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DETERMINATION OF STANNOUS TIN IN
RADIOPHARMACEUTICAL "COLD KITS"

BY

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ABSTRACT

Two simple titrimetric methods for determining stannous tin in radiopharmaceutical cold kits are described. Of the materials tested, albumin interferes with the N-bromosuccinimide method and none interfere with the iodometric procedure. At the present state of development neither method is suitable for determining stannous tin in kits containing aggregated albumin.

INTRODUCTION

Two methods for determining stannous tin in "cold kits", used for the preparation of Tc-99m labelled radiopharmaceuticals, have been developed. Both are based on the direct titration of the SnII in solution. In the first method titration is with N-bromosuccinimide (1). Of the materials commonly used as cold kits only albumin has been found to interfere with the determination. The second method is a standard iodometric titration in which starch is used as indicator. None of the materials tested interfere with this procedure.

The N-bromosuccinimide method is the method of choice as the reagent, a solid, can be used without prior standardization. Iodine solutions must be standardized daily.

An estimate of the amount of SnII in radiopharmaceutical "cold kits" is desirable. The role of the SnII in the kit is to reduce the pertechnetate added to the reaction vial and so enable in-house preparation of the radioactive radiopharmaceutical. If insufficient SnII is present (through e.g. having been oxidised to SnIV - which occurs rapidly in the presence of air) the product will be of inadequate radiochemical purity. To date the SnII content of kits has been determined indirectly by measuring the radiochemical purity of the labelled product. This is of limited use as a marked reduction in stannous tin will not be evident from such tests, but would manifest itself in a reduction of shelf life for the product.

The paper describes in detail the methods used and gives examples of kits in which the SnII levels have been determined using the described procedures.

DESCRIPTION OF PROCEDURES

Method 1

(a) Purification and determination of purity of N-bromosuccinimide (NBS).

NBS may be purified by recrystallization from a ten fold weight equivalent of water at 75-80°C, then washed with cold water, and ethanol and dried in vacuo. NBS purified as above was assayed by direct titration with sodium thiosulphate. A known volume of 0.1% w/v NBS solution was added to 1 g potassium iodide in 10 ml 3% v/v acetic acid solution. Two

ml of freshly prepared starch solution (1% w/v) was then added and the solution titrated with 0.1M sodium thiosulphate solution to a colourless end point. The determination was carried out in an atmosphere of nitrogen.

$$\text{Percentage purity of NBS} = \frac{\text{ml} \times \text{M} \times 0.089 \times 100}{\text{vol} \times 0.1}$$

where ml = titre of thiosulphate

M = molarity sodium thiosulphate

vol = volume 0.1% NBS solution used

The recrystallized material was found to be 100% pure and stable when stored in a dark well-sealed container. Thus standard solutions may be prepared directly from the solid as required.

(b) Titration of tin II standard

Known volumes of stannous tin standard solution (prepared by dissolving an accurately weighed amount of granulated tin in concentrated hydrochloric acid, in an atmosphere of nitrogen, and making the solution to volume with deoxygenated water) were added to vials containing 10 ml 1M hydrochloric acid solution and 2 drops methyl red indicator solution, and immediately titrated with NBS solution to a colourless end-point. Reproducible results (see Table 1) were obtained which gave a slightly high estimate of the SnII content present.

Table 1. Standardization of Method 1

Tin standard		130 µg/ml	
NBS solution		0.0098%	
Titration Blank		0.20 ml	
1 ml 0.01% NBS	=	66.7 µg stannous tin	
Vol. Standard (ml)	Titre (ml)*	Calculated µg SnII/ml	Percentage Measured
0.5	0.98	126	96.9
1.0	2.1	137	105.4
2.0	4.3	140	107.7
3.0	6.4	139	106.9
*corrected for titration blank, mean of at least 3 values.			

Method 2

(a) Preparation and standardization of iodine solution

A 0.001N iodine solution is required. This may be prepared either by quantitative dilution of a standardized volumetric iodine solution, or by dissolving approximately 0.4 g potassium iodide and approximately 0.12 g solid iodine in 25 ml water in a 1 litre volumetric flask. After all the iodine has dissolved the solution is made to volume with water and then standardized by conventional iodometric procedures (2). Arsenic trioxide is a convenient primary standard.

(b) Titration of tin II standard

Known volumes of a stannous tin solution were added to vials containing 1 ml 1M hydrochloric acid and 0.2 ml starch indicator solution. These were titrated immediately with iodine solution to a blue end-point. Results obtained are shown in Table 2.

Table 2. Standardization of Method 2

I ₂ solution		0.000976N
Titration blank		0.02 ml
1 ml 0.001 N I ₂		= 59.4 µg stannous tin
Vol. Std (ml)	Titre (ml)*	Relative SnII content detd.
0.5	0.60	104
1.0	1.15	100
2.0	2.33	101
3.0	3.46	100

*Blank subtracted, mean of at least 3 values.

Comparison of methods

Both procedures are considered sufficiently reliable and reproducible for determining SnII levels in cold kits.

The accuracy of both methods is estimated as $\pm 10\%$ of the observed value for the concentration range to 400 μg stannous tin. Improved technique should reduce this error.

The methods have high precision. Replicate determinations of 1.0 ml aliquots of SnII solutions of concentration approximately 100 $\mu\text{g}/\text{ml}$ were performed. For these determinations:

	<u>Method 1</u>	<u>Method 2</u>
Titrating solution	0.009% NBS	0.0015N I_2
No. of replicate samples	6	8
Mean	1.82 ml	1.17 ml
Range	0.10 ml	0.07 ml
Standard deviation	0.04	0.03

The limit of detection of both methods is considered to be equivalent to the titre of the blank viz. 0.20 ml of 0.01% NBS or 0.02 ml of 0.001N iodine. This corresponds to 14 μg stannous tin for Method 1 and 2 μg stannous tin for Method 2. Barakat and Doweidar (1) consider their method applicable to the determination of 100 μg to 100 mg stannous tin in a sample.

Interfering materials

All the common active materials supplied as cold kits were tested for potential interference with both procedures.

Materials tested were added to 1.0 ml of standard tin II solution (100 $\mu\text{g}/\text{ml}$) and this solution titrated in the normal manner. Levels which approximate to the quantity of each material commonly found in a vial of commercial preparation were used for each test. Any substance Y does not interfere at the levels shown when the titre for SnII solution + Y was within 5% of the titre for SnII alone.

Table 3. Compounds tested for interference

<u>Compound</u>	<u>Amount (mg) added</u> <u>per test</u>	<u>Interference</u>	
		<u>Method 1</u>	<u>Method 2</u>
Stannic tin	5	X	X
Polyphosphate	50	X	X
Pyrophosphate	50	X	X
Methylenediphosphonate	40	X	X
Ethylenehydroxydiphosphonate	50	X	X
Orthophosphate	50	X	X
Diethylenetriamine penta- acetic acid	30	X	X
Ethylenediamine tetra- acetic acid	40	X	X
Phytic acid	50	X	X
Calcium gluconate	20	X	X
Human serum albumin	5	J	X

From Table 3 we see that the only observed interference was with human serum albumin in Method 1. Any oxidant/reductant would be expected to interfere with both procedures.

Procedure for determining SnII in radiopharmaceutical cold-kits

Either method may be used to determine, by direct titration, the amount of stannous tin in any radiopharmaceutical cold kit except those containing aggregated albumin. The problem of albumin containing cold kits will be returned to later.

Note: Stannous tin solutions are very labile and for all determinations contact with atmospheric oxygen must be minimized. For this reason all solutions should be prepared with deoxygenated water (viz. water through which nitrogen gas has been bubbled while heated to boiling for approximately 1 hour and nitrogen purged while cooling to room temperature) and determinations carried out in an atmosphere of nitrogen whenever possible. All solutions are unstable and should be used only on the day of preparation.

(a) Method 1

To the vial containing the lyophilised material add 0.3 ml 1M hydrochloric acid and 2 drops methyl red indicator solution using a syringe. Shake to ensure complete dissolution of the lyophilised material and then titrate directly with 0.01% w/v NBS solution - to a colourless end point, using a 5.0 ml microburette with a large bore syringe needle attached to the tip. During titration the vial should be agitated with a vortex mixer, and evacuated as necessary to maintain a partial vacuum within the vial.

Determine the reagent blank by titrating 0.3 ml 1M hydrochloric acid and 2 drops methyl red indicator solution in a vial with the NBS solution.

The amount of SnII in the kit is given by

$$\mu\text{g SnII per vial} = \frac{66.7 \times P \times (t_k - t_b)}{0.01}$$

$$\text{or } \mu\text{g SnCl}_2 \cdot 2\text{H}_2\text{O per vial} = \frac{126.7 \times P \times (t_k - t_b)}{0.01}$$

where

P = strength of NBS solution as per cent w/v.

t_k = volume NBS solution consumed by the cold kit.

t_b = volume NBS solution of reagent blank.

(b) Method 2

The procedure is as described for Method 1. Starch solution (0.2 ml) is used as indicator and the titration is with a prestandardized 0.001N iodine solution to a blue end point. A reagent blank should be determined.

The amount of SnII in the kit is given by

$$\mu\text{g SnII per vial} = \frac{59.4 \times N \times (t_k - t_b)}{0.001}$$

$$\text{or } \mu\text{g SnCl}_2 \cdot 2\text{H}_2\text{O per vial} = \frac{112.8 \times N \times (t_k - t_b)}{0.001}$$

where N = Normality of iodine solution.

t_k = volume I_2 solution consumed by the cold kit

t_b = volume I_2 solution of reagent blank.

Direct titration was possible into all vials, including those darkened through gamma-irradiation. Occasionally the total volume of titre exceeded the capacity of the sample vial. In these cases the partially titrated solution was transferred to a larger capacity vial which had been purged with nitrogen, and the titration completed as rapidly as possible.

Determination of SnII levels

A selection of cold kits, current and expired, from various manufacturers were titrated using the above methods. Clear end points were obtained for all materials.

The percentage of the stated SnII content found in various kits are given in Table 4. The observed range of SnII content per vial was 1-133% of stated. This is slightly misleading as most manufacturers give a large actual range of acceptable SnII content in the release specifications for their kit, and the amount of SnII required to effect labelling in the product is very small. What is of greatest interest is not the absolute amounts of SnII present but rather that the kits, even batches which expired some years ago, still contain substantial stannous tin levels. This shows that the formulations are inherently stable.

All the products tested, with one exception, contain acceptable levels of stannous tin. The exception, a batch of AAEC Pentastan (expiry date May 1976) contained a negligible stannous tin content. However, it was obvious that these vials had been inadequately lyophilised or sealed, since they had lost their vacuum and the samples were not dry.

In most cases replicate results were within 10% of each other (for different vials).

Table 4. SnII content of radiopharmaceutical kits with Method 1

Pharmaceutical	Kit	Stated SnII Content	Expiry Date of Batch Used	Per cent of stated stannous tin found*
Pyrophosphate	Mallinckrodt PYP	3.4 mg SnCl ₂	Feb 78	X 91
	AEC Skeitec II (RM7)	95 µg SnCl ₂ ·2H ₂ O	June 75 March 77	84, 67, 63 65, 85, 83
Methylene-diphosphonate	Radiochemical Centre M.D.P.	0.34 mg SnF ₂	May 78	62, 50, 60
	Frosstimage (Mallinckrodt)	1 mg SnCl ₂ ·2H ₂ O	Oct 78	72, 76
	New England Nuclear Osteolite	0.85 mg SnCl ₂ ·2H ₂ O	Aug 78	133, 131
Diethylenetriamine pentaacetic acid	Diagnostic Isotopes (4300)	0.25 mg SnCl ₂ ·2H ₂ O	April 77	101, 96
	AEC Pentastan (RM5)	130 µg SnII	Feb 76	94, 43
			May 76	4, 10, 4, 1, 10
			April 77	111, 111
March 79	116, 112			
Aug 79	Ø 93, 92			

* In August 1978

X Each determination is a separate vial

Ø By Method 2 in December 1978

Method 1 cannot be used to determine the SnII levels in albumin-containing preparations because of the observed interference by albumin. This interference occurs even when the albumin is denatured, and is probably due to reaction of the NBS with the amino acids in the albumin. The SnII content of 3 different kits containing aggregated albumin has been determined using Method 2. Results are given in Table 5.

Table 5. SnII content of albumin containing kits

Kit	Stated SnII content	Expiry Date of Batch Used	Per cent of stated stannous tin found
3M microspheres	100-250 μg SnII	Mar 78	23 of minimum
Mallinckrodt MAA	120 μg $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$	Aug 78	0
C.I.S. TCK-8	160 μg SnCl_2	Sept 78	29

In all cases the observed SnII content was significantly lower than the stated. At present the reason for these low values is not known. Immediately prior to assay all kits were lyophilised and showed no sign of physical deterioration. In addition all gave products of acceptable radiochemical purity, as shown by chromatographic procedures, when labelled with sodium pertechnetate solution. Thus it appears that only part of the SnII in the aggregated albumin kits is "available" and much of the SnII cannot be directly titrated using the method described. This stannous tin is probably occluded within the aggregate. If the total quantity of SnII in the vial is to be determined it will be necessary to leach the trapped stannous tin out of the aggregate before titration. Work is continuing on this problem. A procedure for determining total tin (SnII plus SnIV) in albumin preparations, based on reaction with pyrocatechol violet (3), is being investigated.

Conclusion

Both described procedures are suitable for determining SnII levels, in the presence of SnIV, in radiopharmaceutical cold kits. The method using N-bromosuccinimide is the procedure of choice as this reagent can be prepared as a solution which needs no standardization.

The process of direct SnII determination has been successfully tested for kits containing pyrophosphate, methylenediphosphonate and diethylenetriamine pentaacetic acid. Work is continuing on the determin-

ation of SnII in aggregated albumin preparations.

The method will be used as a routine part of the Australian Radiation Laboratory quality assurance programme.

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References

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