JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 231–239.
DOI: 10.1002/jlcr.550

Research Article

Solid phase preparations of ^{99m}Tc labeled radiopharmaceuticals

Ponugoti S. Rao, Hongyu Li, Kishan C. Reddy and
Mathew L. Thakur*
Thomas Jefferson University, 1020 Locust Street, Suite 359JAH, Philadelphia, PA 19107, USA

Summary

For the preparation of most ^{99m}Tc radiopharmaceuticals, $SnCl_2$ has remained the agent of choice for reduction of Tc^{7+} to lower valency states, which facilitates its chelation by compounds of diagnostic importance. We have developed a simple technique in which $SnCl_2$ lyophilized in a glass vial, either alone or on a solid matrix of polymeric microspheres (beads), was used. Tin-113 ($t_{1/2}$ – 115 d) was used as a tracer, which facilitated quantitative assessment of loss or release of tin in the reaction mixtures. The feasibility and efficacy of this technique were examined for preparations of four ^{99m}Tc- labeled peptides, in which $SnCl_2$ was used as a reducing agent for radiolabeling, a procedure well established in our laboratory. Labeling efficiencies for all four peptides using $SnCl_2$ on solid phase was greater than 95%, as compared to less than 90% ($P = 0.05$) for $SnCl_2$ lyophilized without the solid matrix. Colloid formation was less than 3% in either case. The stability of $SnCl_2$ was greater than six months for solid matrix, and less for that without the microspheres. The ^{113}Sn measured as a daughter product ^{113m}In indicated that release of $SnCl_2$ from microspheres in reaction mixture was $85 \pm 3\%$, as compared to $80 \pm 5\%$ lyophilized alone. The recovery of ^{99m}Tc in solution from microspheres was 95–100%. The large size of the microspheres used (649 μm) eliminated the risk of drawing them through

*Correspondence to: M. L. Thakur, Thomas Jefferson University, 1020 Locust Street, Suite 359JAH, Philadelphia PA 19107, USA. E-mail: mathew.thakur@mail.tju.edu

Received 29 August 2001
Revised 24 October 2001
Accepted 15 November 2001

a needle in a syringe used for injection of a preparation.

Key Words: solid phase $SnCl_2$; ^{99m}Tc reduction; ^{99m}Tc-labeled compounds

Introduction

Because of its suitable physical characteristics (γ-140 keV – 90%, $t_{1/2}$ – 6 h) and convenient availability from a longer-lived generator (^{99}Mo – $t_{1/2}$ – 2.8 d), ^{99m}Tc has become the workhorse of nuclear medicine since the inception of this modality in early 1960. Today greater than 90% of all nuclear medicine procedures use ^{99m}Tc as a tracer. Almost all ^{99m}Tc labeled radiopharmaceuticals contain the nuclide in a reduced form from its oxidation state 7^+ in which it is eluted from ^{99}Mo generators. A large number of reducing agents have been used for reduction of $^{99m}Tc^{7+}$ to lower valency states,[1,2] but stannous chloride has remained the agent of choice in the preparations of most ^{99m}Tc labeled radiopharmaceuticals used either clinically or experimentally.

Most stannous salts are hygroscopic and prone to a rapid hydrolysis in solutions of a wide pH range. They must be stored in a dessicator and at the time of use for radiopharmaceutical preparation, must be weighed quickly and dissolved in O_2 free (0.05–2 M) hydrochloric acid. Stannous chloride in such solution cannot be reused, and also requires a pH adjustment with basic solutions to render the final product in a pH range of 6.5–7.5. Despite careful handling, on many occasions radiopharmaceutical preparations fail due either to incomplete reduction of ^{99m}Tc or to formation of unacceptable quantity of colloids.

In order to eliminate these problems, many investigators use kit preparations such as glucoheptonate, methylene diphosphate (MDP), or hydroxyethylene diphosphate (HEDP), as coligand, which contain weakly chelated reduced ^{99m}Tc.[3–6] This can increase the cost of preparation, is inconvenient, and can add unwanted chemicals.

In order to resolve these difficulties, we evaluated the use of lyophilized $SnCl_2$, either alone or coated onto polymeric microspheres, to prepare experimental ^{99m}Tc labeled radiopharmaceuticals. We also estimated the useful shelf life of lyophilized $SnCl_2$, determined the release of Sn^{2+} in the reaction mixture using ^{113}Sn as a tracer, and assessed the loss, if any, of ^{99m}Tc on microspheres. We present here a

novel solution to the problem which we believe is simple, inexpensive, convenient, and reliable.

Experimental

Polymeric microspheres

The microspheres, as they are called by the manufacturer (catalog number BB04N/002649) were obtained from Bangs Laboratories, Inc. (Fishers, IN). These were made from polystyrene containing 2% divinylbenzene and should not be confused with the macroaggregated albumin (MAA) microspheres, routinely used in nuclear medicine laboratories. These microspheres were 649 μm in diameter and white in color. There were approximately 6500 microspheres per gram, each with a surface area of 1.2 mm^2. Their density was 1.062 per cm^3. The microspheres were washed with 0.1 M HCl, thoroughly rinsed with deionized water, and dried at 50°C in an oven.

In a clean, dry 10 ml glass vial (I.D. 1.7 cm, Wheaton, Millville, NJ), 15 mg ($\sim$100) microspheres were weighed and kept ready for use.

Lyophilization of $SnCl_2$

Accurately weighed $SnCl_2$-$2H_2O$ (Sigma Chemicals, St. Louis, MO, Analar grade) was dissolved in nitrogen-purged 0.05 M hydrochloric acid in such a way that the concentration of $SnCl_2$ was 5 mg/ml. An accurately known volume (100 or 150 μg $SnCl_2$) of this solution was carefully dispensed on the top of the microspheres in a vial described above. The vials were then placed in an acetone/dry ice bath for 15 min and transferred into a pre-primed lyophilizer (GeneVac, Boston, MA). These quantities of $SnCl_2$ were then lyophilized for 4 h, vials were filled with oxygen-free nitrogen, sealed, labeled, and stored at 8°C. In addition, the same quantities of $SnCl_2$ were also lyophilized in glass vials, which did not contain the microspheres.

Determination of Sn^{2+} release

In order to determine the release of tin quantitatively from the microspheres into subsequent reaction mixtures, ^{113}Sn($t_{1/2}$ – 115 d, Perkin Elmer, Boston, MA) was used as a tracer. Ten micro Curi of ^{113}Sn chloride solution was mixed with the $SnCl_2$ solution that was

dispensed in each vial for lyophilization, carried out as described above. The 115 day half-life of the radionuclide became useful for quantitative determination of Sn^{2+} from day one until the end of six months, the time we chose to study the useful shelf life of lyophilized Sn^{2+}.

Tin-113, however, with its 79 kev (0.6%) gamma ray energy (I.T. 91%), is difficult to measure in an ionization chamber or in a gamma counter. However, it decays to $^{113-n}In$ ($t_{1/2}$ – 100 min, γ-393 keV, 64%), which comes to equilibrium very quickly and permits its measurements very conveniently and efficiently.

To determine the release of Sn^{2+}, ^{99m}Tc radiopharmaceuticals were prepared as described below and ^{99m}Tc and any ^{113m}In that may have been separated from the microspheres in solution was allowed to decay for one week. Indium-113m that remained in equilibrium with ^{113}Sn, either in the radiopharmaceutical solution or that remained on the microspheres, was then measured and its percentage calculated with respect to the total ^{113}Sn added to the microspheres initially. This allowed us to determine the quantity of tin released in the reaction mixture.

Preparation of ^{99m}Tc peptides

Glass vials containing $SnCl_2$ lyophilized without microspheres, with microspheres, or with added ^{113}Sn as a tracer were used for these preparations for up to six months after lyophilization. In order to ensure the versatility of the use of lyophilized Sn^{2+} as a reducing agent, we labeled four different peptides with ^{99m}Tc. Peptides were chosen randomly from the groups of peptides routinely used in our laboratory and had a molecular weight ranging from 850 to 3871 D[a]. These peptides were modified at either the C or N terminus by adding a group of amino acids Gly–(D)Ala–Gly–Gly as a chelating moiety that permitted N_4 configuration for ^{99m}Tc binding. Each peptide was labeled with ^{99m}Tc periodically on several occasions. We chose peptides as representative of experimentally used radiopharmaceuticals because their batchwise, non-kit-based preparations used ^{99m}Tc as a tracer, for the reduction of which $SnCl_2$ in solution was regularly used.[7–10] The vials with an appropriate quantity of lyophilized Sn^{2+} were allowed to warm to room temperature, and peptides were labeled using a method described previously.[7–10] Briefly, 10 μg of a chosen peptide was added to the vial followed by the addition of 5–40 mCi of ^{99m}Tc in 200 μl 0.9% NaCl and 600 μl 0.05 M Na_3PO_4 solution pH-12. The reaction mixture

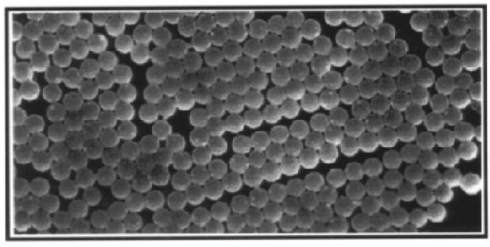
was then mixed and heated for 10 min in a water bath at 90°C. The solution was then allowed to cool and the pH of the reaction mixture was raised to 6–6.5 by the addition of 1 ml 0.05 M, NaH_2PO_4, pH-4.6.

Quality control

For each peptide labeled with ^{99m}Tc using lyophilized $SnCl_2$, HPLC analysis was performed using Rainin HPLC (Woburn, MA), reverse phase C-18 microbond column, 0.1% TFA in H_2O (solvent A) and 0.1% TFA in acetonitrile as solvent B. The gradient was such that the solvent B was 10% at 0 min and 90% at 28 min. The HPLC was equipped with NaI (Tl) radioactivity detector and a variable wavelength ultraviolet monitor. In addition to HPLC, each preparation was also analyzed using two types of instant thin layer chromatographic procedures, in which silica gel impregnated fiberglass paper (Gelman Sciences, Ann Arbor, MI) was used as a stationary phase, methyl ethyl ketone as a mobile phase for determination of free ^{99m}Tc ($R_f = 1.0$) and pyridine : acetone:water (3 : 5 : 1) as a solvent for quantification colloids ($R_f = 0.0$), if any. All data were analyzed using student's t test.

Results and discussion

The uniformity and other characteristics of microspheres used are given in Figure 1. Each peptide preparation was successful, with labeling efficiencies of greater than 95% and colloid formation of less than 3%. These data are provided in Table 1 and a typical HPLC elution profile of a labeled peptide is shown in Figure 2 Using $SnCl_2$ that was lyophilized without microspheres there were two differences. Firstly, the labeling efficiency for all peptides was slightly but consistently less than that with Sn^{2+} lyophilized on microspheres ($P \leqslant 0.05$), and secondly, the shelf life for $SnCl_2$ lyophilized alone was less than six months, as compared to six months for $SnCl_2$ lyophilized on the microspheres. This is presumably because $SnCl_2$ thinly spread on the surface of the microspheres helped to eliminate water molecules during lyophilization, as compared to that which was lyophilized without microspheres forming a small lump. As a result, at the end of six months, the labeling efficiencies using $SnCl_2$ lyophilized without microspheres decreased from approximately 90% to approximately 85%. The high yields we achieved by using $SnCl_2$ lyophilized with microspheres were nearly



Vendor: Bangs Lab, Inc., IN

Polymer: PS/2% DVB

Diameter: 649 micrometers

Color: White

Number of microspheres /g: 6500

Surface area:
1.2 mm^2/microsphere

Density: 1.062 g/ml

Figure 1. Characteristics of polymeric microspheres

Table 1. Efficacy in labeling peptides (with microspheres)[a]

Peptide	% Bound	% Free ^{99m}Tc	% Colloid
TP 850 ($n = 5$)	98 ± 2	2 ± 2	1.3 ± 0.7
TP 1458 ($n = 5$)	98 ± 2	2 ± 2	1.5 ± 0.3
TP 3652 ($n = 4$)	97 ± 2	3 ± 2	1.5 ± 0.4
TP3871 ($n = 4$)	97 ± 2	3 ± 2	1.5 ± 0.5

[a] Results of second (pyridine:acetone : water : 3 : 5 : 1) solvent system. Only % colloid values are given. The % remaining (100-% colloid) was the ^{99m}Tc bound to the peptide.

quantitative and equivalent to those that we usually achieved in our laboratory when we use $SnCl_2$ solutions prepared freshly.[7–10] These yields are also consistently higher than those obtained in methods in which ^{99m}Tc-glucoheptonate is used as a transchelating agent to label peptides using a variety of chelating moieties,[11] and even to label antibodies.[12] Such preparations then require HPLC purification before the agents are used for subsequent evaluation.[13] Because the labeling yields in the present investigation are nearly quantitative, such purification was not necessary.

The recovery of ^{99m}Tc peptides from microsphere vials was also greater than 95%, and higher (Table 2) than the vials in which $SnCl_2$ was lyophilized without microspheres ($<92\%$). The release of $SnCl_2$ in solution from microspheres, as measured by ^{113m}In, was $85 \pm 3\%$ as

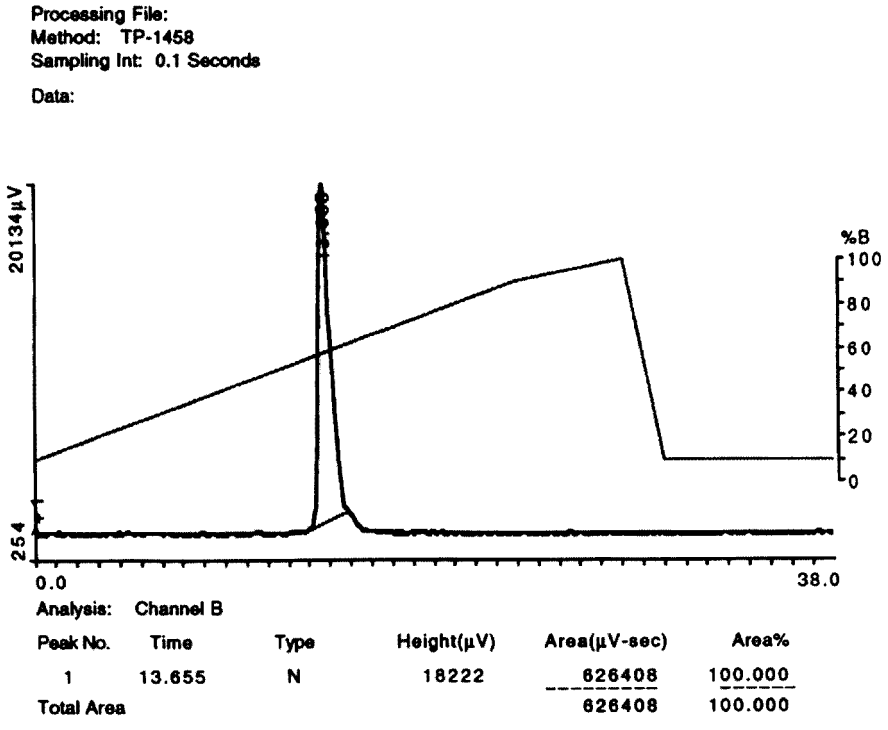


Figure 2. HPLC elution profile of one of the peptides, TP-1458, labeled with ^{99m}Tc using polymeric microsphere-$SnCl_2$ as a reducing agent. A single peak with 100% labeling efficiency is seen at $R_t = 13.6$ min. Free ^{99m}Tc appears at $R_t = 3.5$ min. The diagonal line shows the percentage of gradient composition

Table 2. Recovery of ^{99m}Tc (with microspheres)

Peptide	% In solution	% In vial
TP 850 ($n = 5$)	95 ± 3	2 ± 1.5
TP 1458 ($n = 5$)	97 ± 3	2 ± 1.5
TP 3652 ($n = 4$)	96 ± 3	2.5 ± 1.5
TP 3871 ($n = 4$)	98 ± 2	1.5 ± 1.2

compared to $80 \pm 5\%$ without microspheres ($P \leqslant 0.01$). These data suggest that microspheres may not be porous, retain all $SnCl_2$ on their surface, and make it available for reduction of $^{99m}TcO_4^-$ added to the reaction mixture.

Stannous chloride lyophilized on microspheres makes it possible to dispense only a required number of microspheres containing a

calculated quantity of $SnCl_2$ needed for a given preparation. This is an additional advantage of such a solid matrix. The assumption in such a case, however, will be that $SnCl_2$ was uniformly distributed on each microsphere. In case of such a partial removal of microspheres, for batchwise use, care must be taken to flush the vial containing remaining microspheres with an oxygen and moisture free nitrogen and carefully stored for the future use of the remaining microspheres.

Our data indicate that solid matrix $SnCl_2$ provides a simple, inexpensive, and reliable solution to the preparation of experimental ^{99m}Tc radiopharmaceuticals. In principle, its use for the preparation of other ^{99m}Tc pharmaceuticals should also be feasible. Similarly, the technique should also be applicable to label compounds with such radionuclides as ^{94m}Tc, ^{186}Re, or ^{188}Re, which need to be reduced of their valency states for radiolabeling.

Although $SnCl_2$ lyophilized without microspheres also reduced ^{99m}Tc, comparatively it was less efficient and had less shelf life than that was lyophilized with microspheres. However, it is possible that lyophilization for a longer period of time may have eliminated this problem. The use of microspheres may permit withdrawal from a stock, a predetermined number of microspheres that will provide a required quantity of $SnCl_2$ for the preparation of a given radiopharmaceutical. The diameter, 649 μm, of each microsphere is such that a radiopharmaceutical prepared in the vial can be withdrawn for injection, using even a large (18 G, diameter 127 μm) needle without a risk of drawing a microsphere with the radiopharmaceutical in a syringe used for injection. The microspheres are durable, do not disintegrate by use as described here, and in fact, can be reused after washing the microspheres with 0.1 M HCl followed by deionized water and drying them in an oven. However, microspheres are inexpensive, and for sterility reasons, their repeated use for pharmaceutical grade preparations may not be advisable.

In summary, this simple technique eliminates the numerous drawbacks associated with $SnCl_2$, a commonly used reducing agent in the experimental preparations of a variety of radiopharmaceuticals labeled with ^{99m}Tc. The technique is simple, efficient, and reliable. Stannous chloride lyophilized with a solid matrix of polymeric microspheres is easy to use and provides a longer shelf life and high yields. The technique is applicable not only to preparations of ^{99m}Tc pharmaceuticals, but is also applicable to the preparation of other radiopharmaceuticals in which ^{94m}Tc, ^{186}Re, or ^{188}Re may be used as tracers.

Acknowledgements

This work was presented at the 48th Annual Meeting of the Society of Nuclear Medicine held in Toronto, Canada in June 2001. The generous supply of ^{113}Sn from Perkin Elmer is gratefully acknowledged. Our thanks are due to Ms. Kate Musselman for preparation of the manuscript.

References

1. Richards R, Steigman J. *Radiopharmaceuticals*. The Society of Nuclear Medicine: New York, 1975.
2. Thakur ML, Richard MD, White FW III. *J Nucl Med* 1988; **29**: 1817–1925.
3. Babich JW, Fischman AJ. *Nucl Med Biol* 1995; **22**: 25–30.
4. Maina T, Stolz B, Albert H, Burns C, Maecke H. *Eur J Nucl Med* 1994; **21**: 437–444.
5. Liu S, Edwards DS, Looby RJ. *Bioconjugate Chem* 1996; **7**: 63–71.
6. Baidoo KE, Lever SZ. *Bioconjugate Chem* 1990; **1**: 132–137.
7. Pallela VR, Thakur ML, Consigny PM, Rao PS, Vassileva-Belnikolovska D, Shi R. *Thrombosis Res* 1999; **93**: 191–202.
8. Thakur ML, Marcus CS, Saeed S, Pallela VR, Minami C, Diggles L, Pham HL, Ahdoot R, Kalinowski EA. *J Nucl Med* 2000; **41**: 107–110.
9. Thakur ML, Pallela VR, Consigny PM, Rao PS, Vassileva-Belnikolovska D, Shi R. *J Nucl Med* 2000; **41**: 161–168.
10. Rao PS, Pallela VR, Vassileva-Belnikolovska D, Jungkind D, Thakur ML. *Nucl Med Comm* 2000; **21**: 1063–1070.
11. Stalter MA, Bansal S, Hider R, Mather SJ. *Bioconjugate Chem* 1999; **10**: 130–136.
12. Lei K, Rusckowski M, Chang F, Qu T, Mardirossian G, Hnatowich DJ. *Nucl Med Biol* 1996; **23**: 917–922.
13. Chen JQ, Giblin MF, Wang N, Jurrison SS, Quinn TP. *Nucl Med Biol* 1999; **26**: 687–893.

