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Indirect Iodometric Procedure for Quantitation of Sn(II) in Radiopharmaceutical Kits

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A method of quantitating stannous ion [Sn(II)] suitable for radiopharmaceutical kits, based on indirect iodometric titration, is described. The method is based on the oxidation of Sn(II) using a known excess of iodine and the excess unreacted iodine determined using thiosulphate by potentiometric titration. The titration cell is a beaker and the titrations are done conveniently under air using an autotitrator in approx. 4 min.

The method is accurate and is linear in the range of approx. 10 μg to approx. 6 mg of Sn(II). Several radiopharmaceutical kits were analysed for their Sn(II) content using the method including those containing antibodies or other proteins. The studies indicate that the procedure is rapid, simple and accurate for routine quantitative estimation of Sn(II) in radiopharmaceutical preparations during development, manufacture and storage.

Introduction

Stannous ion [Sn(II)] is used as an active ingredient in the vast majority of radiopharmaceutical kits. Sn(II) facilitates the reduction ^{99m}Tc(VII) to a suitable lower oxidation state when the radiopharmaceutical kits are reconstituted with pertechnetate with the eventual complexation of reduced ^{99m}Tc to the ligand of interest. The extent of radiolabeling and the potential formation of side products determine the suitability of the reconstituted product as an injectable which is dependent on the critical amount of Sn(II) present in the kit (Gallez *et al.*, 1988; Srivastava and Richards, 1983; Srivastava *et al.*, 1985). Similarly, the shelf life of the kit (frozen or freeze-dried) is determined by the amount of Sn(II) present at the end of manufacturing. More recent investigations (Srivastava *et al.*, 1985; Oster *et al.*, 1985) have indicated that the tin-complexes probably play an additional role apart from being a reducing agent in that the chemistry and metabolic fate of the ^{99m}Tc labeled compound is also altered. In view of the importance of Sn(II) in radiopharmaceutical kits, it is necessary to quantitate the amount as a quality control procedure during development, manufacture and storage.

A number of different methods have been reported in the literature for the quantitative determination of Sn(II) in radiopharmaceutical kits (Gallez *et al.*, 1988; Srivastava and Richards 1983; Chervu *et al.*, 1982; Hambright *et al.*, 1977; Lin *et al.*, 1981; McBride *et al.*, 1977; Meinken *et al.*, 1980; Oster *et al.*, 1985). The most commonly adopted method is the direct iodometric method (Srivastava and Richards 1983) with the end point determined either by starch indicator or by the use of a potentiometric redox electrode (Chervu *et al.*, 1982). Since Sn(II) is highly sensitive to air oxidation, direct titration data would be reliable only if all the necessary precautions are taken to exclude oxygen from the titrant solution and reactant and also if an oxygen-free atmosphere above the solution is ensured during the actual titration. The need for such extensive deoxygenation makes this simple method quite cumbersome and needs appropriately trained personnel if reliable data is to be obtained. It has also been reported (Scott, 1990) that direct potentiometric titration is not suitable for determination of Sn(II) in the presence of proteins and that the time requirement for each analysis is about 15–20 min.

In order to overcome the drawbacks of direct titration methods, there have been suggestions (Scott, 1990; Collins and Nebergall, 1962) that the titrations be done by indirect methods. The current indirect method (Collins and Nebergall, 1962) involves reacting an excess of ferric chloride with Sn(II) to instantaneously oxidize the Sn(II) thereby forming an equivalent amount of Fe(II) which is more stable towards air oxidation and can be estimated by

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titrating with dichromate. It is reported (Collins and Nebergall, 1962) that for the determination of Sn(II) content of samples containing either fluoride or phosphate, it is necessary to add an amount of iron(II) chloride considerably greater than the calculated theoretical amount because both fluoride and phosphate ions form colorless complexes with iron(III) and such complexes do not take part in the reaction with Sn(II). The formation of iron(III) complexes lowers the magnitude of the rise in potential observed at the equivalence point. Therefore their method, it is suspected, may not be suitable for samples containing low amounts of Sn(II) in the presence of high molar concentrations of phosphate ions due to the formation of ferrous-phosphate complexes combined with the decreased change in potential at the equivalence point with dichromate. Similar complex formation is expected with organic ligands and/or proteins if present.

In this paper we propose an alternate indirect approach for quantitation of Sn(II) in radiopharmaceutical kits and radioimmunopharmaceutical kits. The method is based on the oxidation of Sn(II) to Sn(IV) instantaneously using a known excess of iodate or iodine and the unreacted excess conveniently determined by titrating with thiosulfate. This methodology thus overcomes the drawbacks of the direct iodometric method. Preliminary experiments indicated that this methodology yields consistent results and the recoveries were quantitative. In view of this, the method was investigated further and automated for routine use using an autotitrator.

In this paper, we have described the method and standardization of a technique for routine quantification of Sn(II) in radiopharmaceutical kits. The results indicate that the method is simple, rapid (~4 min for each analysis) and Sn(II) recoveries are linear in the range of approx. 10 μg to 6 mg.

Experimental

A combination massive platinum billet electrode (Catalog No. 209-2810-7, Brinkman) or a separate platinum metal electrode (Catalog No. 6-0330-100, Brinkman) in combination with a separate Ag/AgCl reference electrode (Catalog No. 6-0726-100, Brinkman) with SGJ sleeve diaphragm and bridge electrolyte, was used in conjunction with a SET-MET 702 Titrino autotitrator. All titration apparatus were purchased from Brinkmann. The titration cell was a 100 mL beaker.

Sodium thiosulfate ($Na_2S_2O_3$; 0.1 N), potassium iodate (KIO_3; 0.1 N) and potassium dichromate ($K_2Cr_2O_7$; 0.1 N) were all NIST traceable standard solutions from Anachemia Sciences. Potassium iodide (KI; AR grade), sodium potassium tartrate (AR grade) were from BDH. Hydrochloric acid (HCl; 1.0 N; Laboratory grade, Sigma), HCl (5.0 N; AR grade) and stannous chloride dihydrate crystals ($SnCl_2 \cdot 2H_2O$ crystals; AR grade) from Anachemia Sciences were used.

Human Serum Albumin (Sigma), Transferrin (Sigma) and Human IgG (Cutter) were used. Monoclonal antibody MAb-170, and MAb-B80 were from Biomira Inc. Deionized double distilled water (ddd H_2O) was used for preparation of all solutions. Stir cells (100 mL) from Wheaton were used for some experiments. The stock solution of ~0.005 N $Na_2S_2O_3$ was prepared by diluting the 0.1 N standard in the ratio of 1:20 with ddd H_2O. A stock solution of 0.01 N KIO_3 was prepared by diluting the 0.1 N NIST standard with ddd H_2O.

A standard stannous chloride solution (0.5 M) was prepared by selectively weighing 1.130 ± 0.015 g of clear crystalline (~2–6 mm dia) $SnCl_2 \cdot 2H_2O$ crystals and dissolving in 10 mL of 1.0 N HCl. Lower molarity solutions [0.25 M, 0.1 M and 0.05 M Sn(II)] were prepared by dissolving the same amount of the crystals in 25 mL, 50 mL and 100 mL of 1.0 N HCl.

A standard stannous tartrate solution containing 238 μg/mL of Sn(II) was prepared as follows: A 0.1 M buffer solution of sodium potassium tartrate was prepared by weighing 2.822 ± 0.035 g of the salt with dissolution in 100 mL of ddd H_2O. The solution was deoxygenated by purging the solution with nitrogen at ~3 psi for 45–50 min in a Wheaton cell stir apparatus. To the deoxygenated buffer 0.4 mL of 0.5 M stock stannous chloride solution was added and stirred. One (1) mL aliquots of the standard solution were transferred into 5 mL nitrogen purged vials and stored at −20°C. At the time of use, the vial contents were thawed and aliquots of 50 and 200 μL assayed for Sn(II) content.

Protein/antibody interference studies were performed by determining the Sn(II) content in the presence and absence of these substances and the mean values compared. Reagent blanks were also determined in a similar fashion.

A typical analysis consisted of the addition of the required reagents to the titration cell, containing a teflon coated stirring bar operated on a magnetic stir plate and the volume made up to ~60 mL. The electrode and the burette tip was introduced into the cell. The solution was gently stirred avoiding any vortex formation. Appropriate pre-stored methods in the memory of the autotitrator were called up and titrations started.

Standardization of titrant ($Na_2S_2O_3$) was done on a weekly basis using 0.1 N $K_2Cr_2O_7$. A solution of 0.5 mL of 0.1 N $K_2Cr_2O_7$ was transferred into the titration cell. To this 2 mL of 10% KI and 4 mL of 5.0 N HCl was added. The solution was diluted to ~60 mL with water and titrated with standard $Na_2S_2O_3$.

Daily standardization of titrant $Na_2S_2O_3$ was done using 0.01 N KIO_3. A solution of 0.5 mL of KIO_3 was transferred into the titration cell. The solution was diluted to ~60 mL with ddd H_2O. To this solution, 1 mL of 1.0 N HCl and 1 mL of 10% KI was added and titrated against $Na_2S_2O_3$.

titrating with dichromate. It is reported (Collins and Nebergall, 1962) that for the determination of Sn(II) content of samples containing either fluoride or phosphate, it is necessary to add an amount of iron(II) chloride considerably greater than the calculated theoretical amount because both fluoride and phosphate ions form colorless complexes with iron(III) and such complexes do not take part in the reaction with Sn(II). The formation of iron(III) complexes lowers the magnitude of the rise in potential observed at the equivalence point. Therefore their method, it is suspected, may not be suitable for samples containing low amounts of Sn(II) in the presence of high molar concentrations of phosphate ions due to the formation of ferrous-phosphate complexes combined with the decreased change in potential at the equivalence point with dichromate. Similar complex formation is expected with organic ligands and/or proteins if present.

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Daily standardization of titrant $Na_2S_2O_3$ was done using 0.01 N KIO_3. A solution of 0.5 mL of KIO_3 was transferred into the titration cell. The solution was diluted to ~60 mL with ddd H_2O. To this solution, 1 mL of 1.0 N HCl and 1 mL of 10% KI was added and titrated against $Na_2S_2O_3$.

Recovery studies were done by transferring a known excess of 0.01 N KIO_3 (0.5–11 mL) into the titration cell and diluted to about 60 mL with ddd H_2O. To this, 1 mL of 10% KI and 1 mL of 1 N HCl was added. Known aliquots of standard solution containing Sn(II) in the range of approx. 10 μg to 6 mg were transferred into the titration cell containing the oxidizing solution using a calibrated micro pipette or Hamilton syringe. The pipette or syringe needle was placed well within the oxidizing solution during transfer. The excess unreacted iodine was then titrated with standardized $Na_2S_2O_3$. From the titre value the volume of 0.01 N KIO_3 reacted with Sn(II) was calculated and the Sn(II) equivalent amount calculated using the relationship: 1 mL of 0.01 N KIO_3 = 0.594 mg Sn(II).

Of the radiopharmaceutical kits used in this study, MDP, DTPA, Albumin, HIDA and glucoheptonate were obtained from one manufacturer and MDP, pyrophosphate and MAA were from a second manufacturer. To the freeze-dried/frozen sealed kit a known excess of KIO_3 (0.01 N) was transferred using a Hamilton syringe, followed by 1 mL of 1.0 N HCl and 1 mL of 10% KI. The solution was mixed well and allowed to stand for 2–3 min. The kit vials were opened and the contents carefully and quantitatively transferred into the titration cell. The vials were washed with water and the washings transferred into the cell. The volume in the titration cell was made up to (if necessary) ~60 mL and the excess unreacted iodine determined by titrating it with $Na_2S_2O_3$ and the Sn(II) calculated as above.

Results and Discussion

The loss of Sn(II) from a stannous tartrate solution exposed to air is shown in Fig. 1. It is clear from the data that Sn(II) in this solution is rapidly oxidized within about 15 min. In view of such rapid degradation, it is not surprising that when low amounts (15–300 μg) of Sn(II) containing solutions (such as in radiopharmaceutical kits) are directly titrated even under controlled nitrogen atmosphere that variable results are obtained. This is due to uncontrolled exposure of the solution to air during transfer and manipulation. In order to overcome the problem of air oxidation, the indirect iodometric method was developed. In the methodology described in this paper, the Sn(II) is instantaneously oxidized using an excess of iodine. The chemical reactions are as follows:

$$Sn^{2+} + I_2 \text{ (known excess)} \rightarrow Sn^{4+} + 2I^- + I_2 \text{ (excess unreacted iodine)} \quad (1)$$

The iodine in the above reaction is produced *in situ* by the following reaction:

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O \quad (2)$$

The excess unreacted iodine is determined by titrating with $S_2O_3^{2-}$ for which the chemical reaction is:

$$I_2 \text{ (excess unreacted)} + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^- \quad (3)$$

The titration corresponding to equation (3) and the end point determination are done potentiometrically using a platinum electrode and Ag/AgCl reference electrode. The voltage change (~80 mV) towards the end point is sharp for this system (Metrohm Product Application Notes, 1992).

The advantage of the method is that Sn(II) in the initial vial is instantaneously oxidized to Sn(IV) and the resulting reaction mixture is not sensitive to air oxidation and thus low amounts of Sn(II) can be quantitatively and reproducibly determined. The accuracy of the method is controlled by:

(a) $Na_2S_2O_3$ standardization,
(b) the volume of thiosulfate required for a given volume of 0.01 N KIO_3 used in the presence and absence of Sn(II); and
(c) loss of iodine from the system during analysis.

In order that the $Na_2S_2O_3$ used has a known accurate concentration, it was routinely standardized using the NIST $K_2Cr_2O_7$ and KIO_3. The reason for using two standard reagents was to establish an internal control and check on a day to day basis for example to monitor the in-house dilution of the NIST

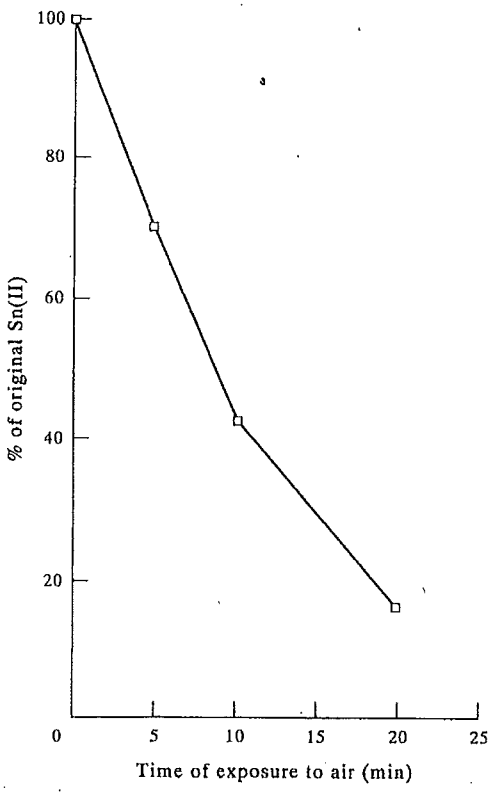

Fig. 1. Sn(II) tartrate solution of 0.5 mL aliquots in 5 mL vials exposed to air analyzed at different time periods

Table 1. Critical parameter information for indirect Sn(II) determination method

Parameter	Value
Thiosulfate standardization using: (a) NIST 0.1 N $K_2Cr_2O_7$ (weekly) mean ± SD: (b) NIST 0.01 N KIO_3 (daily) mean ± SD:	$4.75 \times 10^{-3} \pm 0.05 \times 10^{-3}$ N (*n = 13) $4.74 \times 10^{3} \pm 0.07 \times 10^{-3}$ N (*n = 42)
Daily Control limits (95% confidence limits)	$4.60–5.00 \times 10^{-3}$ N
Acceptable range for daily triplicate analysis	0.15×10^{-3} N
Blank Sn(II) equivalent mean ± SD:	0.5 ± 1.2 μg (*n = 29)
Detection limit (blank mean +3 SD)	4.1 μg
Quantitation limit (blank mean +6 SD)	7.7 μg

*Each n is a mean of three determinations.

standard, to check for any deterioration arising as a result of cross contamination during handling and storage of the reagents and to identify any malfunctioning of the electrodes. This is required as $Na_2S_2O_3$ is a key reagent for Sn(II) quantification and the changes in its concentration as a result of storage etc. needs to be closely monitored. The results of $Na_2S_2O_3$ standardization for a large number of different preparations are shown in Table 1. The data indicates very little variation in concentration for different preparations used during the course of our investigations. Based on the results we have set-up the control limits as shown in Table 1. The reagent blank data obtained over four months is also shown in Table 1. Based on the mean and standard deviation (SD) of the blank values, the Sn(II) detection limit of the method has a value of 4.1 μg and quantification limit of 7.7μg (Long and Winefordner 1983).

In the present studies, the $Na_2S_2O_3$ concentration was chosen as ~0.005 N with the aim of obtaining a larger volume change for a given amount of Sn(II). For example, the volume change for Sn(II) equivalent to $Na_2S_2O_3$ for a typical Sn(II) amount of 25 μg is 84 μL if the concentration is 0.005 N and 42 μL if the concentration is 0.01 N.

In order to avoid any loss of iodine from the system the iodine solutions are diluted considerably during the titration in the presence of a large excess of I^- which leads to rapid conversion of I_2 to I_3^-. All our titrations were done within 5–10 min of mixing of the reagents and the standardization of different preparations of titrant during the course of this investigation (Table 1) under these experimental conditions gave consistent data indicating the problem of iodine loss is not significant. However, the effect of delay between addition of reagents and the titration of the mixture has not been investigated beyond this time period.

As a second practical observation it should be noted that excess KIO_3 should be added, the actual overage depending upon the Sn(II) to be determined. For a typical Sn(II) amount between 10 and 250 μg the volume of KIO_3 used is 0.5 mL. Titration methods were setup for routine use and stored in the memory of the autotitrator. The method included the calculation of results (Schreider and Tinner 1992).

The Sn(II) linearity/recovery study data is shown in Fig. 2 (A) and (B). The theoretical and experimental Sn(II) amounts show a linear correlation in the stannous range studied with an excellent correlation coefficient (r = 1.000) [cf Fig 2(A)]. The percent recovery throughout the Sn(II) range studied is shown in Fig. 2.(B). It is evident that for Sn(II) amounts >600 μg the recovery is 100–105%. For Sn(II) amounts between 100 and 300 μg the recoveries are approx. 93% and for the 60 μg level the recovery is 87%. These studies were done using stock Sn(II) solutions of 0.5, 0.25, 0.1 and 0.05 M and indicate that preparation of dilute solutions of Sn(II) in 1 N HCl leads to oxidation of stannous during preparation. In order to confirm the hypothesis that it is air oxidation and not a problem of the method, we

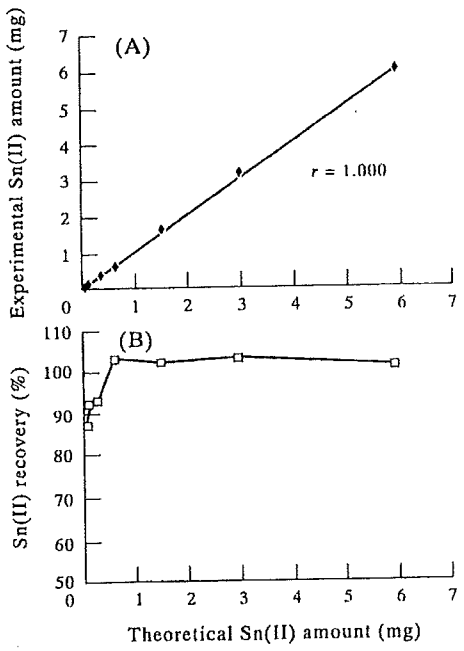

Fig. 2. Sn(II) linearity/recovery studies: Plot of (A) theoretical vs experimental Sn(II) amounts; (B) theoretical vs % recovery of Sn(II) for different Sn(II) amounts.

Table 2. Recovery of Sn(II) at low concentration (<300 μg) using Sn(II) tartrate solution prepared under controlled conditions

	Sn(II) Amount (μg)			
Number	Theoretical	Experimental mean ± SD (n†) (accuracy)	Recovery (%)	CV (%)* (Precision)
1	238.0	236.0 ± 5.2 (3)	99.6	2.1
2	119.0	119.6 ± 3.4 (3)	100.5	2.5
3	47.6	46.9 ± 6.2 (14)‡	98.5	13.4
4	23.8	25.4 ± 3.4 (2)	1.06.7	11.8
5	11.9	12.1 ± 3.6 (14)‡	102.1	28.0

*Coefficient of variation.
†Each *n* is a mean of three determinations.
‡Mean data of stannous tartrate stored at −20°C and analyzed over 90 days.

prepared a Sn(II) standard solution of low concentration (238 μg/mL) under more controlled conditions and estimated Sn(II) in such preparations. The recovery is shown in Table 2. Recovery was quantitative, even at this low concentration, which implies that preparation of Sn(II) at lower concentration requires controlled conditions to avoid air oxidation. On the other hand, the concentrated stock solution of 0.5 M Sn(II) prepared in 1.0 N HCl and stored in a 10 mL stoppered volumetric flask was stable for 2 h as shown in Table 3. The stannous tartrate solutions prepared and stored at −20°C under nitrogen were analyzed over a period of three months and the accuracy and precision of the assay at Sn(II) concentrations of ~50 and ~10 μg/mL are shown in Table 2. The method has an accuracy of 46.9 μg (or recovery of 98.5%) and 12.1 μg (or a recovery of 102%) at theoretical Sn(II) amounts of 47.6 and 11.9 μg, respectively. The precision of the method for the same amounts were ±6.3 μg (13.4%) and 3.4 μg (28%).

Table 4 shows the results of the analysis of radiopharmaceutical kits. The recoveries for glucoheptonate, HIDA and MDP are in the range of 90%, while for the DTPA and albumin kits the values are 76.1 and 82.2% respectively. These recovery values are calculated based on the amount of stannous chloride indicated on the label. The MDP and pyrophosphate kits show quantitative recovery (99.0 and 100.8%, respectively) and the MAA kit 86.5%. These values from the kits obtained from a second manufacturer are compared with the recovery values obtained by the manufacturer's in-house analysis method. The method adopted by the manufacturer is direct iodometric method as reported in the literature (Chervu *et al.*, 1982; Scott, 1990). It is interesting to note the difference in the recovery values for MAA. The direct iodometric method gives a low recovery of 29.2% compared to our present method of 86.5%. This in itself is an indication of the suitability and advantage of the method reported in this paper especially for estimation of Sn(II) in protein containing products. The interference study data for quantification of Sn(II) in the presence and absence of antibody/protein is shown in Table 5. The data indicates that Human IgG, MAb-170, MAb-B80 and transferrin do not show any interference, however, human serum albumin shows a higher blank and thus it is inferred that HSA does interfere but at these Sn(II) levels the error is <10%. This interference could be due to the presence of reducing species, possibly sulfydryl groups in the protein. Reducing agents like ascorbic acid will also interfere with the estimation of Sn(II) as is the case with direct iodometric method. The last column in Table 5 shows the Sn(II) amounts present in the kit after correction for the blank. Comparison of the Sn(II) amounts in samples with the control stock Sn(II) indicate that accurate values are obtained by the method described in this paper even in the presence of interfering ions if appropriate blanks are used.

Table 3. Stability of stock 0.5 M Sn(II) solution in 1.0 N HCl.

Time post preparation (min)	Sn(II) amount (mg) Theoretical (100 μL stock)	Experimental (mean ± SD; n = 3)	Recovery (%)
0	5.948	6.097 ± 0.032	102.5
30	5.948	6.092 ± 0.000	102.4
60	5.948	6.025 ± 0.064	101.3
90	5.948	6.038 ± 0.072	101.5
120	5.948	6.138 ± 0.072	103.2

During the course of the present studies, it was observed that the use of a combination platinum redox electrode led to high blank values (negative) for the analysis of antibody/protein containing samples, however, the electrode behaved normally if kept standing for 15–20 min. This has been identified as due to blockage of the diaphragm causing a hindrance to the flow of electrolyte. On standing the flow of electrolyte was restored as was indicated by the formation of electrolyte crystals on the diaphragm. This problem is very severe in protein samples which precipitate. In order to overcome these problems, it was decided to use a platinum electrode in combination with a separate Ag/AgCl reference electrode with a sleeve type diaphram with ground joints which can readily be cleaned. We have not encountered any problems since switching over to this type of electrode.

Conclusions

An indirect iodometric method of quantitating Sn(II) is described. The method described is simple to perform and requires no extra precautions such as extensive deoxygenation and maintenance of inert atmospheric conditions at the time of titration. All operations can be conveniently performed under ambient atmosphere. The method is simplified and automated if an autotitrator is used. The method is rapid as each analysis takes only about 4 min.

Sn(II) solutions of 0.5 M concentrations can be conveniently prepared and stored in a non-deoxygenated solution of 1 N HCl. For Sn(II) solutions of lower concentration (<500 μg) it is recommended

Table 4. Data on the analysis of Sn(II) in radiopharmaceutical kits obtained from two different manufacturers

Manufacturer	Kit tested (Lot No.)	Sn(II) amount (μg) Theoretical	Sn(II) amount (μg) Experimental (mean ± SD; $n = 3$)	% Recovery Current method	% Recovery Manufacturer's data†	Sn(II) as $SnCl_2$ (amt quoted on label in mg)
I	Glucoheptonate	526.0	507.2 ± 13.9	96.4	n/a	1.0
	HIDA	526.0	468.6 ± 18.4	89.0	n/a	1.0
	MDP	578.0	419.1 ± 16.4	89.8	n/a	1.1
	DTPA	184.0	140.1 ± 3.7	76.1	n/a	0.35
	Albumin	105.0	86.5 ± 1.4	82.2	n/a	0.2
II	MAA	648.0	592.0 ± 11.1	86.5	29.2	1.3
	MDP	1195.0‡	1183.3 ± 79.8	99.0	96.0	2.0 (+0.25AA*)
	Pyrophosphate	2068.0	2104.2 ± 15.4	100.8	98.3	4.0

*AA = ascorbic acid.
†n/a = not available.
‡Includes Sn(II) equivalent of 0.25 mg AA.

Table 5. Interference of antibodies/proteins in the determination of Sn(II)

Sample I.D.	Protein or antibody amount (mg)	Sn(II) amount/ equivalent (mean ± SD; $n = 3$) μg	Sn(II) amount (μg) (Sample-blank)
I			
Reagent blank		−0.1 ± 1.5	0.0
Stock Sn(II)		37.7 ± 0.4	37.8
Stock Sn(II) + MAb 170	2	38.8 ± 1.6	38.0
MAb-170 blank	2	−00.8 ± 1.9	0.0
Stock Sn(II) + MAb B-80	2	37.7 ± 0.9	35.2
Mab B-80 blank	2	−2.5 ± 0.1	0.0
Stock Sn(II) + H IgG	2	40.0 ± 1.4	39.3
H IgG blank	2	−0.7 ± 2.0	0.0
II			
Reagent blank		2.9 ± 0.3	0.0
Stock Sn(II)		95.5 ± 2.4	92.6
Stock Sn(II) + transferrin	2	93.9 ± 2.8	91.0
Transferrin blank	2	2.1 ± 2.8	0.0
Stock Sn(II) + HSA	2	104.1 ± 3.8	96.4
HSA blank	2	7.7 ± 1.0	0.0

that the solution be deoxygenated by bubbling nitrogen for at least 45–50 min at a nitrogen pressure of approx. 3 psi to assure reasonable accuracy.

It is suggested that a separate platinum electrode in combination with a sleeve type Ag/AgCl electrode be used for samples containing proteins/antibody or if the samples shows precipitation or colloidal formation under the experimental conditions described in this paper.

The method developed has a detection limit of 4.1 μg and the method is accurate in the Sn(II) amount range studied (~10 μg to 6 mg). The precision of the assay is a function of the amount of Sn(II) to be determined but at typical Sn(II) levels (0.1–2 mg) it is approx. 5%. The method is suitable for quantification of Sn(II) in radioimmunopharmaceutical kits and in radiopharmaceutical kits currently in use.

In view of the simplicity and rapidity of the method it can be adopted for routine use in a radiopharmaceutical development and manufacturing operations and in nuclear medicine centers as a quality control measure.

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References

Chervu L. R., Vallabhajosyula B., Mani J., Chun S. B. and Blaufox M. D. (1982) Stannous ion quantitation in ^{99m}Tc-radiopharmaceutical kits. *Eur. J. Nucl. Med.* **7**, 291.

Collins R. W. and Nebergall W. H. (1962) Indirect procedure for the determination of tin(II) by potentiometric titration. *Anal. Chem.* **34**, 1511.

Gallez B., Osinski P., Adline J. and Dumont P. (1988) Rapid and precise micro-methods for quantitating active components in commercial bone scintigraphy kits— I. Stannous tin. *Appl. Radiat. Isot.* **39**, 705.

Hambright P., Adabi A., Reid J., Maitland D., Press H. C. Jr and Cherry M. (1977) Rapid spot test for stannous tin levels in ^{99m}Tc kits. *Nucl. Med. Technol.* **5**, 88.

Lin T. H., Garret M. D., Khentigan A., Lum., Lamb J. F. and Winchell H. S. (1981) Instant spot test for Sn(II) in Tc-99 m radiopharmaceuticals and kit reagents. *J. Nucl. Med.* **22**, 72.

Long G. L. and Winefordner J. D. (1983) Limit of detection—a closer look at the IUPAC definition. *Anal. Chem.* **55**, 712A.

McBride M. H. D., George R. E., Kessier W. V. and Shaw S. M. (1977) Stannous ion quantification in pyrophosphate and polyphosphate radiopharmaceutical kits using differential pulse polarography. *J. Pharm. Sci.* **66**, 870.

Methrohm, Product Application Note (1992) 702 Sm Titrino, Application 702 Manual.

Meinken G. E., Srivastava S. C. and Richard P. (1980) Determination of microgram amounts of stannous tin in technetium labeling kits. *J. Nucl. Med.* **21**, 78.

Oster Z. H., Som P., Srivastava S. C., Fairchild R. G., Meinken G. E., Tillman D. Y., Sacker D. F., Richards P., Atkins H. L., Brill A. B., Knapp Jr F. F. and Buttler T. A. (1985) The development and in vivo behaviour of tin—II. Autoradiographic and scintigraphic studies in normal animals and in animal models of bone disease. *Int. J. Nucl. Med. Biol.* **12**, 175.

Schreider W. and Tinner V. (1992) A small high performance titrator. *Am. Lab.* **24**, 48.

Scott J. R. (1990) Stannous ion assay of Tc-99 m kits. *Canadian Association of Radiopharmaceutical Scientists Newsletter* **25**, 2.

Srivastava S. C. and Richards P. J. (1983) Technetium labeled compounds. In *Radiotracers for Medical Applications*, Vol. I. C.R.C. Press, West Palm Beach, FL.

Srivastava S. C., Meinken G. E., Richards P., Som P., Oster Z. H., Atkins H. L., Brill A. B., Knapp Jr F. F. and Butler T. A. (1985) The development and in vivo behaviour of tin containing radiopharmaceuticals —I. Chemistry, preparation and biodistribution in small animals. *Int. J. Appl. Radiat Isot.* **12**, 167.