The Paper Spot Test: A Rapid Method for Quantitating Stannous Concentrations in Radiopharmaceutical Kits

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We have developed a simplified, semiquantitative test for the determination of stannous tin in pyrophosphate and other tin-containing radiopharmaceuticals, excluding those stabilized with ascorbic acid and MAA preparations. The test involves the formation and disappearance of a positive red color complex in the presence of Sn(II) and an acidified porphyrin solution. With this technique, the time of spot disappearance is directly proportional to the Sn(II) concentration spotted. The procedure is easy to use, requiring only a high-intensity light source (30-watt light bulb) and a timing device. The test is accurate, reproducible, and sensitive to Sn(II) levels as low as 40 μg/ml. Because the procedure is rapid (requiring less than 5 min), it can easily be incorporated into the routine radiopharmaceutical quality-control program of any nuclear medicine facility.

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The need to establish a rapid yet quantitative test for measuring the stannous-tin concentrations in radiopharmaceuticals is becoming increasingly important. This is particularly true with in vivo red-cell labeling using stannous ion and pertechnetate, where the stannous concentration may be critical, depending on the quantities of stannous pyrophosphate used (*1*). Yet quantitative determinations of the stannous levels are not routinely performed before injection. This is partly because many of the currently used analytical methods for determining stannous levels are relatively tedious or cumbersome (*2,3*). Recently, a sensitive colorimetric test has been developed using a light-activating procedure in conjunction with a porphyrin, tetra(4-N-methylpyridyl) porphine tosylate (*4*). While the presence of stannous ion is readily detected by this procedure, quantitative data are difficult to obtain, requiring multiple serial dilutions. Another drawback of this method is the difficulty in interpreting borderline colorimetric results. Our laboratory has attempted to minimize these problems by developing a convenient, semiquantitative spot test for the determination of stannous levels in radiopharmaceuticals. It could easily be incorporated into the daily radiopharmaceutical quality-control program of any nuclear medicine department.

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MATERIALS AND METHODS

Increasing concentrations of tetra(4-methylpyridyl) porphine* solutions, ranging from 0.5–4.0 mg/ml, were prepared by dissolving the porphyrin in 5.0 *M* HCl, producing a green color. These porphyrin solutions were then used for all subsequent experimental work.

The experimental procedure consisted of spotting 5 μl of the solution to be assayed (either stannous solution or normal saline) on Whatman 31ET chromatography paper, followed immediately by the addition of 10 μl of the porphyrin solution onto each of the previous spots. The paper strip was then placed under a high-intensity light source (30-watt light bulb) and the formation of a red spot indicated the presence of Sn(II). With the light source still on, the time to disappearance of the red spot was recorded as the "positive spot-disappearance time."

A stock solution of stannous chloride was prepared daily by dissolving between 10 and 20 mg of anhydrous stannous chloride in concentrated HCl and adding enough nitrogen-purged 0.9% NaCl to make a final stannous concentration of 1.00 mg/ml. All subsequent stannous concentrations were made by dilution of the stock solution.

The optimum porphyrin concentration was determined by spotting various concentrations of Sn(II), ranging from 20–200 μg/ml, on Whatman 31ET paper. This was followed by addition of porphyrin solutions in concentrations ranging from 0.5–5.0 mg/ml, to the previous spots. The degree of red color formation,

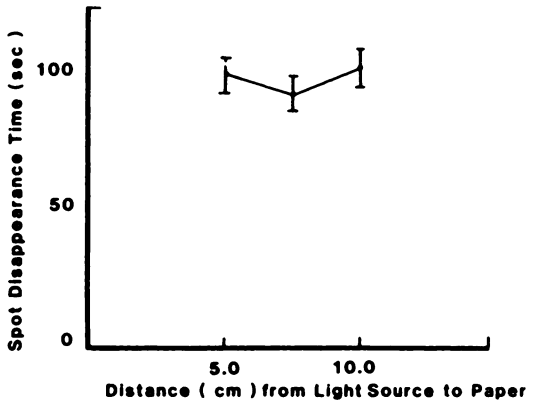

FIG. 1. Effect of varying light-source distance on positive spot-disappearance time.

the case of detection, and the uniformity of spot-disappearance time were observed. At porphyrin concentrations less than 4.0 mg/ml, the red-spot formation was not readily detectable, while no observable improvement in test readability was observed with porphyrin concentrations greater than 4.0 mg/ml. For these reasons, a porphyrin concentration of 4.0 mg/ml was chosen for all subsequent experiments.

The effect of the light-source distance on the positive spot-disappearance time was determined by varying the distance from light source to paper from 5.0–10.0 cm. Five replicate samples were taken at each distance and the data analyzed using a t-test.

The effect of various stannous concentrations on positive spot-disappearance time was tested by placing five replicate spots (5 μl per spot) of each specific stannous concentration (20–180 μg/ml) on Whatman 31ET paper, immediately followed by adding 10-μl samples of the porphyrin solution (4.0 mg/ml) onto the previous spots. The paper was placed 5 cm from the high-intensity light source, and the positive spot-disappearance time determined as previously described. Linear regression analysis of the data was used to establish a standard curve for all subsequent stannous determinations.

The accuracy and reproducibility of determining stannous levels was tested by spotting known standard stannous concentrations (five replicate samples for each specific concentration) and determining the positive spot-disappearance time as previously described. From these data, the stannous concentrations were calculated, utilizing the standard curve, and compared with the standard value. This procedure was repeated 1 wk later, and the results of the data compared.

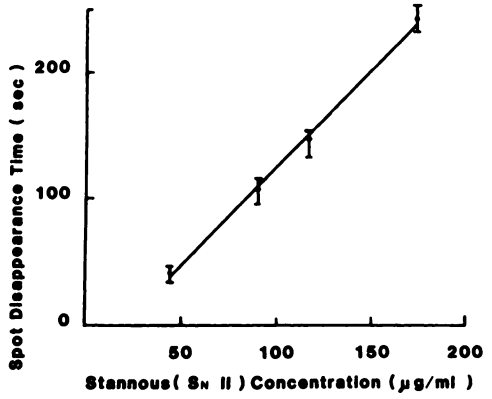

FIG. 2. Effect of increasing Sn(II) concentrations on positive spot-disappearance time.

TABLE 1. ACCURACY AND REPRODUCIBILITY OF THE STANNOUS TEST USING A 4.0 mg/ml SOLUTION

Time after porphyrin solution formulation (days)	Stannous concentration (mg)	Observed stannous concentration (mg)
1	0.62	0.75 ± 0.23*
	1.10	1.03 ± 0.22
	2.10	2.18 ± 0.30
8	0.50	0.40 ± 0.12
	1.09	0.98 ± 0.26
	1.99	1.85 ± 0.25

* Mean and standard deviation. N = 5.

The stannous concentrations of several commercially available tin-containing radiopharmaceutical kits were determined by the method described. Each vial was reconstituted with 2.5 ml of normal saline. If the positive spot-disappearance time of the specific kit was outside the range of the standard curve, appropriate dilutions were made and the dilutions analyzed for stannous content. The measured stannous levels were then compared with the stannous levels supplied by the manufacturer.

RESULTS

Figure 1 plots the positive spot-disappearance time as a function of distance from the high-intensity light source. No statistically significant differences were observed when the light-source distance was increased from 5–10 cm. A distance of 5 cm was chosen for all subsequent stannous determinations.

Figure 2 shows the effect of increasing stannous concentrations on the positive spot-disappearance time. The lower detection limit of the test was approximately 40 μg/ml. Linear regression analysis of the data provided the following equation for the line of best fit: $y = 1.55x - 31.50$. The coefficient of correlation, $r = 0.99$, confirms the satisfactory fit of the data.

The accuracy and reproducibility of the stannous test is shown in Table 1. No statistically significant differences were found be-

TABLE 2. STANNOUS DETERMINATIONS ON COMMERCIAL TIN-CONTAINING RADIOPHARMACEUTICAL KITS

Commercial kit	Manufacturer's stated stannous concentration (mg)	Observed stannous concentration (mg)
Sn-diphospho-nate	0.26	0.19 ± 0.02
		0.18 ± 0.02*
Sn-DTPA	0.26	0.27 ± 0.02
Sn-MDP	0.44	0.43 ± 0.04
Sn-pyrophos-phate	2.12	2.19 ± 0.16

* Value repeated for second vial in same lot. N = 5.

TABLE 3. PROCEDURE FOR DETERMINING STANNOUS CONCENTRATION IN RADIOPHARMACEUTICALS

1. Add 5 μl of the stannous sample to be tested to Whatman 31ET chromatography paper.
2. Immediately add 10 μl of the porphyrin solution (4 mg/ml) to the spot formed in Step 1.
3. Place the paper approximately 5 cm from the high-intensity light source, turn the light on, and record the 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.

tween the observed and the standard stannous levels: all observed values were within 10% of the actual stannous values, demonstrating that the test was reliable. The stannous test was also reproducible, as demonstrated by the low standard deviations associated with the mean stannous levels. The accuracy and reproducibility of the stannous test were maintained at 8 days after porphyrin solution formulation, signifying that no significant degradation of the solution occurred within that interval.

The results of determining the Sn(II) levels in commercial kits are shown in Table 2. Three of the four kits tested were within 10% of the stannous values stated by the manufacturer. For one commercial preparation, Sn-diphosphonate, the observed Sn(II) level was significantly lower than the value reported by the manufacturer. A repeat on another vial within the same lot of Sn-diphosphonate, yielded a low value similar to that of the first vial.

DISCUSSION

The basis of the spot formation has been described previously (*4*). In the presence of an acidic solution of Sn(II), the porphyrin, tetra(4-N-methylpyridyl) porphine tosylate, is reduced to the dihydroporphyrin producing a color change. Reoxidation of the dihydroporphyrin then ensues, resulting in the disappearance of the color complex. The total time required for the oxidation process is proportional to the initial concentration of Sn(II).

Based on the data presented, the procedure outlined in Table 3 is currently used in our laboratory to assess stannous concentrations in radiopharmaceuticals. Initially, a porphyrin solution is formulated (4.0 mg/ml), and a standard curve prepared using known stannous concentrations. This curve is then used to derive stannous concentrations for unknown samples.

This procedure is easy to use, requiring only a high-intensity light source and a stopwatch. The test is accurate, reproducible, and sensitive, detecting stannous concentrations as low as 40 μg/ml. As most commercially available stannous kits contain higher concentrations of tin, ranging from approximately 0.5-2.0 mg per vial, the test is suitable for evaluation of stannous content in these products.

In our laboratory, the primary use of the testing procedure is to determine the stannous content in stannous pyrophosphate solutions intended for in vivo RBC labeling. At present, the only FDA-approved product contains ~2.1 mg of Sn(II) per vial. After reconstitution, an aliquot of the pyrophosphate preparation is removed and a tenfold dilution made. Using the dilution, the spot disappearance time is tested and the stannous concentration determined. If lower stannous levels are obtained, the patient doses can be individualized in order to optimize red-cell labeling.

The spot test is semiquantitative and has distinct advantages over other analytical methods. It is faster and easier to perform than other methods, thus allowing it to be incorporated into a routine daily radiopharmaceutical quality-control program. With the existing porphyrin solution test (*4*), only qualitative results are obtained unless multiple serial dilutions are carried out. Furthermore, stannous concentrations at the lower detection limits of the porphyrin solution test are difficult to visualize, thus inviting errors.

Another useful function of the test is to evaluate quickly the stannous content of Tc-99m(Sn) radiopharmaceuticals (see Table 2). Low stannous levels in these kits can result in poor labeling efficiencies when pertechnetate is added. The stannous test, however, will not give accurate results for some stannous-containing preparations. Diphosphonate and methylene diphosphonate preparations containing ascorbic acid as a stabilizer will give spuriously high stannous results because the ascorbic acid, as a reducing agent, will produce a red color complex with the porphyrin solution. Macroaggregated albumin (MAA) kits will also yield higher results because the particles will clump together when spotted on the paper instead of dispersing throughout the spot.

We are currently using the spot test to evaluate the effects of various chemicals on the Sn(II) levels in radiopharmaceutical preparations.

FOOTNOTE

* Man-Win Coordination Chemicals, Washington, DC.

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