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Synthesis of Radioactive Tributyl[^{113}Sn]tin of High Specific Activity for Use in Environmental Fate Studies.

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A method of synthesis of tributyl[^{113}Sn]tin, $(n/C_4H_9)_3{}^{113}Sn$(IV), from commercially available inorganic ^{113}Sn(IV) is presented. Inorganic tin is first extracted in diethyl ether and reacted with C_4H_9MgCl to produce tetrabutyltin, $(C_4H_9)_4{}^{113}Sn$, which is then debutylated with $HgCl_2$. The resulting tributyl[^{113}Sn]tin chloride is isolated from the reaction mixture by successive extractions with hexane and aqueous $Na_2S_2O_3$. The yield is 40–60% and the product obtained is >98% pure. It has the same specific activity as the starting ^{113}Sn(IV), i.e. up to 550 MBq mg^{-1} Sn, making it suitable for use in environmental fate and toxicology studies at concentrations relevant of those found in the aquatic environment. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

We are currently working on the fate of tributyltin, TBT, in aquatic ecosystems. In order to study its bioaccumulation kinetics and tissue distribution in biota, we needed to use radiolabelled TBT. An economic approach to obtaining radioactive organometals is to synthesize them from the corresponding radioactive metal. A few methods of synthesis of ^{113}Sn-labelled TBT have been published,[1,2,3] but in none of them is ^{113}Sn used in the form in which it is at present available from most suppliers, $^{113}SnCl_6{}^{2-}$ in 4–6 M HCl. Furthermore, these methods are intended for the synthesis of 0.1–2 g TBT and need the addition of non-radioactive tin to the radioactive metal. The resulting specific activity of the final product, 0.04–1.1 MBq mg^{-1} Sn, is much too low for fate studies at realistic concentrations (nanomolar and lower). As 37 MBq ^{113}Sn represents 60–500 μg of metal, depending on the specific activity, a way to synthesize microquantities of radioactive tributyltin without diluting the starting ^{113}Sn needed to be found.

Tetra-alkyltin(IV) can react with mercuric chloride to yield the corresponding trialkyltin(IV) and monoalkylmercury(II) compounds.[4,5] A method

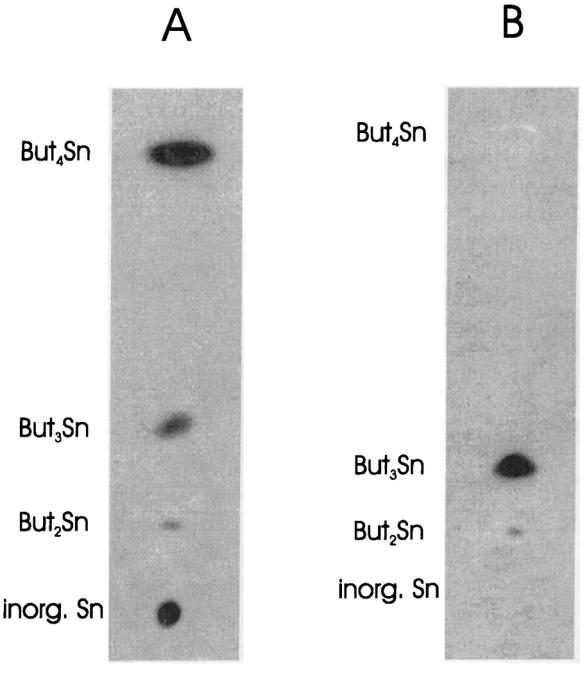

Figure 1 Autoradiograms of thin-layer chromatograms of $(C_4H_9)_4{}^{113}Sn$ after reaction of inorganic ^{113}Sn(IV) with C_4H_9MgCl (A) and of the final tributyl[^{113}Sn]tin solution (B).

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of synthesis using this type of reaction was developed to produce radioactive tributyltin of high specific activity from the radioactive $^{113}SnCl_6^{2-}$ commercially available. It can be summarized by Eqns [1]–[4].

$$^{113}SnCl_{6\,(HCl,4M)}^{2-} \longrightarrow {}^{113}SnCl_{4\,(diethylether)} + 2Cl^-_{(aq)} \quad [1]$$

$$^{113}SnCl_4 + 4C_4H_9MgCl \xrightarrow[diethylether]{} (C_4H_9)_4{}^{113}Sn + 4MgCl_2 \quad [2]$$

$$(C_4H_9)_4{}^{113}Sn + HgCl_2 \xrightarrow[methanol]{} (C_4H_9)_3{}^{113}SnCl + (C_4H_9)HgCl \quad [3]$$

$$(C_4H_9)_3{}^{113}SnCl + (C_4H_9)HgCl \xrightarrow[-(C_4H_9)HgCl]{\text{hexane, aq. } Na_2S_2O_3} (C_4H_9)_3{}^{113}SnCl_{(hexane)} \quad [4]$$

MATERIALS AND METHODS

The first part of the synthesis process was done in a glove box, under a dry nitrogen atmosphere. It began by extraction of 100 µl of the aqueous radioactive $^{113}Sn(IV)$ solution (Amersham or New England Nuclear) with 3 ml of anhydrous diethyl ether (Et_2O) in a 15-ml Kimax glass tube with a Teflon-lined screw cap. After being mixed for 1 min on a Vortex mixer, the Et_2O fraction was transferred to another glass tube containing 1 g $MgSO_4$, previously dried overnight at 120 °C. The extraction was repeated twice and the Et_2O fractions were combined in the same tube, which was then shaken on a Vortex mixer for 1 min. The ^{113}Sn-containing Et_2O was transferred to a thoroughly dried glass tube and evaporated with a gentle stream of dry nitrogen to approx. 2 ml. Then, 3 ml of the Grignard reagent C_4H_9MgCl (2 M in Et_2O) was added and the system was allowed to react for 1 h at room temperature.

At the end of the Grignard reaction, the tube was removed from the glove box and placed in a water–ice bath, and 3 ml of 4 M HCl was slowly added. The Et_2O phase was separated and placed in a clean tube. The aqueous phase was extracted with 3 ml of n-pentane, as above. The combined organic phases, containing $(C_4H_9)_4{}^{113}Sn$, were then evaporated to 0.1–0.2 ml, 2 ml of absolute methanol containing 10 mg $HgCl_2$ ml^{-1} was added, and the tube was closed and placed in a water bath at 40–45 °C for 16–20 h.

After the tube had cooled, 1 ml of 1 M HCl was added and tributyl[^{113}Sn]tin chloride was extracted three times with 3 ml n-hexane, as above. Then, 1 ml of $Na_2S_2O_3$ 0.01 M in water was added to the combined organic phases, mixed for 1 min to remove C_4H_9HgCl, and the organic phase was transferred to a clean tube. Finally, the tributyl [^{113}Sn]tin solution in hexane was evaporated to 2 ml and placed in a suitable vial for storage.

Chemical purity was assayed by TLC against a standard containing tetra-, tri-, di-, and monobutyltin, using 0.2 mm silica-gel plates with hexane:acetic acid (12:1, v/v) for elution. Plates were then submitted to Br_2 vapour for 30 min and developed with 1% dithizone in CH_2Cl_2. After processing, TLC plates were applied to an X-ray film (Hyperfilm MP, Amersham) for autoradiography. The spots were scraped from the plate and their radioactivity was measured with a Cobra Auto-gamma counter (Packard).

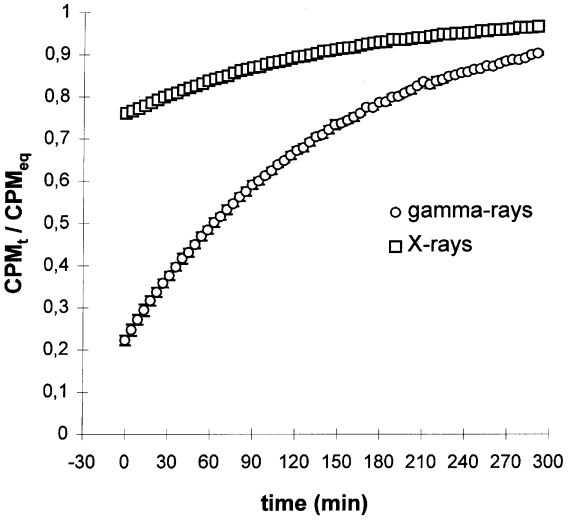

Figure 2 X-ray and γ-ray activities of 5-µl samples of tributyl[^{113}Sn]tin in hexane. Time 0 represents the time at the beginning of measurements, approximately 30 min after the final extraction step of the synthesis. Data are expressed as the ratio of activity at time *t*, CPM_t, and equilibrium activity, CPM_{eq} (CPM = counts per minute). Each point represents the mean ± S.D. of three samples. Equilibrium activity was measured one day after sampling.

RESULTS AND DISCUSSION

The initial extraction of inorganic ^{113}Sn relied on the presence of a large excess of Et_2O relative to the water phase to allow the formation of the neutral $SnCl_4(Et_2O)_2$ complex, which was efficiently extracted (>95%). However, a small amount of water was also extracted, but not the Cl^- ions. Tin(IV) chloride is readily hydrolysed by water in the absence of chloride to yield chemically inert hydrated tin(IV) oxide.[6] In preliminary tests, the yield of the Grignard reaction appeared to be dependent upon the time elapsed between extraction and addition of the Grignard reagent, a shorter time being better. This is probably attributable to the partial hydrolysis of Sn(IV). The above extraction procedure, which takes 45–60 min inclusive of the evaporation of Et_2O before Grignard reagent addition, is the most rapid we were able to find. Although not perfect, drying with $MgSO_4$ is essential to maximize the yield of the Grignard reaction. Use of anhydrous Et_2O instead of ordinary ether also proved better. Use of tubes with leak-proof screw caps is essential to avoid spillage during mixing with the Vortex mixer.

The yield of the Grignard reaction is excellent, at least with tin that is still reactive. An autoradiogram from a thin-layer chromatogram of the organic phase after neutralization of the Grignard reagent (Fig. 1A) showed that most of the reactive ^{113}Sn had formed tetrabutyltin (71%), with some tributyl- (7%) and dibutyltin (1%). The spot with a retention factor of 0 is unreacted tin (21%).

The debutylation reaction is quantitative under the reaction conditions described above, i.e. with up to 500 μg Sn (1500 μg $(C_4H_9)_4Sn$). TLC shows that the radioactive tributyltin produced contains traces of dibutyltin, and occasionally tetrabutyltin (Fig. 1B). However, quantification of the radioactivity of the different spots reveals that the tributyl[^{113}Sn]tin spot represents >98% of the total radioactivity on the plate. Preliminary tests also confirmed that C_4H_9HgCl, which can be seen on the TLC plate as a bright yellow spot with an R_f of about 0.5, is completely removed by $Na_2S_2O_3$ (TLC results not shown). When stored in the dark and in a tightly capped vial, the tributyl[^{113}Sn]tin solution in hexane is stable for three months at room temperature.

The final yield is variable, 40–60% usually being achieved. Most of the losses are due to the presence of inert tin, which is unable to react with the Grignard reagent. However, the yield decreases rapidly after the opening of the vial containing the inorganic ^{113}Sn solution. Actually, we observed that the yield falls below 10% when the synthesis is performed more than a week after the first opening of the vial. Thus, vials containing inorganic ^{113}Sn should be opened only shortly before the beginning of the synthesis process.

Potential users must be warned about precautions to be taken concerning the quantification of tributyl[^{113}Sn]tin by γ-ray spectrometry to determine the synthesis yield or the quantity of tributyltin in material to be used in experiments. When measuring the activity of samples of the final tributyl[^{113}Sn]tin solution immediately after the final extraction step, we observed that the activity at the energies of both γ-rays and X-rays increased with time (Fig. 2). A steady level was reached less than a day after sampling and activity decreased with a half-life corresponding to that of ^{113}Sn thereafter. The disintegration of this radioisotope is not a single-step phenomenon; it proceeds as shown in Eqn [5].

The 391.6-keV γ-ray is often use to quantify

$$^{113}\mathrm{Sn} \xrightarrow[t_{1/2}=115.1\mathrm{d}]{\text{electron capture}} {}^{113\mathrm{m}}\mathrm{In} + \text{In X-rays} \xrightarrow[t_{1/2}=1.658\mathrm{h}]{\text{internal transition}} {}^{113}\mathrm{In\ (stable)} + \text{In X-rays} + \gamma\ (391.6\,\mathrm{keV}) \qquad [5]$$

^{113}Sn. Actually, it is not emitted by ^{113}Sn, but by ^{113m}In, although in handbooks this γ-ray emission is associated with both isotopes. It can easily be calculated[7] that the ^{113m}In/^{113}Sn ratio is 6×10^{-4} at secular equilibrium, which is reached within 12 h. In the synthesis process of ^{113}Sn-TBT, ^{113m}In is separated from ^{113}Sn during the successive water–solvent extractions because of its different chemical properties, so that the latter is not in equilibrium with the former at the end of the synthesis. The half-life of equilibration was calculated from data shown in Fig. 2[7] to be 1.654 ± 0.054 h. This clearly proves that the increase of measured activity is due to the re-establishment of secular equilibrium between ^{113}Sn and ^{113m}In. We also observed this equilibration phenomenon when preparing tributyltin-contaminated food from the hexane solution, indicating that ^{113m}In produced by ^{113}Sn disintegration is not in a chemical form that is soluble in organic solvents. Thus, any process causing the separation of these two isotopes may result in the under-

estimation of sample activity, unless they are left to stand for at least 12 h before counting. The quality of activity data can be controlled with the ratio of X-ray to γ-ray activity, which has a constant value once secular equilibrium is achieved. With our γ-counter, this ratio is 3.77 ± 0.02 (counting windows: 15–120 keV for X-rays, 200–450 keV for γ-rays).

The relatively low yield of this method of synthesis may appear disappointing at first, but it was not possible to do better due to the instability of the chemical form in which inorganic ^{113}Sn is supplied. Nevertheless, the final product is pure, and more importantly it has a specific activity identical to that of the starting inorganic ^{113}Sn, up to 550 MBq mg^{-1} Sn. With such a high specific activity, sub-nanogram quantities of tributyl[^{113}Sn]tin are easily detectable by γ-counting. Thus, this radiolabelled compound is suitable for use in environmental fate studies at concentrations relevant of those found in the aquatic environment, i.e. 1–100 ng l^{-1} in water and $<$1–1000 ng g^{-1} in sediment or food. The availability of ^{113}Sn-labelled tributyltin also allows the use of radioisotopic techniques such as autoradiography. We used this radiolabelled compound to study the kinetics and the body distribution of dissolved and dietary tributyltin in echinoderms.[8,9] It has also proved useful for studying the kinetics of dietary tributyltin in fish and crustaceans with γ-counting *in vivo* (C. Rouleau, C. Gobeil and H. Tjälve, unpublished results), provided that the half-life measured is greater than 1 d. A half-life shorter than 1 d should be interpreted with caution as it might not be possible to make a distinction between ^{113}Sn- and ^{113m}In-related processes if secular equilibrium has been disrupted in some way.

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