Final Report

for the Period of:

July 1, 2010 through September 30, 2012

Project Title:

Production of Astatine-211 for U.S. Investigators
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Principal Investigator on Project:

D. Scott Wilbur, Ph.D.
University of Washington, Box 355016
Department of Radiation Oncology
Benjamin Hall Interdisciplinary Research Building
616 N.E. Northlake Place
Seattle, WA 98105
206-616-9246
dswilbur@uw.edu

Report Provided to:

Dennis Phillips, Ph.D.
U.S. Department of Energy
Office of Science Office of Nuclear Physics
Isotope Development and Production for Research and Applications
301-903-7866
Dennis.Phillips@science.doe.gov

Mailing Address:

U.S. Department of Energy
Germantown Building/SC-26.2
1000 Independence Ave., S.W.
Washington D.C. 20585-1290

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I. Summary of Research Results

The *overall objective* of the proposed research effort was to obtain methods and materials that allow personnel in the Department of Radiation Oncology at the University of Washington (UW) to *supply the alpha-particle emitting radionuclide astatine-211 (^{211}At) to U.S. investigators at other academic sites or companies for their research interests*. To accomplish the *overall objective*, we: (1) optimized production on the UW cyclotron, (2) developed a robust wet chemistry method for efficiently of ^{211}At from the irradiated target, (3) evaluated a semi-automated system for isolation of ^{211}At, and (4) conducted a trial shipment of ^{211}At to confirm that all of the required procedures were in place for handling and shipping ^{211}At. Optimization of the irradiation parameters determined that ^{211}At could be produced by irradiation of bismuth targets at 29 MeV running at beam amperages of up to 55 μA. Using those irradiation parameters, we demonstrated that a 4-hour irradiation could produced >100 mCi of ^{211}At. Isolation of the ^{211}At from the irradiated bismuth target by a "wet chemistry" approach was optimized, providing ~80% decay corrected recovery or 60% actual yield from beginning of isolation. Obtaining highly purified ^{211}At required a final distillation, which resulted in ~50% overall isolated yields. Our attempts to develop a "semi-automated" isolation process were fraught with difficulties. While we did show that the approach worked, it was deemed that the available instrumentation was inadequate to obtain a robust method. Thus, we have submitted a follow-up funding application in collaboration with investigators at PNNL (Matt O'Hara) to develop a fully automated system which takes advantage of novel separation and purification approaches. Finally, an ^{211}At shipment was made to the University of Missouri (Dr. Tom Quinn) to demonstrate that shipments could be made. At this time, the requisite paperwork from DOE to set up an agreement for supplying ^{211}At through their distribution system has not been put into place. When that agreement is in place we will be ready to produce and ship ^{211}At to investigators at other institutions.

II. Research Results

This final report covers the entire period of funding, July 1, 2010 thru September 30, 2012, for the research project. The report lists the milestones for the project, status of reaching the milestones, information on experiments conducted, and results obtained to reach the specific objectives of the grant. The information is provided in the following pages under each **specific objective** of the project.

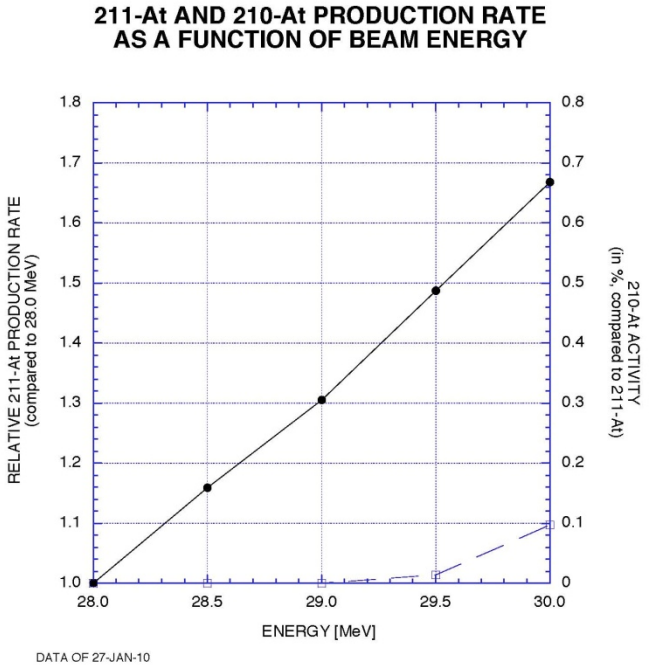
Specific Objective 1: Optimize ^{211}At production on the UW cyclotron.

Milestones:
(a) Determine settings required on cyclotron for optimum beam
(b) Evaluate Production of ^{211}At & ^{210}At at various beam energies
(c) Demonstrate optimal beam characteristics for production of ^{211}At
(d) Conduct irradiations at increasing μA values
(e) Evaluate the amount of ^{211}At that can be made with 4 & 8 h irradiations

Milestones "a" through "d" focused on a common goal; obtaining a stable beam and optimizing its characteristics to maximize production of ^{211}At. However, one parameter took precedence in evaluation and optimization over the others, the energy of the beam used for irradiations. It

was important that the maximum beam energy used for the irradiation produce no, or an undetectable quantity of, ^{210}At. Production of ^{211}At contaminated with ^{210}At ($t_{1/2}$ = 8.1 h) is undesirable because ^{210}At decays to ^{210}Po. ^{210}Po is a long-lived (138 d) alpha-particle emitting radionuclide that has a propensity to localize to kidney, spleen and liver. Irradiations of bismuth targets were conducted at 28.0, 28.5, 29.0, 29.5 and 30.0 MeV energies. The irradiated targets were evaluated on a Ge/Li detector system for the presence of ^{210}At (γ photon emission at 1181.4 keV). The results obtained are presented in Figure 1 below. We determined the production of ^{210}At contaminant to be <0.005% at 29.0 MeV and ~0.01% at 29.5 MeV.

Figure 1



Initially, our irradiations were performed with a beam energy of 28.0 MeV. However, based on the results of these experiments we have run all subsequent irradiations at 29.0 MeV, as this results in an undetectable amount of ^{210}At production. The very low levels of ^{210}At production at 29.5 MeV is suggestive that it might also be used if the additional 20% production rate were important (i.e. not being able to produce enough). *Changing from conducting the irradiations at 28.0 MeV to 29.0 MeV increased the production rate by 30%* (see figure above).

With the beam energy optimized, beam current was addressed. The question was whether higher current alpha-beams could be used to produce ^{211}At and if so, how much of an increase in production rate would be obtained. Our initial beam current was established at 50 μA. However, when we began studies to increase the current to 55 and 60 μA respectively difficulties were encountered in the cyclotron with a batch of bad halfnium-carbide cathodes (suffered a catastrophic failure, i.e. cracked), so new halfnium-carbide cathodes were made. We then evaluated an increase in current (55 μA and 60 μA) to determine if the cyclotron would run stably at higher currents. We were successful in generating a stable beam at both 56 and 58 μA, which are close to the 55 and 60 μA values that were our targets. However, the increases in production were not substantial enough to risk further batches of cathode buttons so, the beam current of 50 μA is used for production of ^{211}At.

Testing production capabilities with long irradiation times were deferred until the end of the project funding period. The primary reason for this was that we wanted to optimize the isolation and purification method to be used prior to producing larger quantities of ^{211}At. Optimization of the final distillation step was not conducted until close to the end of the funding period. As we wanted to be sure that the final distillation yield would provide adequate recovery before conducting the longer irradiation and subsequent isolation, the long irradiations were not conducted until near the end of the funding period. We initially planned to conduct a 4-hour and an 8-hour run. However, once the chemistry was refined, budget constraints forced us to modify the milestone to run a 2 h irradiation and a 4 h irradiation. Those irradiations generated 57 and 108 mCi of ^{211}At, respectively. While the irradiation times were shorter than initially listed, we believe the shorter irradiation times provide adequate information to make it possible to project how much might be made in an 8 h irradiation.

Specific Objective 2: Evaluate and optimize ^{211}At-isolation from irradiated targets.

Milestones:

(a) Evaluate methods to make adjustments of pH in final solution easier
(b) Adjust wet chemistry steps to obtain ^{211}At without bismuth and aluminum
(c) Determine how small a volume can be obtained for the final ^{211}At solution

A combination of initial difficulties with the dimensions of our aluminum-backed bismuth target and the inconsistent yields obtained with dry distillation, lead us to pursue a wet chemistry approach to isolate ^{211}At from the irradiated bismuth target. This approach however, presented its own unique challenges in that we experienced difficulty obtaining a pH in the final aqueous product that would be acceptable to end-users (e.g. pH 10) in a time period that allows preparation for shipment. Several attempts at shortening the time to obtain the target pH of the final product by manipulating experimental parameters were tested. In those experiments non-irradiated bismuth metal was dissolved in nitric acid and the resulting solution was treated with the same work-up as an irradiated target. The standard work-up includes removal of the nitric acid by distillation, dissolution of the resulting solid (oxides of At and Bi) in hydrochloric acid, extraction of astatine species from the hydrochloric acid using diisopropyl ether (DIPE), and back extraction of astatine into sodium hydroxide.

Titrations with sodium hydroxide were conducted on several runs to determine how much hydrochloric acid was left behind in the DIPE. The problem was that varying amounts of hydrochloric acid were removed because of its apparent miscibility with DIPE. In an attempt to mitigate the acid loss we freshly distilled DIPE (due to some concerns about stabilizers) and pre-equilibrated it with the hydrochloric acid immediately prior to each target work-up. These additional steps appeared to work fairly well, however we eventually found that we needed to pre-equilibrate the hydrochloric acid with DIPE as well for the same reasons. It was further determined that a set amount of hydrochloric acid must be removed from each DIPE wash step to obtain consistent neutralization (with sodium hydroxide). Additional studies were conducted, evaluating the use of lower molarity hydrochloric acid to wash the DIPE, as that would result in less sodium chloride being present in the final product.

Although it was not initially included in in our project proposal we found it important to address the nature of the isolated species produced, and contaminants introduced by, the wet chemistry approach. We thought it was important to be certain that the ^{211}At is present as a

single anionic species, [^{211}At]astatide, as it could potentially affect the final isolated yields. Additionally, we thought it relevant to analyze the amounts of bismuth (from the target) and aluminum (from the target backing) present in the final ^{211}At solution. Principally because the chemicals used in the wet chemistry approach can introduce contaminants, including a lot of NaCl. The presence of metals such as bismuth and aluminum could be very problematic in obtaining a pure isolate.

Predicting the quantity NaOH required to neutralize the hydrochloric acid present in the DIPE after the wash steps was problematic. This appeared to be in part due to the removal of varying quantities of HCl remaining in the DIPE after separation from the bulk HCl. A systematic evaluation of ^{211}At activity was made in different steps of the experiment to help understand where an appreciable amount of ^{211}At activity (1.5 – 3 mCi) is lost in the HCl wash steps. Adjustment of the pH of the final solution by adding NaOH was difficult to standardize because of the widely varying amounts of HCl remaining with the diisopropylether (DIPE) after separation of layers. Some of the problem was due to variation between 3 operators. It is still not clear where the differences came from, but the operators varied in the amount of HCl they leave in the separation vessel, and how they handled pipetting DIPE. It was later found that use of less DIPE (4 mL vs. 8 mL) made it a lot easier to neutralize the resulting isolate. This result suggests that the quantity of HCl that can be inadvertently removed is directly proportional to the quantity of DIPE used, which in turn made finding the amount of NaOH to neutralize it easier.

Given the issues in the separation step, it was thought that removal of the DIPE by distillation might make the neutralization more consistent. Non-radioactive experiments incorporating the distillation of DIPE were conducted in the same manner as with the ^{211}At isolation, except that non-irradiated bismuth was used. The non-radioactive experiments showed that more consistent amount of base could be used in the neutralization step. However, when this was done using ^{211}At, losses (into a charcoal filter) were too large to make this approach of value.

We then evaluated using a aqueous distillation approach to remove NaCl and trace metals (e.g. bismuth) from the ^{211}At. Unfortunately, the distillation conditions used to isolate the ^{211}At free of salts appear to be highly dependent on controlling its oxidation state. Under acidic conditions the astatide was converted to astatate, even under an argon atmosphere. This resulted in low isolation yields in our initial attempts to isolate ^{211}At by distillation. Based on those results, we evaluated distilling from both acid and base, but saw no advantage at either high or low pH. Interestingly, the best recovery yields were at neutral pH. Alternatively, we tried putting several reducing agents into the ^{211}At solution prior to distillation, but that gave even lower isolated yields (presumably through interaction of the reducing agent with the ^{211}At). Alterations in the distillation setup were pursued in an attempt to get the activity to come over faster (giving it less time to undergo oxidation); which did not significantly improve the recovery yields, even when a bulb-to-bulb distillation was conducted.

Other chemistry based modifications were also attempted. One was to use chloramine-T as an oxidant of [^{211}At]astatide to produce [^{211}At]AtCl, which is quite volatile. Under HPLC analysis it was observed that introduction of chloramine-T into a mixture containing both [^{211}At]astatide and [^{211}At]astatate, resulted in a single product. It was believed that the [^{211}At]AtCl would readily distill, but we found that the yield was even lower (~13% isolated) than was obtained without the chloramine-T addition (~37% isolated). Another modification evaluated was plating

the ^{211}At on platinum and silver. The thought was that if that were found to be effective (which was not the case), a simple distillation from the metal (disk) might be possible.

A change in our isolation and processing procedure resulted in a highly efficient distillation. The distillation, performed under reducing conditions, i.e. [^{211}At]astatide + 1.5M H_2SO_4 + 0.75M $FeSO_4$, is carried out as the last processing step. The distillation process yields pure [^{211}At]astatide, as identified by HPLC on an ion exchange column, in high radiochemical yield (>75%) regardless of the operator. These changes have resulted in finding an efficient distillation process for the final step. This has resulted in our being able to develop a method for isolation of ^{211}At to obtain pure ^{211}At (i.e. without salts) for shipment to other investigators. Our studies using ion chromatography HPLC have provided evidence that the final (distilled) product is obtained as sodium [^{211}At]astatide in >98% purity.

Decreasing the volume of the final ^{211}At solution was studied by heating the vial under a stream of argon (running through a charcoal filter). Our studies showed that only a small amount of NaOH (pH 9 or greater) is needed to retain the ^{211}At in the vial. Interestingly, it appears that a small amount of activity is released irrespective of pH. It is thought that this is due to formation of an organic ^{211}At in the isolation process. Additionally, we evaluated taking the ^{211}At to dryness and re-dissolving in a set amount of water (e.g. 50 – 100 uL). While most investigators will be able to use an aqueous solution in their astatination reactions, some will likely want to conduct labeling reactions in non-aqueous solutions. Therefore, we have begun to evaluate heating the basic ^{211}At solution to dryness and examining its re-dissolution into MeOH. We plan to continue to evaluate radiolabeling in vials that have dried ^{211}At solutions.

The optimized isolation and purification protocol is outlined below. The majority of the ^{211}At isolation studies conducted over the last year of the grant were done without the final distillation step, so we have very good data on the experimental reproducibility of isolation yields to that point. We have conducted six ^{211}At isolation runs that included a final distillation process, allowing us to estimate an average recovery yield for that step. The wet chemistry isolation process is outlined step-by-step below and the isolated yields for the final distillation step follow.

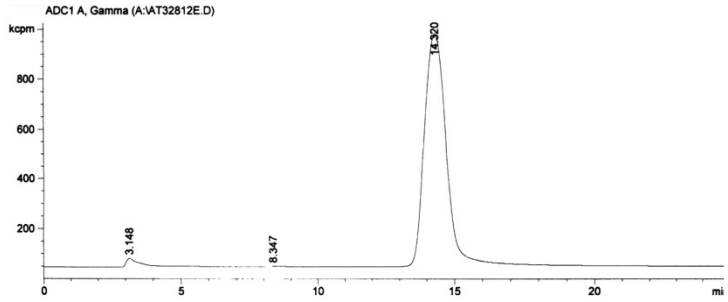
1. The irradiated target is measured in a dose calibrator to estimate the amount of ^{211}At
2. The top layers of bismuth target are dissolved in 10 mL conc. HNO_3 for 10 minutes
3. The HNO_3 solution is transferred to a 50 mL round bottom flask
4. The bismuth target is rinsed with an additional 5 mL of HNO_3 to remove remaining ^{211}At
5. The HNO_3 rinse is transferred to the round bottom flask
6. In this step, (*a*) the round bottom flask is connected to a cold water jacketed distillation head, (*b*) it is lowered into a 50 mL round bottom aluminum heating block, (*c*) the distillation head is covered with aluminum foil and (*d*) the HNO_3 is distilled at 305°C with gentle stirring
7. When all HNO_3 has been distilled into the receiving flask, a colorless residue remains in the distillation flask. The distillation flask is removed the heat source and allowed to cool for 10 minutes
8. An 8 mL quantity of 8M HCl is added to the residue in the distilling flask, and that mixture is agitated to ensure complete dissolution
9. After dissolution, the solution is transferred via pipet to a 20 mL scintillation vial
10. The distilling flask is rinsed with an additional 2 mL of 8M HCl as a wash, and it is transferred to the scintillation vial

11. A 4 mL quantity of freshly distilled HCl-equilibrated diisopropyl ether (DIPE) is added to the scintillation vial and that biphasic mixture is stirred for 10 minutes
12. The aqueous HCl layer is removed and counted in a dose calibrator
13. A 5 mL quantity of DIPE-equilibrated 8N HCl is added to the DIPE in scintillation vial, and that biphasic mixture is stirred for 5 minutes
14. The aqueous HCl layer is removed via pipet and counted in a dose calibrator
15. Steps 13 and 14 are repeated an additional three times (4 washes total)
16. The DIPE solution in the scintillation vial is counted in a dose calibrator to determine the ^{211}At activity remaining
17. An 800 µL quantity of 4M NaOH is added to the DIPE in the scintillation vial, and the vial is agitated for 10 minutes (color should change to pale yellow when pH rises above 7)
18. The aqueous layer (containing the ^{211}At) is removed and both layers are counted separately in the dose calibrator to determine the amount of ^{211}At isolated (typical decay-corrected radiochemical yields are 80% of starting activity)
19. The pH of the resulting basic aqueous ^{211}At solution is checked (should be very basic-13+)
20. To a 25 mL round bottom flask containing 9.19 mL DI-water, 2.085g ferrous sulfate-heptahydrate, and 0.81 mL conc. H_2SO_4 is added the basic ^{211}At solution.
21. The ^{211}At solution is agitated to re-dissolve the cloudy greenish precipitate, then the flask is connect to a distillation head that has a 10 mL round bottom receiver flask precharged with 100 µL of 1M sodium hydroxide
22. The distillation assembly is lowered into a 25 mL round bottom flask aluminum heating block (and an ice bath for the receiving flask), the distillation head is covered with aluminum foil and the solution is distilled at 200°C with gentle stirring. Isolation yields are shown in Table 1. An ion exchange HPLC chromatogram of the isolated product (sodium [^{211}At]astatide) is shown in Figure 2.

Table 1: ^{211}At Distillation Yields

Date	Yield
12/21/11	80.6%
2/1/12	84.7%
2/8/12	60.8%
2/29/12	74.1%
3/14/12	72.3%
3/29/12	71.8%
Average	74.1%

Figure 2: Radio-HPLC chromatogram of Na[^{211}At]At from distillation



It has been observed that as much as 85% of the activity will distill in the first 1.5 mL (fraction one), the remaining distillate is then collected in an additional round bottom flask precharged with 100 µL of 1M sodium hydroxide (fraction two). However, fraction two is collected in an appreciable volume (e.g. 3 mL), which further dilutes the specific concentration of the final product. Our experiments have shown that [^{211}At]astatide can be distilled as a pure product and will remain in the reduced form in the presence of sodium hydroxide in a volume up to 5 mL. Since it is important to decrease the final product volume to 100 µL or less, we have investigated methods of volume reduction. We have been successful in reducing the volume of basic ^{211}At solution with a stream of argon while heating at 80°C. This results in a reduction to dryness. The Na[^{211}At]At obtained is free of impurities and quite basic (13+) ensuring the ^{211}At distillate is in the desired [^{211}At]astatide form. Six ^{211}At isolation runs have had

distillations performed under the conditions described as the last step to isolate high purity ^{211}At. The distillation step has been performed by two individuals. The individuals obtained similar radiochemical yields of high purity ^{211}At (i.e. astatide rather than astatate) as assessed by HPLC (see chromatogram in Figure 2). Importantly, all of the ^{211}At activity is brought back into solution with 100 µL of deionized H_2O. Activity loss due to volatilization is minimal and can be contained with a charcoal filter; and further, the amount of salt (sodium hydroxide in this case) can be easily calculated by the end-user. This method also allows us to keep the specific concentration high enough that minimal manipulation by the end-user will be necessary prior to usage. No change is seen in the nature of the ^{211}At (i.e. sodium astatide). Additional studies were conducted to determine the yields obtained in the final distillation step. In six ^{211}At distillations, there was an average yield of 74%; 77% in 5 distillations without any problems (Table 1). Thus, in the overall isolation process we are able to obtain ~60% (decay corrected) yield of isolated Na[^{211}At]At. The 2-3 hour processing time makes the actual isolated yield is ~50% of the amount in the target at workup.

Specific Objective 3: Automation of ^{211}At-isolation process.

Milestones:
(a) Evaluate automation of dissolution of irradiated targets
(b) Evaluate automation of HNO_3 distillation & residue dissolution
(c) Evaluate automation of 8M HCl transfer and DIPE extraction & washings
(d) **Evaluate automation of NaOH neutralization and separation**
(e) Evaluate automation of ^{211}At distillation and basification

milestone (d) dropped from studies after optimization of the isolation process made it unnecessary.

We believe that one way to circumvent some of the problems in isolation of the ^{211}At is to automate the isolation process. Making the process very reproducible and simplified should circumvent the problems encountered with reproducibly in adjusting the final pH. As a proof of concept, various automated (computer-driven) experiments were successfully conducted where the transfer of solutions in the isolation process were achieved using a syringe pump to move materials from one vessel to another. Rather than obtaining pieces of the automation equipment and evaluating each step separately, we purchased the equipment necessary and evaluated the individual steps with the entire setup in place. A charcoal-filtered glove box was purchased to house the automation system. The other equipment purchased include: a dose calibrator; a Hamilton computer-driven dual syringe pump (50 mL capacity on each syringe); three Hamilton computer-driven MVP multi-valve systems; and a laptop computer with software to run the syringe pumps and electronic valves.

Our first goal was to demonstrate that the computer-driven syringe pumps and valves would move liquids (using colored water as a surrogate for actual compounds) into the appropriate containers and move them between containers. We used chemical surrogates to determine if there were weak points in the system (i.e. valves or joints) before evaluating it with more caustic agents [i.e. conc. HNO_3, 8N HCl and 4N NaOH). Overall there are (>40) liquid transfer steps conducted in the isolation process. Each step has to be programmed into the computer with times and (precise) quantities to make the automation work. We began the assembly and programming process outside of the glovebox to aid in ease of modification of the system. The preliminary completed setup of the automated system can be found in the photograph below

(see Figure 3). The preliminary setup was then placed in a glovebox for testing, system refinement, and optimization.

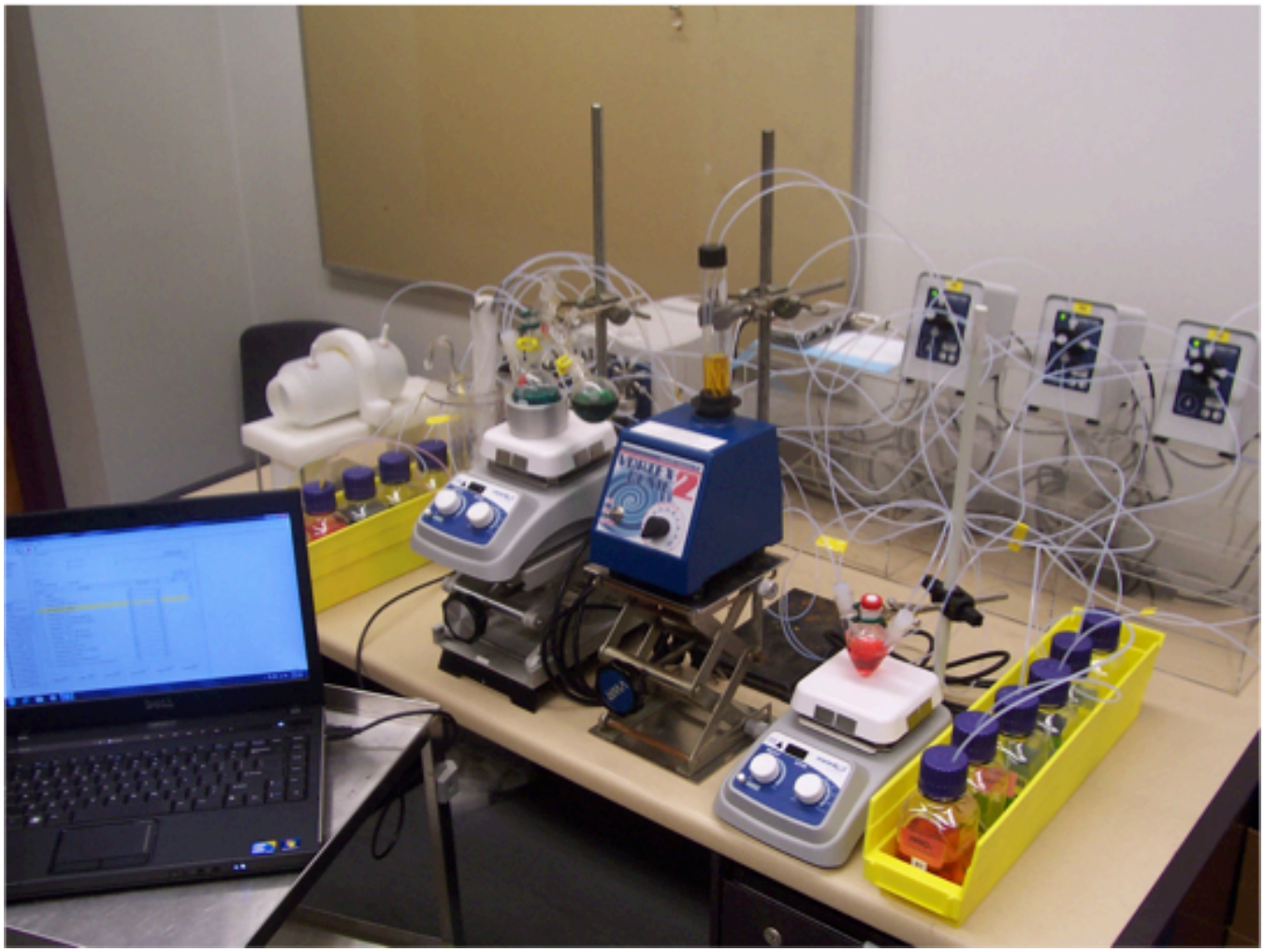


Figure 3. Photograph of setup for semi-automation of ^{211}At isolation. A dual-syringe pump (Hamilton ML560; behind stirrer in middle of picture) is driven by a computer program (laptop that will be outside of glovebox). That pump moves the reagents from one vessel to another and into and out of the dose calibrator (not shown). Solvent bottles are in yellow tray at left and waste bottles are in yellow tray at right. Three computer-driven valves (Hamilton MVP) used to redirect the reagents being moved are shown on the shelf at the right.

With the above model we were able to show that:

1) All of the liquid moving steps work as designed with colored water solutions (up to last distillation step)
2) HNO_3 can be transferred from a reagent vial to the dissolution chamber containing bismuth metal. After the 10 min dissolution period, the conc. HNO_3 solution containing dissolved bismuth metal (up to 1 g) can be readily transferred into the distillation vessel, and the dissolution chamber can be rinsed with HNO_3.
3) After distillation of HNO_3, 8M HCl can be added to the vessel for dissolution of the residue, and the resulting solution can be transferred to the extraction vessel (containing DIPE).
4) After vortex stirring, the DIPE can be rinsed with 8M HCl.

5) Between all steps, the solutions transferred can be stopped in a dose calibrator to assess the amount of ^{211}At in the solution.

Initially, we were able to demonstrate that the computer-driven syringe pumps and valves moved liquids (using the colored water) to and from the appropriate storage containers and reaction vessels. Secondary lines moved the solutions to a dose calibrator, which was initially incorporated into our setup. While a dose calibrator was not to be used in the final construction of the automation process, at this early stage it was hoped that this would allow us to determine how much of the ^{211}At activity was being transferred in each step. The order of the semi-automation studies changed from that outlined in the proposal. This was because we did not want to contaminate the setup with ^{211}At (or daughter ^{207}Bi) before all of the operations were running smoothly. When the trial (mock) isolation runs were conducted with non-irradiated targets a number of small issues were found. Some of the findings are listed below.

1) In the non-radioactive runs difficulties were encountered in delivering the desired amounts of reagents to the reaction vessels. Adjustments were made in the computer program to provide measured volumes very close to those desired. The problem appeared to be caused by the fact that some of the transfer solutions remain in the transfer tubing, altering the volumes delivered (particularly in the first run of the day). This was not a major problem with regards to the transfer of ^{211}At activity, as each step included a rinse of the tubing. However, it was problematic in that it effected the amount of NaOH or HCl delivered. The extraction of ^{211}At from DIPE was quite dependent on having a high basicity (pH >12), so it was imperative that most of the HCl be removed prior to addition of the NaOH. Removal of varying quantities of HCl was studied to better understand how the amount of HCl remaining affected the quantities of NaOH required. In the end it was important to make the extraction solution basic enough for efficient extraction without having a large excess of NaOH to carry into the distillation step.
2) A separate computer program for the neutralization process was implemented, as the computer program to move set amounts of solution from one vessel to another was inadequate for that task. (The neutralization step will not be used in future runs)
3) There was concern that residual HCl and NaOH solutions could cause problems with the syringe pump valves and directional valves used for moving the solutions from vessel to vessel. Therefore, a “clean-up procedure” was implemented to flush the lines with water and air.

Experimental Steps: (see Figure 4 for diagram of setup)

1. Dissolve the bismuth metal target using a concentrated HNO_3 immersion (DB chamber-top middle of Fig. 4)
2. Move the HNO_3 solution to a distilling flask (DS1-bottom left of Fig. 4)
3. Distill HNO_3 to give residue
4. Move 8M HCl into DS1 and dissolve the residue
5. Move the 8M HCl solution to a separation flask (SV-bottom right of Fig. 4) precharged with diisopropyl ether (DIPE)
6. Mix the DIPE and 8M HCl (vortex) in SV, and remove 8M HCl layer to waste
7. Add more 8M HCl as a wash and repeat #6 a total of 2 times
8. Add 4M NaOH to make strongly basic biphasic solution (back extraction into the aqueous phase)

9. Move the basic aqueous phase to a round bottom distillation flask (DS2-bottom right in Fig. 4)
10. The basic solution is then distilled in the presence of 1.5M H_2SO_4 and 0.75M $FeSO_4$ into a receptacle containing 50 µL 4M NaOH
11. The high purity solution (containing [^{211}At]astatide) can be reduced to an acceptable volume for shipment

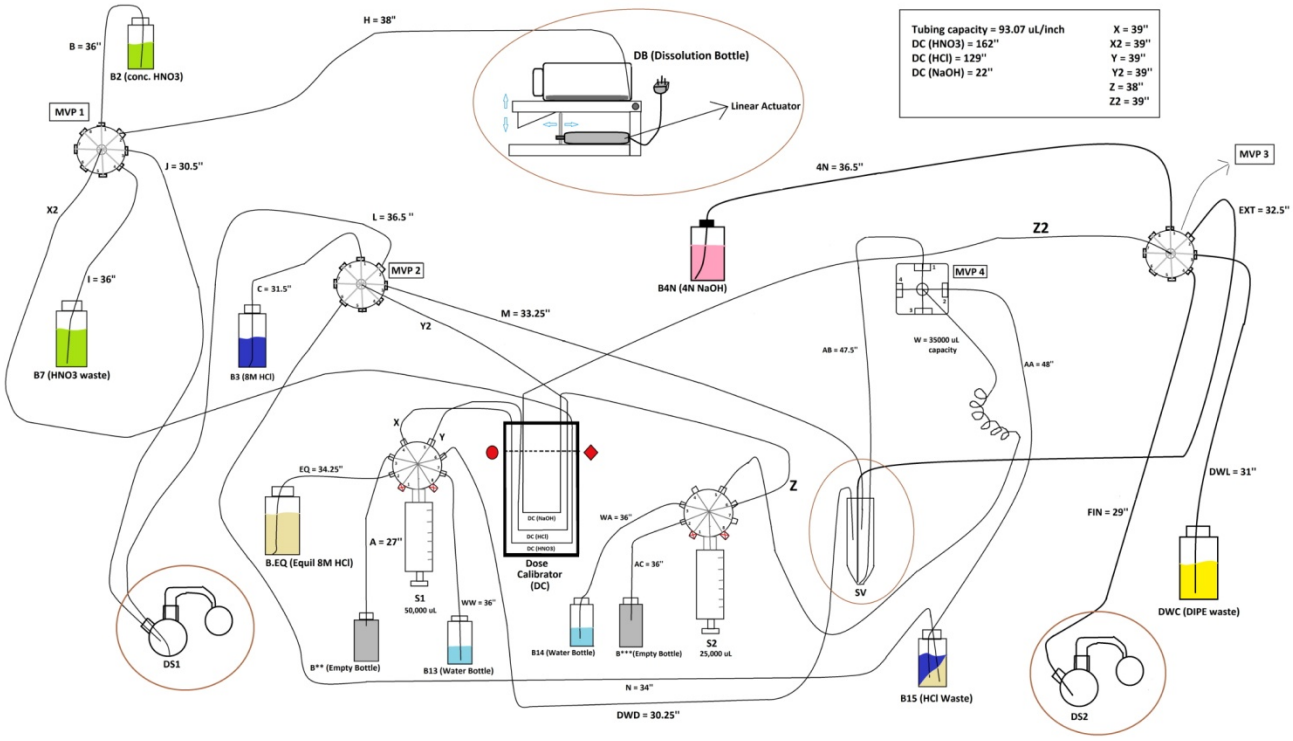


Figure 4: Schematic of semi-automated process (note that computer control of syringe pumps and valves is not shown). Syringe pumps are noted as S1 (left of Dose Calibrator) and S2 (right of Dose Calibrator). Directing valves are noted as MVP1 thru MVP4. Solvent and waste bottles are designated by the letter B in name. Tubing sections are noted as alphabet letters. Containers used in the processing include: DB, dissolution bottle (upper middle); DS1, distillation setup 1; SV, separation vial (right of S2); DS2, distillation setup 2 (lower right).

Following the above experiments we conducted a mid-project assessment. The results of the assessment follow. Automation of any chemical separation presents a unique set of challenges. As opposed to manual processing, with the current software troubleshooting a reaction that deviates from expectations is generally not an option in an automated system. Although the expectation is that the reaction will occur in the same manner every time it is run, once an automated technique is set up it will run from beginning to end using the same parameters each time regardless of the success or failure of each individual step. Importantly, the physical properties of each solvent or solvent mixture, like changes in liquid phase interfaces, reagent vapor pressures, and surface tension have the potential to present unique challenges when liquids are pumped from reaction vessel to reaction vessel. It is therefore understandable that translation to semi-automated processing began with challenges not found in the development of the manual method of ^{211}At isolation, and needed to be addressed in a different manner. The main challenges encountered with manual target processing were

as follows:

1. Assessing the number of acidic washes necessary to efficiently separate the isolated ^{211}At from the residual bismuth target material.
2. Assessing the efficiency of the extraction of ^{211}At from HCl into DIPE and the back extraction from DIPE into NaOH.
3. Controlling the formation and isolation of the [^{211}At]astatide, since [^{211}At]astatate and [^{211}At]astatide behave very differently and their formation is pH dependent.

Simplified to its most basic function, the semi-automated ^{211}At separation and isolation technique, hereafter referred to, as "the automated technique" was nothing more than a series of liquid handling steps. Using Hamilton's *Microlab 500 Series* software to control the twin barreled Hamilton ML-560 syringe pump and associated Modular Valve Positioners (MVP), the system could be thought of in terms of two different sets of commands, one set allowed reagents to be delivered to the various reaction vessels while the other allowed for the removal of a reaction mixture from a vessel. Combinations of these two types of commands allowed the procedure to accomplish each step outlined in the manual isolation technique.

Four computer programs were utilized to accomplish the operations needed to isolate ^{211}At, retain the ^{211}At as [^{211}At]astatide, and rinse the setup after running. All of the programs had multiple sub-routines and more than 200 individual (computer) steps were involved. The function of each program is outlined below.

Program 1: (Primary target dissolution procedure) **1a:** target dissolution; **1b:** movement of HNO_3 solubilized ^{211}At to distillation vessel #1 (DS1)

Program 2: (Primary isolation and extraction procedure)- **2a:** dissolution of ^{211}At residue with 8M HCl followed by subsequent rinses and transfer of solution to the DIPE pre-charged separation vessel (SV) via the dose calibrator for verification of transferred activity
2b: removal of initial HCl and addition and removal of subsequent pre-equilibrated HCl washes

Program 3: (NaOH back extraction) **3a:** addition of 4M NaOH to the separation vessel (SV) to ensure basicity; **3b:** transfer of aqueous layer to distillation vessel #2 and the organic layer to waste; both via the dose calibrator for verification of transferred activity

The final distillation or isolation of pure [^{211}At]astatide was still performed manually due to space and manipulation constraints.

Program 4: *(Clean-up procedure)* **4a:** line/tubing clearance to waste; **4b:** line/tubing H_2O flush and air purge

Liquid handling was accomplished by the syringes delivering and removing predetermined quantities through selected valve ports and multiple addition lines. Individual lines were used for removal and addition of reagents so there was no cross-contamination, additionally each solution could be diverted to an appropriate waste container. While the initial quantities of reagents or solutions were programmed based on the manual process, the volumes entered into the computer program were refined by experiments that determined the actual amount

delivered. In the initial trials, monitoring of the radioactivity being processed in the system was accomplished by the use of an in-line dose calibrator. More specifically, as the reaction mixture was transferred from one reaction vessel to another its line passes through the well of a dose calibrator (with a brief stop) so the processed activity can be quantified. The majority of the experiments conducted were directed at demonstrating the computer-driven movement of solvents and solutions occurred as designed, and at refining the volumes delivered so that they were accurate. Several tests were conducted to verify the volume precision of the system from experiment to experiment. While the accuracy of the volume of solvent delivered was calculated to be ≤ 2% and the previously reported larger variances that were obtained in moving solutions from one vessel to another (through dose calibrator) due to droplets being left in the tubing appeared to be resolved. The precision and repeatability of the system continued to be of concern. Modifications to the quantities of reagents delivered had to be made, which led to large systematic variances. Attempts were made to compensate for the system's variance by constantly testing, evaluating, and subjecting the system to refinement for the sake of precision and accuracy.

One of the major issues was that mixtures of HCl and DIPE caused problems with moving the requested amounts over the tubing distances in our system. Another issue that came up was the fact that the current computer-driven valve system did not operate as designed under a negative atmosphere (i.e. vented glovebox), causing the liquid transfers to back up in the system. A third issue was the type of motor (step motor) used to move the syringe pump. More information on how we addressed these issues follows.

The most significant issue that we had to address was that of moving aqueous basic and acidic mixtures containing DIPE. We had two catastrophic syringe failures resulting from caustic reagent corrosion (NaOH), which led us to install a system of tubing loops so that only equilibrated HCl ever made contact with the interior of a syringe barrel. All other reagents were either pre-charged into their respective vessels prior to experiment initiation or were directly delivered via the modular valve positioners. Initially, it was thought that this corrected the instrumentation failures and drastically improved precision. While that was true for the steps leading up to and including the transfer of the ^{211}At in HCl to the separation vessel, the subsequent transfer steps, which have mixtures of DIPE and HCl or DIPE and NaOH, were not improved by the looped tubing system. By forcing the manipulation of the liquids through a significant length of tubing, it appeared that a substantial pressure differential was created within the closed system, making any steps where DIPE was present nearly impossible to complete with any degree of reproducibility. This issue, moving liquids that have two somewhat miscible liquids with different vapor pressures, was addressed by a redesign of the glassware. We believed that redesigning the liquid flow can be done in a way that the mixtures are separated in a separation funnel using gravity flow (less automation). Our hope was that by utilizing a modified piece of glassware as a makeshift separation funnel we could more easily and reliably achieve gravity based separation and removal of the aqueous layers in the wash steps. Any movement of the DIPE-containing solutions would be based on gravity flow, and would be controlled by a stopcock. This approach was designed to eliminate moving the biphasic solutions under pressure or vacuum. We believed that the system re-configuration would also allow for a reduction in the number of washes (which it did not) and a more accurate delivery and removal of the wash reagent (which it did), both of which contribute to the overall precision and accuracy of the system.

The second issue, that of valve leakage in a negative atmosphere, was successfully addressed by allowing enough air into the glovebox (through a charcoal vented line) to keep negative pressure from developing when the venting fan is operating. The third issue, that of having a step motor for the syringe delivery and removal of reagents was addressable as well. The problem stemmed from the precision of the delivery from a syringe pump being related to the size of syringe and the length (volume) of the tubing used. Thus, if a 50 mL syringe is used, the precision for delivering 1 mL of reagent is not high. Initially we used 50 mL syringes to minimize the number of steps involving transfer of reagent into the syringe (from reagent bottles). While there are a number of liquid addition and transfer steps in the wet chemistry isolation process, it appears that the most appropriate size of syringe to obtain higher precision in the liquid delivery and transfer processes is 25 mL.

The previously reported fundamental issues with the automated system consisted of: (1) difficulties with the syringe pump delivering and removing the programmed amounts of reagents in certain steps. (2) A lack of ability to adjust the volume of the liquids delivered and/or removed from certain steps within acceptable tolerances. (3) The system of pumps and valves appeared to have problems handling liquids that have two somewhat miscible liquids with different vapor pressures. Additionally, the computer-driven valve system did not appear to be operable under a negative atmosphere (i.e. vented glovebox), causing inaccurate liquid transfers.

The combination of these problems left us with a system that was inadequate for our use in that there was absolutely no degree of certainty that the results would ever be reproducible from one experiment to the next. Our only option appeared to be to undertake a complete redesign and rebuild of the system. Rebuilding of the system encompassed the bulk of our budget and time for the final 3 quarters of project funding. Conceptually, the system is the same in that it involves simply transferring liquids from one vessel to another. In practice however, what began as making only two significant changes to the system turned into a complete change in system design. The change in design was, however, limited by the fact that we could only indirectly affect the accuracy, precision, and calibration of the syringe pumps being used without completely replacing them at considerable expense, which was not an option.

The following significant changes to the system design resulted in an operable system with results listed below.

(1) Fluid delivery lines - all loops and excessively long pieces of tubing were removed to reduce the internal pressure differential of the closed system and allow it to operate normally under a slightly negative atmosphere without impeding the flow of the liquids in the lines. This also, improved the accuracy of the volumes delivered/removed from the individual steps.

(2) Separation vessel - the series of extractions conducted in the separation vessel led to the discovery that DIPE or mixtures of solvents that contain DIPE could not be pumped with any degree of reproducibility (at least in this system). To address this issue we re-designed the glass separation vessel. We believed that a custom piece of glassware (resembling a classical separatory funnel with a stopcock) for gravity based separation (less automation) would allow more control over the liquid flow and efficiency of the overall aqueous/organic phase separations. Additionally, we replaced the mechanical

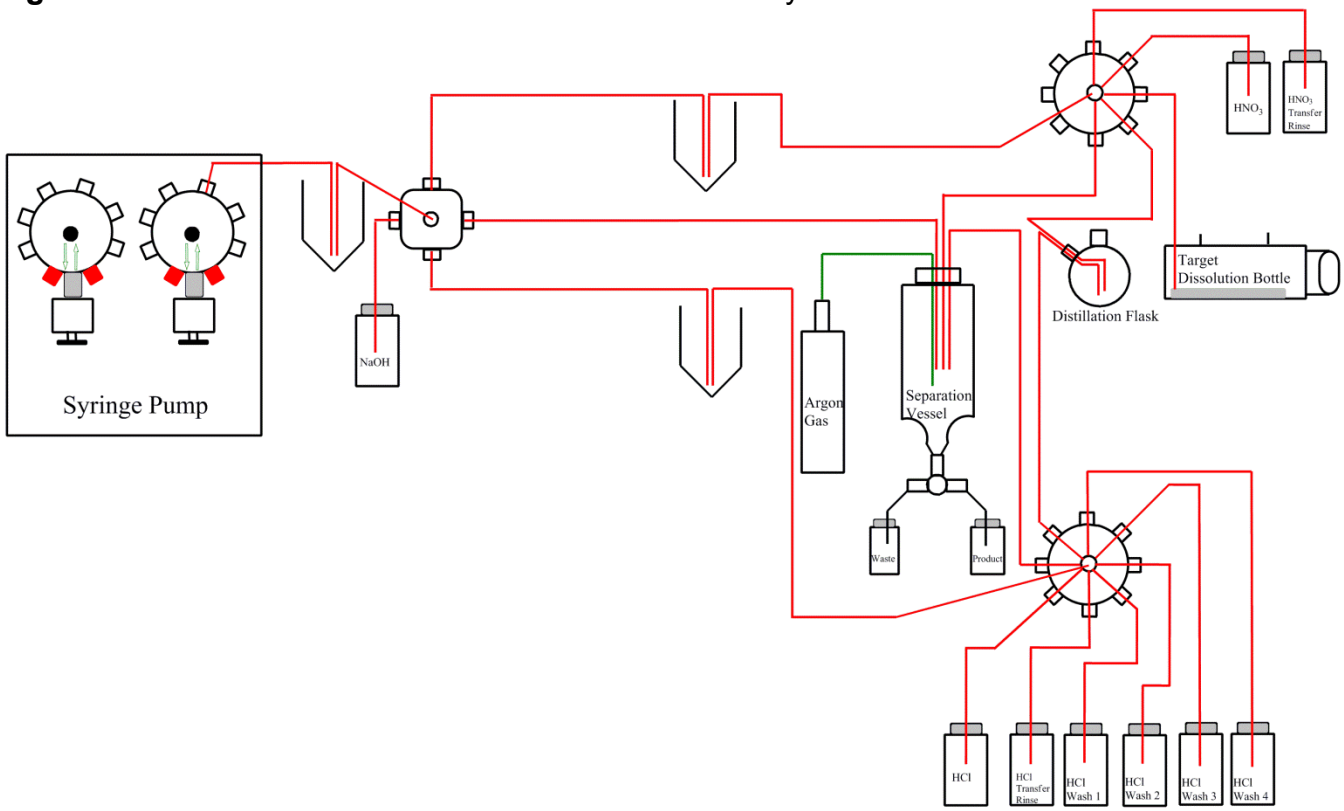
agitation of the solvent mixture in the separation vessel with an adjustable stream of bubbled argon gas for more efficient and reliable liquid disruption. Ideally we thought these changes could also translate to an overall reduction in the total number of HCl wash steps for removal of bismuth, but this was not the case. The glassware was fabricated at the glass shop on the UW campus.

(3) The stream of argon gas, while ideal as an agitation replacement, was ruled a safety hazard due to the potential for volatizing the ^{211}At species, and therefore was not incorporated into the final system design. The reasoning behind the design change was that, in the case of a catastrophic system failure, having a carrier gas with continuous flow would serve to transport rather than contain the ^{211}At. This could result in an accidental release of ^{211}At. Instead, the system was configured to use an open port on the syringe pump to quickly cycle and repeatedly deliver 25 mL of air (circulating inside the glovebox) during each wash step. The result was a system capable of agitating the aqueous/organic mixture throughout the extraction step, but it does so in discrete increments and can be controlled by the computer (speed of delivery and cycling time along with the number of cycles to complete). The resulting modification is safer and its efficiency has been experimentally validated, in that it provides sufficient agitation to facilitate chemical extraction or back-extraction as the step demands.

(4) Given the calibration issues encountered previously, greater efficiency in liquid transfers (removal/delivery or solvents and products) was sought. We believed this could be achieved by removing the total contents of a vessel as opposed to discrete quantities out of a stock bottle or reaction vial.

(5) The preceding system modifications were incorporated into the semi-automated ^{211}At extraction system. The changes are visible in the schematic below (Figure 5). It should be noted that all “V-shaped” vials are 20 mL syringes with silicone stoppers in the tops as opposed to plungers. Delivery lines were inserted in the stopper along with acid-neutralizing sodium bicarbonate filters. The solvent removal lines were attached to the luer® slip tip of the syringe using luer® fitting adapters. All reagents were added in discrete quantities to the corresponding syringe and complete transfers are achieved using positive pressure displacement generated by the syringe pump. No liquids ever directly contacted the barrels of the syringes and air was pumped in quantities that far exceeded the volumes of the liquids being transferred. This modification yielded fluid transfer volumes with an error margin of ± 200 µL (4% or less depending on volume), which is well within our level of tolerance. We conducted one ^{211}At isolation experiment using an irradiated target. The amount of ^{211}At present in the target was determined to be 18 mCi. Following the semi-automated isolation 4 mCi were recovered, corresponding to a 22% decay corrected recovery. The typical manual recovery is approximately 80% decay corrected. However, of particular note is the amount of ^{211}At lost in the wash steps, which is similar to that typically seen in the manual isolation. A survey of the apparatus following the experiment indicated that the bulk of the activity remaining resided in the distillation head used for removal of concentrated HNO_3. We believe this result came from the distillation head being placed in a less than optimal position to efficiently and expediently drive off the nitric acid.

Figure 5: Schematic of a Functional Semi-Automated System



Specific Objective 4: Put into place methods and materials for shipment of ^{211}At.

Milestones:

(a) Identify UW Radiation Safety and DOE requirements for shipping ^{211}At
(b) Set up schedule to meet requirements for shipping ^{211}At
(c) Complete 50% of required tasks to meet requirements for ^{211}At shipments
(d) Complete 75% of required tasks to meet requirements for ^{211}At shipments

This specific objective was to be accomplished by putting into place standard operating procedures for the wet chemistry isolation process, measurement of ^{211}At activity, labeling of vials, and setting up procedures with Radiation Safety for shipping ^{211}At from UW. The proof that we are ready to sell to customers through DOE was to be shown by making a trial ^{211}At shipment. The following describes the work on this specific objective.

Since our goal is to make ^{211}At shipments through the DOE Isotope Development Center (NIDC). DOE headquarters was contacted about who might be able to help with regards to DOE requirements for shipment of ^{211}At. We were told to contact Mitch Ferren at Oak Ridge National Laboratory. A teleconference with Mitch Ferren (ORNL), Jeff Shelton (ORNL), Don Hamlin (UW) and Scott Wilbur (UW) was conducted, during which the DOE Ordering and Shipping processes were explained. It was noted that shipment training might be required and then later determined not to be the case as UW Radiation Safety was to conduct all shipments. They are already trained/certified for shipping radioactive materials.

It was also suggested that we look into commercial sources for (returnable) shipping containers that are already approved to handle radioactive materials. We determined that the available "Bexxar Shipping System" (Biodex) would work for all quantities of ^{211}At activity that we might ship. The shipping system contains a Unit Dose lead container which is placed in a converted ammunition carrier (overall weight is 31 lb). The Bexxar Shipping System complies with USA DOT 7A type A Radioactive Material Requirements and IATA Dangerous Goods Regulations for shipping containers. The system meets DOT II Type A packaging requirements when shipping 160 mCi of ^{131}I Bexxar. Since it is unlikely that we would ship over 100 mCi of ^{211}At, and ^{211}At has very soft photons (i.e. 79 keV) compared with ^{131}I. The container was found to be a suitable option for our shipments.

Having an isolation procedure for making highly purified ^{211}At for shipment and having identified the appropriate shipping vials and shipping containers, we had a good idea of what it would take to make ^{211}At shipments from UW to an investigator at another institution. So in quarter 7 of the funding period we were ready to begin making the trial shipments to other investigators subject to their obtaining institutional approval to receive the radionuclide. Dr. Thomas Quinn at the University of Missouri arranged to receive (and use) some ^{211}At for his peptide studies. To assist Dr. Quinn, we also shipped to him a *closo*-decaborate(2-) reagent for modifying his peptide so that it would label with ^{211}At. Initial discussions with Mitch Ferren and Dr. Marc Garland about what it would take to conduct the trial shipments provided guidance. However, an investigator purchase price for ^{211}At must be set and accepted by DOE before trial shipments can be made. After further discussions with Dr. Marc Garland at DOE, it was determined that the best approach was to make the trial shipment of ^{211}At independent of the DOE isotope sales program. The rationale for making the shipment independent of DOE was that an agreement needed to be put into place between the UW and DOE to have DOE sell ^{211}At produced at UW. While another factor was that a price still needed to be set for the ^{211}At. Although we had determined a cost to prepare ^{211}At for shipment, without having the agreement in place between UW and DOE a final price could not be set.

When Dr. Quinn was ready to conduct an astatination of his peptide with ^{211}At, we made a shipment to him. As Dr. Quinn's proof of principle experiments by radioiodination of his peptide conjugate were previously successful he was ready to try an astatination. Following that, an ^{211}At shipment was made on September 13, 2012. We isolated and shipped 12 mCi of ^{211}At in 0.1N NaOH in a volume of 600 µL via FedEx courier. The shipment was logged as "ready for pick-up" from the University of Washington radiation safety office at 10:30 am (PDT) on September 13, 2012, and 1.35 mCi of ^{211}At (11% of the shipped quantity) was delivered at 11am (CDT) on September 14, 2012. The shipment represents the successful completion of this Specific Objective.

Specific Objective 5: Work with DOE to make ^{211}At available to other US investigators.

In this specific objective we planned to work with the DOE Isotope Development and Production for Research and Applications Program to make ^{211}At available through their service. As this has not been done previously at the University of Washington, agreements between the UW and DOE are required before the program can be instituted. It was anticipated that paperwork would need to be completed to arrange to receive orders from

DOE, and to get paid for the shipments. One of the issues was that a price for selling the ^{211}At had to be set and that could not be done without knowing the optimal conditions for irradiation and for isolation of ^{211}At from the targets. After those parameters were determined, we began to explore what it takes to make the ^{211}At available through the DOE system. Dr. Marc Garland at DOE informed us that there would be legal paperwork that had to be signed by UW and DOE, and that DOE would send those documents to the UW to start the agreement process. We are now ready to provide the ^{211}At, but know that the signing of legal documents can take some time. We eagerly await the agreement paperwork and the opportunity to collaborate with DOE on this production effort.