

Evaluation of Formamidine Sulfinic Acid and Other Reducing Agents For Use In The Preparation of Tc-99m Labeled Radiopharmaceuticals

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Various reducing agents have been evaluated for their potential usefulness in the preparation of ^{99m}Tc labeled radiopharmaceuticals for use in nuclear medicine. Adequate labeling of various radiopharmaceuticals was accomplished using formamidine sulfinic acid. Nitrogen-purging of solutions is not required, which is an advantage for in-house preparation. Tagging requires heating, however, so heat-labile material cannot be used. Various compounds that could not be labeled when stannous chloride was used, could be tagged with ^{99m}Tc when formamidine sulfinic acid was used as the reducing agent.

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Technetium-99m as pertechnetate requires reduction as part of the process of binding the Tc-99m to various carriers in radiopharmaceuticals. Stannous chloride (SnCl₂) (1), the most commonly used reducing agent, is rapid and effective at room temperature. However, it is easily oxidized to stannic ion by oxygen and is rapidly hydrolyzed to a colloidal stannous hydroxide which effectively binds Tc-99m. In addition, residual stannous salts in the body may cause localization of pertechnetate in subsequent brain scans (2-4).

These considerations have encouraged attempts to find improved alternatives. Electrolytic reductions using tin electrodes (5) and zirconium electrodes (6) have been reported, but each involves an intermediate reducing agent. Other metal reducing agents used include ferric chloride coupled with ascorbic acid (7), copper salts (8), and chromous salts (9). In addition, the use of stannous pyrophosphate as an example of a stable stannous complex has been suggested (10). These alternatives, however, offer only limited solutions. Since the ligand requirements of technetium and other metals used as reducing agents are likely to be different, the use of such metallic reducing agents will limit the choice of ligands to those that effectively bind both the technetium and the other metal. Complications also arise with or-

ganic reducing agents. For example, the use of thiols often results in stable complexes that prevent formation of the desired complex (11).

Therefore, the following study was undertaken in an attempt to find a reducing agent that was not influenced by these shortcomings. Using either nitrilotris(methylene)-triphosphonic acid or phenylphosphonic acid, various reducing agents were evaluated in terms of their potential for promoting binding between technetium and these ligands.

MATERIALS AND METHODS

Preparative procedures. Reducing agent trials. Solutions of the ligands, phenylphosphonic acid, or nitrilotris(methylene)-triphosphonic acid, were prepared at 30-50 mg/ml and adjusted to pH 7.4-7.8. Then the reducing agent was added and the effect of various parameters (such as concentration, time and temperature) on ligand binding to Tc-99m were studied.

*Preparations with formamidine sulfinic acid (FSA).** A solution was prepared in a solvent sys-

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tem suitable for injection as required by the ligand and then an aliquot from a stock solution of FSA (10–20 mg/ml, pH adjusted to 7.8–8.0) was added to give a final concentration of 0.75–1.25 mg/ml. After mixing, the preparation was heated in a water bath at 60° for 30–45 min, or for 10–15 min in a boiling water bath. Since concentrated stock FSA solutions were slightly turbid, those used in preparations for injections into animals were Millipore-filtered (0.22 μ).

Rate studies using HEDP as the ligand were done in a constant-temperature shaker bath at 49°C ($\pm 0.75^\circ\text{C}$ variation observed). Samples were equilibrated for 4 min before Tc-99m as pertechnetate was added. Aliquots were removed at stated intervals and the percentage bound was immediately determined by thin-layer chromatography.

Analysis. The complexes were chromatographed on silica gel impregnated glass fiber strips (Gelman ITLC) in the solvents saline, 1-butanol, and others as required. After developing in the appropriate solvent, the strips were cut into 1-cm sections and counted in a well scintillation counter.

Clinically used radiopharmaceuticals such as DTPA, HEDP and pyrophosphate were prepared with FSA and injected (1 mCi) intravenously into 2.5- to 4.0-kg white New Zealand rabbits for comparison with stannous preparations. Scans were performed on a scintillation camera at appropriate intervals.

Toxicity. The toxicity of FSA was determined by injection of a 30 mg/ml solution (pH 7.5) into the tail veins of mice. A minimum of six mice were used for each level studied. The volume of injection was varied from 0.1 to 0.5 ml and the animals were observed for one week following the injection.

RESULTS

Since many different reducing agents were investigated, it was convenient to group them by classes in order to summarize the results. The agent of choice, FSA, and other sulfinates are covered last in a more detailed study.

1. Stannous salts. Several stannous salts were studied to find one that would be effective at reduction, but stable to hydrolysis. Despite encouraging reports, stannous acetate (12) and stannous formate (13) hydrolyzed easily at neutral pH to form a stannous colloid. In addition, the same behavior was observed for the oxalate and tartrate. These stannous salts provided no advantage over stannous chloride.

2. Sulfur oxygen radicals.

(a) Sodium dithionite: At room temperature, pH 7.5, and 5 mg/ml of reducing agent, the results were

42% bound technetium and 13% hydrolyzed or as TcO_2 . Increasing the temperature to 60°C increased the TcO_2 to 82%.

(b) Sodium bisulfite: At 5 mg/ml reducing agent and pH 7.5, no binding was observed at room temperature. After 30 min at 60°C there was 8.2% TcO_2 15.5% free pertechnetate, and 76.3% bound technetium.

(c) Sodium thiosulfate: At 5 mg/ml reducing agent and pH 7.5, less than 1% binding was observed after 15 min at room temperature.

3. Hydrazines.

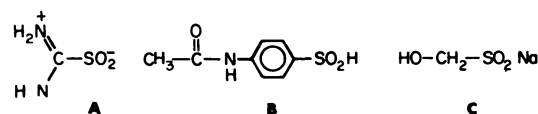
(a) Hydrazine: At 2 mg/ml reducing agent and pH 7.5, there was 1.4% bound after 30 min at room temperature and a mixture of 32.0% bound, 5.2% TcO_2 , and 62.8% free after 30 min at 95°C.

(b) Benzoylhydrazine: No binding was observed up to 95° for 30 min at 30 mg/ml.

(c) Hydralazine (1-hydrazinophthalazine): At 30 mg/ml and pH 7.5, there was 1% bound Tc after 30 min at room temperature and 12.7% bound after 30 min at 95°C. TcO_2 formation was negligible.

4. Miscellaneous. A variety of miscellaneous reducing agents were also evaluated with the results summarized in Table 1. Only dithiothreitol gave significant binding, and it was also accompanied by the formation of TcO_2 .

5. Sulfinic acids. Formamidine sulfinic acid (A), p-N-acetylphenylsulfinic acid (B), and sodium formaldehyde sulfoxylate (C) were studied. No Tc binding resulted from the p-N-acetylphenylsulfinic acid after 30 min at 95°C. However, binding was observed with sodium formaldehyde sulfoxylate, but only after heating to 95°C, in which case a mixture of 31% bound and 53% TcO_2 resulted. FSA gave satisfactory results using the trial ligands, with little unbound pertechnetate or TcO_2 .



In view of the encouraging preliminary results with FSA, and its suitability for use with current $^{99\text{m}}\text{Tc}$ radiopharmaceuticals, its potential for general applicability was studied.

Various radiopharmaceuticals, currently prepared with stannous chloride, were prepared with FSA and their properties compared.

Ethane-1-hydroxy-1, 1-diphosphonic acid, disodium salt (HEDSPA) (14). The complex was prepared by heating a mixture of HEDSPA at 31.3 mg/ml and 1.2 mg/ml FSA at pH 7.4 for 1 hr. The results were 1.2% free pertechnetate, 0.2% TcO_2 ,

TABLE 1. RESULTS OF REACTIONS BETWEEN $^{99m}\text{TcO}_4^-$ AND VARIOUS REDUCING AGENTS IN THE PRESENCE OF PHENYLPHOSPHONATE OR NITRILOTRIS(METHYLENE)-TRIPHOSPHATE

Reducing agent	T °C	% bound	% TcO_2	% TcO_4^-
Sodium borohydride	25	50	35	15
Cyanoborohydride	95	0	0	100
Sodium nitrite	25	0.3	0	99.7
Sodium amalgam	25	7.0	2.0	91.0
Dithiothreitol	58	84.0	13.5	2.5
Propionaldehyde	25	41.0	1.5	57.5

and 98.6% bound Tc, and were identical after 30 min and 24 hr.

Scans of a rabbit injected with the preparation indicate good bone uptake.

The rate of reduction and binding was slow enough at 49–50°C to permit convenient monitoring of the progress of the reaction. The results are shown in Table 2 for HEDP as ligand and 1.0 mg/ml FSA. Nearly complete binding is observed after 30 min. When the reaction is carried out at 95°C, binding is 99% in less than 5 min.

Pyrophosphate. The complex was prepared at concentrations of 26.8 mg/ml pyrophosphate and 1.2 mg/ml FSA. After adjusting pH to 7.4 and heating at 60°C for 30 min, there was 6.3% TcO_4^- , 0.3% TcO_2 , and 92.9% bound Tc. After an additional 24 hr, there was 5.7% free TcO_4^- , 0.3% TcO_2 , and 94.0% bound Tc.

Rabbit scans showed bone uptake and some liver uptake. This has also been reported for pyrophosphate preparations made with stannous chloride (15).

Diethylenetriaminepenta-acetic acid (DTPA) (16). Binding was more than 99% complete after 5 min at 95°C, with less than 0.2% TcO_2 . Stability of the complex was concentration-dependent, as shown in Table 3.

TABLE 2. RATE OF REDUCTION AND BINDING OF ^{99m}Tc TO HEDP WITH FORMAMIDINE SULFINIC ACID AT 49–49.50°C*

Time (min)	% bound
5	63.0 ± 7.1
10	91.1 ± 2.1
15	97.2 ± 0.9
30	99.2 ± 0.3
75†	98.9 ± 1.3

* Results are expressed as an average of six runs. Error limits represent one standard deviation. Samples were equilibrated for 4 min at the stated temperature before the addition of activity.

† Chromatography indicated 0.12% TcO_2 at this time.

TABLE 3. CONCENTRATION-DEPENDENCE OF THE STABILITY OF ^{99m}Tc DTPA PREPARED WITH FSA*

DTPA = 6.0 mg/ml			
Time (hr)†	% TcO_4^-	% TcO_2	% bound Tc
0	0.3	0.3	99.4
3.25	0.5	0.2	99.3
10.0	2.1	0.0	97.9
22.0	25.8	0.1	74.1
DTPA = 9.0 mg/ml			
0	0.9	0.2	98.9
3.25	0.3	0.2	99.5
10.0	0.3	0.1	99.6
22.0	7.2	0.2	92.6

* Complexes were prepared with 1.0 mg/ml FSA at pH 7.5 and heated at 58° for 20 min.

† Delay in analysis after preparation of the complex.

Rabbit scans and renograms compared favorably with those using the Ca DTPA/ SnCl_2 kit as prepared for clinical use.

Several other complexes of ^{99m}Tc have been prepared with both FSA and SnCl_2 as reducing agents. The complex formed with 8-hydroxyquinoline-7-carboxylic acid (bioquin-7CA) localizes in the liver and is excreted into the biliary tract (17). It exhibits the same lipophilic chromatographic and distribution properties when prepared with either agent. Other preparations exhibited difficulty when prepared with SnCl_2 using conditions of 0.05–0.50 mg/ml concentrations. Table 4 shows the results of a comparison study of FSA and SnCl_2 with nicotinic acid as the ligand. Nicotinic acid binds the stannous ion weakly, as evidenced by increasing amounts of colloid with increasing concentrations. At a level of 0.033 mg/ml SnCl_2 , the colloid percentage may be acceptable, but the completeness of reduction would be expected to be a problem. Increasing the level to 0.060 mg/ml and 0.090 mg/ml increases the colloid binding to 16–20% and 22–27%, respectively. At the higher levels, a fine colloidal suspension was also visible. With FSA and a lower concentration of nicotinic acid, the net binding was 94–97% over a 5-hr period.

No deaths were observed from intravenous injection of FSA into mice. The poor solubility of the FSA limited the dose that could be given in a reasonable volume. Russian investigators (18) reported an i.p. LD_{50} of 423 mg/kg in rats.

DISCUSSION

Of the diverse types of reducing agents studied, few yielded encouraging results. However, FSA, the

TABLE 4. COMPARISON OF FSA AND SnCl_2 FOR THE PREPARATION OF $^{99\text{m}}\text{Tc}$ -NICOTINIC ACID COMPLEX*

Reducing agent	Conc. mg/ml	Nicotinic acid M	pH	Time	% Free	% Colloid
FSA	0.5	0.27	6.4	5 min	2.7	1.5
FSA	0.5	0.27	6.4	5 hr	1.6	1.4
FSA	1.0	0.27	6.4	5 min	3.0	3.2
FSA	1.0	0.27	6.4	5 hr	1.8	3.1
SnCl_2	0.033	0.54	6.0	15 min	20.4	8.6
SnCl_2	0.033	0.54	6.0	5 hr	4.2	7.1
SnCl_2	0.060	0.54	6.0	15 min	7.8	20.0
SnCl_2	0.060	0.54	6.0	5 hr	4.2	16.2
SnCl_2	0.090	0.54	6.0	15 min	0.4	21.8
SnCl_2	0.090	0.54	6.0	5 hr	2.9	26.6

* Time is after preparation. Results are the average of duplicate preparations. FSA preparations were heated at 75–80°C for 15 min. Analysis was performed on Gelman ITLC strips. The complex had an R_f of 1 in saline and 0 in 1-butanol.

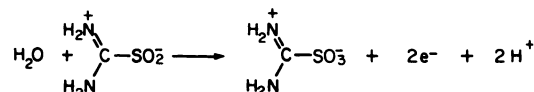
most promising agent, gave clean binding using simple preparative procedures, and appears to have potential general applicability. The scan properties of several different clinical preparations, including DTPA, pyrophosphate, HEDP and bioquin-7CA (a hepatobiliary agent), were unchanged whether FSA or SnCl_2 was used as the reducing agent.

Preliminary experiments without nitrogen-purging indicated no differences and purging was not done in any of the tests presented here. The temperature is not critical, although reasonable rates are observed only above 50°C. In early experiments with compounds that were not heat-sensitive, a boiling-water bath was used so that 10 min would be sufficient for complete reduction.

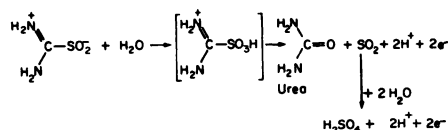
Preparation of Tc-99m complexes using FSA is simplified since purging of oxygen from solutions is unnecessary. In addition, one is limited only by the ability of the ligands to bind to technetium, since the binding of other metal ions such as Sn(II) is not involved. Several preparations, including a long-chain phosphonate and nicotinic acid, were mentioned as exhibiting difficulty when prepared using 0.05–0.50 mg/ml of SnCl_2 . On the other hand, conditions consisting of 10–20 mg/ml of ligand and 0.5–1.0 mg/ml of FSA resulted in a high percentage of bound and little hydrolyzed technetium with the preceding ligands. These conditions yielded adequate binding for a large number of compounds surveyed as $^{99\text{m}}\text{Tc}$ carriers, including amino acids, phenols, hydrazines, complex phosphonates, and carboxylic acids. The heating step during the reduction would be a disadvantage for carriers that are heat-sensitive. The time required for complete reduction, however, is only about 10 min in a boiling-water bath and poses no difficulty for laboratories already preparing kits that require heating.

It has been suggested (19,20) that FSA functions

as a reducing agent through formation of the corresponding sulfonate (Eq. 1).



The inability of p-n-acetylphenylsulfonic acid to reduce pertechnetate, whereas both FSA and sodium formaldehyde sulfoxylate did so at temperatures at which decomposition occurs, suggests, however, that the simple oxidation to the corresponding sulfonate does not occur. Moreover, the only other reducing agent that approached FSA in promoting binding to technetium was bisulfite ion. This suggests that FSA would function by initially decomposing to sulfur dioxide (SO_2) and urea (Eq. 2) and the SO_2 would then act as the reducing agent. Other workers (21) have stated that the decomposition is required before reduction can occur.



The effectiveness of some reducing agents, or lack thereof of others in this study, suggests that a requirement for effectiveness is the ability to bind the technetium during the reduction process. There is the supporting observation that electrolytic reduction of pertechnetate on platinum electrodes, in the presence of phenylphosphonate as ligand at neutral pH, resulted in only TcO_2 deposition on the electrodes. Without this ability the technetium either hydrolyzes or is reoxidized to pertechnetate. The ability of FSA to promote binding of Tc-99m may lie in its ability to bind the technetium weakly for a long enough period

so that transfer to a more stable complex can occur. An analogous situation is the use of acetate in the preparation of ^{113m}In complexes (22). Acetate ion is added to protect the indium from hydrolysis by forming a weak complex that can be displaced by the desired, more stable one.

The results of this study indicate the potential utility of formamidine sulfinic acid as an alternative reducing agent to SnCl_2 . In addition to producing clean binding of technetium to chelating agents currently in use, its greater stability makes it more convenient and the lack of colloid binding problems reduces the variability of radiopharmaceutical preparations. In our toxicity studies no deaths resulted from doses up to 750 mg/kg. Surprisingly, such doses are considerably higher than the LD_{50} of 423 mg/kg reported in rats. Unfortunately, experimental details are not available for comparison. Using the lower value and a maximum injection of 5 mg of FSA, however, a large safety margin of 3000–5000 results for a 70-kg man. Finally, the ability to prepare technetium complexes that are not possible with SnCl_2 enables one to consider a wider variety of compounds as possible carriers of technetium. The fact that heating is required deters slightly from its general applicability to nuclear medicine, but this is not a serious drawback for the majority of radiopharmaceuticals.

FOOTNOTES

* Formamidine sulfinic acid, phenylphosphonic acid, and nitrilotris-(methylene) triphosphonic acid were obtained from Aldrich Chemical Co., Milwaukee, Wisc.

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