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THE CHEMISTRY OF ^{99m}TC LABELING KITS*†

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THE CHEMISTRY OF ^{99m}TC LABELING KITS

Numerous problems have recently been reported with the use of kit-produced ^{99m}Tc radiopharmaceuticals. Anomalous biodistribution results mainly because of the presence of undesired forms of Tc such as free pertechnetate and reduced hydrolyzed Tc. The origin of these problems is twofold: (a) the source of Tc and (b) the stannous kits themselves.

Present research was undertaken to better understand the chemistry involved in various stannous kit systems. The interrelationship between Tc, tin, and the ligands used to complex the reduced Tc was investigated in order to find solutions to existing problems of unreliable performance of kit-produced Tc radiopharmaceuticals.

(Slide 1). The source of Tc as shown in this slide may give rise to complications due to the presence of oxidants or other reactive contaminants as well as due to presence of significant amounts of carrier Tc. While most existing generator systems for Tc reportedly contain no added oxidants, radiolytic reactions could produce oxidizing radicals. Instant Tc could have the same problem. When high concentrations of ^{99}Mo are allowed to decay for a long time before using the ^{99m}Tc, a significant build up of Tc atoms results, sometimes enough to exceed the reducing capability of the stannous tin in the kits.

The chemistry of Tc reductions in various stannous kit systems is not well understood, especially at the radiopharmaceutical concentration levels. The reducing intensity, i.e., the reduction potential of the system, and the kinetics of reduction and complexation of Tc with the desired ligand are factors that have a bearing on the <u>in-vitro</u> stability and the <u>in-vivo</u> performance of the final radiopharmaceutical preparation.

The problems that arise because of the use of stannous ion itself are described in the next slide (2). These may occur during kit formulation or at the time of reconstitution. Hydrolysis and oxidation of stannous tin may result due to an improper control over conditions. If hydrolyzed tin is present in the kits, reduced Tc can coprecipitate with it and form an undesirable colloid. If an unfavorably low ratio of ligand to tin is used, especially in the case of weakly complexing systems, stannous ion can enter into side reactions. All these factors cut down on the amount of stannous tin available in the useful chemical form at reconstitution. This "usable" tin can be defined as the stannous tin available in the appropriate chemical form at reconstitution to effectively reduce Tc to the required valence state for complexation, without any side reactions of either tin or Tc. If the usable tin in the kits gets diminished drastically, the total Tc atoms in the ^{99m}Tc sample becomes an important factor.

Many commercial stannous kits, bone and lung agents in particular, fail in many of the above ways. To evaluate the usable tin in these kits we developed an <u>in-vitro</u> titration procedure. Results are shown graphically in the following slide (3). The method involves reconstitution of a series of kits with saline containing gradual stoichiometric increments of ^{99}pertechnetate. Trace ^{99m}pertechnetate is added for counting purposes. Aliquots are analyzed by sephadex G25 filtration to give the amount of Tc in various forms: the desired complex, unreduced pertechnetate, and reduced hydrolyzed Tc. As we see here, a log-log plot of the number of Tc atoms added <u>vs</u> the complex yield provides the Tc capacity of the kit, i.e., the maximum Tc that could be added to give a 95% or better yield of the sought-after complex.

(Slide 4). Once the Sn saturation point of a kit has been determined, the usable tin in the kit is evaluated by using a theoretical standard curve, such as the one shown in this slide. This curve presumes a four-electron transfer reaction between stannous and pertechnetate in the DTPA and pyrophosphate media. This is based on some recent work of Steigman, Meinken, and Richards, carried out at the millimolar level. An extrapolation of the findings at the millimolar level to concentrations used here is not unjustified.

A model procedure for preparing stannous kits was developed. Various factors such as the optimum quantity of tin, ligand-to-tin ratio, etc., have to be evaluated for individual kit systems because of the differences in their chemistry. Desirable conditions are to use no more than the minimum required quantity of tin, an excess of the complexing agent, and keep all the tin available in the usable form at reconstitution. Stannous solutions are prepared by dissolving electrochemically plated tin metal in concentrated hydrochloric acid, and all operations of ligand addition, mixing and dispensing, etc., are carried out under strict exclusion of air. Solutions are quick-frozen and lyophilized and the final kits are stored under N_2. We prepared and evaluated model kits using DTPA, pyrophosphate, and etiylidene diphosphonate using this procedure.

(Slide 5). Typical results on the evaluation of usable tin in the model as well as commercial kits are shown in this slide. Column 1 shows the original tin in the kits and the last column shows the usable tin at reconstitution. As seen here, a high ratio of ligand-to-tin results in a higher percentage of usable tin in the kits. The one with 131 μg was a commercial kit with a ratio DTPA/tin = 9, and it showed 11-21% of the original tin in the usable form upon titration.

The next slide (6) represents comparable data in the pyrophosphate system. Kinetics requires use of a higher level of tin in this system; approximately 25 µ mol was found to be optimum.

For reliable performance, then, stannous kits should be prepared using extreme caution to keep most of the tin available in the usable form. Clinical levels of Tc require so little tin for reduction that the solution would be to use minimum tin and large excess of complexing agent to tin in the kit systems. These parameters should be optimized by a systematic investigation of individual kit systems.

The problems due to the source of Tc are more difficult to control. However, to repeat our observations, good stannous kits prepared under optimum conditions can minimize the existing problems of unreliable performance to within acceptable limits. Availability of the tin in the non-hydrolyzed, non-oxidized form at reconstitution would mean a favorable reduction potential, greater resistance of the Tc complex to oxidation, no Tc hydrolysis and, therefore, a uniform performance of the radiopharmaceutical preparation.

In our experience, oxidation of the Tc complex to produce free pertechnetate upon prolonged standing under nitrogen or air post-reconstitution is not a major problem in most of the kit systems.

Slide 1

(A) PRESENCE OF OXIDIZING IMPURITIES (RADIOLYSIS?)

(B) REACTIVE CONTAMINANTS

(C) REDUCED HYDROLYZED TECHNETIUM SPECIES

(D) BUILD UP OF [illegible] TO DUE [illegible] DECAY OF LARGE ACTIVITIES OF [illegible]

POSSIBLE REASONS FOR ANOMALOUS BEHAVIOR OF TC PHARMACEUTICALS PREPARED USING SN(II) KITS

(A) PARTIAL OXIDATION AND/OR HYDROLYSIS OF SN(II) DURING FORMULATION.

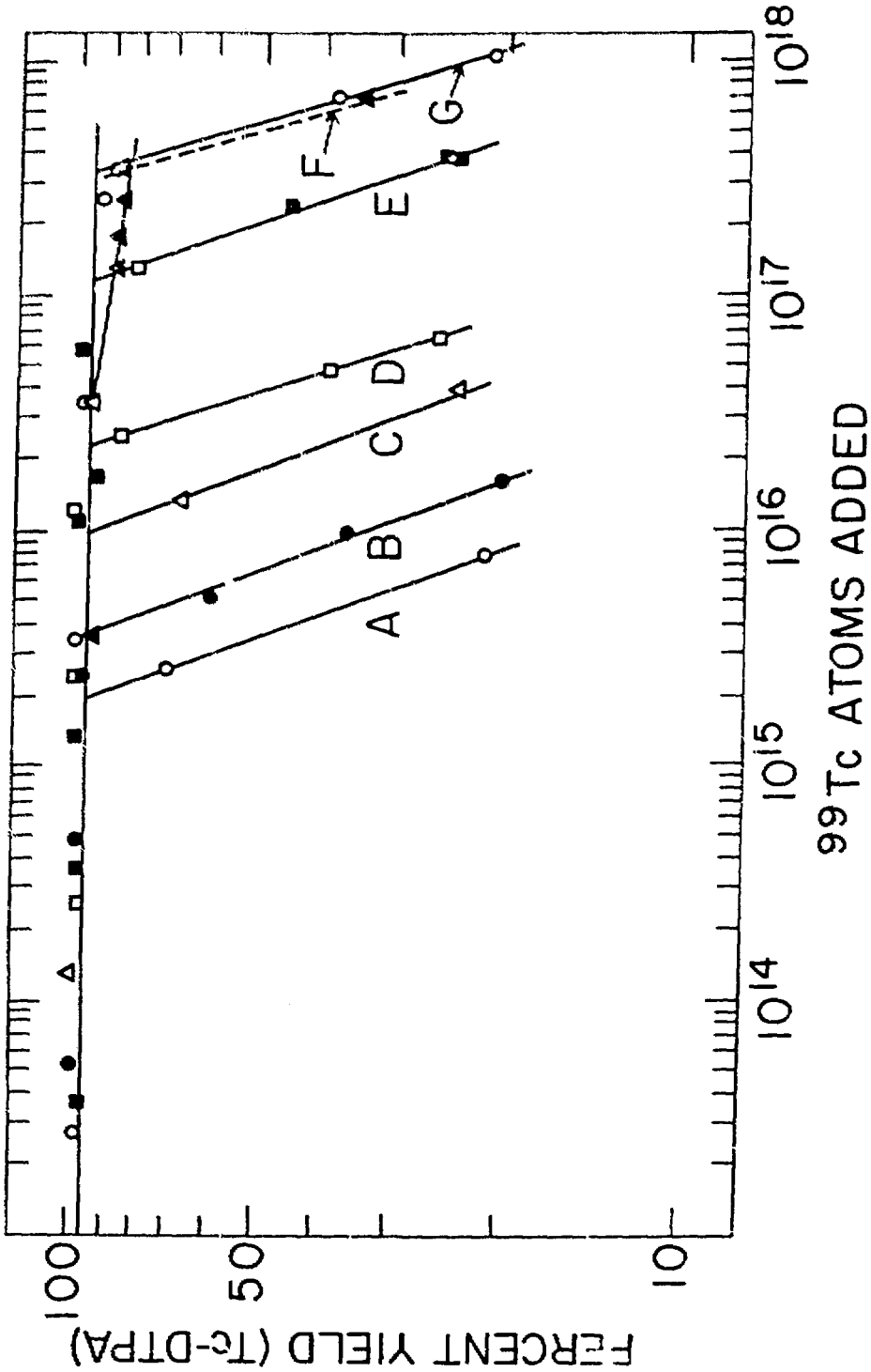
(B) LOW STOICHIOMETRIC RATIOS OF COMPLEXING AGENT TO SN(II), ESPECIALLY IN WEAKLY COMPLEXING SYSTEMS.

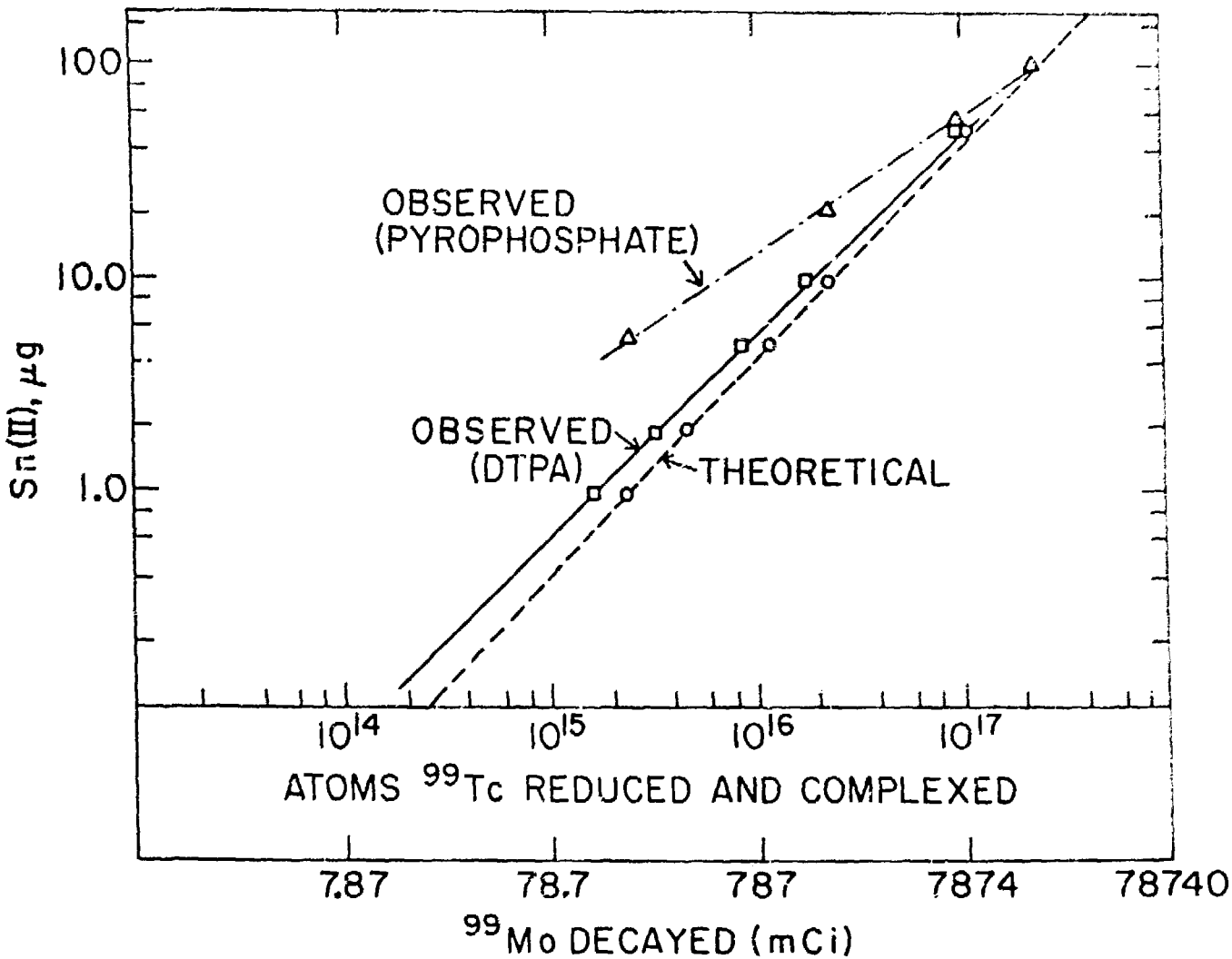
(C) UNDESIRABLE SIDE REACTIONS OF TIN AND TECHNETIUM DUE TO ABOVE.

(D) ONLY A FRACTION OF THE ORIGINAL SN(II) AVAILABLE IN THE DESIRED USEFUL FORM AT RECONSTITUTION.

(E) THE CARRIER CONTENT OF ^{99m}TC SOLUTIONS WHICH MAY EXCEED THE REDUCTIVE CAPABILITY OF STANNOUS ION, ESPECIALLY WHEN KITS HAVE A VERY SMALL QUANTITY OF "USABLE" SN(II).

Slide 3


PERCENT YIELD (Tc-DTPA)
100
50
10
10^{14}
10^{15}
10^{16}
10^{17}
10^{18}
^{99}Tc ATOMS ADDED
A
B
C
D
E
F
G


100
10.0
1.0
Sn(II), μg
OBSERVED
(PYROPHOSPHATE)
OBSERVED
(DTPA)
THEORETICAL
10^14
10^15
10^16
10^17
ATOMS 99Tc REDUCED AND COMPLEXED
7.87
78.7
787
7874
78740
99Mo DECAYED (mCi)

TITRATION RESULTS USING SN(II)-DTPA KITS

ORIGINAL SN(II) IN KIT, µG	MOLAR RATIO DTPA/SN(II)	"USABLE" SN(II) AT RECONSTITUTION[1]	
		µG	% OF TOTAL
0.96	890	0.8	81
1.92	890	1.6	81
4.8	890	4.0	83
9.6	890	9.4	98
48.0	890	47.5	99
131.0[2]	9	15 - 28	11 - 21
134.3	9	16 - 35	12 - 26

[1] FROM THEORETICAL STANDARD CURVE, AFTER DETERMINING THE TC SATURATION POINT OF KIT (≥ 95% COMPLEX YIELD)

[2] COMMERCIAL KIT

TITRATION RESULTS USING SN(II)-PYROPHOSPHATE KITS

ORIGINAL SN(II) IN KIT, µG	MOLAR RATIO PYROPHOSPHATE/SN(II)	"USABLE" SN(II) AT RECONSTITUTION[1]	
		µG	% OF TOTAL
5.1	196	1.1[2]	22
20.4	196	11.0	54
51.1	196	39.7[2]	78
102.2	196	102.2	100
	13.7	102.2	100
108.2[3]	14.1	60	56

[1] FROM THEORETICAL STANDARD CURVE, AFTER DETERMINING THE TC SATURATION POINT OF KIT (≥ 95% COMPLEX YIELD)

[2] EXTRAPOLATED VALUES

[3] COMMERCIAL KIT