A NEW PREPARATION METHOD FOR ^{99m}Tc-PHYTATE

Isao Ikeda, Osamu Inoue, and Kunio Kurata

Dainabot Radioisotope Laboratories, Tokyo, Japan

A new method is described in which Pt–Sn or Sn–Sn electrodes are used to activate ^{99m}TcO$_4^-$. The ^{99m}Tc is incorporated into phytic acid by stannous ion released from a tin anode by the corrosive reaction. The most suitable pH for labeling phytic acid by this method was below 5 and the ^{99m}Tc-phytate formed could be precipitated with Ca^{+2} at a pH above 3.5. Though ^{99m}Tc-phytate is soluble in aqueous solution, it forms an insoluble species with Ca^{+2} in vivo and is trapped in the reticuloendothelial system. More than 93% of the ^{99m}Tc-phytate localized in the liver of mice; here its biologic half-time is about 112 hr. The ^{99m}Tc-phytate prepared by this method promises to be useful as a liver-scanning agent. Since our method does not require an applied electric potential, it appears to be one of the most convenient methods for labeling compounds with ^{99m}Tc.

The electrolysis method using a zirconium or tin anode for labeling compounds with ^{99m}Tc has been studied by several investigators (1–4). Further studies indicate that the labeling efficiency depends upon the amount of stannous ion formed during the electrolysis (5). Stannous ion can also be formed in an electrolyte solution by a corrosive reaction on a metallic tin electrode without an applied electric potential. We have found that the small amounts of stannous ion formed in this manner are sufficient to reduce the low levels of pertechnetate ion typically present in the eluate from a ^{99}Mo–^{99m}Tc generator to a lower oxidation state for the labeling of some potential radiopharmaceutical compounds. The basic principle of the corrosion method is essentially the same as the other conventional reductive methods, such as the use of stannous chloride and electrolysis.

Labeling phytic acid with ^{99m}Tc requires only a small amount of stannous ion and lends itself to this corrosion method. In the corrosion method, which we will call the electrochemical method, Pt–Sn or Sn–Sn electrodes are used to label phytic acid, which forms an insoluble salt with Ca^{+2} in the blood and localizes in the liver. The possible use of ^{99m}Tc-phytate prepared by this method in liver scanning was examined in animal experiments.

MATERIALS AND METHODS

The Pt–Sn and Sn–Sn electrodes were made by attaching pieces of analytic-grade platinum or tin to the ends of magnetic stirring bars and coating them with polypropylene as shown in Fig. 1. The sodium phytate was obtained from Sigma Chemical Co. (St. Louis, Mo.), and the ^{99m}Tc was eluted from the generator of Dainabot Radioisotope Laboratories (Tokyo, Japan) as a 0.9% NaCl solution of sodium pertechnetate.
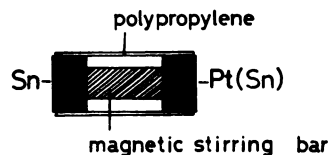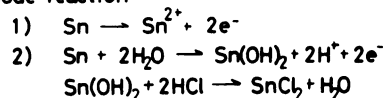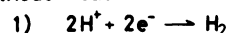
FIG. 1. Magnetic stirring bar with Pt–Sn or Sn–Sn electrodes for electrochemical reaction. Anode and cathode reactions show processes for stannous-ion formation in electrolyte solution.

This NaCl solution, contained in a 10-ml glass vial, was used as the electrolyte. Its pH was adjusted with 0.1 N HCl or 0.1 N NaOH before adding the magnetic bar with the Pt–Sn or Sn–Sn electrodes to the reaction vial. The vial was then placed on a magnetic stirrer and stirred gently. The amount of stannous ion dissolved from the tin anode was determined as a function of time.

Quantitative analysis of the total tin. The total tin dissolved from the anode was measured by a modification of the method of Ross and White (6). One-half milliliter of 0.1 N HCl, 1 ml of pyrocatechol violet solution (0.05% W/V), and 5 ml of 0.2 M acetate buffer solution (pH 4.5) were added to 0.5 ml of the sample. After the samples were allowed to stand for 30 min, the optical density was determined at 555 nm using a Hitachi-124 spectrometer (Tokyo, Japan).

Reduction yields of ⁹⁹ᵐTcO₄⁻. One milliliter of sodium phytate solution (10 mg/ml) was added to 4 ml of ⁹⁹ᵐTcO₄⁻ solution (0.5–10 mCi), and the pH was adjusted with 0.1 N HCl or 0.1 N NaOH. A magnetic stirring bar with Pt–Sn electrodes was placed in the solution and stirred gently. Further, the same experiment was performed without sodium phytate or Pt–Sn electrodes in order to evaluate the reduction of ⁹⁹ᵐTcO₄⁻. The reduction yields of ⁹⁹ᵐTcO₄⁻ were determined by thin-layer chromatography on Eastman silica-gel sheets, using acetone as the developer. In this system the R_f value of ⁹⁹ᵐTcO₄⁻ was 0.95, and various reduced species (⁹⁹ᵐTc-phytate, unidentified ⁹⁹ᵐTc-complex, ⁹⁹ᵐTc-stannous colloid, or ⁹⁹ᵐTcO₂) remained at the origin.

Yield of phytic acid labeled with ⁹⁹ᵐTc. The ⁹⁹ᵐTc-stannous colloid and/or ⁹⁹ᵐTcO₂ formed in the reaction mixture of technetium and phytic acid was measured by filtration through a 0.025-μm membrane filter. More than 99% of ⁹⁹ᵐTc-stannous colloid and/or ⁹⁹ᵐTcO₂ remained on the filter, whereas more than 99% of ⁹⁹ᵐTcO₄⁻ solution or ⁹⁹ᵐTc-phytate passed through the filter. Then, 1 ml of calcium chloride solution (100 mg/ml in barbital buffer solution at pH 7.5) was added to the filtrate and stirred for 5 min to precipitate ⁹⁹ᵐTc-phytate completely. The reaction mixture was filtered again through a 0.45-μm membrane filter and the radioactivity of the precipitate was attributed to ⁹⁹ᵐTc-phytate. The ⁹⁹ᵐTcO₄⁻ and unidentified ⁹⁹ᵐTc complexes in the final filtrate were determined by thin-layer chromatography. More than 99% of the initial ⁹⁹ᵐTc activity could be recovered in the final filtrate. Thus, neither change of the chemical form of unreacted ⁹⁹ᵐTcO₄⁻ nor coprecipitation with Ca^{+2} occurred during the separation procedure.

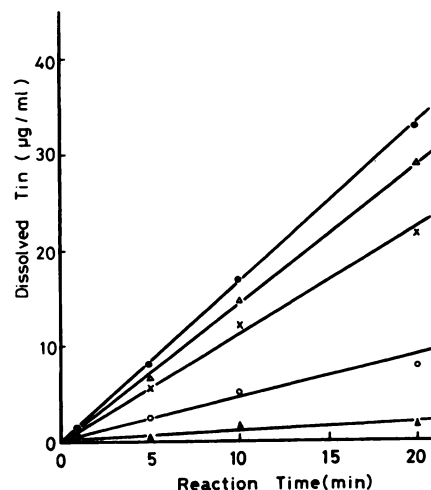**Reactivity of ⁹⁹ᵐTc-phytate with Ca^{+2}.** Pure ⁹⁹ᵐTc-

FIG. 2. Anodic dissolution of tin from Sn–Sn electrodes in acidified 0.9% NaCl solution as function of reaction time. (●) pH 1.0; (△) pH 1.5; (X) pH 2.0; (○) pH 2.5; (▲) pH 3.0.
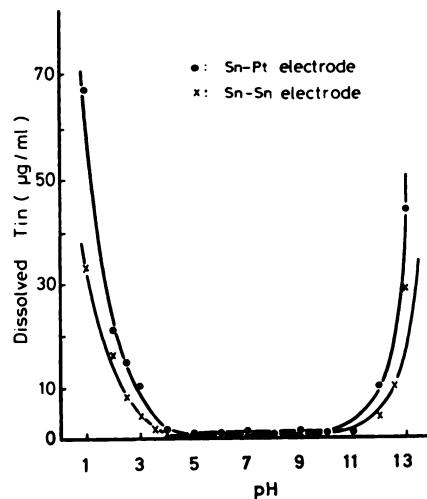

FIG. 3. Anodic dissolution of tin in 0.9% NaCl solution as function of pH (reaction time, 20 min).

phytate was prepared by the stannous chloride method as follows. After adding 1 ml of sodium phytate solution (10 mg/ml) to 4 ml of ⁹⁹ᵐTcO₄⁻ solution, the pH was adjusted to 1.0. One milliliter of stannous chloride solution (1 mg/ml) was added and the solution was stirred gently for 5 min. The reaction mixture was filtered through a 0.025-μm membrane filter to remove the ⁹⁹ᵐTc-stannous colloid. The labeling yield was checked as above: more than 99% of ⁹⁹ᵐTc was found to be incorporated into phytic acid. One milliliter of calcium chloride solution (100 mg/ml) at pH 1.0 was added, the solution was brought to the desired pH with 0.5 N NaOH, and the amount of ⁹⁹ᵐTc-phytate precipitated by the Ca^{+2} was determined.

Animal experiment. Female d-d mice (7), weighing 20–25 gm (Shiraishi Co., Japan), were used in all the animal experiments. One-twentieth milliliter of 99mTc-phytate was injected intravenously within 30 min of preparation. The mice were killed from 5 min to 24 hr after injection, and the radioactivities of the liver and lung measured. Three mice were used in each determination. The biologic half-time in the liver was calculated by the least-squares method from the liver clearance curve.

RESULTS

As shown in Figs. 2 and 3, anodic dissolution of stannous ion depends on the pH of the electrolyte. Tin is easily dissolved in the pH range of less than 4 or more than 11. Below pH 4, the stannous ion can be utilized to label compounds. In the pH range of physiologic saline solution, the anodic dissolution takes place more slowly.

Table 1 shows the yields for pertechnetate reduction in solutions of various pH. Though the amount of stannous ion dissolved from the tin anode depends upon the pH of the solution, the rate of $^{99m}TcO_4^-$ reduction is not critically dependent on pH equilibrium within 10 min. Without sodium phytate in a reaction vial, only a few percent of $^{99m}TcO_4^-$ was reduced in 10 min, and technetium was not reduced without the Pt–Sn or Sn–Sn electrodes in the sodium phytate–technetium mixture.

The reactivity of ^{99m}Tc-phytate with Ca^{+2} does depend upon the pH. More than 98% of ^{99m}Tc-phytate reacts with Ca^{+2} to form insoluble particles in the pH range above 3.5 (Fig. 4).

The ^{99m}Tc-labeling reaction proceeds rapidly and reaches its equilibrium state within 5 min. Figure 5 shows the labeling efficiency of ^{99m}Tc-phytate, once equilibrium has been reached, as a function of pH. No ^{99m}Tc-stannous colloid or ^{99m}TcO$_2$ was observed in the reaction mixture. More than 99% of the activity in the reaction mixture filtered through a 0.025-μm filter. Labeling yields above 90% were
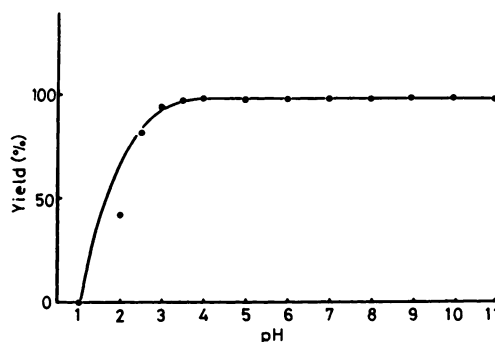

FIG. 4. Precipitaiton yield of ^{99m}Tc-phytate with Ca^{+2} from solutions of varying pH.
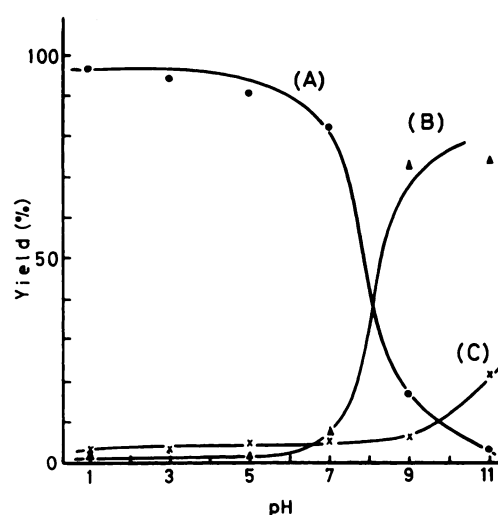

FIG. 5. Composition of electrochemical reaction mixture as function of pH (reaction time, 10 min): (A) ^{99m}Tc-phytate; (B) unidentified ^{99m}Tc complex; (C) ^{99m}TcO$_4^-$. Pt–Sn electrodes and 10 mg of sodium phytate in 1 ml of 0.9% NaCl solution were used.

obtained for pH below 6. Free ^{99m}TcO$_4^-$ gradually increased as the solution pH was increased.

A large amount of unidentified ^{99m}Tc complex, thought to be a reduced species of ^{99m}TcO$_4^-$, was produced at high pH values. The labeling efficiency

TABLE 1. REDUCTION OF PERTECHNETATE (% OF TOTAL ACTIVITY)*

Reaction time (min)	pH = 1			pH = 3			pH = 5			pH = 7			pH = 9			pH = 11		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	66.5	0	0	68.2	0	0	71.7	0	0	85.0	0	0	72.7	0	0	37.2	0	0
3	78.7	0	0	60.5	0	0	91.0	0	0	89.4	0	0	80.8	0	0	71.7	0	0
5	85.5	1.3	0	84.7	1.7	0	91.5	0	0	89.7	0	0	83.2	0	0	74.1	0	0
10	91.2	7.1	0	91.5	4.2	1.1	95.8	1.0	0	94.2	1.3	0	83.3	2.1	0	79.6	5.4	0
15	91.7	5.6	0	96.3	9.3	1.0	96.3	7.2	0	96.4	3.8	1.6	92.8	7.3	0	81.2	12.0	0

* Each value is the mean of three samples. (A) Reaction of sodium phytate and ^{99m}TcO$_4^-$ with Sn–Sn electrodes; (B) reaction of ^{99m}TcO$_4^-$ with Sn–Sn electrodes; (C) reaction of sodium phytate and ^{99m}TcO$_4^-$ without electrodes.
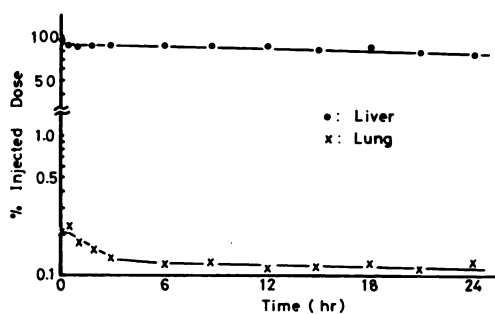
FIG. 6. Liver and lung clearances of ^{99m}Tc-phytate after administration to mice. Biologic half-time is ~112 hr.
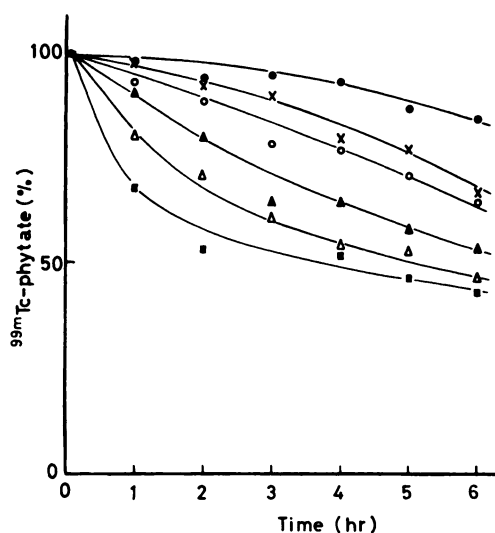

FIG. 7. Stability of ^{99m}Tc-phytate (electrochemical method) as function of temperature. (●) 10°C; (X) 20°C; (O) 28°C; (▲) 37°C; (△) 50°C; (■) 68°C.

was independent of both the amount of sodium phytate over the range of 1–50 mg and the volume of ^{99m}TcO$_4^-$ solution over the range of 2–10 ml (0.1–20 mCi).

More than 93% of ^{99m}Tc-phytate localized in the liver 5 min after intravenous administration in mice, and less than 0.5% was found in the lungs. Figure 6 shows the clearance curves of ^{99m}Tc-phytate from the liver and lungs. The ^{99m}Tc-phytate was slowly excreted from the liver with a biologic half-time of about 112 hr. The unidentified reduced form of ^{99m}TcO$_4^-$, shown in Fig. 5, did not react with Ca^{+2} either in vivo or in vitro and was rapidly excreted through the kidney when injected intravenously.

The stability of the ^{99m}Tc-phytate depends upon the temperature, with the amount of free ^{99m}TcO$_4^-$ increasing as the temperature rises (Fig. 7).

DISCUSSION

There are many methods for labeling compounds with ^{99m}Tc. Most chemical methods use stannous

chloride solution for reduction labeling (8); however, the stannous ion is unstable in aqueous solution and is readily oxidized by small amounts of oxygen. These characteristics can cause poor labeling efficiencies. In the electrochemical method, the tin is dissolved into the solution from both Pt–Sn and Sn–Sn electrodes (Fig. 3). In the case of Pt–Sn electrodes (Fig. 1), tin acts as the anode due to the difference of electrode potential between tin and platinum. In the case of Sn–Sn electrodes, however, microscopic highly localized potential cells form at inhomogeneities on the surface of metallic tin in electrolyte solution. The corrosive reaction therefore proceeds through anode–cathode couplets on the metallic tin surface, stannous ion is released into the solution from the anode and can be used to reduce the ^{99m}TcO$_4^-$. The amount of stannous ion produced depends greatly not only on the pH of the solution (Fig. 3), but also on the surface area of the tin, the volume of the solution, and the speed of stirring. Our labeling method is based on reduction of ^{99m}TcO$_4^-$ by stannous ion, which is essentially the same as in more conventional methods. However, since metallic tin is not oxidized readily when it is stored in a reaction vial packed with nitrogen gas, consistently high labeling efficiencies can easily be obtained. When only trace amounts of stannous ion are needed, this electrochemical method can be utilized for labeling with ^{99m}Tc.

We have previously reported labeling EDTA with ^{99m}Tc by electrolysis (5). In this case the electrochemical method cannot be applied because EDTA forms a stable complex with tin. Only a few percent of ^{99m}Tc-EDTA was obtained at pH 3.5 for a 30-min reaction time. The labeling probably fails here due to the formation of a stannous ion–EDTA complex having a stability constant of log K = 22.3 (9). Thus, labeling such strong chelating agents as EDTA with ^{99m}Tc will require the addition of large amounts of stannous ion. The electrolysis method is therefore more effective for labeling EDTA because the amount of stannous ion can easily be varied by controling the quantity of electricity passed through the sample.

In the electrochemical method, ^{99m}Tc-phytate was easily prepared without formation of stannous colloid. Though ^{99m}Tc-stannous colloid can also be used for liver scanning, its particle size is difficult to control because of aggregation after preparation. Subramanian et al have shown the possibility of using ^{99m}Tc-phytate for liver scanning (10), but they present little information regarding reactivity with Ca^{+2}, optimal labeling conditions, and organ distribution. We found that the labeling should be done below pH 5 and that the ^{99m}Tc-phytate produced is

precipitated with Ca^{+2} quantitatively by adjusting the pH above 3.5 (Fig. 4). Therefore, once the labeled product is injected intravenously, insoluble calcium salt forms immediately in the blood. However, when Ca^{+2} solution is added to ^{99m}Tc-phytate before injection, aggregates of ^{99m}Tc-phytate and calcium gradually form, and these can be trapped in the lungs.

Although the chemical state is not proven for the present, the unidentified reduced species (Fig. 5) was not considered to be either ^{99m}Tc-phytate or ^{99m}Tc-stannous colloid because of its nonreactivity with Ca^{+2}, its filtration through a 0.025-μm membrane filter, and its low affinity for the reticuloendothelial system in mice.

The biologic half-time for cleaning ^{99m}Tc-phytate from the liver is longer than that of ^{99m}Tc-stannous colloid, used routinely for liver scanning. The ^{99m}Tc-stannous colloid has a 32-hr half-time (5); it is gradually oxidized in the liver and excreted. The ^{99m}Tc-phytate precipitated with Ca^{+2} is stable and seems to be metabolized more slowly.

The stability studies (Fig. 7) indicate that free ^{99m}TcO$_4^-$ is released from the ^{99m}Tc-phytate prepared by the electrochemical method with increasing time after preparation. However, ^{99m}Tc-phytate prepared by either the electrolysis or the stannous chloride reduction method is more stable than that prepared by the electrochemical method (11). This phenomenon was also observed with ^{99m}Tc-tetracycline (12). Since the stability of ^{99m}Tc-phytate increases as the amount of tin used for labeling is increased and since the amount of stannous ion used in the electrochemical method is very small, ^{99m}Tc-phytate prepared by this method should be injected within 2 hr after preparation.

Since the electrochemical method does not require either an applied potential or the addition of a chemical reducing agent, it should be a very useful method for labeling certain compounds with ^{99m}Tc.

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