(2-hydroxyethyl)ethylenediaminetriacetic acid, NTA—nitrilotriacetic acid, DCTA—trans 1,2-diaminocyclohexane-N,N,N',N'tetraacetic acid.

TABLE 2. YIELDS OF BIOMEDICAL PRODUCTS LABELED WITH  $^{99m}\text{Tc}$

Reducing agent ( $\mu\text{M}$ )	Chelate ( $\mu\text{M}$ )	Conditions	Percent $^{99m}\text{Tc}$ appearing in chelate fraction	Percent $^{99m}\text{Tc}$ appearing in $\text{TcO}_4^-$ fraction	Percent $^{99m}\text{Tc}$ adsorbed on Sephadex*
36 $\text{Fe}^{2+}$	106 DTPA	$\text{Fe}^{2+}$ dissolved in $\text{TcO}_4^-$ solution, 0.35 ml conc. $\text{H}_2\text{SO}_4$ added, chelate added, pH raised to 6, and heated at 100°C for 20 min.	98	2	0
36 $\text{Fe}^{3+}$	106 DTPA		0	99	1
18 $\text{Fe}^{2+}$	222 o-phenanthroline	↓	0	100	0
18 $\text{Fe}^{2+}$	284 ascorbic acid		43	—	57
18 $\text{Fe}^{2+}$	284 deoxyascorbic acid		34	9	55

\* Percent adsorbed is the fraction of the  $^{99m}\text{Tc}$  activity which was not eluted from a 35-cm Sephadex G25 column in 80 ml nitrogen purged isotonic saline.

extracted from acid solutions by a chloroform solution of tetraphenylarsonium chloride (TPA) (3). TPA extractions of acid solutions of zirconium or ferrous ion with pertechnetate indicate that technetium

is partially present as pertechnetate. However, it can be misleading to look at the chemical form of technetium in the acid solution before chelation because we have found a dependence of technetium chelate yield on the chelate added. For instance, in the case of ferrous ion which has a potential in water too negative to reduce technetium, formation of the ferrous technetium complex with DTPA occurs in high yield because the DTPA increases the ferrous redox potential whereas phenanthroline lowers the ferrous redox potential and forms high-yield iron chelates but does not incorporate technetium. Technetium reduced with concentrated HCl/HI is incorporated in phenanthroline. Table 2 contains the results of gel chromatography analysis of ferrous reduced  $^{99m}\text{Tc}$  chelates.

**Gel chromatography.** A strong indication that technetium is present in a reduced state in the commonly known biomedical products is the behavior of the  $^{99m}\text{Tc}$ -labeled compound on gel chromatography. Technetium mixed with either  $\text{NaBH}_4$  or a mixture of concentrated HCl and HI (4) is adsorbed strongly on Sephadex G25 whereas pertechnetate is eluted in a reasonable volume. Figure 1 shows typical runs on Sephadex G25. Run A shows the elution characteristics of pertechnetate alone. Run B is  $^{99m}\text{Tc}$ -Fe-citrate at 24 hr in air from Table 3. By integrating the citrate and pertechnetate peaks, it can be seen that 100% of the activity is not recovered. That fraction that is not eluted is the unchelated species. Using the commonly employed chromatographic systems for  $^{99m}\text{Tc}$ -chelates, anion exchange chromatography (5), and, for  $^{99m}\text{Tc}$ -HSA, paper chromatography in 85% methanol and anion exchange chromatography (1), this unchelated  $^{99m}\text{Tc}$  component cannot be separated from the respective chelate. This may cause the yield of the desired product to appear erroneously high because the unchelated  $^{99m}\text{Tc}$  travels with the  $^{99m}\text{Tc}$ -HSA in both paper chromatography in 85% methanol and anion exchange chromatography and with the  $^{99m}\text{Tc}$ -chelates in anion exchange chromatography. However, the third component is observed on Sephadex G25 using weak chelating agents, and this component is adsorbed in the same fashion as the technetium mixed with  $\text{NaBH}_4$  or a HCl-HI mixture. It is therefore clear from gel chromatography that the unchelated  $^{99m}\text{Tc}$  is not in the form of pertechnetate but is a reduced species. Table 3 shows the yield data for two weak chelating groups, ascorbate and citrate. These data illustrate the argument concerning the chemical state of technetium. They also illustrate the practical importance of using gel chromatography to separate all components of a system.

An alternate explanation of the gel chromatogra-

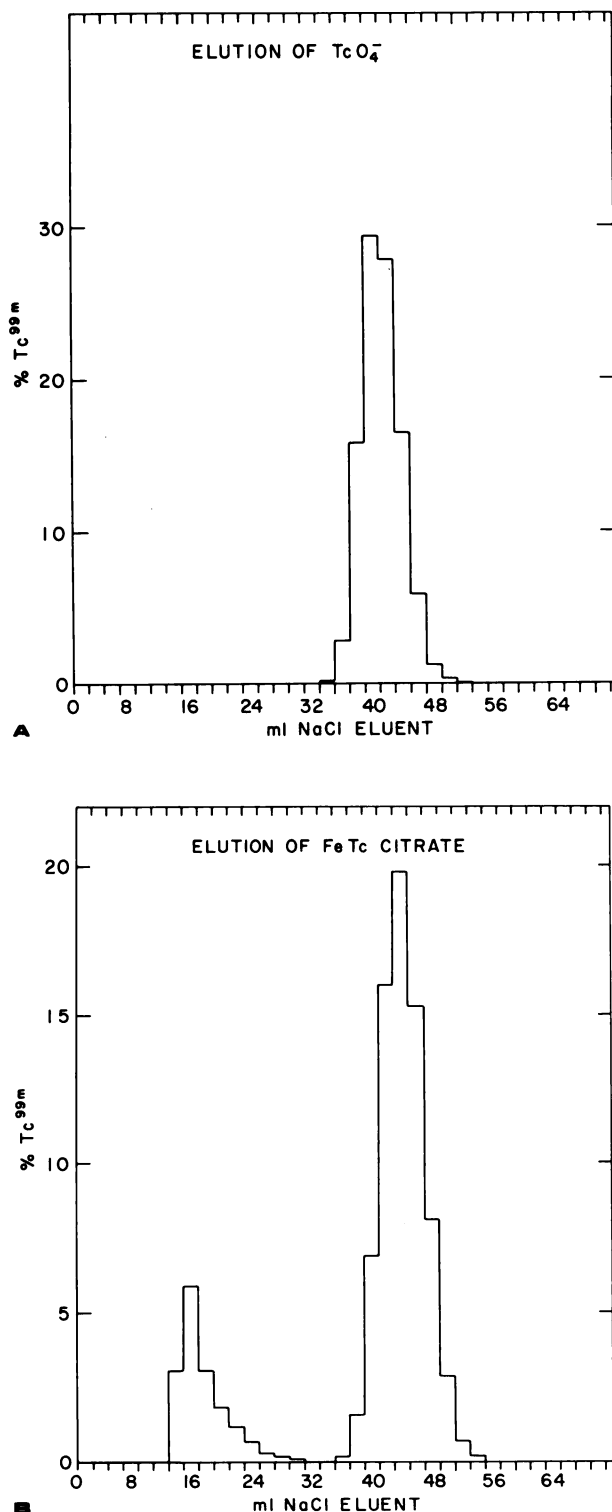


FIG. 1. Separation of  $^{99m}\text{Tc}$ -Fe-citrate by gel filtration on Sephadex G25 (1  $\times$  35 cm column) eluted with 0.15 N NaCl. A is for  $\text{TcO}_4^-$  alone. B is for  $^{99m}\text{Tc}$ -Fe-citrate.

phy results—chemisorption of pertechnetate on a metal hydroxide—is not likely with either zirconium (2) or iron (6). Technetium can be reduced by non-metallic reducing agents and successfully incorporated into chelates. Table 4 illustrates technetium reduced by a HI/HCl mixture and incorporated into chelates. This method of technetium incorporation argues against a metal pertechnetate complex.

### RESULTS

The results of the electrolysis experiments and the gel chromatography work indicate that technetium is indeed reduced in biomedical products. Since this reduced technetium can be oxidized to pertechnetate by oxygen ( $\text{TcO}_2 + 2\text{H}_2\text{O} = \text{TcO}_4^- + 4\text{H}^+ + 3\text{e}^-$ ;  $E^\circ = -0.738$ ), the stability of the chelate can be affected by the presence of oxygen depending on the amount of excess reducing agent present. In the iron

ascorbate system the <sup>99m</sup>Tc compound is stable for reasonable lengths of time because of the large excess of ascorbic acid as shown in Table 3, but in a <sup>99m</sup>Tc compound with minimal residual reducing power, e.g., a <sup>99m</sup>Tc compound containing <sup>99m</sup>Tc reduced by  $\text{NaBH}_4$ , the stability toward oxygen would be negligible.

The thermodynamic stability of the reduced technetium can also be affected by the strength of the chelating agent. As shown earlier for the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  redox potential, use of EDTA as a chelating agent raises the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  potential from  $-0.771$  to  $-0.12$  whereas the use of phenanthroline lowers the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  potential from  $-0.771$  to  $-1.120$ . Similar changes in redox potential would be expected for technetium although no measurements have been made to date. As can be seen in Table 3 by comparing the <sup>99m</sup>Tc-citrate data in air and in nitrogen, the reduced technetium seems more stable to oxida-

TABLE 3. YIELD DATA FOR <sup>99m</sup>Tc-ASCORBATE AND <sup>99m</sup>Tc-CITRATE

Time (hr)	<sup>99m</sup> Tc-ascorbate*				<sup>99m</sup> Tc-citrate†				
	0	1	3	23	0	1	2½	4	23
Percent <sup>99m</sup> Tc appearing in chelate fraction	26	33	38	64	86	47	27	20	3
Percent <sup>99m</sup> Tc appearing in $\text{TcO}_4^-$ fraction	3	2	2	—	2	2	3	3	2
Percent <sup>99m</sup> Tc adsorbed on Sephadex‡	71	65	60	36	12	51	60	77	95
Time (hr)	0	1	3	24	0	1	4	24	
Percent <sup>99m</sup> Tc appearing in chelate fraction	24	21	34	52	81	61	41	16	
Percent <sup>99m</sup> Tc appearing in $\text{TcO}_4^-$ fraction	4	1	2	2	9	2	5	72	
Percent <sup>99m</sup> Tc adsorbed on Sephadex‡	72	78	64	46	11	37	54	12	

\* <sup>99m</sup>Tc-ascorbate preparation: to 0.1 ml  $\text{TcO}_4^-$  solution, add 10 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 2 ml 1 N  $\text{H}_2\text{SO}_4$ , then add 100 mg ascorbic acid, stir for 5 min and raise pH to 6.5. Store in 25-ml test tube in stated atmosphere.

† <sup>99m</sup>Tc-citrate preparation: to 4 ml  $\text{TcO}_4^-$  solution, add 10 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , then add 15 mg citric acid, stir for 5 min and raise pH to 7.5. Store in 25-ml test tube in stated atmosphere.

‡ Percent adsorbed is that fraction of <sup>99m</sup>Tc activity which was not eluted from a 35-cm Sephadex G25 column in 80-ml nitrogen purged isotonic saline.

TABLE 4. YIELDS OF BIOMEDICAL PRODUCTS LABELED WITH <sup>99m</sup>Tc

Reducing agent (μ M)	Chelate (μ M)	Conditions	Percent <sup>99m</sup> Tc appearing in chelate fraction	Percent <sup>99m</sup> Tc appearing in $\text{TcO}_4^-$ fraction	Percent <sup>99m</sup> Tc adsorbed on Sephadex*
conc. HCl/HI	120 DCTA	Reduce <sup>99m</sup> Tc in small volume conc. HCl and HI. Add 0.2 ml of reduced <sup>99m</sup> Tc to chelate solution and raise pH to 7. Heat at 100°C for 20 min.	55	42	—
↓	400 citric acid	↓	18	52	29
	400 HEDTA		54	16	29
	222 o-phenanthroline		26	24	50
	284 ascorbic acid		44	7	49

\* Percent adsorbed is the fraction of the <sup>99m</sup>Tc activity which was not eluted from a 35-cm Sephadex G25 column in 80 ml nitrogen purged isotonic saline.

tion in the citrate chelate form than in the reduced unchelated hydroxide form.

In any case it seems that the exclusion of oxygen in  $^{99m}\text{Tc}$  compounds is a reasonable precaution.

#### CONCLUSION

Although technetium can exist as a cationic species, at least for  $^{99m}\text{Tc}$  carrier (7), there has been some question concerning its chemical state in biomedical products (2,8). It appears that in spite of the iconoclastic appeal of Benjamin's pertechnetate complex theory, the technetium is probably reduced and chelated as a cationic species. The practical implications resulting from this study are that solutions of  $^{99m}\text{Tc}$  compounds should be prepared oxygen free and analyzed by gel chromatography to better understand and control the  $^{99m}\text{Tc}$  products.

#### ACKNOWLEDGMENT

The authors wish to acknowledge the assistance in these studies given by L. Newman, Department of Applied Science, BNL. This work was performed under the auspices of the United States Atomic Energy Commission.

#### REFERENCES

1. STERN H, MCAFEE J, ZOLLE I: Technetium-99m albumin. In *Radioactive Pharmaceuticals*, Andrews GA,

Kniseley RM, Wagner HN, eds, CONF-651111, Springfield, Va., National Bureau of Standards, pp 359-382

2. BENJAMIN P: A rapid and efficient method of preparing  $^{99m}\text{Tc}$ -human serum albumin: Its clinical applications. *Int J Appl Radiat* 20: 187-194, 1969; BENJAMIN P, REJALI A, FRIEDEL H: Electrolytic complexation of  $^{99m}\text{Tc}$  at constant current: Its applications in nuclear medicine. *J Nucl Med* 11: 147-154, 1970

3. FERRADINI C, CARLIER R, GENET PJ: Effets chimiques associés à l'émission  $\beta^-$ . III. Etude du technetium 99m forme par désintégration  $\beta^-$  de  $^{99}\text{Mo}$ . *Radiochim Acta* 12: 1-4, 1970

4. PEACOCK RD: *The Chemistry of Technetium and Rhenium*, New York, Elsevier Publishing Co., 1966, pp 63-71

5. HARPER P, LATHROP K, GOTTSCHALK A: Pharmacodynamics of some technetium-99m preparations. In *Radioactive Pharmaceuticals*, Andrews GA, Kniseley RM, Wagner HN, eds, CONF-651111, Springfield, Va., pp 335-358

6. ANDERS E: *The Radiochemistry of Technetium*, Washington, D.C., National Academy of Sciences-National Research Council, 1960, pp 4-5

7. GORSKI B, KOCH H: Zur chemie des Technetium in wässriger Lösung—I. Über den Zustand des vierwertigen Technetium in wässriger Lösung. *J Inorg Nucl Chem* 31: 3565-3571, 1969

8. DAVIS M: Chemistry and physiology of  $^{99m}\text{Tc}$  iron hydroxide macroaggregates. *J Nucl Med* 11: 313-314, 1970