Radiopharmacy

Stannous Tin Levels in Commercial Stannous Pyrophosphate: Effect of Different Preparation Methods

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We determined the stannous tin levels of commercial stannous pyrophosphate preparations in order to evaluate the effects of different preparation methods on the stability of stannous pyrophosphate. Preparation methods included maintaining stannous pyrophosphate at room temperature, refrigerating (2–8° C) preparations after formulation, reconstituting stannous pyrophosphate vials with nitrogen-purged saline, and introducing air into preparations after formulation. Stannous tin levels were determined from 0.5 to 48 hr after formulation using a rapid stannous spot test. Results show that stannous tin levels in all stannous pyrophosphate preparations were within 10% of the manufacturer's stated levels up to 8 hr after formulation. The only exception was when air was introduced into the preparations; this resulted in a significant reduction in the stannous tin levels within 2 hr after formulation. High stannous tin levels in stannous pyrophosphate preparations can be maintained for up to 8 hr if careful radiopharmaceutical preparation and dispensing techniques are used.

In the last few years blood pool imaging agents have become more and more important in nuclear medicine. The most prevalent blood pool agent is T-99m red blood cells (RBCs). Labeling is accomplished in vivo using consecutive injections of stannous pyrophosphate and [^{99m}Tc] pertechnetate (*1*). Using this method of RBC labeling the stannous ion concentration may be critical, yet quantitative determinations of the stannous levels are not routinely done before injection. In addition, the effect of different preparation methods on the stannous tin levels in stannous pyrophosphate kits is not well described. We undertook a determination of the stannous tin levels in commercial stannous pyrophosphate preparations in order to evaluate the effects of varying preparation conditions on the stannous tin content using an easy, semiquantitative spot test.

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Materials and Methods

The stannous tin spot test used has been described elsewhere (*2*). The basis of the spot test involves the porphyrin, tetra (4-N-methylpyridyl) porphine, which is reduced in the presence of acidic Sn(II) solutions to dihydroporphyrin, producing a red color change. Reoxidation of dihydroporphyrin then ensues resulting in the disappearance of the color complex. The total time required for the oxidation process is proportional to the initial Sn(II) concentration. Initially, a ten-fold dilution of unknown stannous pyrophosphate preparation (Mallinckrodt Diagnostics, St. Louis, MO) is made and the procedure outlined in Table 1 is followed. A standard concentration curve is made by using known amounts of stannous ion. An example of a standard curve is found in Fig. 1. The stannous ion of an unknown sample can be calculated from the spot's disappearance time using the standard curve.

The initial Sn(II) content in commercial stannous pyrophosphate kits (Mallinckrodt) was determined by performing the stannous tin spot test on aliquots of the preparations within 1 hr after formulation. Four vials from one lot and five vials from a second lot were tested.

TABLE 1. Procedure for Determining Stannous Tin Concentrations

1. Add one drop (5μl) of stannous sample to be tested to Whatman 31ET chromatography paper.
2. Immediately add one drop (10μl) of porphyrin solution (4 mg/ml) to spot formed in step 1.
3. Place paper approximately 5 cm from the high intensity light source, turn light on, and record time.
4. A deep red spot is formed if the test is positive and the light remains on until the red spot disappears. The total time of disappearance is recorded.
5. The unknown stannous concentration is calculated from the spot disappearance time using a standard concentration curve.

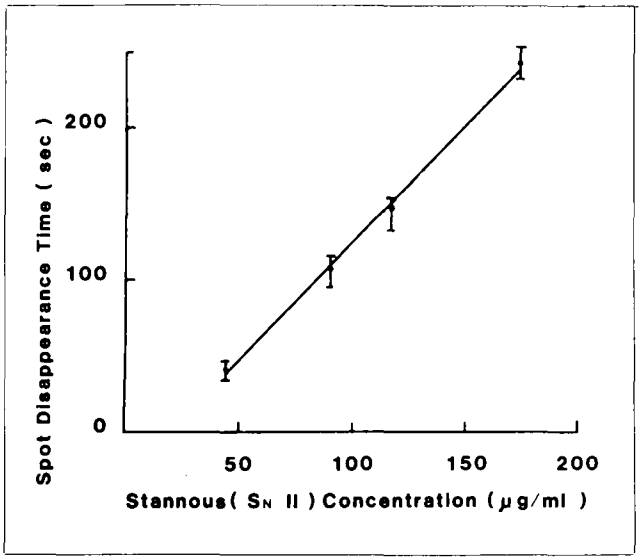

FIG. 1. Standard stannous concentration curve associated with stannous spot test.

For each specific stannous pyrophosphate preparation, five replicate determinations were made and the data statistically summarized.

The effects of altering the preparation methods on the stannous levels in stannous pyrophosphate preparations were tested by adding 2.5 ml of normal saline to each of three vials and vortexing to allow for complete mixing. One vial was maintained at room temperature and the second vial was refrigerated (2–8° C) throughout the study. In the third stannous pyrophosphate vial air was injected into the vial immediately after preparation and the vial maintained at room temperature throughout the study. A fourth vial of stannous pyrophosphate was prepared by adding 2.5 ml of nitrogen-purged normal saline, vortexing, and incubating the solution at room temperature. Nitrogen-purged normal saline was prepared by continuously purging a 30-ml normal saline solution with nitrogen for approximately 5 min. At specific time intervals after preparation, ranging from 0.5 to 48 hr, an aliquot of the specific preparations was removed, a ten-fold dilution was made, and five replicate stannous concentration determinations were performed on the dilutions as previously described. The data was statistically analyzed using Student's *t*-test.

Results

The initial stannous concentration of commercial stannous pyrophosphate kits is shown in Table 2. According to the manufacturer, the amount of Sn(II) in each vial is 2.12 mg. As can be observed from the data, all values were within 10% of the manufacturer's stated values. The Sn(II) values for the vials in lot 1 appeared to be lower than those for lot 2; however, no statistically significant differences were observed between lots or among vials within each specific lot.

TABLE 2. Stannous Tin Concentrations in Commercial Stannous Pyrophosphate Kits

Lot	Stannous Concentrations (mg) in each vial (mean and standard deviation)
1	2.1 0.3
	1.9 0.2
	2.1 0.2
	2.1 0.1
2	2.4 0.2
	2.4 0.3
	2.3 0.3
	2.2 0.2
	2.2 0.2

The stability of a stannous pyrophosphate preparation incubated at room temperature is shown in Fig. 2. Within the first 8 hr after preparation, a slight decrease in the stannous levels was observed; however, these decreases were not statistically significant. At 24 hr after formulation, a statistically significant reduction in the stannous tin (21%) was observed ($p<0.02$). A further reduction in the stannous levels was observed at 48 hr after formulation (47%), which was also statistically significantly lower than the initial stannous concentration ($p<0.001$).

The effect of using nitrogen-purged saline on the Sn(II) levels in stannous pyrophosphate preparations is shown in Fig. 3. No statistically significant differences were observed between the stannous levels of stannous pyrophosphate preparations formulated with normal saline and those formulated with nitrogen-purged saline.

The effect of refrigeration on the Sn(II) levels in

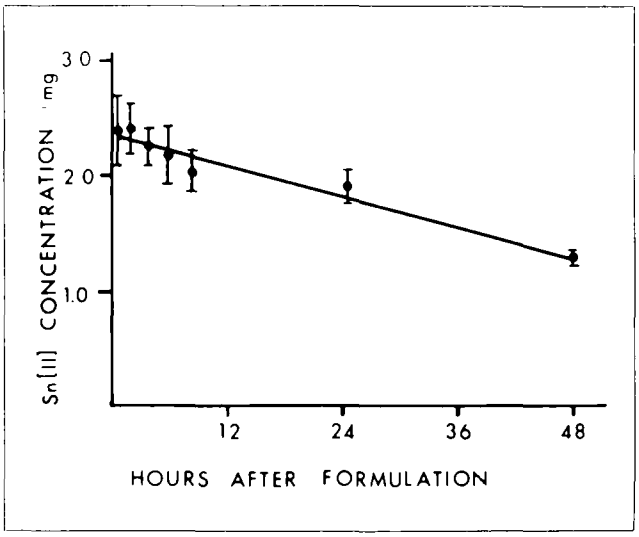

FIG. 2. Stannous concentration in stannous pyrophosphate solution prepared with normal saline and stored at room temperature.

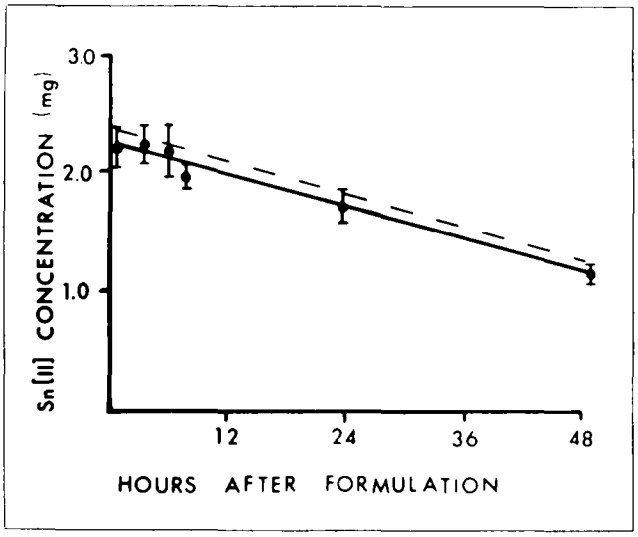

FIG. 3. Stannous concentrations in stannous pyrophosphate solutions: solution prepared with nitrogen-purged saline is indicated by solid line; solution prepared with normal saline is indicated by broken line.

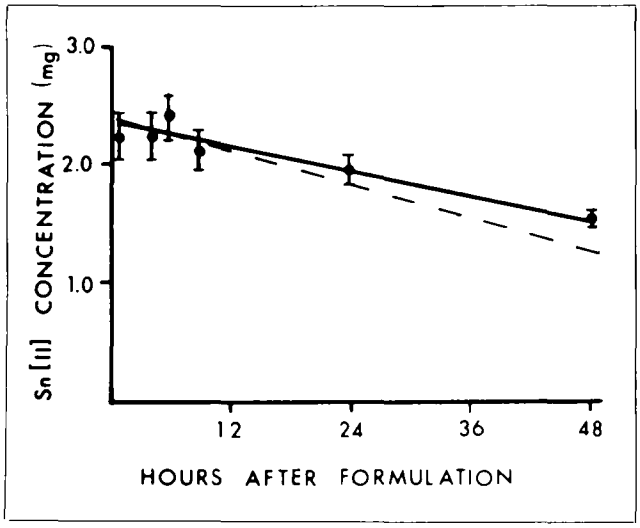

FIG. 4. Stannous concentrations in stannous pyrophosphate solution: solution incubated at 2–8°C is indicated by solid line; solution incubated at room temperature is indicated by broken line.

stannous pyrophosphate preparations is shown in Fig. 4. No statistically significant differences were observed between the refrigerated and room temperature stannous levels within 24 hr after formulation. However, at 48 hr after formulation, the stannous levels of the refrigerated stannous pyrophosphate preparation were significantly higher than the stannous levels of the room temperature stannous pyrophosphate preparation ($p<0.001$).

The effect of air on the stannous levels in stannous pyrophosphate preparations is shown in Fig. 5. A dramatic reduction in the stannous levels was observed within 2 hr after formulation ($p<0.001$). A 36% reduction in the stannous levels was observed at 2 hr after formulation, and the reduction in the stannous tin levels increased to 72% at 24 hr after formulation. At 48 hr after formulation, no Sn(II) levels were observed in the stannous pyrophosphate vial containing air.

Discussion

It is general knowledge that tin in the reduced state (stannous) can easily be oxidized to the stannic state (oxidized form) by such oxidizing agents as air. In the in vivo RBC labeling procedure, the stannous ions participate in the labeling process, and any oxidation to the stannic state before injection will reduce the degree of red cell labeling. For this reason it is important to obtain a quantitative index of the stannous concentration in stannous pyrophosphate preparations before patient injection.

The initial stannous concentration in stannous pyrophosphate kits was within 10% of the manufacturer's stated stannous levels; no statistically significant differences were observed between and within specific lots. This tends to confirm the manufacturer's careful quality control program. However, it is possible that air could be introduced into a vial during the manufacturing process, resulting in a loss of stannous activity within the vial.

The effect of altering the preparation and incubation of stannous pyrophosphate vials demonstrated that minimal oxidation of the stannous tin levels occurred within 8 hr after formulation if no air was introduced into the vial. With the introduction of air into the vial, a rapid oxidation process ensued resulting in a dramatic decrease in the stannous levels within 2 hr after preparation.

A decrease in the stannous levels (21%) occurred 24 hr after formulation in the preparation incubated at room temperature (Fig. 2). Refrigeration or nitrogen-purged saline did not significantly retard stannous oxidation. Optimum RBC labeling could be achieved with a 24-hr

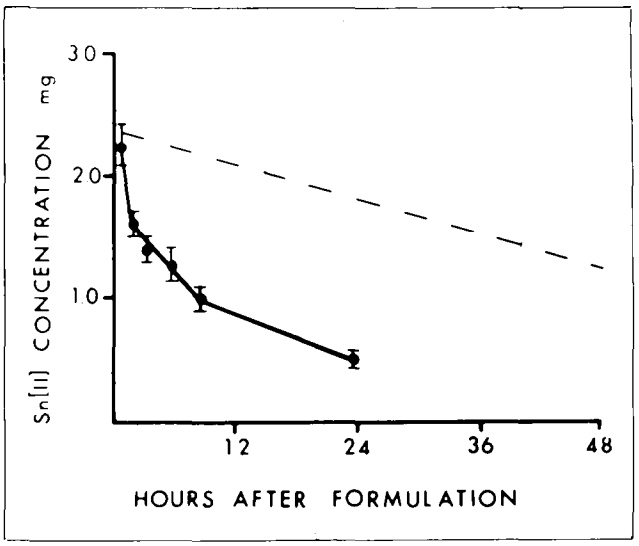

FIG. 5. Stannous concentrations in stannous pyrophosphate solutions: solution with air added to vial after formulation is indicated by solid line; solution with no air added to the vial is indicated by broken line.

stannous pyrophosphate preparation by compensating for the amount of oxidized tin.

At 48 hr after formulation, the stannous levels were reduced by 47% in the solution maintained at room temperature, and a significant reduction in stannous oxidation was noted with the refrigerated preparation.

The data indicate that stannous tin levels in stannous pyrophosphate preparations remain stable for at least 8 hr if the proper technique is used—regardless of incubating conditions. However, if air is introduced into the preparation, either in the initial formulation or in the process of dispensing, a rapid decrease in the stannous tin levels can result. With longer incubation periods, refrigeration of the stannous pyrophosphate preparation is recommended to retard Sn(II) oxidation. The use of nitrogen-purged saline was not effective in retarding Sn(II) oxidation throughout the incubation period (up to 48 hr), indicating that endogenous oxygen is not responsible for stannous oxidation.

The stannous spot test, as outlined, is easy to use and rapid, taking less than 5 min to complete. As such, it can easily be incorporated into the radiopharmaceutical quality control program.

References

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2. Zimmer AM, Spies SM. The paper spot test: A rapid method for quantitating stannous concentrations in radiopharmaceutical kits. *J Nucl Med* (in press).