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Article

Evaluation of a Wet Chemistry Method for Isolation of Cyclotron Produced [^{211}At]Astatine

Ethan R. Balkin, Donald K. Hamlin, Katherine Gagnon [†], Ming-Kuan Chyan, Sujit Pal [‡], Shigeki Watanabe [§] and D. Scott Wilbur *

Radiochemistry Division, Department of Radiation Oncology, University of Washington, 616 N.E. Northlake Place, Seattle, WA 98105, USA; E-Mails: erbalkin@u.washington.edu (E.R.B.); dkhamlin@u.washington.edu (D.K.H.); kgagnon1@ualberta.ca (K.G.); mchyan@u.washington.edu (M.-K.C.); sujit@u.washington.edu (S.P.); shwata@u.washington.edu (S.W.)

[†] Current Address: Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta T6H 5Y1, Canada

[‡] Current Address: Department of Radiology, University of Washington, Seattle, WA 98105, USA

[§] Visiting Scholar from Medical Radioisotope Application Group of the Japan Atomic Energy Agency, Takasaki, Gunma 370-1292, Japan

* Author to whom correspondence should be addressed: E-Mail: dswilbur@uw.edu; Tel.: +1-206-616-9246; Fax: +1-206-616-8798.

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Abstract: A "wet chemistry" approach for isolation of ^{211}At from an irradiated bismuth target is described. The approach involves five steps: (1) dissolution of bismuth target in conc. HNO_3; (2) removal of the HNO_3 by distillation; (3) dissolution of residue in 8 M HCl; (4) extraction of ^{211}At from 8 M HCl into DIPE; and (5) extraction of ^{211}At from DIPE into NaOH. Results from 55 "optimized" ^{211}At isolation runs gave recovery yields of approximately 78% after decay and attenuation corrections. An attenuation-corrected average of 26 ± 3 mCi in the target provided isolated (actual) yields of 16 ± 3 mCi of ^{211}At. A sixth step, used for purification of ^{211}At from trace metals, was evaluated in seven runs. In those runs, isolated ^{211}At was distilled under reductive conditions to provide an average 71 ± 8% recovery. RadioHPLC analyses of the isolated ^{211}At solutions, both initial and after distillation, were obtained to examine the ^{211}At species present. The primary species of ^{211}At present was astatide, but astatate and unidentified species were also observed.

Studies to determine the effect of bismuth attenuation on ^{211}At were conducted to estimate an attenuation factor (~1.33) for adjustment of ^{211}At readings in the bismuth target.

Keywords: astatine-211; wet chemistry isolation; radioHPLC; reductive distillation; bismuth attenuation factor

1. Introduction

Several research groups are evaluating the use of α-particle-emitting radionuclide astatine-211 (^{211}At) for targeted radionuclide therapy of cancer [1–3]. While there have been few clinical studies with ^{211}At-labeled radiopharmaceuticals [4,5], preclinical studies have been directed at treatment of several different cancer types, including ovarian cancer [6,7], glioma [8], leukemia [9,10], lymphoma [11], breast cancer [12–14], squamous cell cancer [15,16], and prostate cancer [17]. The high interest in use of targeted α-emitters comes from potential advantages of their very high local cytotoxicity brought about by the α-particle's short path length and its high linear energy transfer (LET) [18]. These and other properties of α-emitters, such as high cytotoxicity independent of tissue oxygen concentration and dose rate [19,20], provide an advantage over β-emitting radionuclides for some cancer therapy applications. Of the α-emitting radionuclides, ^{211}At is particularly attractive, as it has negligible high-energy photon emissions and does not have any long-lived α-emitting daughter radionuclide(s). Further, its half-life (7.21 h) is long enough to allow ample time for preparation, quality control and administration before an appreciable amount has decayed, but short enough for its use in an outpatient setting. Most importantly, due to its physical and radiobiological properties, ^{211}At is very attractive for use in targeted treatment of disseminated (metastatic) cancer, blood-borne cancers, minimal residual disease, and disease isolated in compartmental spaces (e.g., ovarian and pancreatic cancers in the peritoneal cavity). An effective treatment of these conditions could greatly improve the five-year survival rates in advanced stages of cancers [21].

Production of ^{211}At can be readily accomplished by irradiating a natural abundance bismuth target (*i.e.*, 100% ^{209}Bi) with a cyclotron-produced α-particle beam [22]. We have used this ^{209}Bi(α,2n) reaction to produce the ^{211}At in all of our studies. Isolation of the ^{211}At from the irradiated bismuth target is generally accomplished by dry distillation [22–26]. The dry distillation method for isolation of ^{211}At can be relatively efficient, providing up to 70% recovery if parameters such as temperature of oven, inert gas flow rates, and size and design of glassware are optimized. We optimized the parameters of the dry distillation isolation approach [27,28] and used that method of isolation for nearly two decades. Even with process optimization, the recovered yields from the dry distillation varied greatly at times. In addition to that problem, more recently our bismuth target was increased in size to produce higher quantities of ^{211}At required to undertake clinical studies [29]. The increase in the size of bismuth target (and its aluminum backing) resulted in having to greatly increase the size of quartz tube used in the high temperature oven. Attempts to remove the bismuth from the aluminum backing mechanically or through heat were deemed too dangerous. An alternate method of cutting the aluminum backing with a small band saw in a charcoal filtered Plexiglas glovebox was used for a short time, but this was also deemed too dangerous for routine application.

An alternate method for isolation of ^{211}At from bismuth targets is referred to as the "wet chemistry" isolation method [22]. In this method, the bismuth target is dissolved in nitric acid, and in subsequent steps, the ^{211}At is separated from the bismuth. The wet chemistry approach was used in early studies for isolation of ^{211}At [30,31] and has been employed more recently by other research groups [32,33]. This approach was attractive for our purposes as it appeared to: (a) be an approach where there was control over the ^{211}At recovery at each step, such that more consistent yields might be obtained; (b) provide high recovery yields; (c) be scalable to clinical and commercial levels of ^{211}At production; and (d) ultimately allow automation of the isolation process. For these reasons, we undertook an investigation of the wet chemistry approach for isolation of ^{211}At from bismuth targets. The results from our investigation are reported herein.

2. Experimental Section

2.1. General

Reagents used were obtained from commercial sources as analytical grade or better and were used without further purification, except for diisopropyl ether (Sigma-Aldrich, St. Louis, MO, USA), which was distilled within 24 h of use to purify and remove any stabilizers. Standard methods for safely handling radioactive samples were employed. Handling and processing of the irradiated target was conducted in a glovebox (Innovative Technologies, Inc., radioisotope glovebox, Amesbury, MA, USA) vented through a charcoal filter on the glovebox exhaust, and subsequently vented through tubing into a second charcoal-filtered Plexiglas enclosure (12" × 16" × 21"; Biodex 112-038) within a radiochemical fume hood. A general purpose rate meter (model 3A) with an alpha detection probe (model 43-2) and a second general purpose rate meter (model 3A) with a gamma detection probe (model 44-3) were placed on a shelf at the inside back of the glovebox for monitoring use during the isolation procedures.

2.2. Dose Calibrator Measurements and Calibration

Measurements of ^{211}At were conducted on either a Capintec CRC-15R or a CRC-55t Radioisotope Calibrator (Capintec Inc., Ramsey, NJ, USA) using the calibration number of 046 for ^{211}At (calibration # calculated by KG). Initial measurements were conducted by insertion of the irradiated target into the dose calibrator, aligning the target (in a plastic zipper bag) into the same position for run-to-run comparisons. All other measurements were obtained by placing glass or plastic vials containing ^{211}At into the dose calibrator using the Plexiglas vial/syringe dipper.

The Capintec dose calibrator calibration setting for ^{211}At was empirically determined by cross calibration against a high purity germanium (HPGe) detector (AMETEK, Oak Ridge, TN, USA). The HPGe detector, coupled to a PC-based multichannel analyzer, provided spectra that were analyzed using Maestro-32 software (ORTEC, Oak Ridge, TN, USA). As the HPGe detector efficiency varies substantially in the ~80 keV range (*i.e.*, the most abundant photons of ^{211}At), the less abundant 687.0 keV (I_γ = 0.261%) photon of ^{211}At, and the 596.65 (I_γ = 0.311%) and 897.8 (I_γ = 0.320%) photons of the short-lived ^{211}Po daughter (branching ratio of 58.20% applied above) were selected for quantification. The HPGe detector was calibrated using ^{133}Ba, ^{137}Cs, and ^{60}Co at a sample-detector

distance of 17 cm in the presence of a 2 mm sheet of copper and a 3 mm sheet of steel placed adjacent to the sample. This material was applied to allow for assaying of a substantial quantity of ^{211}At while maintaining reasonable dead-time by attenuating the high abundance ~80 keV photons of ^{211}At.

To obtain the cross calibration, 100 μL of purified ^{211}At was aliquoted into the tip of a microcentrifuge tube and assayed for approximately 2 min on the HPGe detector. The dead-time was <16%, the statistical error of each photopeak was <2%, and the activity at the start of counting was determined to be 26.6 ± 0.7 MBq (~718 μCi) when calculating the average and standard deviation of the three photopeaks. Within the hour, the same microcentrifuge tube was re-assayed in Capintec models CRC-55tR and CRC-15R dose calibrators, whereby a calibration number of 046 was found to be necessary to display the HPGe-based decay-corrected ^{211}At activity.

2.3. Preparation and Irradiation of Bismuth Targets

The University of Washington Medical Center (UWMC) houses a Scanditronix MC-50 positive-ion source cyclotron capable of accelerating and extracting α-particles with an energy range of 27.0–47.3 MeV and currents of up to 70 μA. A 0° beam extraction line and external target station were designed and installed to scale-up to clinical production levels of ^{211}At [29]. Irradiation of a bismuth target (34 mm × 160 mm × 7 mm) placed on a 10° incline with a 50 μA, 29 MeV α-beam routinely produces 19+ mCi of ^{211}At (as measured in the dose calibrator) in roughly a 45 min irradiation period.

The ^{209}Bi metal target (99.999%, Aldrich) was prepared by melting >5 g of Bi into a machined cavity in the aluminum target body (18 mm × 120 mm × 0.30 mm) on a hot plate at 330 °C. During the melting process, a porcelain spatula was used to constantly scratch the aluminum surface below to eliminate air bubbles and improve adherence of Bi. After cooling to room temperature, the uneven bismuth surface was machined flat to yield a bismuth (and surrounding aluminum) thickness of 0.17 mm. At the specified depth (0.17 mm), width (18 mm) and length (120 mm) a mass of bismuth of ~3 g can be calculated, but the tolerances for milling the cavity in the aluminum backing can change the mass significantly. The bismuth target was installed on the beamline as previously described [29].

2.4. Procedure for Wet Chemistry Isolation of Na[^{211}At]At

The wet chemistry isolation process is conducted in a charcoal-filtered radiochemical glovebox designed to prevent any release of ^{211}At into the atmosphere. A picture of the glovebox is shown in the Supplemental Material (Figure S1). Most reagents, glassware and equipment (e.g., heater-stirrers, lab jacks, *etc*.) required for the isolation are placed in the glovebox prior to introduction of the irradiated target. A list of those items is provided in the Supplemental Material (List S2). Prior to the introduction of the plastic bag containing the irradiated target into the glovebox, the fan removing air through the charcoal filter is turned on to provide negative pressure in the glovebox. The exhaust from the fan passes through a charcoal filter within the glovebox and then through tubing, which is vented into a Plexiglas box that has two stacked charcoal filters. The exhaust-facing surfaces of both charcoal filters are examined to determine if ^{211}At has been released from the glovebox. A picture of the Plexiglas enclosure with the vent tubing is shown in the Supplemental Material (Figure S3).

Just prior to beginning the procedure, the irradiated bismuth target, inside of a plastic zipper bag used while transporting from the cyclotron vault to the radiochemistry laboratory, was placed in a dose

calibrator to estimate the amount of ^{211}At produced. It was then passed through the antechamber into the glovebox. There is a second dose calibrator in the glovebox, so the target is read in that dose calibrator as well. Any ^{211}At measurements performed on material within the glovebox are compared with the target reading obtained from the dose calibrator within the glovebox.

Step 1: Dissolution of Bi target in HNO_3. The irradiated bismuth target was removed from the plastic zipper bag and placed bismuth face down in a 1L modified polypropylene bottle. A 10 mL aliquot of conc. HNO_3 (ThermoFisher, Waltham, MA, USA) was then added by pipet to the bottle. The dissolution of the bismuth target was allowed to proceed for 10 min. The HNO_3 solution was transferred by a pipet to a 50 mL round bottom flask. An additional 5 mL of HNO_3 was added to the dissolution bottle to rinse the target and container. After gently shaking the flask, the wash HNO_3 was also transferred to the round bottom flask.

Step 2: Removal of HNO_3. The 50 mL flask containing Bi and ^{211}At dissolved in conc. HNO_3 was connected to a short-path distillation head. That glassware was lowered into a 50 mL round bottom aluminum heating block on a hot plate at ~300 °C. The distillation flask and neck of distillation head were covered with aluminum foil. After rapidly heating the HNO_3, it was distilled with gentle stirring over a 20–30 min period. The distillation was stopped when no further HNO_3 distillate drops were visible. At that time the distilling glassware was raised from the heating block, and the white residue in the distillation flask was allowed to cool for 10 min.

Step 3: Dissolution in HCl. The 50 mL distillation flask containing the white residue was removed from distillation head. To that flask was added 8 mL of 8 M HCl (ThermoFisher, Waltham, MA, USA), and the mixture was agitated until all solid was dissolved (5–10 min). The solution was transferred via pipet to a 20 mL glass scintillation vial. The distillation flask was rinsed with an additional 2 mL of 8 M HCl as a wash, and that solution was also transferred to the scintillation vial.

Step 4: DIPE Extraction and HCl Washes. A 4 mL aliquot of freshly distilled diisopropyl ether (DIPE) (Sigma-Aldrich, St. Louis, MO, USA) that had been saturated with 8 M HCl was added to the scintillation vial and the resulting biphasic mixture was stirred for 10 min. Following that, the aqueous layer was removed and counted in a dose calibrator. A 5 mL quantity of DIPE-saturated 8 M HCl was added to the DIPE in the scintillation vial, and that biphasic mixture was stirred for 5 min. Following agitation, the aqueous HCl layer was again removed via pipet and counted in a dose calibrator. The preceding two steps were repeated an additional three times (for a total of 4 washes). The DIPE solution in the scintillation vial was counted in a dose calibrator to determine the ^{211}At activity remaining.

Step 5: Back-Extraction into NaOH. A 600–700 μL quantity of 4 M NaOH (ThermoFisher, Waltham, MA, USA) was added to the DIPE in the scintillation vial, and the vial was stirred for 10 min. The pH of the basic aqueous ^{211}At solution was checked to confirm that it was strongly basic (pH > 13). The aqueous layer (containing the ^{211}At) was then removed and both layers were counted separately in the dose calibrator to determine the total amount of ^{211}At isolated. If the ^{211}At was to be used without a further purification, the isolated NaOH solution was neutralized (brought to pH 6.8–7.0) by addition of aliquots of HCl or NaOH solutions (4 M, 1 M, 0.5 M and 0.1 M) as required.

Step 6: Distillation under reductive conditions. To a 25 mL round bottom flask containing 9.19 mL DI-water, 0.75 M ferrous sulfate heptahydrate (Sigma-Aldrich, St. Louis, MO, USA), in 1.5 M H_2SO_4 (ThermoFisher, Waltham, MA, USA) was added the basic ^{211}At solution. The resultant ^{211}At solution was stirred to re-dissolve the characteristic cloudy greenish precipitate, and the flask was connected to

a cold-water jacketed distillation head. To the receiving side of the apparatus was attached a 10 mL round bottom flask pre-charged with 100 μL of 1 M NaOH. The distillation assembly was lowered into a 25 mL round bottom aluminum heating block (and an ice bath for the receiving flask), the distilling flask and neck of the distillation head were covered with aluminum foil, and the ^{211}At solution was distilled at 200 °C (hot plate temperature) with gentle stirring. The collected ^{211}At, free of bismuth metal and other contaminants from the wet chemistry procedure, was used for radiolabeling experiments. In some studies, the aqueous solution was reduced in volume while heating (e.g., 80 °C) under an argon stream to provide a higher concentration of ^{211}At.

2.5. HPLC Analysis of Isolated Na[^{211}At]At

Analyses of the identity of ^{211}At species and purity of the isolated Na[^{211}At]At solutions, both from the initial isolation and the solutions of distilled ^{211}At were performed by radioHPLC. Two radioHPLC systems were used. The HPLC system used in the studies was a Hewlett-Packard model 1050 HPLC (Hewlett-Packard Company, Palo Alto, CA, USA) with a Beckman Model 170 Radioisotope Detector (Beckman-Coulter, Brea, CA, USA) to detect the radioactive species present. The radionuclide solution was evaluated with a YMC J'sphere ODS-M80 reversed-phase column (YMC America, Allentown, PA, USA) under isocratic conditions using a 1:1 mixture of 0.05 M TBAP (tetrabutylammonium phosphate) at pH 4.5 and acetonitrile [34]. A second analytical radioHPLC method was conducted on the HPLC equipment described using a Dionex IonPac AS-20 anion exchange column with a Dionex AG-20 guard column (Dionex, Sunnyvale, CA, USA) eluting with an isocratic solvent system consisting of 50 mM NaOH and a flow-rate of 1 mL/min.

2.6. Evaluation of Bismuth Attenuation in Quantification of ^{211}At

The effect of bismuth attenuation on the dose calibrator reading of ^{211}At for a fixed volume was examined by taking readings in the presence of varying amounts of Bi in HNO_3. In the experiment, an aliquot of isolated ^{211}At was added to 5 mL of concentrated HNO_3 in a 20 mL glass vial. This vial was assayed (CRC-55tR), after which ~200 mg pellet of bismuth was dissolved, the solution re-assayed, and this process repeated until the total reached ~1.5 g total bismuth was dissolved (average pellet size: 219 ± 32 mg). In plotting the data, all readings were normalized to the no bismuth added measurement and decay-corrected to account for the time lapsed during bismuth dissolution.

The effect of sample volume was examined by increasing the volume of the above solution with addition of 1 mL aliquots to a total volume of 15 mL (assaying after each addition). Although an increase in measured activity was noted with increasing volume, it was not conclusive as to whether this change was attributed to a change in geometry and/or an overall decrease in bismuth concentration. To assess the effect of geometry, a 0.3 mL sample of isolated (*i.e.*, no bismuth added) ^{211}At was similarly brought to a total volume of ~15 mL (in 1 mL steps).

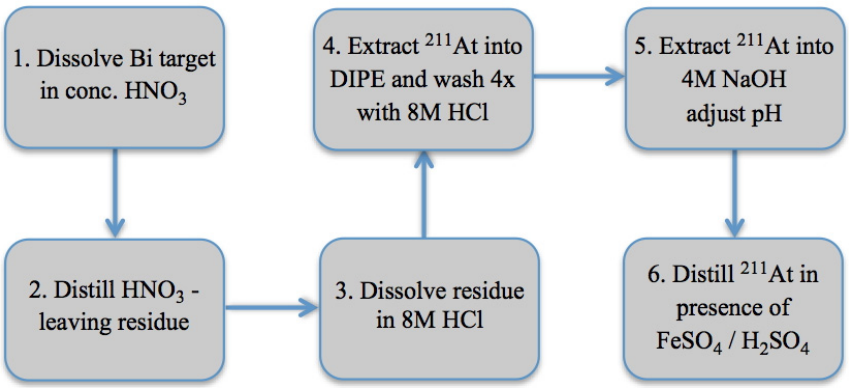
3. Results

3.1. Wet Chemistry Isolation of ^{211}At

The steps involved in the wet chemistry ^{211}At isolation approach are outlined in Figure 1. The first step in the isolation process is to remove the bismuth and ^{211}At from the aluminum target backing by

dissolution in concentrated HNO_3. This is a very efficient process, where >99% of the ^{211}At activity is transferred to the round bottom flask used in the next (distillation) step. Pictures of the target (bismuth face down) placed in the modified 1 L bottle are provided in the Supplemental Material (Figure S4). In this step it can be noted that reddish-brown gas (NO_x fumes) is released upon dissolution of the bismuth. It was somewhat surprising to find that this process does not release significant quantities of volatile ^{211}At. The release of the NO_x gas, however, is damaging to the stainless steel interior of the glovebox, requiring covering the surface with adhesive PTFE sheet (Bytac, Sigma-Aldrich, St. Louis, MO, USA). Studies were conducted to replace the open dissolution chamber with a closed chamber system in which a stream of air (or N_2) was used to sweep the NO_x fumes through a scrubbing bubbler system.

Figure 1. Steps involved in the "wet chemistry" isolation and purification of ^{211}At from a ^{209}Bi target. Note that the isolation process is usually stopped at step 5, but an additional distillation under reductive conditions can be conducted to obtain ^{211}At solutions without bismuth and other chemical contaminants from the wet chemistry process.



The HNO_3 distillation was part of the system, as NOx fumes are also released during distillation. A picture and scheme for the scrubber system is shown in the Supplemental Material (Figure S5). Ultimately, this approach was not used since it would occupy too much space in the glove box.

The second and third steps in the isolation process involve removal of the conc. HNO_3 and dissolution of the residue in 8M HCl. Although it had been shown that having some HNO_3 present did not cause problems in the subsequent DIPE extraction step, the fact that HNO_3 is efficiently extracted into DIPE [35] caused problems with loss of At so all of the HNO_3 was removed by distillation to give a colorless residue. To obtain an efficient HNO_3 distillation, a heat block temperature of ~300 °C is required and the distilling flask needs to be covered with aluminum foil. Once the HNO_3 is removed the white residue was readily dissolved in 8 M HCl. This process is also very efficient with 93%–98% of the activity transferred into the 8 M HCl. The glassware setup is shown in the Supplemental Material (Figure S6).

The fourth step in the isolation process is extraction of ^{211}At from 8 M HCl into diisopropyl ether (DIPE). In this step the bismuth remains in the 8 M HCl allowing a separation of the bulk quantities of bismuth from the ^{211}At. To further decrease the levels of bismuth, several washes with 8 M HCl are required. When carrying out the separations, there were difficulties with removing specified amounts of DIPE after mixing due to volume changes, as the DIPE and 8 M HCl are somewhat miscible

making it difficult to quantitatively isolate either reagent. Therefore, as with many of the earlier investigators, the DIPE and 8 M HCl were mixed prior to their use to minimize the loss of reagent due to miscibility. The miscibility is problematic as some ^{211}At is present in each wash (probably in the miscible DIPE). The number of washes required to obtain low levels of Bi was investigated. It was found that four washes were required to obtain a low level of bismuth in the isolated ^{211}At solutions.

The fifth step in the isolation process is back-extraction of the ^{211}At from DIPE into 4 M NaOH. This step is thought to hydrolyze the ^{211}At species in the DIPE to obtain a different species that is soluble in aqueous base. Although it is likely that other species are intermediates, the hydrolysis in strong base ultimately leads to formation of [^{211}At]astatide [36]. The efficiency of extraction from DIPE was high. Direct use of this isolated ^{211}At solution for radiolabeling biomolecules was hampered by the fact that varying concentrations of NaOH were present due to varying amounts of 8 M HCl present in the DIPE. Although many attempts were made, including pre-saturating the DIPE with 8 M HCl and carefully separating and aliquoting the 8 M HCl, we were unsuccessful in obtaining consistent concentrations of NaOH. Therefore, the basic solution is generally brought to near neutral pH, so that the pH of the ^{211}At-labeling reaction mixtures can be easily controlled. Because there are varying concentrations of NaOH, the neutralization can take varying amounts of time. Despite this, good recovered yields have been obtained. The production, isolation, and recovery yields for experiments, where ~45 min irradiations (108 isolation runs), a single 2 h irradiation and a single 4 h irradiation were conducted, are summarized in Table 1. The ~45 min irradiation experiments have been split into the first 53 experiments and the second 55 experiments, as there were small adjustments in the procedure used that improved the yields (labeled as "optimized") in the later experiments. It should be noted that the time to isolation in the most recent 27 runs is 125 ± 9 min. Spreadsheets showing data from individual experiments used in obtaining the average yields in Table 1 are provided in the Supplemental Material (Tables S7 and S8).

Table 1. Wet chemistry ^{211}At Isolation times, activity and yields *.

^{211}At Isolation Runs (most ~0.75 h irrad.)	Activity Produced (mCi) **	Time to Isolation (min)	Isolated Activity (mCi)	Decay Corrected Recovery (%) ***
Non-optimized (n = 53)	20.7 ± 1.7	171 ± 46	13.6 ± 2.3	87 ± 15
Optimized (n = 55)	19.6 ± 2.0	155 ± 50	15.9 ± 2.7	104 ±15
Preparative Run (2 h)	54.0	118	43.2	97
Preparative Run (4 h)	100.5	114	72.0	86

* Values for multiple runs (steps 1–5) are average ± standard deviation; ** The activity produced is a dose calibrator measurement of ^{211}At in the bismuth target. This value is systematically low due to bismuth attenuation of the dose calibrator reading (see later section); *** The decay corrected recovery yields are high due to the systematically low measurement of ^{211}At activity produced. See Table 3 for attenuation corrected yields.

Concern that low levels of bismuth and trace levels of other metal ions might be problematic in some ^{211}At-labeling reactions, inclusion of a final purification step, Isolation Step 6, was evaluated in several ^{211}At isolation runs. Inclusion of a final distillation step was also of interest as a potential means for circumventing the time-consuming neutralization after back-extraction into NaOH. Initial distillations were conducted with ^{211}At at pH 8.5, 6.5–7, and 3. The highest yield (~45%) was obtained with the solution at pH 6.5–7. The distillation at pH 8.5 provided ~24% recovery, and the distillation at

pH 3 gave only ~16% recovery. Unfortunately, the amount of ^{211}At that distilled under acidic pH appeared to be limited by the formation of $^{211}AtO_3^-$ (by radioHPLC). This led to evaluation of several reductive conditions, all of which gave low recovery yields. Johnson *et al*. previously reported distillation of ^{211}At under a variety of conditions, including distilling under reductive conditions (solution containing 0.05 M $FeSO_4$ in 0.5 M H_2SO_4) [37]. They found that only the distillation under reductive conditions provided high recovery (97%) of ^{211}At. It seemed that this reducing agent would work for our purification, but since the isolated ^{211}At solutions were highly basic (with varying amounts of NaOH for reasons previously explained), we chose to have a much higher quantity of H_2SO_4 to offset the base. After some initial studies with lower amounts of reductant and acid, it was found that a solution of 0.75 M $FeSO_4$ in 1.5 M H_2SO_4 routinely provided a highly acidic solution which resulted in good ^{211}At recovery yields. The reductive conditions keep the ^{211}At as astatide during the distillation process. It is presumed that hydroastatic acid is distilled. To assure that the distilled ^{211}At remain as astatide, the distillate was collected in a vessel precharged with a volume of 0.1 mL of 1 M NaOH. A compilation of the results from the seven reductive distillations employing solutions containing 0.75 M $FeSO_4$ in 1.5 M H_2SO_4 is provided as Table 2. It was noted that over 3 mL of distillate was required to obtain >80% recovery of activity, but that the majority of activity >60% was obtained in the first 1.2 mL of distillate. By collecting only the first 1.5–2.0 mL of activity the time for the final distillation step was shortened considerably. This shortening of time came about from the shorter time for the distillation, but also from not having to concentrate the final ^{211}At solution prior to use.

Table 2. Isolation yields after reductive distillation.

Run [#]	^{211}At Isolate to Distill (mCi)	^{211}At Activity Purified (mCi)	Volume Collected (mL)	% ^{211}At Recovered
1	14.3	12.2	3.4	85
2	8.0	4.8	1.5	61
3	12.3	9.1	1.5	74
4	7.5	5.4	1.2	72
5	15.1	10.8	1.8	72
6	16.5	10.5	1.8	64
7	34.0	23.0	2.0	67

[#] Average ± SD 70.7 ± 7.9.

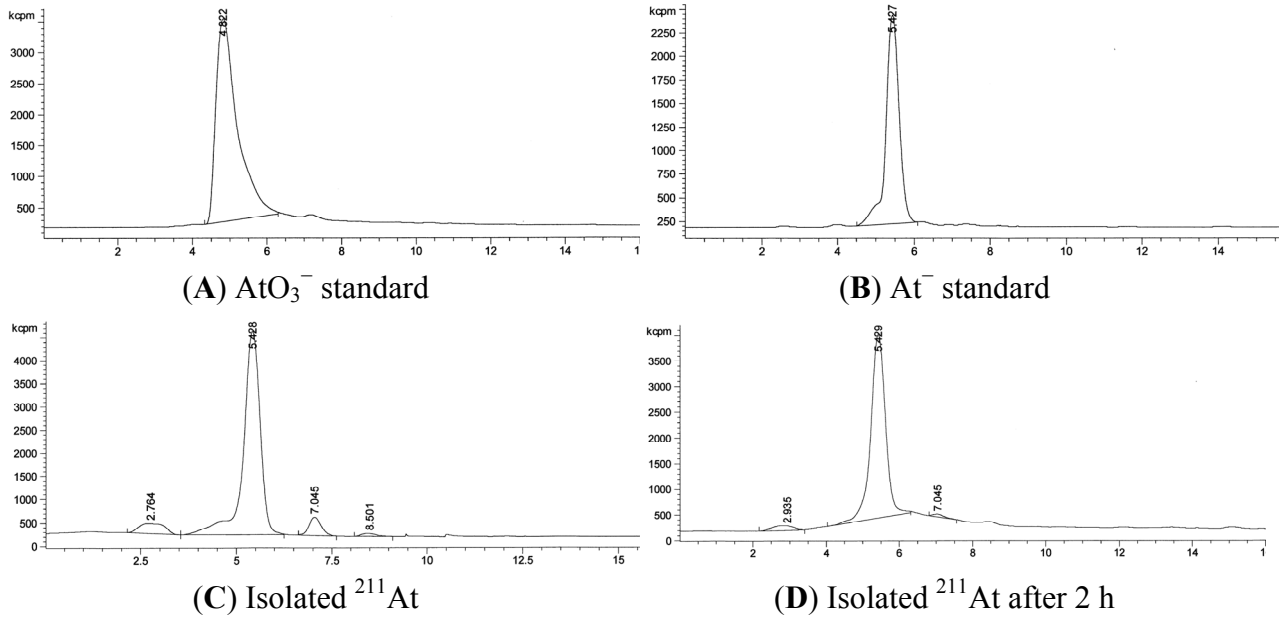
Reasonable average isolation yields were obtained when the first 1.5–2.0 mL of distillate are used, but higher overall recovery yields may be obtained if larger quantities of distillate are isolated. More rapid and efficient methods for removal of water from the isolated aqueous ^{211}At solution are being investigated.

3.2. RadioHPLC of ^{211}At Solutions

The nature of the ^{211}At species produced by the wet chemistry approach was of high interest in our investigation, as the method of isolation may affect the species obtained for radiolabeling. The initial step of the wet chemistry ^{211}At isolation approach is highly oxidizing, most likely resulting in highly oxidized form of ^{211}At. The fact that the ^{211}At species produced is not volatile indicates that may be the case. In addition to the oxidized forms of ^{211}At, use of 8 M HCl may produce a number of interhalogen

species [24,36,38,39]. There was concern that oxidized forms of ^{211}At might be obtained in the final isolated solution (except where the distillation was conducted under reductive conditions). Therefore, a HPLC method for identifying the ^{211}At species present was sought. Initial radioHPLC studies were conducted on a YMC J'sphere ODS-M80 C-18 reversed-phase column, previously reported for separation of periodate, iodate and iodide [34]. This column has a very high surface area with high coating (14%) on 4 μm size silica particles with 80Å pore sizes. It has a usable pH range of 2.0–7.5. As only a small amount of the injected ^{211}At was recovered when the HPLC eluent was acidic (*i.e.*, <10%), it was determined that the column should only be used when the samples were neutralized. Radiochromatograms showing retention of $^{211}AtO_3^-$ (panel A), $^{211}At^-$ (panel B), crude reaction product mixture at pH 8 (panel C), and the same product mixture after setting at room temperature for 2.5 h (panel D) are shown in Figure 2. The At^- (t_R = 5.4 min) was obtained under reductive conditions to assure that the species was astatide. As a HPLC standard, Na[^{125}I]I eluted at t_R = 6.2 min. The $^{211}AtO_3^-$ (t_R = 4.8 min) was produced from $^{211}At^-$ using the following oxidation conditions; $^{211}At^-$, $NaIO_4$, 0.1N H_2SO_4, 80 °C (microwave). The corresponding Na[^{125}I]IO_3, produced under the same oxidation conditions (except at 120 °C) had a t_R = 3.7 min. A number of ^{211}At species have been observed in the radiochromatograms that could not be identified. In many cases the unidentified species appear to be converted to either astatide or astatate with time (e.g., Figure 2, panel D). Although the retention times for the isolated ^{211}At species were the same when the pH was constant, slight variations in pH resulted in small changes in peak retention times making it difficult to identify species run-to-run with certainty. This was problematic as the pH of the isolated product varied from batch-to-batch.

Figure 2. Examples of radiochromatograms obtained by eluting ^{211}At solutions on a YMC J'Sphere ODS-M80 column using isocratic conditions eluting with 1:1 CH_3CN:50 mM TBAP @ 1 mL/min. Panel **A** shows a radiochromatogram of Na[^{211}At]AtO_3. Panel **B** shows a radiochromatogram of Na[^{211}At]At. Panel **C** shows a radiochromatogram of ^{211}At species from an isolation run (from step 5). Panel **D** shows a radiochromatogram of ^{211}At species in the same solution as panel **C**, after setting for 2.5 h.

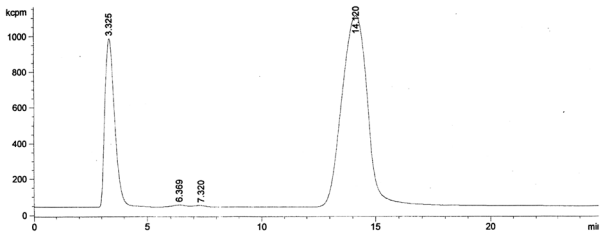


(**A**) AtO_3^- standard (**B**) At^- standard

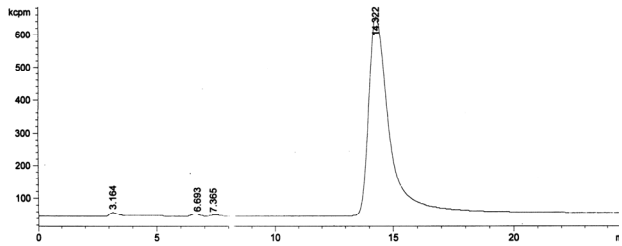
(**C**) Isolated ^{211}At (**D**) Isolated ^{211}At after 2 h

Rössler *et al*. had previously shown that a strongly basic anion exchange column (BioRad Aminex A 27) could be used to separate ^{211}At from ^{125}I [40]. More recently, Sabatié-Gogova *et al*. described the use of a Dionex IonPak AS-20 column (with AG-20 guard column) in their studies to characterize the astatine species in solution [41] and Champion *et al*. used the same column in their evaluation of the hydrolyzed At(III) species under oxidizing and acidic conditions [42]. Discussions with technical personnel at Dionex Corporation also led us to use this chromatographic column for separating At species. It had previously been shown that this column separates chloride from chlorate and perchlorate, bromide from bromate and iodide from iodate (ThermoFisher product guide) using a hydroxide eluent, so it seemed likely that it could be used for separation of ^{211}At species. This column is a polymer-bound anion exchange resin that is very stable under the highly basic conditions that the ^{211}At solutions are obtained in, making it particularly attractive for our use. Examples of chromatograms obtained with the Dionex column are shown in Figure 3. The radiochromatogram peak at ~14.3 min (Figure 3, panel A) is [^{211}At]At^{-} and the peak at 3.3 min is an unidentified species. It is interesting to note that after neutralization of the NaOH solution, the peak at 3.3 min is not observed (Figure 3, panel B), and after setting at room temperature, the ^{211}At species do not change further (Figure 3, panel C). Distillation in the presence of $FeSO_4$ in H_2SO_4 in some runs provided a single ^{211}At species (see Supplemental Material, Figure S9), but in this example (Figure 3, panel D) two additional unknown species were present. Monoclonal antibody radiolabeling reactions using distillation purified ^{211}At solutions from step 6 of the isolation generally gave slightly lower labeling yields than those obtained with the non-purified ^{211}At solutions from step 5 (data will be presented elsewhere). It is not clear why this is the case, but it is thought to be due to a higher dilution of activity obtained after purification, not the presence of additional ^{211}At species.

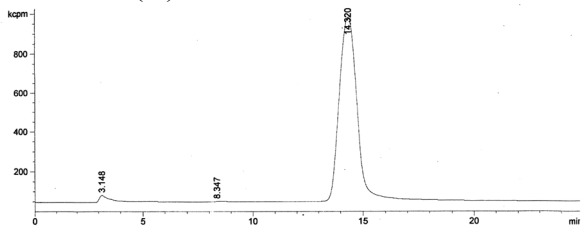
Figure 3. Radiochromatograms obtained by eluting ^{211}At solutions on a Dionex IonPac AS20 column using isocratic conditions with 50 mM NaOH @ 1 mL/min. (**A**) radiochromatogram of ^{211}At in NaOH solution after isolation; (**B**) radiochromatogram of neutralized solution; (**C**) radiochromatogram of neutralized ^{211}At after setting at room temperature for 2 h; (**D**) radiochromatogram of ^{211}At species after distillation.



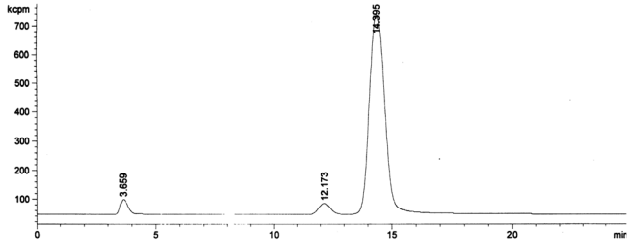
(**A**) Before neutralization



(**B**) After neutralization



(**C**) Neutralized, after 2 h



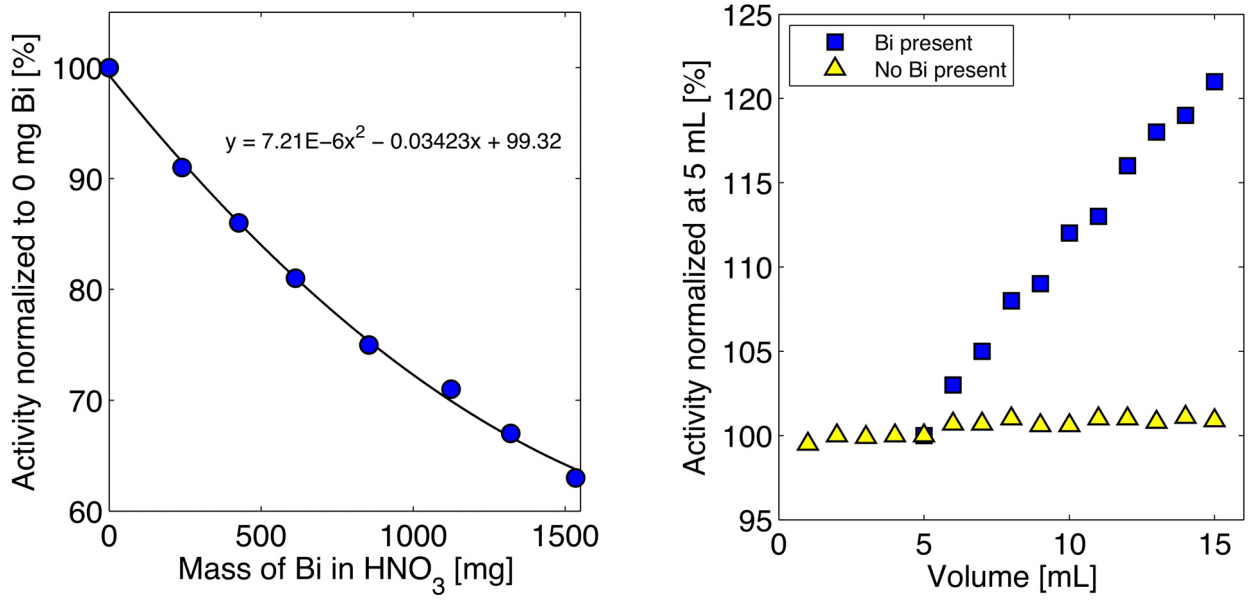
(**D**) After distillation

3.3. Quantification of ^{211}At in the Presence of Bismuth

It is apparent that bismuth can attenuate the 76.9 and 79.3 keV X-rays used to quantify ^{211}At in the target and in solution, but the extent of attenuation was not investigated until our average decay-corrected wet chemistry isolated yields became greater 100% (see Table 1: "Optimized" runs had an average of 104% ± 15% decay-corrected yields). Additionally, high variability in the dose-calibrator yields for samples within a particular run suggested that attenuation in samples containing bismuth was larger than we anticipated. Therefore, two sets of experiments were conducted to understand the variability by testing the hypothesis that the dose calibrator measurements observed during the extraction process may be susceptible to bismuth attenuation and/or geometric effects due to varying sample volumes.

In the first experiment, dose calibrator readings were taken for a set amount of ^{211}At, when the quantity of bismuth was increased in a constant volume of HNO_3. The experiment was conducted by dissolving increasing amounts of bismuth metal (0–1.5 g) in 5 mL of conc. HNO_3 containing ^{211}At. The data obtained is plotted in Figure 4, panel **A**. All dose calibrator readings were normalized to the measurement with no bismuth added and decay-corrected to account for the time lapsed during bismuth dissolution. It is clear that the dose calibrator readings were significantly decreased due to attenuation of bismuth. At the highest amount of bismuth added (1.5 g), where the concentration of bismuth in solution was 300 mg Bi/mL HNO_3, an attenuation of ~38% was noted.

Figure 4. Graphs depicting the change in dose calibrator reading of a fixed amount of ^{211}At when the concentrations of bismuth are changed. (**A**) Dependence of bismuth concentration *vs*. dose calibrator reading for a fixed (5 mL) quantity of HNO_3; (**B**) Dependence of sample volume *vs*. dose calibrator reading in the presence and absence of bismuth. Values in each graph have been decay corrected and normalized to; (**A**) the no added bismuth reading, or (**B**) the 5 mL reading.



A **B**

In the second experiment, the change in dose calibrator reading of ^{211}At was examined when the volume was increased from 5 mL to 15 mL by 1 mL additions of H_2O. Although an increase in measured activity was noted with increasing volume, it was not conclusive as to whether this change was attributed to a change in geometry and/or an overall decrease in bismuth concentration. To further assess the effect of geometry, a 0.3 mL sample of isolated (*i.e.*, no bismuth added) ^{211}At was similarly brought to a total volume of ~15 mL (in 1 mL steps). The results are presented in Figure 4, panel B. That graph clearly shows that the change in reading is almost exclusively attributed to the decrease in bismuth concentration, and not to the change in sample volume. Combining the results of panel A (*i.e.*, ~38% reduction in activity when adding 1.5 mg of Bi), along with the increase of ~21% when diluting this solution to 100 mg Bi/mL (1.5g in 15 mL), the dose calibrator reading for such a bismuth concentration and volume is expected to be ~75% of that achieved for the case where no bismuth is added (*i.e.*, underestimated by ~33%).

The simple experiments to determine the attenuation effect that bismuth has on dose calibrator readings of ^{211}At show that significant changes in readings are due to attenuation by bismuth. If an approximate attenuation value can be obtained, a more accurate estimate of the recovered yields can be made. Measurement of the amount of ^{211}At activity in an irradiated target after the HNO_3 dissolution has shown that <1% of the activity remains, so it is possible to say that the amount of activity in the target is essentially the same as that of the conc. HNO_3. Calculations suggest that after milling to 0.17 mm thickness, the targets hold ~3 g of bismuth metal. Unfortunately, this value can vary considerably based on the tolerances of the machining (weights suggest variances may be as large as 1 g). Fortunately, in the dissolution process only part of the bismuth is removed, suggesting that the top portion of the bismuth contains the ^{211}At. Again, it is not possible to know exactly how much bismuth metal is dissolved, but it seems likely that it may be in the range of 1.5–2.5 g. Also, differences in the weights of bismuth targets before being milled (removing some bismuth and aluminum) and after milling are highly variable. With these variances and the fact that there were no good estimates of the amount of bismuth dissolved from the target, it is not possible to use the data obtained in the graphs to estimate a single global attenuation factor.

Another way to get an approximation of the attenuation factor is to measure a solution of ^{211}At in the isolation process that does not have bismuth in it. That measurement, corrected for loss of ^{211}At activity in each step/transfer to that point, and corrected for decay during the steps, can provide a value that approximates the original amount of ^{211}At activity in target. Once the number is calculated, division of the ^{211}At target measurement by that number (×100) can provide an estimate of the percent the target reading is of the actual amount. The first solution in the isolation process that does not have bismuth in it is the DIPE solution (prior to NaOH extraction). Fortunately, measurement of the ^{211}At in the DIPE solution is routinely taken. The calculations that are described above were performed for 13 recent isolation runs. Although it is likely that the bismuth mass was highly variable in the runs, the average percent of (approximated) original ^{211}At in the 13 runs was 74% ± 4%, which is on par with data of Figure 4. Thus, a bismuth attenuation factor may be approximated to be 1.33. We believe that adjusting the target reading upward by 33% can provide a reasonable estimate of the amount of ^{211}At activity in the irradiated target, and that value can provide a better estimate of the percent ^{211}At recovered from the target than obtained without using an attenuation factor.

Adjustment of the amount of ^{211}At produced and the decay- and attenuation-corrected recovery yields are provided in Table 3. Spreadsheet showing data from individual experiments used in obtaining the average yields in Table 3 are provided in the Supplemental Material (Tables S10 and S11).

Table 3. Wet chemistry ^{211}At isolation yields corrected for decay and attenuation *.

^{211}At Isolation Runs (most ~0.75 h irrad.)	Corrected Act. Produced (mCi) **	Isolated Activity (mCi)	Est. Attenuation* & Decay Corrected Recovery (%) ***
Non-optimized (n = 53)	27.6 ± 2.3	13.6 ± 2.3	65 ± 11
Optimized (n = 55)	26.1 ± 2.7	15.9 ± 2.7	78 ± 11
Preparative Run (2 h)	72	43.2	73
Preparative Run (4 h)	134	72.0	65

* An attenuation correction of ~1.33 has been applied to the measured bismuth target value based on an average difference between the ^{211}At reading in DIPE (corrected for decay and losses) and the initial target reading; ** Estimated mCi of ^{211}At produced in bismuth target based on approximation that the reading was 75% of actual; *** Estimated recovery yields if decay and attenuation of ^{211}At emissions are factored into calculations.

4. Discussion

This investigation of a wet chemistry approach for isolation of ^{211}At was prompted by difficulties in our production of ^{211}At brought about by a large increase in size of the bismuth target used. The increase in target size came about when designing and implementing an external target station for increasing our ^{211}At production levels [29]. It should be noted that our cyclotron situation is somewhat unique, as an external target was required rather than an internal target [43–45] because the cyclotron had to remain in daily operation for fast neutron therapy of cancer patients. In addition to circumventing difficulties with the size increase of target, we were also interested in determining whether the wet chemistry method could provide more consistent ^{211}At isolation yields than previously obtained with dry distillation. It is our belief that a robust isolation method will be required when conducting clinical studies.

The wet chemistry approach described herein is not new. In 1949, Johnson *et al*. reported that bismuth targets could be dissolved in conc. HNO_3 with little loss of ^{211}At when the HNO_3 was distilled away [37]. Eight years later, Neumann described a similar procedure using extraction of ^{211}At into DIPE and back-extracting into NaOH [30]. In 1973, Neirincks and Smit reported extracting ^{211}At into DIPE, but used aqueous hydroxylamine to back-extract [31]. More recently Yordanov *et al*. described a similar approach using DIPE or butylether for the extraction of ^{211}At [32], and Bourgeois *et al*. described an approach using the DIPE extract directly for radiolabeling of *m*-Me_3Snbenzoate *N*-hydroxysuccinimidyl ester [46]. Zona *et al*. also described a similar wet chemistry approach to obtain ^{211}At on silver foils [33]. Interestingly, Alliot *et al*. reported on an investigation of the extraction mechanism when ^{211}At is extracted into DIPE [35].

Our goals in investigating the wet chemistry approach were: (1) to evaluate previous approaches and optimize conditions to efficiently isolate ^{211}At from the large amounts of bismuth (up to 3 g) in our targets; and (2) to demonstrate reproducibility of the optimal approach. The data presented herein support our achieving those goals. A secondary goal is to further refine the wet chemistry approach such that it can be automated. Isolation of ^{211}At by an automated wet chemistry isolation system is

currently under investigation. While all of the steps in the described isolation approach can be readily accomplished, the miscibility issues with DIPE separations requiring pre-saturation of reagents is not favorable for automation, so alternative separation approaches are being sought.

The chemistry of radiolabeling with ^{211}At is not always straightforward [47,48]. Arguments can be made that some of the radiolabeling difficulties could be due to having species of ^{211}At present that are not reactive or react differently than expected/desired. In this investigation we examined two radioHPLC methods for separation and identification of ^{211}At species so that we could use them for identification of the desired Na[^{211}At]At, and for quality control of the isolated ^{211}At. Both a C-18 reversed-phase separation method and an ion exchange separation method were evaluated. The ion exchange radioHPLC method has the advantage that it provides good separation of ^{211}At species when taking samples directly from the highly basic isolation solution, whereas the separations on reversed-phase were best conducted by injection of ^{211}At solutions that had been neutralized. Our radioHPLC studies identified At species we believe were astatide (At^-) and astatate (AtO_3^-) based on using known reactions to obtain these species. Minor amounts of other unidentified species were also seen, but we have demonstrated that the wet chemistry approach can provide high radiochemical purity astatide as a final product.

In addition to knowing the nature of the species in the final product, it is of interest to know the nature of the astatine species obtained in each step of the isolation steps. Unfortunately, this has not been established and is difficult to ascertain. It is generally assumed that **step 1**, dissolution of the bismuth target in concentrated nitric acid provides At(0) in solution [33], but if that is the case it is surprising that no volatile astatine is released in this open-air vigorous dissolution. Irrespective of the initial At species in step 1, based on the fact that iodine (I_2) is converted to iodate (IO_3^-) under heating in concentrated HNO_3 [49] it seems highly likely that in **step 2** At(0) or other species present will be converted to astatate (AtO_3^-). The fact that the HNO_3 distillation can be heated to dryness without release of volatile astatine species supports formation of a species such as astatate. Neumann suggested that the use of 8 M HCl to dissolve the residue from the HNO_3 distillation in **step 3** provides either $AtCl_2^-$ or $AtCl_4^-$ [30]. The hypothesis was based on the fact that ICl_2^- and ICl_4^- are known species and it was known that ICl_2 could be extracted into ethers from HCl solutions. In experiments conducted it was demonstrated that the species formed was efficiently extracted DIPE (as in **step 4**), but was not extracted into benzene or CCl_4. The later fact ruled out the formation of neutral species such as AtX (where X is a halide). Norseyev and Khalkin later reported on the stability constants of chloride complexes of At formed from varying concentrations of HCl with oxidants present and concluded that $AtCl_2^-$ was the likely species formed [50]. Interhalogen species, including $AtCl_2^-$, were also prepared by Meyer and Rössler via distillation methods [24]. Back-extraction with 4 M NaOH in **step 5** produces a species that has very different properties to the DIPE extracted species. Initial evaluation by reversed-phase and anion exchange radioHPLC can have a number of species present, but with time the minor species disappear leaving only one species, which appears to be astatide. It seems likely that the species in DIPE (perhaps $AtCl_2^-$) is hydrolyzed with a strong base to intermediate species before obtaining astatide. It was not the purpose or focus of this investigation to study the At species formed, so the forgoing discussion on potential At species in each step of the isolation process is meant only for consideration. Definitive experiments to identify At species will have to be conducted as separate studies.

As stated before, one of our goals was to optimize the wet chemistry isolation procedure. We were pleased in our initial studies where the recovery yields were estimated to be >80%, but became concerned with our data when the average yields were 100% or higher. In hindsight, it was not too surprising that average optimized yields might be 100% or more, as we knew that the ^{211}At readings of the irradiated target were low due to attenuation. Yet, it was surprising that the attenuation was so high (readings estimated to be only ~75% of actual). We believe these studies may be the first to evaluate the level of attenuation in the irradiated bismuth target, or in solutions of ^{211}At that also contain bismuth ions. The fact that an attenuation factor was not used (to the best of our knowledge) in calculating some previously reported ^{211}At isolated yields for dry distillations means that those reported yields may be higher than they actually were.

5. Conclusions

We have demonstrated with over 100 ^{211}At isolation runs that the wet chemistry method for isolation of ^{211}At described in this report is relatively easy to conduct, it provides good recovery yields, is robust and quite reproducible. The average time for an investigator to conduct the wet chemistry isolation (without final distillation) is ~2 h if he/she is familiar with the procedure. In our experience, the 2 h time period is similar to the time it takes to setup and conduct the dry distillation approach. The use of the wet chemistry ^{211}At isolation rather than the dry distillation will be dependent on an investigator's unique situation, and perhaps their personal preference, but we have shown that it is an alternative worth considering.

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Conflicts of Interest

The authors declare no conflicts of interest.

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