Final Report

for the Period of:

July 1, 2013 through June 30, 2016

Project Title:

Evaluation of Novel Wet Chemistry Separation and Purification Methods to Facilitate Automation of Astatine-211 Isolation

Award #: DE-SC0010502

Principal Investigator on Project (UW Studies):
D. Scott Wilbur, Ph.D.
University of Washington, Box 355016
Department of Radiation Oncology
616 N.E. Northlake Place
Seattle, WA 98105
206-616-9246
dswilbur@uw.edu

Report Provided to:
Dennis R. 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 Project:

This research is a collaborative effort between the research groups of the PIs, Dr. D. Scott Wilbur in the Department of Radiation Oncology at the University of Washington (UW) and Matthew O'Hara at the Pacific Northwest National Laboratory (PNNL). In this report only those studies conducted at UW and the budget information from UW will be reported. A separate progress and financial report will be provided by PNNL.

This final report outlines the experiments (Tasks) conducted and results obtained at UW from July 1, 2013 thru June 30, 2016 (2-year project with 1 year no-cost extension). The report divides the information on the experiments and results obtained into the 5 specific objectives of the research efforts and the Tasks within those objectives. This format is used so that it is easy to see what has been accomplished in each area. A brief summary of the major findings from the studies is provided below.

Summary of Major Findings from Research/Training Activities at UW:

- Anion and cation exchange columns did not provide adequate ^{211}At capture and/or extraction results under conditions studied to warrant further evaluation.
- PEG-Merrifield resins containing mPEG350, mPEG750, mPEG2000 and mPEG5000 were synthesized and evaluated.
- All of the mPEG resins with different sized mPEG moieties conjugated gave similar ^{211}At capture (>95%) from 8M HCl solutions and release with conc. NH_4OH (~50-80%), but very low quantities were released when NaOH was used as an eluent.
- Capture and release of ^{211}At when loading [^{211}At]astatate appeared to be similar to that of [^{211}At]astatide on PEG columns, but further studies need to be conducted to confirm that.
- Capture of ^{211}At on PEG columns was lower (e.g. 80-90%) from solutions of 8M HNO_3, but higher capture rates (e.g. 99%) can be obtained when 10M HNO_3 is mixed with an equal quantity of 8M HCl.
- Addition of reductants to the ^{211}At solutions did not appear to change the percent capture, but may have an effect on the % extracted.
- There was some indication that the PEG-Merrifield resins could be saturated (perhaps with Bi) resulting in lower capture percentages, but more studies need to be done to confirm that.
- A target dissolution chamber, designed and built at PNNL, works well with syringe pumps so it can be used in an automated system.
- Preliminary semi-automated ^{211}At isolation studies have been conducted with full scale target dissolution and ^{211}At isolation using a PEG column on the Hamilton automated

system gave low overall recoveries, but HNO_3 was used (rather than HCl) for loading the ^{211}At and flow rates were not optimized.

- Results obtained using PEG columns are high enough to warrant further development on a fully automated system.
- Results obtained also indicate that additional studies are warranted to evaluate other types of columns for ^{211}At separation from bismuth, which allow use of HNO_3/HCl mixtures for loading and NaOH for eluting ^{211}At. Such a column could greatly simplify the overall isolation process and make it easier to automate.

II. *Specific Objective 1: Evaluation of novel methods for liquid/liquid extraction of ^{211}At from irradiated bismuth targets to facilitate automation of the currently used manual wet chemistry isolation method.**

In this specific objective a tandem in-line liquid/liquid extraction apparatus and a tandem in line microreactor apparatus was built and their efficiency at extracting/recovering ^{211}At from solutions of 8M HCl was evaluated.

SO 1a: Evaluate tandem in-line liquid/liquid extraction apparatus (PNNL)
***Task 1**: Evaluation of In-Line Liquid/Liquid Extraction Methods (PNNL)*

SO 1b: Evaluate tandem in-line microreactor apparatus (PNNL)
***Task 2**: Evaluation of Tandem Microreactors with Permeable Organic Membrane Phase Separators (PNNL)*

**Results from this SO will be reported separately by Matt O'Hara, PNNL*

III. *Specific Objective 2: Evaluation of solid-phase extraction and metal adsorption approaches as possible replacements of the liquid/liquid extraction step to greatly simplify automation of the wet chemistry ^{211}At isolation method.*

In this specific objective alternate methods for separation of ^{211}At from bismuth were evaluated as an approach to simplify the wet chemistry isolation method.

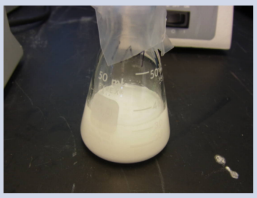
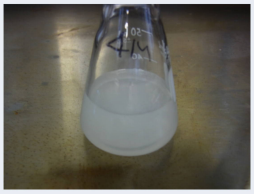
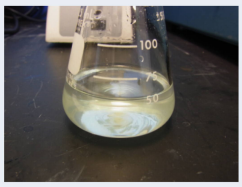
(a) SO 2a: Evaluate solid-phase extraction approaches for separation of ^{211}At from Bi targets (UW)

i. Bismuth solubility in acid and effect of reductants

Important parameters when considering solid-phase column separation of ^{211}At from bismuth are the solubility of bismuth in the acid solutions employed and the ability to add reductants to keep the ^{211}At in reduced form (i.e. astatide). The normal approach to wet chemistry isolation of ^{211}At from bismuth starts by dissolving the bismuth target in concentrated nitric acid, the removing the nitric acid through distillation, followed by redissolving the residue in 8M HCl. If the $FeSO_4/H_2SO_4$ reduction is to work the residue must be dissolved in H_2SO_4. So, some initial studies were conducted to determine if bismuth could be dissolved in H_2SO_4. The results from those studies are shown as photos in **Table 1**. It can be noted in **Table 1** that dissolution of $BiCl_3$ (used as a surrogate for $Bi(NO_3)_3$) was very poor in 1M H_2SO_4 but was possible in 50 mL of 4M H_2SO_4. (note: in latter studies 8M HNO_3 or higher was used to keep the volume to a minimum). We thought that it might be possible to reduce the ^{211}At away from the $Bi(NO_3)_3$, so we conducted a reduction study where non-radioactive I_2 was placed in solutions of 0.75M $FeSO_4$/1M H_2SO_4 with/without the addition of $BiCl_3$. The results of that reduction study are shown in **Table 2**. As the pictures in **Table 2** show, the reduction can occur in the presence of Bi, but it takes quite a long time. Therefore, another reductant was sought. The reductant that is used normally to reduce radioiodine to radioiodide is sodium metabisulfite ($Na_2S_2O_5$). This is a strong reductant as it decomposes in acid to form SO_2. A study using $Na_2S_2O_5$ to reduce iodine in 1M H_2SO_4 with/without Bi ($BiCl_3$) was conducted. The results of that study are shown in **Table 3**. It can be

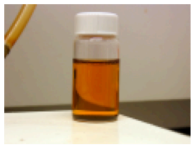
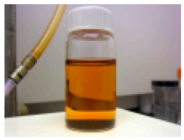
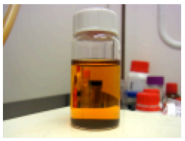
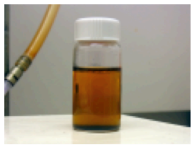
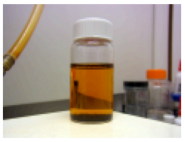
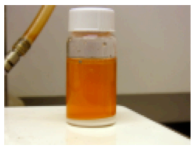
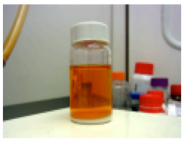
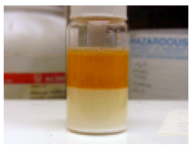
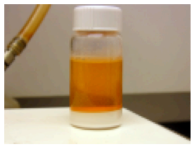
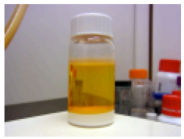
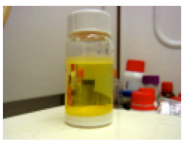
readily seen in **Table 3** that sodium metabisulfite reduces iodine rapidly, even in the presence of $BiCl_3$. Thus, in all subsequent studies, sodium metabisulfite was used for reduction of ^{211}At in the presence of Bi salts.

Table 1: Dissolution of $BiCl_3$ in H_2SO_4 solution*

	5 mL	10 mL	20 mL	30 mL	50 mL
1 M H_2SO_4	Not dissolved			 Not dissolved at all	
4 M H_2SO_4	Hardly dissolved			 Some of $BiCl_3$ is dissolved.	 Dissolved

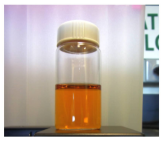
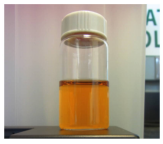
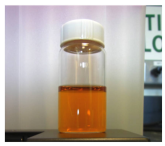
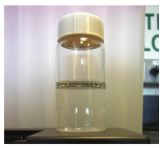
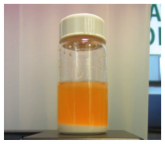
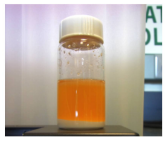
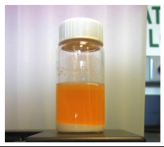
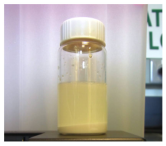
*Total volume of acid used to dissolve $BiCl_3$ is shown across the top row

Table 2: Reduction of I_2 by 0.75M $FeSO_4$/1M H_2SO_4 solution

	0 min	15 min	1 h	2 h
1 M H_2SO_4				
0.75M $FeSO_4$ 1 M H_2SO_4				
$BiCl_3$ 1 M H_2SO_4				
$BiCl_3$ 0.75M $FeSO_4$ 1 M H_2SO_4				

Total volume: 15 mL; Iodine in MeOH: 0.5 mL (1 mg)

Table 3: Reduction of Iodine by Sodium Metabisulfite in Acid

	Before adding $Na_2S_2O_5$	After adding $Na_2S_2O_5$
1 M H_2SO_4 (10 mL)		
1 M H_2SO_4 (10 mL) $Na_2S_2O_5$ (3eq. to I_2)		
$BiCl_3$ (2.7 g) 1 M H_2SO_4 (10 mL)		
$BiCl_3$ (2.7 g) 1 M H_2SO_4 (10 mL) $Na_2S_2O_5$ (3eq. to I_2)		

Iodine in MeOH: 1 mg

ii. Ion Exchange Columns:

(Added Task) *Evaluate ^{211}At separation using anion exchange resins (AG1x8 and Silica SAX)*

A. AG1x8 Studies

We were interested in evaluating anion exchange resins as literature reports have shown that ion exchange columns can be used for separation of (radio)halogen species and isolation of radioiodine from tellurium targets. Ion exchange was used to determine the quantity and nature of iodine species in seawater with a strong anion exchange (TSKgel SAX) column on a HPLC [1]. Hao et al. also evaluated the chemical species of iodine present in seawater by passing filtered natural seawater through a strongly basic anion exchange column (Bio-Rad AG1-x4) [2]. Wang and Jiang separated iodine and bromine species in water and urine samples with HPLC using a strong anion exchange resin (Hamilton PRP-X100) column [3]. Bruchertseifer et al. also analyzed iodine species in aqueous solutions using strong anion exchange column (Dionex AG11) on a HPLC [4]. Interestingly, in that study it was shown that the iodine species could be evaluated on the column over a range of pH 5 – 10.

Early studies by Rössler, Tornau and Stöcklin demonstrated that using a radioHPLC with a strong anion exchange resin (Aminex A-27) provided separations of radioiodine and ^{211}At species when eluting with 1N $NaNO_3$/0.1N Na_2SO_3 at 80°C [5]. We [6], and other investigators [7], have shown the anion exchange resin Dionex S20 can be used with radioHPLC to separate and quantify radioiodine and ^{211}At species.

Chattopadhyay and Das used an anion exchange column to separate ^{131}I from tellurium oxide targets [8]. To make the separation, the TeO_2 target was dissolved in 4N NaOH, diluted to make 0.01N NaOH and passed over a Dowex1x8 column. A mean of 96% of the ^{131}I was captured on the column. Recovery of the ^{131}I from the column was accomplished using tetrabutylammonium bromide (TBAB) in methylene chloride.

All of the prior literature reports suggest that a strong anion exchange resin might be used to separate ^{211}At from a bismuth target. Thus, we evaluated a strong anion exchange resin (AG1x8) in some initial experiments. Some of the conditions used to place the ^{211}At on the column and to elute from that column were based on the prior literature examples.

The first AG1x8 experiments were conducted with ^{125}I to determine if that radiohalogen could be efficiently trapped by, and subsequently recovered from, the column. Boric acid solution was used as eluent, so that both acidic and basic conditions could be evaluated. The results of the experiment are provided in **Table 4**. It can be noted that very high trapping efficiency of the ^{125}I was obtained, but there was no recovery of radioiodine except when using a high concentration (500mM) of tetrabutylammonium bromide (TBAB). The same experimental conditions were used with ^{211}At, but different results were obtained, as shown in **Table 5**. Importantly, the trapping efficiency was low and highly variable. Further, the recovery was very low and activity was lost in washes under all conditions studied. These results suggested that some of the astatine may have been in another form rather than the anionic form, and that ^{211}At was bound to the column much stronger than radioiodine.

Table 4: Radioiodine (^{125}I) trapping and recovery from anion exchange resin AG1x8

Solution	Applied activity	**Trap Efficiency**	Wash loss	Recovery			
				0.2M NaOH	1M NaOH	3 mM TBAB	**500 mM TBAB**
0.2M Boric acid-borate buffer (pH 5.3)	63.4 µCi	**>99%**	0%	0%	0%	0%	-
H_2O (pH 7.0)	61.6 µCi	**>99%**	0%	0%	0%	0%	**82.1%**
0.2M Boric acid-borate buffer (pH 8.0)	63.6 µCi	**>99%**	0%	0%	0%	0%	-
0.1M Borate buffer (pH 10)	67.9 µCi	**>99%**	0%	0%	0%	0%	-

Resin volume: 200 mg, I-125:

Table 5: Astatine (^{211}At) trapping and recovery from anion exchange resin AG1x8

Solution	Applied activity	Trap Efficiency	Wash loss	Recovery			
				0.2M NaOH	1M NaOH	3 mM TBAB	500 mM TBAB
0.2M Boric acid-borate buffer (pH 5.3)	141 µCi	69%	5.0%	**0.48%**	**0.44%**	**0.25%**	5.8%
H_2O (pH 7.0)	145 µCi	91%	1.1%	**0.17%**	**0.15%**	**0.11%**	4.3%
0.2M Boric acid-borate buffer (pH 8.0)	146 µCi	69%	6.7%	**0.44%**	**0.44%**	**0.19%**	5.4%
0.1M Borate buffer (pH 10)	145 µCi	77%	3.1%	**0.30%**	**0.26%**	**0.21%**	3.4%

The results obtained in the studies put into question the nature of the ^{211}At species that was loaded onto the column. Our earlier studies had shown that the $FeSO_4$ in 1M H_2SO_4 was efficient at keeping the ^{211}At reduced as [^{211}At]astatide, so it was important to evaluate whether reductive conditions could be used to assure that the ^{211}At was present as an anion for column isolation.

An experiment with AG1x8 was conducted to determine if ^{211}At could be captured from the 8M HCl solution used to dissolve the Bi/^{211}At after HNO_3 distillation in the wet chemistry isolation procedure. In that experiment, a small aliquot (283 µCi) of the 8M HCl solution (from solid dissolution) was placed on a AG1x8 column (392 mg). The column was washed 4x with 2M HCl. Essentially quantitative trapping was obtained on the AG1x8 column and no activity was eluted in the subsequent 4 washes. Unfortunately, recovery of the ^{211}At from the resin with conc. NH_4OH was very low (5.8%). The results are shown in **Table 6**.

At that point we felt it was important to review our results with the anion exchange resin, and to decide whether further studies with the resin were warranted. The review results are shown in **Table 7**. For comparison, the results of ^{211}At capture from 8M HCl (99%) and from 4M H_2SO_4 are listed with the results obtained using the AG1x8 resin with boric acid buffers at different pH.

The data in **Table 7** indicate that the trapping efficiency from 8M HCl and H_2O are high enough that the resin could be useful. However, the amount of ^{211}At released from the resin using 500 mM tetrabutylammonium bromide (TBAB) or concentrated NH_4OH were not encouraging. The facts that there was; (1) a very high capture of ^{211}At was obtained with the AG1x8 resin when 8M HCl was used (the solvent in the wet chemistry isolation approach), and (2) excellent retention of the ^{211}At on the column when washing with 2M HCl to remove bismuth, suggested that we should investigate the removal of the ^{211}At from the AG1x8 resin under additional experimental conditions (i.e. higher temperature, longer contact time, etc.).

Table 6: Summary of Astatine (^{211}At) capture and recovery from AG1x8 and Methoxy-Merrifield resin.

Resin	Lot #	Resin volume [mg]	Loading Solution	Loading activity [μCi]	Trapping Efficiency [%]	Wash loss [%]	Overall recovery Efficiency* by 15M NH_4OH [%]
Methoxy	MKC 18-94	387	8 M HCl	283.3	**11.1**	**88.9**	-
Ion exchange	AG 1x8	392	8 M HCl	283.3	**>99.9**	**0.0**	**5.8**

Table 7: Summary of Astatine (^{211}At) trapping and recovery from AG1x8 resin.

Resin	Eluant	Trapping Efficiency	Wash loss	Eluent to recovery	**Recovery Efficiency**
AG1x8	0.2M Boric acid-borate buffer (pH 5.3)	69%	5.0%	500 mM TBAB	**5.8%**
	H_2O (pH 7.0)	91%	1.1%	500 mM TBAB	**4.3%**
	0.2M Boric acid-borate buffer (pH 8.0)	69%	6.7%	500 mM TBAB	**5.4%**
	0.1M Borate-NaOH buffer (pH 10)	77%	3.1%	500 mM TBAB	**3.4%**
	4M H_2SO_4	59.6%	2.8%	8M NaOH	**8.2%**
	8M HCl	99%	0.0%	15M NH_4OH	**5.8%**

The results from the forgoing experiments were disappointing, but we felt that better results might be obtained if metabisulfite was used to reduce the [^{211}At]astatine species to [^{211}At]astatide. So, an additional experiment using the AG1x8 resin was conducted with ^{211}At. In that experiment, metabisulfite was used in the eluent, either with or without TBAB as co-eluent. The results from that study are shown in **Table 8**. It can be noted that much higher ^{211}At trapping efficiencies were obtained, but recovery yields were again very low.

Table 8: Astatine (^{211}At) trapping and recovery from anion exchange resin AG1x8 when metabisulfite is used by itself or in addition to TBAB as an eluent

Condition	Charged activity	Trap efficiency	Wash loss	Recovery
Eluted by 500 mM TBAB	154 µCi	**>99%**	0.2%	**2.0%**
Eluted by 1 mg/mL metabisulfite	150 µCi	**>99%**	0.3%	**0.2%**
Reduced by metabisulfite in prior to separation Eluted by 500 mM TBAB	154 µCi	**>99%**	0.9%	**3.0%**
Eluted by 500 mM TBAB on 9/24/13	146 µCi	**91%**	1.1%	**4.3%**

In the initial studies of AG1x8 resin, the ^{211}At was in reagents that are not used in the wet chemistry ^{211}At isolation approach. Further, a literature report by Nelson and Kraus [9] indicated that Bi(III) could be stripped from an anion exchange column (Dowex-1) with 1M H_2SO_4. This result was suggestive that 1M or higher solution with the anion resin AG1x8 might be used for elution. Therefore, another experiment was conducted where $BiCl_3$ was added and metabisulfite was used to reduce the species from the nitric acid distillation step, presumably from [^{211}At]astatate, to [^{211}At]astatide. The experimental procedure used was as follows:

(Procedure) Bismuth trichloride (2.7 g) was dissolved in 40 mL of 4M H_2SO_4. That solution was aliquoted into two equal portions. To one portion was added 20 mg of $Na_2S_2O_5$ (for reduction of the radiohalogen). Na[^{125}I]I was added to the solutions, and the solutions were passed over AG1x8 columns to trap the radioiodine, then 40 mL of H_2O was passed over each column as a wash step. In an identical experiment, ^{211}At was evaluated. The results of the trapping of radiohalogens on the AG1x8 resin were poor, as shown in **Table 9**.

A critical question in all of the strong anion exchange resin studies was whether the correct counterions were being used. If one evaluates the selectivity factors, there may be reasons that sulfuric acid did not provide higher trapping efficiencies. That is, HSO_4^- (in H_2SO_4) has a selectivity factor of 85 relative to iodide's selectivity of 175 (relative to hydroxide ion at 1.0) [BioRad Strong Anion Exchange Resin Instruction Manual], which is only a factor of 2x. It might be assumed that [^{211}At]astatate would have a higher selectivity factor than iodide, but the difference is probably relatively small (e.g. less than 2x). In contrast, chloride (in HCl) has a selectivity factor of 22, which may be 10x less than ^{211}At. Thus, it is not surprising that the best trapping results are obtained using HCl solvent. It seems likely that weak anions such as salicylate might release astatide much better than hydroxide ion, but then it would have to be further purified. Thus, *the use of a strong anion exchange resin does not seem to be good approach to isolation of ^{211}At from bismuth metal*.

Table 9: Radioiodine (^{125}I) and astatine (^{211}At) trapping from 4M H_2SO_4 onto the anion exchange resin AG1x8 with/without metabisulfite in the eluent

Nuclide	Solution	Applied activity	Trap Efficiency	Wash loss
I-125	$BiCl_3$ in 4 M H_2SO_4 + $Na_2S_2O_5$	85 µCi	**19.3%**	**1.9%**
I-125	$BiCl_3$ in 4 M H_2SO_4	95 µCi	**12.5%**	**1.2%**
At-211	$BiCl_3$ in 4 M H_2SO_4 + $Na_2S_2O_5$	293 µCi	**27.0%**	**7.4%**
At-211	$BiCl_3$ in 4 M H_2SO_4	287 µCi	**59.6%**	**3.8%**

B. Monospin Silica SAX Studies (studies to circumvent the HNO_3 distillation step)

These studies were conducted by loading the ^{211}At/Bi in HNO_3 in an effort to circumvent the HNO3 distillation step in the wet chemistry approach. Studies with HCl loading were not conducted.

In collaborative studies, Dr. Shigeki Watanabe (Japan Atomic Energy Agency, Takasaki, Japan) has reported that a small monospin column SAX (anion exchange; GL Science Co. Ltd.) provided very good recovery of ^{211}At from mixtures with $BiCl_3$. In the experiments he loaded 1 – 4 µCi ^{211}At using (1) water, (2) 8M HCl, (3) 8M HCl + 0-10 mM $BiCl_3$ (200 ug – 2g), or (4) 8M HNO_3 + $BiCl_3$. The capture efficiency ranged from 88% - 97%. For elution of the ^{211}At either 8M NaOH or 0.5 M NaOH was used. The 8M NaOH elutions provided 67 – 94% isolated yields and 0.5 M NaOH provided 95-100% with HCl loading and 62 – 65% with HNO_3 loading. He further showed that efficient isolation could be achieved even with 0.1 M NaOH. These results were very exciting so we wanted to try this in our laboratory. Dr. Watanabe provided samples of two monolith SAX ion exchange columns, pictured in **Figure 1** (B & C). We have obtained a preparative SAX column (D) directly from the manufacturer for our studies. Some studies conducted are described below.

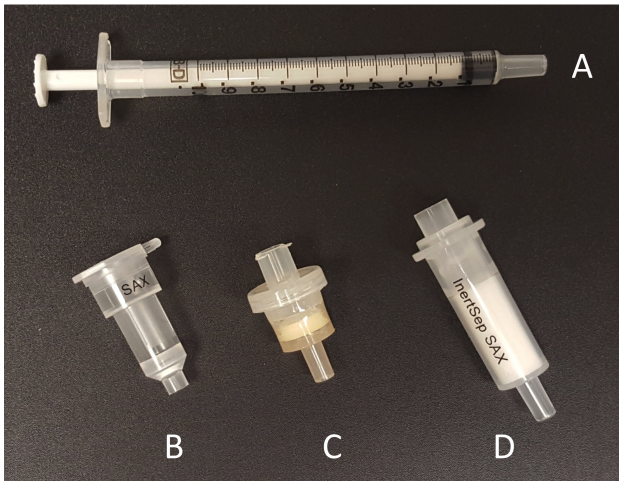


Figure 1: Picture of syringe and monolith SAX columns used; (B) microspin column, (C) modified column (cut down and sealed with leur-lock mechanism), and (D) larger scale SAX column for preparative separations from same manufacturer.

A study to determine if ^{211}At could be separated from bismuth was conducted using a SAX monolith column

that was sent from Japan. The column was washed with 10 mL 2M HCl, followed by 10 mL of 10M HCl. (Experiment 1) A sample containing 1.0 mL of ^{211}At (~0.9 mCi) in concentrated nitric acid diluted with 0.5 mL water (to make 10M HNO_3) was loaded onto (passed over) the column at a rate of ~1 mL / min. A 14 μCi quantity (~2%) remained in the vial, 634 μCi ^{211}At (77%) passed through the column and 178 μCi (~21%) remained on the column. Washing the column with 2 x 10 mL 2M HCl removed 90% of the ^{211}At activity.

A second study was conducted the next day using the same column (as above) that was rewashed with 10 mL 2M HCl, followed by 10 mL 8M HCl. A sample containing 1 mL of conc. HNO_3 containing ^{211}At (68 μCi) diluted with 2 mL water (i.e. 5M HNO_3) was passed over the column. A quantity of 58.1 μCi (86%) was in the pass-through solution and 10 μCi remained on the column. This result confirmed that the ^{211}At was not strongly bound to the column.

A third column study was conducted. The conc. HNO_3 solution was diluted to 1.5 M by adding 11.5 mL water. The study was conducted as above. Greater than 95% of the ^{211}At activity came in the pass-through solution.

These studies suggest that there may be something very different about the ^{211}At activity or reagents used in Japan and the activity/reagents used in our laboratory. In Japan the ^{211}At is obtained by dry distillation, then $BiCl_3$ is added to that solution. In our studies, it is likely that the bismuth is present as the nitrate salt. Further, the ^{211}At may be present as different species. No additional studies were conducted due to time constraints and funds available for studies.

(Added Task) *Evaluate ^{211}At separation using cation exchange resin (AG MP-50)*

A major difficulty in the separation of ^{211}At from bismuth target is removal of the large quantities (2.5 - 5 g) of bismuth from the target. Separation of bismuth from other metals has been accomplished by cation exchange chromatography. Strelow described the separation of lead from gram amounts of bismuth using the strong cation exchange resin AG MP-50 [10]. In that paper, a number of metals were separated from bismuth using different concentrations of acid and methanol. In a second paper by Strelow, it was noted that bismuth eluted with 1M HCl from AG MP-50 [11]. Normally a cation exchange resin would not be used for isolation of ^{211}At, but the fact that the AG MP-50 resin does not retain bismuth when the molarity of HCl is higher than 1M suggested we should evaluate whether ^{211}At is trapped on that column material. Additionally, if all of the bismuth could be captured by the column and the ^{211}At pass through, purification of the ^{211}At that passed through the column might be much easier.

(Procedure) The MP-50 resin (380 mg) was conditioned using 10 mL of 2M HCl solution. Two 1M HCl solutions (2 mL each) were prepared and ^{211}At isolated from the wet chemistry approach was added to each solution. To one of the solutions was added 370 μg sodium metabisulfite to reduce the ^{211}At species present to [^{211}At]astatide. Each solution was loaded onto a column containing the AG MP-50 resin, then eluted with 2M HCl. The amount of radioactivity in the eluent and remaining on the column provides the efficiency of trapping. The data are shown in **Table 10**.

Table 10: Astatine (^{211}At) trapping from 1M HCl onto the cation exchange resin AG MP-50 with/without metabisulfite addition

Resin	Solution	Resin volume [mg]	Loading activity [µCi]	Trap Efficiency [%]
MP-50	1 M HCl $+Na_2S_2O_5$	398	154.5	**15.3**
MP-50	1 M HCl	392	173.7	**29.4**

The results are interesting as the addition of metabisulfite reductant decreases the amount of ^{211}At held on the column. This is suggestive that there may be reduction of some cationic ^{211}At species in aqueous solution that gets reduced to an anionic species that is not retained by the column. The data in **Table 10** clearly show that the cation resin does not capture (isolate from solution). It must be questioned whether this is favorable to a separation where the bismuth is trapped on the column, but it was felt that it would be difficult to be certain that all of the bismuth is trapped during the elution process, so its use as a separation method for ^{211}At and bismuth seemed unlikely. Therefore, no more studies with this resin were conducted.

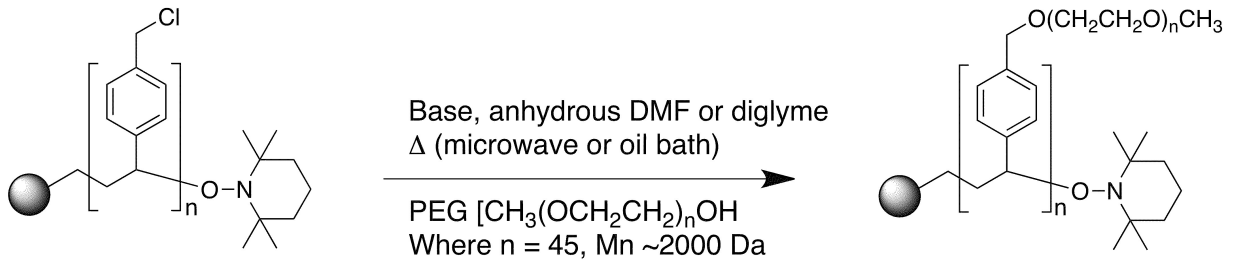
iii. PEG-Resin Columns:

***Task 3**: Prepare resins with PEG attached (UW)*

A. Preparation of PEG Columns:

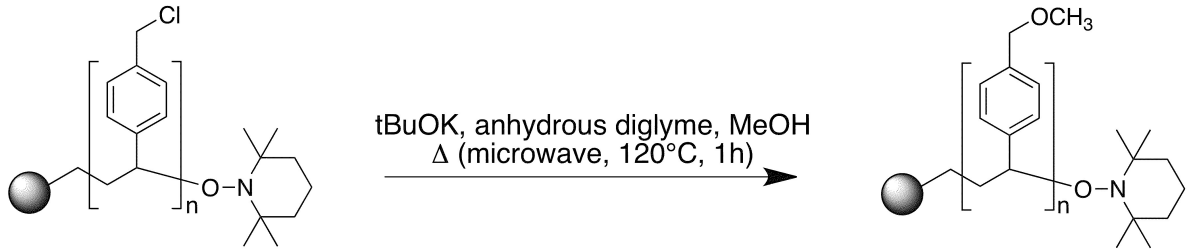
Initial studies on solid-phase extraction were conducted prior to the beginning of the funding period. At that time samples of PEG-modified resins were obtained from researchers at the University of Edmonton and PNNL. There were only small samples of the PEG-modified resins, but data obtained with them suggested that this approach had merit for further study.

A synthetic method for “Preparing Polystyrene-Immobilized PEG Chain” was found in the literature [12]. We chose to use the Merrifield benzyl chloride resin for modification, as it could be obtained from commercial sources at a relatively low cost. The reaction for modification of the resin is shown below.



Merrifield's peptide resin
100-200 mesh, 3.5-4.5 mmol/g Cl^-
1% cross-linked

Initial preparations were conducted using microwave heating to assure that the PEGylation reaction went to completion, i.e. having as many of the PEG units attached to the resin as possible. The use of microwave in PEGylation of Merrifield resins had previously been described in the literature [13]. However, ^{211}At capture studies seemed to indicate that the loading was perhaps too high, or other reactions might have taken place, so milder reaction conditions were sought. While the sealed microwave tubes were still used, the reactions to prepare PEG-coated resins were changed to oil bath heating (75-80°C) for an extended period of time (3 days). This seemed to work better so a preparative run was made using 10 g of resin. The preparative study was conducted to provide ample PEG-modified resin for several experiments (~400 mg/column). Initial inability to release the ^{211}At captured on the PEG-modified column made us wonder if the ^{211}At had reacted with the column, so a control column resin was prepared by reaction with methanol rather than the PEG-OH. The reaction used for making the MeOH-modified resin is shown below.



Merrifield's peptide resin
100-200 mesh, 3.5-4.5 mmol/g Cl^-
1% cross-linked

Additional preparations of PEG-resins were conducted and the experimental conditions used are provided below:

***Resin Prep 1: Merrifield Resin*-mPEG2000 (microwave heating to 120°C/1h)**

Merrifield resin (1.0 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous DMF (15 mL) were mixed in a 20-mL microwave reaction vial and allowed to swell for 30 min. Methoxy-poly(ethylene glycol) (mPEG, MW ~ 2000, 1 g), NEt_3 (0.63 mL) and DMAP (0.055 g) were added, then the resultant solution was stirred and heated by microwave at 120 °C, 1 h. The solution was poured to a beaker containing 200 mL of 0.1 N HCl with stirring, then the solid product was filtered, washed with H_2O, dried under high vacuum to give a light-yellow solid product. Yield 1.57 g

***Resin Prep 2: Merrifield Resin*-mPEG2000 (microwave heating to 160°C/30 min)**

Merrifield resin (1.0 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous DMF (13 mL) were mixed in a 20-mL microwave reaction vial and allowed to swell for 30 min. Methoxy-poly(ethylene glycol) (mPEG, Mn ~ 2000, 0.2, 0.4, 0.6 g) and NaOH (0.16 g) were added, then the resultant solution was stirred and heated by microwave at 160 °C for 30 min. The solution was poured to a beaker containing 200 mL of H_2O with stirring, then the solid product was filtered, washed with H_2O, dried under vacuum to give the light-yellow solid product. Three preparations were made using these conditions: (**18-81A**)Yield 1.064 g (by 0.2 g PEG). (**18-81B**) Yield 1.072 g (by 0.4 g PEG). (**18-81C**) Yield 1.105 g (by 0.6 g PEG).

***Resin Prep 3: Merrifield Resin*-mPEG2000 (microwave heating at 120°C/1h)**

Merrifield resin (1 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (12 mL) were mixed in a 20-mL microwave reaction vial and allowed to swell for 30 min. Methoxy-poly(ethylene glycol) (mPEG, Mn ~ 2000, 2 g) and t-BuOK (1 mL, 1 M in THF) were added, then the resultant solution was stirred and heated by microwave at 120 °C for 1 h. The solution was poured to a beaker containing 200 mL of H_2O with stirring, then the solid product was filtered, washed with H_2O, dried under vacuum to give the light-yellow solid product. Yield 1.266 g.

***Resin Prep 4: Merrifield Resin*-MeOH (microwave heating at 120°C/1h)**

Merrifield resin (1 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (11 mL) were mixed in a 20-mL microwave reaction vial and allowed to swell for 30 min. Anhydrous MeOH (0.324 mL) and t-BuOK (5.19 mL, 1 M in THF) were added, then the resultant solution was stirred and heated by microwave at 120 °C for 1 h. The solution was poured to a beaker containing 200 mL of H_2O with stirring, then the solid product was filtered, washed with H_2O, dried under vacuum to give the light-yellow solid product. Yield 1.115 g.

***Resin Prep 5: Merrifield Resin*-mPEG2000 (oil bath heating at 75°C/3 days)**

Merrifield resin (1 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (15 mL) were mixed in a 20-mL microwave reaction vial and allowed to swell for 30 min. Methoxy-poly(ethylene glycol) (mPEG, Mn ~ 2000, 1.3 g) and t-BuOK (1.3 mL, 1 M in THF) were added, then the resultant solution was stirred and heated (oil bath) at 75 °C for 3 days. The solution was poured to a beaker containing 300 mL of H_2O with stirring, then the solid product was filtered, washed with H_2O, dried under vacuum to give the yellow solid product. Yield 1.347 g.

***Resin Prep 6: Merrifield Resin*-mPEG2000 (oil bath heating @ 80°C/3 days)**

[Preparative Scale] Merrifield resin (10 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (125 mL) were mixed in a 300-mL round-bottom flask and allowed to swell for 30 min at room temperature. Methoxy-poly(ethylene glycol) (mPEG, Mn ~ 2000, 13 g) and t-BuOK (13 mL, 1 M in THF) were added respectively, then the reaction solution was stirred and heated (oil bath) at 80°C for 3 days under argon. After the temperature was cooled to room temperature, the solution was poured slowly to a 500-mL beaker containing 300 mL of H_2O with stirring. The yellowish solid product was then filtered by vacuum, washed with H_2O (3 x 200 mL), dried under vacuum to give the pale-yellow solid product. Yield 13.58 g.

Resin Preps 7, 8 and 9: Merrifield Resins with mPEG-750, -2000 and -5000

One of the questions that arose during the studies is whether there was a difference in trapping and release of ^{211}At with different sizes of mPEG coatings on the resin. To answer that question, three larger-scale preparations (12-18 g product) were conducted to prepare resins with varying lengths of methoxy-terminated PEG (mPEG) on them. In the studies mPEG-OH with MW (averages) of 750 Da, 2000 Da and 5000 Da were conjugated to the Merrifield resin (benzyl chloride). The experimental details of the preparation of the mPEG resins follow:

The Merrifield resin (10 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (125 mL) were mixed in a 300-mL round-bottom flask and allowed to swell for 30 min at rt. Methoxy-poly(ethylene glycol) (mPEG2000, 13 g or mPEG750, 4.84 g or mPEG5000, 32 g) and t-BuOK (13 mL, 1 M in THF) were added respectively, then the reaction solution was stirred and heated at 80 °C for 3 days under argon. After the temperature was cooled to room temperature, the solution was poured slowly into a 500-mL beaker containing 300 mL of H_2O with stirring. The yellowish solid product was then filtered by vacuum, washed with H_2O (3 x 200 mL), dried under vacuum to give the pale-yellow, hygroscopic solid product. Yield 18.18 g (mPEG2000), 15.08 g (mPEG750), 12.18 g (mPEG5000).

Interestingly, while the ratio of moles mPEG-OH to resin was the same in each case, under the conditions used virtually all of the mPEG750 was conjugated, ~60% of the mPEG2000 was conjugated and only ~6% of the mPEG5000. This result appears to come from difficulty in reacting the larger mPEG-oxide moieties with the benzyl chloride on the surface of the resin, presumably due to steric effects. The fact that we were able to fully substitute the benzyl chloride functionalities on the Merrifield resin with mPEG750-OH molecules, suggested that using this resin could provide more reproducibility than would be obtainable with the mPEG2000 or mPEG5000 molecules. Additionally, results from the comparison study this quarter indicated that mPEG750 provided as high trapping yields as the other two PEG-resins used for the separation, so a large-scale preparation of mPEG750 resin was conducted as described above.

Resin Prep 10: Merrifield Resin with mPEG-350

Previous studies have shown that Merrifield resins containing mPEG750, 2000 and 5000 molecular weights have yielded similar ^{211}At isolation yields. From those studies we selected mPEG750, as the dry weight suggested that the resin was fully substituted, which should make it easier to reproduce the columns. Because of a concern that larger mPEG-resins may cause more back pressure than small mPEG-resins, we felt it was important to evaluate whether a shorter length mPEG, MW = 350, would provide the same separation as the mPEG 750. Therefore, a Merrifield resin containing mPEG 350 was prepared as follows.

Merrifield resin (10 g, 3.5-4.5 mmol/g Cl^-, 100-200 mesh, 1% cross-linked) and anhydrous diglyme (125 mL) were mixed in a 300-mL round-bottom flask and allowed to swell for 30 min at room temperature. Methoxy-poly(ethylene glycol) (mPEG350, Mn ~ 350, 2.25 g) and t-BuOK (13 mL, 1 M in THF) were added respectively, then the reaction solution was stirred and heated at 80°C for 3 days under argon. After the temperature was cooled to room temperature, the solution was poured slowly into a 500-mL beaker containing 300 mL of H_2O with stirring. The yellowish solid product was filtered by vacuum, washed with H_2O (3 x 200 mL), and dried

under vacuum to give 12.50 g of a pale-yellow solid. (Note that total weight indicates that all of the mPEG-OH was coupled with the resin).

***Task 4**: Evaluate ^{211}At separation using resins with PEG attached (UW)*

1. Column Loading with 8M HCl:

The goal of this task was to evaluate the capture efficiency and recovery from PEG-coated resins. A sample of a synthesized PEG column material was obtained from Edmonton, Alberta (denoted as Edm in **Table 11**) for comparison. The other PEG-coated supports were prepared by Dr. Ming-Kuan Chyan as described above. An additional column made of a proprietary resin from IBC Advanced Technologies called AnaLig TcO_2 was also evaluated.

(Procedure 1) Approximately 2 mL of 2 M HCl (prepared from ACS grade, conc. HCl) was used to rinse the 1st distillation flask of the "standard" ^{211}At separation procedure to recover residual ^{211}At. This ^{211}At was then added to a solution of $BiCl_3$ (prepared by dissolving 2.712 g $BiCl_3$ [Aldrich, 98+%] in approximately 15 mL of 2 M HCl). Although there may be some differences from the $Bi(NO_3)_3$ obtained when dissolving bismuth targets in 10M HNO_3, addition of $BiCl_3$ was intended to mimic the presence of bulk bismuth from standard target processing. Fractions of ~2.0-2.5 mL of this ^{211}At/Bi solution were then processed as follows:

Three columns each (~200mg) of Edmonton and MKC PEG resins were prepared using empty Mini Spe-ed Cartridges (Applied Separations, Allentown, PA). Resin lot numbers are given in **Table 11**. All columns were conditioned with 10 mL of 2 M HCl. Column conditioning occurred at the start of the entire series of experiments (i.e. not prior to each individual separation study). Columns were immursed in 2M HCl prior to their use.

Table 11: Summary of loading efficiency, loss from washing, and overall recovery of ^{211}At*

Resin	Lot #	Elution	Loading efficiency [%]	Wash loss [%]	Overall recovery 1st elution [%]	Overall recovery 2nd elution [%]	Overall remaining on column [%]	Bi precipitate in washes upon addition of NaOH
Edm**	DRM. 20.22	NaOH	99.6	1.6	20.8	8.2	69.0	Not visible in 3rd & 4th wash
Edm*	DRM. 23.179	NaOH	98.0	2.0	74.6	12.9	8.6	Not visible in 3rd & 4th wash
MKC	MKC 18-79	NaOH	99.1	0.0	0.0	0.0	99.1	Precipitate visible in all washes
MKC	MKC 18-79	NH_4OH	99.2	0.4	8.3	0.0	90.5	Precipitate visible in all washes
Edm*	DRM. 23.179	NH_4OH	94.2	1.8	74.4	5.8	12.1	Not visible in 3rd & 4th wash

*Order listed follows the chronological order in which experiments were performed.
**Small sample of PEG-coated resin provided by researchers at University of Alberta, Edmonton, CA

For each separation study, the column was loaded with the ~2.0-2.5 mL fraction of ^{211}At/Bi solution, washed with 4 x 10 mL fractions of 2 M HCl, and finally, eluted with 2 x 1.0 mL fractions of either 4 M NaOH, or 4 M NH_4OH. Column loading and washing proceeded rapidly, whereas column elution occurred at a rate of approximately 1 drop every 15 seconds. Each of the 6 experiments was completed within approximately 30 minutes. Loading activities varied from 22 – 26 μCi of ^{211}At. Trapping efficiency, loss due to washing, overall elution recovery in

each of the two fractions, and overall activity remaining on the column are reported in **Table 11**. All values reported are *non-decay corrected*. Finally, upon completion of the separation study, NaOH was added to the washes as a qualitative assessment of residual bismuth (if $Bi(OH)_3$ is present, it is seen as a white precipitate).

All of the resins trapped the ^{211}At on them, but the ^{211}At could be recovered from only 1 resin. It should be noted that bismuth metal was effectively removed (i.e. none in washes 3 or 4) from the resins prepared in Edmonton, but not MKC (Resin Prep. 1). This resin was prepared by microwave heating. We were concerned about this result so additional PEG resins were prepared by alternate methods. A similar study to that above was performed (below) to evaluate additional PEG-coated resins prepared under microwave conditions at a higher temperature (160°C vs. 120°C) for a shorter time (30 min vs. 1h).

(Procedure 2) All resins were conditioned with 10 mL 2 M HCl just prior to each separation study (i.e. in contrast to above which occurred at the start of the entire series of experiments). Similar to the above study, ^{211}At was added to bulk bismuth to mimic target conditions. For the first experiments, ^{211}At was added to 1.413 g $BiCl_3$ in ~15 mL 2N HCl and ~2-2.5 mL of solution was used for each experiment (corresponding to 23-30 μCi of ^{211}At). In the experiment PEG resins with varying amounts (0.2g, 0.4g, 0.6g) of mPEG reacted were evaluated. The $BiCl_3$ solution leftover was spiked with ^{211}At final extracted product (day prior), as the residual activity in the $BiCl_3$ solution was too low to ensure adequate statistics. An additional 1 mL 2 M HCl was also added to the ~2-3 mL $BiCl_3$ solution to allow for ~2 mL loading volume. Column capture of ^{211}At and elution efficiencies are reported in **Table 12**. Column loading, washing, and elution proceeded as per conditions described in Procedure 1.

Table 12: Summary of loading efficiency, loss from washing, and overall recovery for ^{211}At

Resin	Lot #	Resin mass [mg]	Elution	Loading efficiency [%]	Wash loss [%]	Overall recovery 1st elution [%]	Overall recovery 2nd elution [%]	Overall remaining on column [%]	Bi precipitate in washes upon addition of NaOH
MKC	MKC 18-81A	200	NaOH	93.7	2.0	6.1	4.3	81.3	Not visible in 3rd & 4th wash
MKC	MKC 18-81B	202	NaOH	96.6	1.4	2.4	1.2	91.6	Slight precipitate in 3rd wash
MKC	MKC 18-81C	201	NaOH	96.8	0.7	3.0	0.2	92.9	Slight precipitate in 3rd wash
AnaLig $Tc0_2$	091019K T28-40	205	NaOH	94.2	3.2	3.0	1.4	86.5	Not visible in 3rd & 4th wash

Again, in this study all of the resins were efficient at trapping the ^{211}At, but the elution with NaOH solutions did not provide free ^{211}At. The resin with the lowest amount of mPEG appeared to provide the best recovery of ^{211}At. The results of these studies did not provide the release of ^{211}At that had been trapped on the column. It was decided that other column materials should be studied, as well as studies to determine the effect the ^{211}At species on the trapping and release of ^{211}At by the columns.

In our earlier studies, a methoxy-Merrifield resin was prepared and evaluated for capture of ^{211}At. The purpose of methoxy-Merrifield resin was to be a control to ensure that chemical binding/bonding to the Merrifield resin was not the mechanism by which the ^{211}At was captured on the synthesized PEG resins. It is clear from the data in **Table 6** that this is not the case.

(Procedure 3) Two 2M HCl solutions containing $BiCl_3$ (0.364 g each) were prepared and a small quantity of ^{211}At (150-160 μCi) was added to each solution. Sodium metabisulfite was added to one solution just prior to placing it on the column. Each column was loaded with 2.0 mL of ^{211}At/Bi solution, washed 4 x 10 mL of 2M HCl, and finally eluted with 1 mL of 15M NH_4OH. Column loading and washing proceeded rapidly, but elution of ^{211}At with NH_4OH occurred at a rate of ~1 drop/15 seconds. The results of the study are shown in **Table 13**. All values in the table were decay corrected. After ^{211}At decayed to background, 10 mL of each wash solution was tested (spotted) on bismuth test paper (last column on right).

Table 13: Astatine (^{211}At) trapping from 2M HCl onto the PEG-coated resins in the presence of $BiCl_3$ with/without metabisulfite addition

Lot #	Resin volume [mg]	Loading Solution	Loading activity [μCi]	Trapping Efficiency [%]	Elution	Recovery Efficiency [%]	Bi check in wash
MKC 19-94	380	2 M HCl +$BiCl_3$ +$Na_2S_2O_5$	163.8	**98.1**	15 M NH_4OH (1 mL)	**68.1**	Not detected in 3rd and 4th wash
MKC 19-94	363	2 M HCl +$BiCl_3$	152.5	**99.3**	15 M NH_4OH (1 mL)	**59.7**	Not detected in 3rd and 4th wash

It can be noted that the trapping efficiency is quite high, the recovery yield is reasonable, and all of the bismuth has been separated from the ^{211}At (eluted from column in washings). There does not appear to be a significant difference in the results when metabisulfite is added. These results were encouraging so a larger quantity of PEG-coated resin was prepared in the same manner for use in the continuing studies.

The results above were obtained by addition of ^{211}At from our wet chemistry isolation procedure. The HPLC analyses suggest that this material is mostly [^{211}At]astatide. However, if we are to simplify the isolation process, the ^{211}At should be obtained directly from the residue after distillation to remove the nitric acid. Although the chemical species of ^{211}At at that point is not established yet, we believe it is [^{211}At]astatate because of the oxidizing capability of boiling concentrated nitric acid. Therefore, it was important to test the capture and release of both [^{211}At]astatine and [^{211}At]astatate by the PEG-coated resin. To do that, [^{211}At]astatide was obtained by the wet chemistry isolation approach and [^{211}At]astatate was obtained by oxidation with IO_4^- using microwave heating.

(Procedure 4) Six 2M HCl solutions containing $BiCl_3$ (0.346 g each) were prepared and a small amount (150-160 μCi) of [^{211}At]astatide was added to 3 of the solutions, while [^{211}At]astatate was added to the other 3 solutions. The columns were loaded with 2.0 mL of ^{211}At/Bi solution, washed with 4 x 10 mL of 2M HCl, and finally eluted with 1 mL of 15M NH_4OH or 12.5M NaOH solution. Column loading and washing proceeded rapidly. For the elution of ^{211}At, the NH_4OH or NaOH solutions were loaded onto the column and allowed to set for 10 min, then eluted rapidly. The results obtained are shown in **Table 14**. The wash solutions from each column were set aside for 2 days, then 10 mL of each wash was spotted on the

bismuth test paper to determine if bismuth was present. As a final check, NaOH was added to the washes to provide another assessment of residual bismuth (precipitate will form if present).

Table 14: [^{211}At]Astatide and [^{211}At]astatate trapping from 2M HCl onto the PEG-coated resins in the presence of $BiCl_3$*

Lot #	Resin volume [mg]	Loading Solution	At species	Loading activity [µCi]	Trapping Efficiency [%]	Elution	Recovery Efficiency [%]	Bi precipitation in wash
MKC 19-94	406	2 M HCl + $BiCl_3$	At^-	168.0	**97.8**	15M NH_4OH (1 mL)	**30.0**	Not detected in 2nd, 3rd & 4th wash
MKC 20-10	400	2 M HCl + $BiCl_3$	At^-	175.7	**98.9**	15M NH_4OH (1 mL)	**45.2**	Not detected in 3rd & 4th wash
MKC 20-10	396	2 M HCl + $BiCl_3$	At^-	175.5	**99.1**	12.5 M NaOH (1 mL)	**4.1**	Not detected in 2nd, 3rd & 4th wash
MKC 19-94	404	2 M HCl + $BiCl_3$	AtO_3^-	177.7	**101.2**	15M NH_4OH (1 mL)	**42.9**	Not detected in 2nd, 3rd & 4th wash
MKC 20-10	398	2 M HCl + $BiCl_3$	AtO_3^-	177.2	**96.4**	15M NH_4OH (1 mL)	**39.1**	Not detected in 3rd & 4th wash
MKC 20-10	401	2 M HCl + $BiCl_3$	AtO_3^-	179.4	**101.8**	12.5 M NaOH (1 mL)	**2.8**	Not detected in 3rd & 4th wash

*All ^{211}At values are decay corrected.

It is interesting that both ^{211}At species can be loaded onto the column and be trapped very efficiently from 2M HCl. It is not clear why the recovery yields were lower in this experiment than in the prior experiment. The difference in elution with ammonium hydroxide vs. sodium hydroxide is striking. It is clear that ammonium hydroxide is the eluent of choice. Why it works better than sodium hydroxide is not known, but it can be speculated that it more readily penetrates the PEG layer to reach ^{211}At.

Our primary goal is to separate the entire amount of ^{211}At/Bi from an irradiated target using a solid-phase column and isolate the ^{211}At from that column in a usable form and high recovery yield. To do that we must capture all (or a high percentage) of the ^{211}At on the column, wash that column to remove the bismuth, then elute the ^{211}At from the resin and remove the eluent to give a basic residue of ^{211}At. Thus, we conducted 2 experiments with PEG-resin (MW ~2000) columns where all of those steps were carried out. Descriptions of the experiments follow.

In one experiment, we evaluated capturing 50% of the 8M HCl solution from a single ^{211}At run on a PEG column (9.3 mCi/~1.5 g Bi). The details of the experiment are shown in **Table 15**. A 7 mL quantity of the bismuth and ^{211}At in 8M HCl, obtained after distillation of conc. HNO_3 and redissolution in 8M HCl, was passed over a ~400 mg of PEG resin, then washed with 4 x 2 mL

of 2M HCl. The column retained 97% of the ^{211}At. Following that 2 mL of conc. NH_4OH was passed over the column, and 78% (7.41 mCi) of the ^{211}At on the column was eluted.

Table 15: Experimental data from large-scale isolation from PEG-resin columns when 8M HCl is used for loading the column.

Activity loaded [mCi]	Trapping efficiency [%]	Wash loss [%]		Isolated activity [mCi]	Recovery Efficiency [%]	Distillation				Final solution	
		2M HCl	H_2O			Left in flask [mCi]	% Left	Distilled [mCi]	% Distilled	Activity [mCi]	% Recovered
9.34	**97.3**	**3.1**	**-**	**7.41**	**78.5**	**0.674**	**8.7**	**5.58**	**75.3**	**0.647**	**6.9**
14.4	**89.6**	**< 1.0**	**36.6**	**2.58**	**18.4**	**2.31**	**89.5**	**0.01**	**<1.0**	**1.09**	**7.6**

Distillation of the NH_4OH solution to dryness resulted in only 8.7% (0.67 mCi) remaining in the distilling flask. The majority of the ^{211}At, 5.58 mCi (75%) distilled into the receiving flask. Distillation of 75% of the ^{211}At into the receiving flask was not the desired outcome, but it may be useful in obtaining ^{211}At without trace metals present.

A 2nd large-scale ^{211}At isolation using a PEG-resin column was conducted. In that experiment (shown in Table 3) the entire 8M HCl solution (~8 mL) was placed on a PEG column (~400 mg) and washed with 3x 2 mL of 2M HCl, followed by a 4th wash with 2 mL of H_2O. Unlike all of the previous PEG column captures, only ~90% of the ^{211}At was captured on the column. It seemed likely that we exceeded the capacity of the 400 mg column, so a larger quantity (e.g. 600 mg) of PEG resin was employed in some of the later studies where the entire 8M HCl solution is used. (It is unclear whether there is a capacity issue if the column is conditioned well before running samples.) We thought that diluting the 2M HCl remaining on the column by washing with H_2O rather than 2M HCl might make the NH_4OH elution of ^{211}At more efficient, as none of the base would be neutralized. As shown in Table 3, a significant amount (~37%) of the radioactivity on the column eluted with the H_2O solution. To avoid that in later studies, 8M HCl and 2M HCl was used for the column washings. In fact, washing the column with H_2O did not improve the elution of ^{211}At using conc. NH_4OH, as only 2.58 mCi (18%) was eluted. In this (2nd) experiment, the NH_4OH eluted solution dripped into a round-bottom flask containing 300 μL of 1N NaOH solution so that the ^{211}At would not distill into the receiving flask when the NH_4OH was removed. Indeed, 90% of the (2.31 mCi; non-decay corrected) activity remaining in the flask and 0.01 mCi (<1%) distilled into the receiver flask. Subsequent dilution of the residue in the distilling flask with H_2O, and transfer of that activity to a separate vial resulted in less than half of the retained activity to be transferred. While this large-scale run did not provide a high yield (only ~ 1 mCi; ~8%), we learned a lot from it. Based on these results, several new experiments were designed.

A 3rd large scale ^{211}At PEG column isolation experiment was conducted by dissolving an irradiated bismuth target containing 17.7 mCi in conc. HNO_3. This was followed by removal of the HNO_3 by distillation, dissolution of the residue in 8mL of 8M HCl, transfer to a scintillation vial, addition of another 2 mL for rinse, then transfer of that solution to the same scintillation vial. The resultant 10 mL ^{211}At/Bi solution was split into 2 x 5 mL aliquots for purification on 2 PEG columns.

(a) A 5 mL sample (~8.8 mCi) was loaded at 2 mL/min onto a 600 mg PEG column that had been *pre-equilibrated with 10 mL 2M HCl*. The column was washed with 4 x 2mL aliquots of 2M HCl. After this process 95% of the activity remained on the column, 3% was in the original pass-through, and 2% was in the 4 x 8M HCl washes. Following this, the column was eluted with 4 mL of conc. NH_4OH. Unlike the previous run, 22% (2.30 mCi) of the activity was found spread between the 8 x 0.5 mL fractions collected. *The column retained 7.94 mCi.* In an attempt to determine if other solvents might strip off the remaining ^{211}At, the column was eluted with 1 mL each of the following solutions: (a) H_2O; (b) 1:1 H_2O:EtOH; (c) 1 mg/mL $Na_2S_2O_5$ in H_2O; and (d) 10 mg/mL $Na_2S_2O_5$ in H_2O. We had previously observed that H_2O could remove some ^{211}At from PEG columns, but that was not the case in this study as <1% was released. We thought that perhaps addition of an organic (EtOH) might assist in removing the ^{211}At, but that did not work either as again <1% was released. We thought that reduction of the species held might release the ^{211}At, but <8% was released with 1 mg/mL $Na_2S_2O_5$ solution and increasing the concentration to 10 mg/mL $Na_2S_2O_5$ only released another 10%. Based on our previous studies, these were disappointing results. So, we decided to run a 2nd PEG column purification.

(b) A 2nd 5 mL sample (4.16 mCi; not adjusted for Bi attenuation) was loaded at 2 mL/min onto a 600 mg PEG column that had been *pre-equilibrated with 10 mL 8M HCl*. Because of the results obtained in the previous study, 8M HCl pre-equilibration was used rather than 2M HCl. The column was then washed with 4 x 2 mL aliquots of 2M HCl. Again ~95% of the ^{211}At was retained on the column, with 3% coming through in the initial pass-through solution and 2% in the washes. The column was then eluted with 4 mL of conc. NH_4OH. In the combined 8 x 0.5 mL elutions, only ~14% of the activity on the column came off.

A fourth PEG column ^{211}At separation/isolation experiment was conducted after reviewing prior experiments. This analysis indicated that *larger volumes of washes (4 x 10mL) might be needed to remove the bismuth* from the column prior to eluting with NH_4OH. So in this study that change was made.

As in all ^{211}At PEG column isolation experiments, the irradiated bismuth target containing 18.4 mCi was dissolved in conc. HNO_3. That solution was heated to remove the HNO_3 by distillation, then the residue was dissolved in 8 mL of 8M HCl, transferred to a scintillation vial. The distillation flask was rinsed by addition of another 2 mL conc. HNO_3, then that solution was transfer to the same scintillation vial. A 10 mL ^{211}At solution was used in the experiment.

The entire 10 mL of solution was loaded at 2 mL/min onto a 800 mg PEG column that had been pre-equilibrated with 10 mL of 2M HCl, followed by 10 mL of 8M HCl. The column was washed with 4 x 10 mL solutions of 2M HCl. After elution **98%** of the activity (23.9 mCi) remained on the column with only ~2% being present collectively in the original flow-through and 4 washes. The column was eluted with 4 mL of conc. NH_4OH in approximately 0.5 mL fractions taken at 1 drop per 15 seconds. ^{211}At activity was found in all 8 fractions, but post the 2nd collected fraction, all later fractions contained smaller amounts than the previous fraction and the last fraction contained only 0.28 mCi. The *total collected* from the 8 NH_4OH fractions was *17.33 mCi, or about **73%** of the activity* originally on the column (not decay corrected).

The results for ^{211}At release from the PEG column in the 4th experiment were much better than obtained in the 3rd experiment. It appears that the primary reason for this is that in experiment

3 only 8 mL HCl solution (total) was used for washing the column whereas 40 mL (total) HCl solution was used in the 4th experiment. The ^{211}At capture yields are as high as we can expect, but we believe that the ^{211}At recovery yields can be further optimized.

To demonstrate reproducibility of our successful runs in previous studies we ran a fifth PEG-resin column (mPEG2000) separation. In that study the entire 10 mL of 8M HCl solution containing ^{211}At (20.5 mCi) and Bi salts was loaded (at ~2 mL/min) onto a 800 mg mPEG2000 column that had been pre-equilibrated in 10 mL of 2M HCl followed by 10 mL 8M HCl. The column was washed with 4 x 10 mL 2M HCl. A total of ~98% of the ^{211}At was captured on the column and the 4 washes only removed ~1% of the activity, so *97% of the activity was on the column after the washes*. At that point we deviated from our earlier studies by passing air over the column to remove residual 2M HCl. This was done so that little 2M HCl would be present when the conc. ammonium hydroxide was added. The hypothesis was that better recovery yields might be obtained if the ammonium hydroxide did not have to neutralize the remaining 2M HCl.

A 4 mL quantity of conc. ammonium hydroxide was added to the column at a rate of 1 drop per 15 seconds, and 4 x 1 mL fractions were collected. Unfortunately, unlike the previous runs the combined 4 x 1 mL fractions collected only accounted for about 3% of the total activity – with 97% remaining on the PEG-resin column. Because this result was contrary to what we had previously obtained, we decided to determine if some other reagents might work to release the ^{211}At from the column (we hypothesized that the oxidation state or nature of the species on the column may have changed during the purging with air). Thus 5M NaOH was evaluated as an eluent. In an attempt to change the oxidation state of the ^{211}At, an aliquot of 300 uL 5M NaOH was placed on the column and that was allowed to set for 5 min before eluting 2 mL (2 fractions of 1 mL). Much less than 1% of the activity came out with that elution. A second reagent, diisopropyl ether (DIPE) was evaluated. Again 300 uL was loaded onto the column and was allow to set for 5 min, then 2 x 1 mL fractions were collected. Much less than 1% of the ^{211}At activity was eluted. The column was re-acidified with 2 x 10 mL of 8M HCl (<1% ^{211}At came off), then eluted with 2 x 1 mL of DIPE as before. We thought that reintroduction of 8M HCl might change the nature of the ^{211}At species back to an extractable species, however, as before <1% of the ^{211}At activity on the column was eluted. At this point we decided to try a different eluent, methanol. The column was eluted with 4 x 1 mL of MeOH at a loading rate of 1 drop per 15 sec. *About 7% of the activity on the column eluted*. With these disappointing results it was decided that removing the 2M HCl from the column using air was not a good idea and should not be done in the future (but we did test that in the next experiment).

One of the questions that we felt needed addressed with the mPEG-resin columns was whether different sizes of mPEG on the resin made a difference with regards to capture of ^{211}At from 8M HCl and its release using the conc. ammonium hydroxide. Therefore, resins containing mPEG750, mPEG2000 and mPEG5000 were prepared (see Task 3, p14). Another question was how to make sure that the same amount of resin was used in experiments when different batches of PEG-resin were used. A hydrated resin was being put into the columns and varying amounts of water may be present, resulting in packing with varying amounts of resin. To alleviate this possibility, it was thought that dry resin should be weighed for each column, then variations in hydration would not matter. Thus, the following column loading experiments were conducted.

Two weighed dry PEG-resin (2000) samples, 300 and 400 mg, were placed in separate Mini

Spe-ed cartridges. A cap was placed on the bottom of the cartridge and DI water was added in 100 µL aliquots until the resin appeared hydrated. It took 500 uL and 600 µL respectively. Bubbles, which slowly made their way to the top, were clearly seen in both samples. Eventually (about 20min) a layer of water could be seen at the bottom of the resin. These columns were sealed upright in scintillation vials and left over the weekend. Both columns looked dry on top with no water visible at the bottom of the resin (swelled to more than the water volume). Both had clearly visible voids on the edges of the column contacting the plastic. An aliquot of 100µL of water was added to each of the two columns and the columns were tapped until the voids were displaced (appeared to be bubbles). Both looked fully hydrated on the top at this point. This experiment demonstrated that we could produce usable columns from dry PEG-resin. From these findings we estimate that the 800 mg of the wet resin previously used in the experiments would equate to about 350 mg of dry resin. Thus, we used weighed samples of 350-400 mg dry mPEG-resin in experiments to assure that the same amount of resin is used in each experiment.

A 6th set of ^{211}At/Bi separations were conducted to evaluate whether the lack of removal of ^{211}At from a column after passing air over it (to remove 2M HCl) and (separately) to determine whether there was a difference in trapping on the column, and release from the column, when the mPEG size was changed from 2000 Da (our usual) to 750 Da or 5000 Da. The variables and yields in the study sets are shown in **Table 16**. It should be noted that the amount of activity loaded on the column is lower than the amount of activity remaining on the column after the rinses (to remove bismuth) due to significant attenuation of gamma radiation by bismuth in the initial readings. It should also be noted that the % recovery from the column was obtained by adding all of the activity in the 1mL elution vials divided by the amount ^{211}At on the column after rinsing the Bi from the column. The percent values are smaller (~3%) than obtained if one simply divides the amount remaining on the column after elution with conc. NH_4OH by the amount remaining after the 4 rinses (e.g. 100 –(0.799/2.11) = 62.1% vs. 58.9% for column #1). This difference may be due to the geometry of measured samples and decay of ^{211}At.

Table 16: Results from comparison of columns for isolation and recovery of ^{211}At*

Column #	Resin	how packed	loaded with	Activity Loaded [contains bismuth] (mCi)	Flow through and washes (%)	Activity Remaining [no bismuth] (mCi))	elute with	Activity Remaining on Column (mCi)	% Recovery from column (%)
1	mPEG2000 (old)	wet	8M HCl	1.87	3.50%	2.11	conc. NH4OH	0.799	58.9%
2	mPEG2000 (old)	wet	8M HCl/air	1.72	4.50%	1.92	conc. NH4OH	0.647	63.7%
3	mPEG2000(new)	wet	8M HCl	1.46	4.73%	1.67	conc. NH4OH	0.71	35.9%
4	mPEG2000(new)	dry	8M HCl	1.16	4.77%	1.31	conc. NH4OH	0.476	62.9%
5	mPEG750(new)	wet	8M HCl	1.43	4.18%	1.56	conc. NH4OH	0.584	61.0%
6	mPEG5000(new)	wet	8M HCl	1.32	5.54%	1.41	conc. NH4OH	0.335	75.5%
7	Grace IC-OH	wet	8M HCl	1.27	3.73%	1.21	2M KNO3 conc. NH4OH	1.13	3.7%

*At-211 quantities were not decay corrected, but only relevant decay occurred during the column loading and isolation (~30 min).

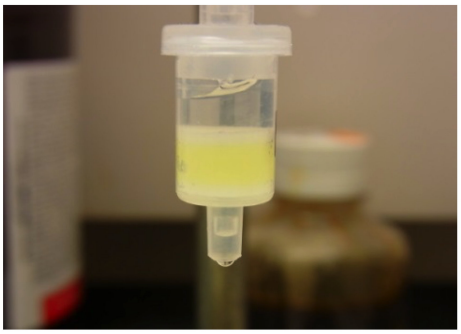
It can be seen that the previous column failure, where air was blown over it, could not be reproduced (see results from column #2). We are uncertain as to why this occurred. An ion exchange column was also evaluated (column #7). We thought that a high concentration of salt (potassium nitrate) might remove the ^{211}At from the column, but that did not work. So conc. NH_4OH was evaluated. Neither of these eluents removed ^{211}At from the column. In short, the

results obtained with this ion exchange column are the same as obtained previously with other anion exchange columns. The use of an anion exchange column is attractive as they are available from commercial sources, but it is unclear what solvent / conditions might improve the elution of ^{211}At.

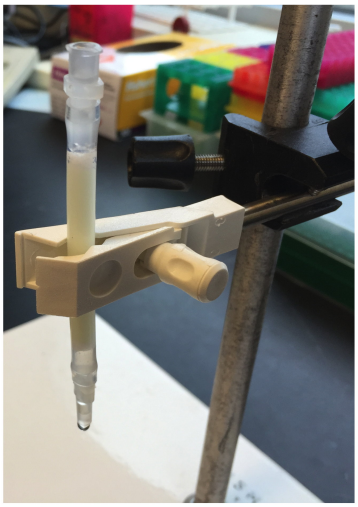
Interestingly, the mPEG5000 column (column #6) appeared to give higher recovery yields than the mPEG750 or mPEG2000 columns. We evaluated this further (i.e. compare mPEG2000 and mPEG5000 columns again) in later studies to confirm this observation. It could be that the lower loading of the mPEG5000 on the column affects the retention of the ^{211}At. It was noted that elution of ^{211}At from the mPEG5000 column was faster, i.e. 50% of recovered activity was eluted in the first 1 mL whereas in the other columns the highest quantity of ^{211}At came out in the 2nd and 3rd 1 mL elution. It may also be that higher quantities of ^{211}At can be obtained from the mPEG750 and mPEG2000 columns if more 1 mL elutions were conducted to recover the maximum amount of ^{211}At. In all of the NH_4OH elutions in **Table 16** a total of 4 x 1 mL were taken. Perhaps increasing that number to 6 x 1 mL will provide similar recovery yields as obtained with mPEG5000. Our preference was (is) to make columns from mPEG750 or mPEG2000, as we believe that more consistent loading of the PEG on the resin can be obtained.

Based on the results obtained with the different PEG-resin columns we ran additional studies to compare the loading and recovery of ^{211}At from mPEG2000, mPEG5000 and mPEG750 resin columns. In the studies, we chose to evaluate the use of another type of resin holder that could be more readily adapted to an automated system, wherein the loading, washing and eluting of the column is carried out using a computer-driven syringe pump. A picture of the resin holders used, denoted as “old column format” or “new column format”, are shown in **Figure 2.** The new column format is easily prepared and should work well in-line when automating the process. As part of the experiment, results obtained from a column (mPEG2000), which had the “old column format”, were compared with another column (mPEG2000) that had the “new column format”. Essentially identical results were obtained, so the column holder does not affect the yield.

Figure 2: pictures of mPEG-resin columns used in the investigations. New column format is preferred as it has less “headspace” for filling when using an automated (syringe Pump) system and appears to have a more uniform loading and elution profile.



“old column format”



“new column format”

In the experiment conducted, all resins were weighed dry to obtain a consistent quantity (e.g. 400 mg) for comparison of mPEG-resin columns. As in all of the studies, the bismuth target was first dissolved in 17 mL conc. HNO_3, then the HNO_3 was distilled off to leave a white residue. That residue was dissolved in warm 8M HCl (10 mL). Once the 8M solution was obtained, 1 mL aliquots were placed on the different PEG-resin columns. The ^{211}At activity on the column was then obtained using a dose-calibrator. It is known that the presence of large quantities of

bismuth salts attenuate the ^{211}At counts, so the measured activity was lower than the actual ^{211}At activity present. Then 2 x 20 mL of 2M HCl was used to wash the bismuth from each column. The ^{211}At activity in the washes was measured, as well as the amount of activity remaining on each column (now without bismuth). Following this, the ^{211}At was eluted from the column(s) using 4-6 mL conc. NH_4OH. The amount of ^{211}At in the NH_4OH solution and remaining on the column after elution was measured to give a recovered yield. The time that it took to conduct the entire process was noted to determine what percentage of the ^{211}At was lost due to decay during the isolation/separation process. The shortest time to complete the process was 32 min (~5% lost to decay) and the longest time was 55 min (~9% lost to decay). The results of the experiment are shown as Experiment #1 in **Table 17**.

Table 17: Re-evaluation of mPEG-resins for isolation of ^{211}At when loading with 8M HCl

Column #	Resin	Activity Loaded [contains bismuth] (uCi)	Flow Through [contains bismuth] (uCi)	Activity in 2M HCl Washes (uCi)	Activity after Washes [no bismuth] (uCi)	Activity on Column [no bismuth] (uCi)	Activity Eluted [no bismuth] (uCi)	Recovery from Column (%)	minutes to load wash and elute
Experiment 1									
1	PEG 2000 (old format)	1240	6	25	1301	313	1038	80%	55
2	PEG 2000 (new format)	1260	10	22	1321	211	1053	80%	45
3	PEG 5000 (new format)	1200	26	28	1227	233	963	78%	42
4	PEG 5000 (new format)	3150	533	84	3260	618	2611	80%	51
5	PEG 750 (new;1 equiv)	1030	14	24	1125	175	938	83%	40
6	PEG 750 (new; 0.5 equv)	1015	18	30	1074	325	717	67%	38
7	PEG 750 (new; 0.25 equiv)	938	18	27	980	312	640	65%	39
Experiment 2									
8	PEG 750 (1 equiv.)	9940	43	170	9740	1630	7771	80%	32
9	PEG 750 (1 equiv.)	11330	47	155	10840	1140	9429	87%	37

Unlike the results in **Table 16**, the comparison results in **Table 17** (experiment 1) indicate that all mPEG resins provide about the same recovered yield (~80%). However, it appears that full saturation of PEG750 on the Merrifield resin is required for optimized recovery. Since we believe that the mPEG750-resin column will be easier to reproduce, that resin has been chosen for future studies.

A second experiment was conducted where larger quantities (~5 mL) of the 8M HCl/bismuth solutions containing ^{211}At were evaluated on mPEG750-resin columns. Thus, the total amount of 8M HCl was split (approximately) into 2 fractions, and those fractions were placed on 400 mg (weighed dry) resin that had been soaked overnight in water, then washed with 10 mL 2N HCl followed by 10 mL 8M HCl (just prior to loading with ^{211}At). The ^{211}At trapping, washing and elution steps were conducted in the same manner as described above. Less than 0.5% of the activity was found in the "pass-through", indicating that 99%+ of the ^{211}At activity was retained on the column. Washing the columns with 2 x 20 mL of 2M HCl removed less than 2% of the column-bound ^{211}At. Importantly, 80-87% of the activity was removed from the column using conc. NH_4OH. These results confirm that an efficient method has been developed for: (1) capturing ^{211}At from an 8M HCl solution containing bismuth, (2) rinsing all of the bismuth from the column, (3) then eluting the ^{211}At from the column in NH_4OH. This however is not the final step as the ^{211}At needs to be removed from the NH_4OH before it is usable for radiolabeling. See later section (p33) for results of the recovery of ^{211}At from the NH_4OH solution.

3. Column Loading with HNO_3

(Added Task) *Evaluate ^{211}At separation using HNO_3 on PEG column*

A high priority was to determine whether the wet chemistry isolation process might be greatly simplified by capturing the ^{211}At from the HNO_3 solution, obtained by dissolution of the target,

rather than obtaining it from 8M HCl after the HNO_3 is removed. If that worked, the time and effort to conduct the distillation to remove HNO_3 and subsequently redissolve the residue in 8M HCl could be eliminated.

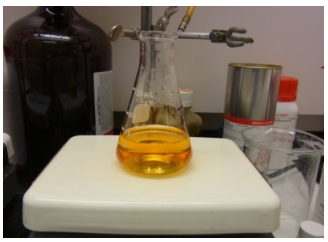
We began the studies of the use of PEG-resin columns to capture ^{211}At from HNO_3/Bi solutions by evaluating whether I_2 in 4M HNO_3 would be trapped on a PEG-resin column. The experiment is shown in the pictures provided in the **Figure 2** below.

Figure 2: Photographs of steps involved in isolation of ^{211}At from HNO_3 solutions using I_2 as a surrogate for At^+

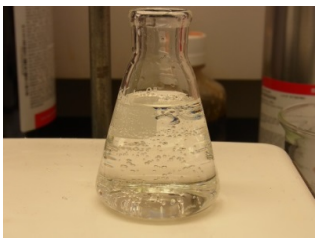
Bi metal (3.20 g) was dissolved in 17 mL conc. HNO_3.

HNO_3 solution was diluted with 50 mL H_2O to 4M (Total volume: 67 mL)

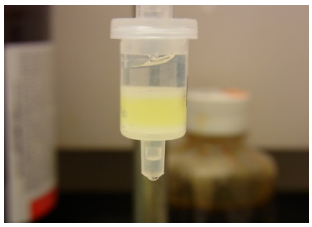
No precipitation was observed.



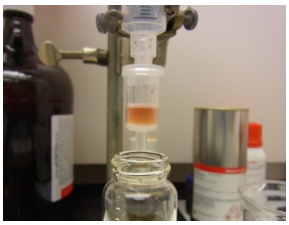
I_2 in MeOH (5 mg) was added.



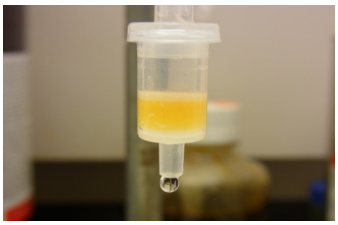
After splitting 2 portions, $Na_2S_2O_5$ (228 mg) was added to one portion for quench.



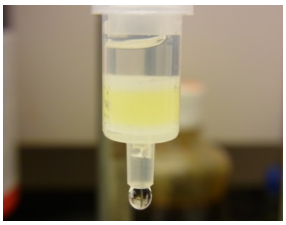
The solution (w $Na_2S_2O_5$) was loaded on the PEG column (400 mg)
followed by washing with 10 mL 2M HCl (x 4)



The other solution <u>(w/o</u> $Na_2S_2O_5$) was also passed through a PEG column (resin volume: 418.2 mg)



Washed with 10 mL 2M HCl (x4)



Eluted with 2 mL 15M NH_4OH

In both cases, Bi precipitation was observed in the pass though and 1st wash.

Complete dissolution of 3.2 g Bi metal (similar to a target amount) required 17 mL of HNO_3. We were concerned that conc. HNO_3 might not be compatible with the PEG-resin column, so the solution was diluted with 50 mL H_2O to bring it to 4M HNO_3. Solid I_2 was added to the solution, then the solution was split into 2 equal portions. In one portion the I_2 was reduced to iodide by addition of sodium metabisulfite (quite a lot was required). The two solutions were then run across 2 PEG-resin columns (~400 mg resin each). Following that, the resins were washed 4x with 10 mL 2M HCl, then they were eluted with 2 mL of conc. NH_4OH. NaOH was added to the wash solutions to determine if bismuth hydroxide was formed. Interestingly, bismuth hydroxide precipitate was observed only in the first wash of the columns. These results encouraged us to conduct ^{211}At isolation studies from HNO_3. The experiments are described in the next section.

Studies were conducted using both conc. HNO_3 and 8M HNO_3 (with and without reductant $Na_2S_2O_5$) as solvent mixtures for loading ^{211}At/Bi solutions onto PEG-resin columns. The results of the studies are shown in **Table 18**. It should be noted that degradation of the column material was apparent when conc. HNO_3 was used, so solutions containing conc. HNO_3 could not be used for loading the PEG columns. We believe that is the reason there was so much

loss of activity in the washes in runs #1 and #2. It is not apparent why the elution with NH_4OH was low in runs 1 and 2, since all had 4 x 2 mL 2M HCl washes. However, the results obtained with 8M HNO_3 were suggestive it might be possible use solutions of 8M HNO_3 to load samples onto PEG-resin columns.

Table 18: Isolation of ^{211}At from HNO_3 solutions using PEG-resin columns.

No.	Lot #	Resin volume [mg]	Loading Solution	Loading activity [µCi]	Trapping Efficiency [%]	Wash loss [%]	Overall recovery Efficiency by 2 mL 15M NH_4OH [%]
1	MKC 20-10	409	conc. HNO_3	276	**89.1**	32.0	**27.6**
2*	MKC 20-10	406	conc. HNO_3 + 5 mg $Na_2S_2O_5$	247	**96.8**	26.0	**32.5**
3	MKC 20-10	403	8 M HNO_3	271	**96.7**	2.0	**66.6**
4	MKC 20-10	394	8 M HNO_3 + 5 mg $Na_2S_2O_5$	245	**95.9**	3.2	**66.6**

*At least 170 mg of the PEG resin was lost during this run.

In a non-radioactive experiment designed to determine if bismuth dissolved in 8M HNO_3 would interact with the PEG-resin columns, 2 different quantities of Bi were evaluated. In the experiments, 1.58 or 3.14 g of bismuth metal was dissolved in conc. HNO_3. PEG-resin columns were prepared with the quantities of PEG resin shown in **Table 19**, then the columns were conditioned with 10 mL 8M HNO_3. Following that, the conc. HNO_3/Bi solutions were diluted to make them 8M HNO_3 and the solutions were run over the PEG-resin columns. The columns were rinsed 4x with 2M HCl, then eluted with 2 mL of conc. NH_4OH. Aliquots of 8M NaOH were added to the pass-through solutions to determine if Bi was present (i.e. precipitate formed). The fact that no precipitate was noted in the 4th wash was encouraging, so further ^{211}At studies were conducted.

Table 19: Evaluation of the Bi elution from PEG-resin columns when dissolved in 8M HNO_3 solution.

Run	Bi metal [g]	PEG resin [mg]	Bi precipitation
1	1.5811 g	605.0 mg	Observed in pass through, 1st, 2nd and 3rd washes
2	3.1371 g	804.7 mg	Observed in pass through, 1st, 2nd and 3rd washes

Following the studies to determine whether Bi interacted with PEG-resin columns, a study was conducted to determine if HNO_3 could be used as the solvent when capturing ^{211}At from an irradiated target. The design of the study was similar to that conducted with 8M HCl previously.

The irradiated bismuth target was dissolved in ~17 mL of HNO_3, then that material was diluted to 34 mL to make 8M HNO_3. The results of the study are shown in **Table 20**. The plan was to conduct 2 separation/isolation runs so the solution containing ^{211}At was split into 2 x 17 mL each.

Table 20: Isolation of ^{211}At in 8M HNO_3 solution by PEG-resin column.

#	Resin volume	8M HNO_3 To load	Loading Activity [mCi]	Trapping Activity [mCi]	Trapping Efficiency [%]	Wash loss [%]	Overall recovery Efficiency by 15M NH_4OH
1	803.4 mg	17 mL	11.81	**2.10**	**17.8**	-	**-**
2	803.5 mg	1 mL	0.697	**0.677**	**99.5**	-	**-**
		2 mL	1.37	**1.20**	**87.6**	-	-
		3 mL	2.03	**1.56**	**76.8**	-	-
3	5.05 g	5 mL	2.57	**2.61**	**>100**	-	**-**
		10 mL	5.09	**4.91**	**96.4**	-	**-**
		14 mL	7.08	**5.90**	**83.3**	3.8*	**16.5***

A major problem with the approach was seen immediately as very little (2.1 mCi) of the loaded activity (11.8 mCi) was trapped on the PEG column. It appeared that the PEG-resin columns were saturated much easier when 8M HNO_3 was used than when 8M HCl was used. To evaluate this possibility, another column was prepared and 3 x 1 mL aliquots of the 2nd 17 mL solution were added to the column. Indeed, with the first 1 mL of solution, essentially all of the ^{211}At was trapped, but with each subsequent 1 mL placed on the column, less of the ^{211}At was trapped. We wondered if a much larger PEG column (~ 5g) would allow us to trap a higher percentage of the ^{211}At. Indeed, when 5 mL (2.57 mCi) of the HNO_3 solution was placed on the column essentially quantitative trapping was obtained. When a 2nd 5 mL quantity (2.52 mCi) was added to the column, the combined trapping was 96%. When a final 4 mL (1.99 mCi) was added to the column, the total amount trapped dropped to 83% (5.90 mCi of the 7.08 mCi). The results obtained indicated that the capacity for ^{211}At trapping on the PEG-resin column was much lower when 8M HNO_3 was used as the solvent. It may be that the ^{211}At species is different in each case. Fortunately, the washes with 2M HCl appeared to simply elute ^{211}At that was not bound in the initial loading (**Table 21**). However, the increased dead volume of column (due to larger column size) required 4 mL elution volumne, and in the study 4 x 4 mL elution with conc. NH_4OH gave a low recovery yield of ^{211}At (**Table 21**).

Table 21: Isolation of ^{211}At in 8M HNO_3 solution by PEG-resin column (non-decay corrected values shown).

Loading Activity [mCi]	**Wash loss with 2M HCl (12 mL x 4)** upper: activity [mCi], lower: efficiency					**Recovery with 15M NH_4OH (4 mL x 4)** upper: activity [mCi], lower: efficiency					**Column Remaining**
	1st	2nd	3rd	4th	**Total**	1st	2nd	3rd	4th	**Total**	
7.08	0.27	<0.01	<0.01	<0.01	**0.27**	0.325	0.565	0.140	0.140	**1.17**	**4.01**
-	4.5%	0%	0%	0%	**3.8%**	4.6%	8.0%	2.0%	2.0%	**16.5%**	**56.6%**

Another study was conducted to determine ^{211}At trapping efficiency from HNO_3 solution when using PEG-resin (mPEG750) and recovery of ^{211}At from that resin when using either 15M

NH_4OH or 4M NaOH. In the study ^{211}At (5.74 mCi) was isolated in 5 mL HNO_3. Due to other tasks being conducted, the experiment was not started until a later time (248 min; 46% remaining). Four column experiments were conducted. Each experiment was conducted using 400 mg (dry weight) of mPEG750-resin in the "new column format". The results of the study are shown in **Table 22**.

Table 22: Isolation of ^{211}At in 8M HNO_3 solution by PEG-resin column (non-decay corrected values shown).

Run #	Loaded Activity [Estimated]* (uCi)	Pass Through Activity** (uCi)	Activity Remaining on Column (uCi)	Activity in 2M HCl Washes (uCi)	Percent Activity Trapped***	Elution Solvent Used	Activity Isolated By Elution (uCi)	Recovery of Trapped Activity	Recovery of Loaded Activity
1	898 [1013]	70.7	890	52.5	87.9%	15 M HNO3	554	62.2%	54.7%
2	795 [847]	77.1	716	54.2	84.5%	15M HNO3	416	58.1%	49.1%
3	745 [796]	43.2	721	31.8	90.6%	4M NaOH	27.9	3.9	3.5
4	618 [715]	87.2	579	48.6	81.0%	4M NaOH	27.1	4.7	3.8

* Loaded activity contains bismuth and attenuates quantity - Estimated amount is added activity remaining on column + pass through + wash

** Pass-through activity is that amount that is not retained (passes through) when loading the sample - note that it has Bi present

***Percent trapped is obtained by total estimated activity divided by activity remaining on column (x100 to get %)

It appears that the significant time delays from isolation of the ^{211}At in conc. HNO_3 until each sample was diluted, and the column isolation experiment was conducted, could have had an impact on the amount of ^{211}At isolated. The first experiment was conducted ~4 hours after the ^{211}At was dissolved in conc. HNO_3, and each subsequent column experiment was conducted ~1 hour after the one prior. From previous studies it had been shown that nearly quantitative trapping was obtained unless the column was overloaded with bismuth (i.e. **Table 22**). It seems likely that another ^{211}At species that is not trapped by the PEG column was formed while in conc. HNO_3 for the extended period of time. Elution with conc. NH_4OH gave similar (slightly lower) results to those obtained previously. Elution with 4M NaOH gave very low recovery yields, as had been observed previously.

A compilation of the isolated yields obtained using the PEG-resin columns is shown in **Table 23**. The most encouraging results for ^{211}At separation/isolation has been with the PEG-resin columns, using 8M HCl as solvent for loading and conc. NH_4OH as solvent for elution. The data obtained indicated that PEG-resins with PEG750, PEG2000 and PEG5000 have similar properties with regards to their ability to capture and release ^{211}At. However, we chose to use the PEG750 modified resin since all of the surface benzyl chloride moieties appear to be modified when that resin is prepared. We reasoned that full modification of the resin would be much easier to obtain consistently than would be obtained with the larger resins, due to the fact that only a portion of the benzyl chloride groups with PEG moieties are substituted

(presumably due to steric hindrance). Since the criteria for selection was full modification of the resin, we wondered whether an even smaller mPEG, mPEG350, might give similar substitution results. Thus, mPEG350 was conjugated with the Merrifield resin as previously described (see pages 17-18), and experiments were conducted to compare resins that had been functionalized with mPEG750 and with mPEG350.

Table 23: Summary of ^{211}At isolation with PEG-resin columns.

Eluant	Trapping Efficiency	Wash loss	Recovery Efficiency
conc.HNO_3	89.1%	32.0%	5.8%
8M HNO_3	96.7%	2.0%	66.6%
8M HNO_3 + $Na_2S_2O_5$	95.9%	3.2%	66.6%
8M HCl	99.7%	4.8%	78.5%
8M HCl + $Na_2S_2O_5$	99.4%	5.6%	60.9%
4M HCl	99.7%	2.5%	71.1%
4M HCl + $Na_2S_2O_5$	99.7%	3.5%	73.4%
2M HCl	99.9%	3.8%	73.5%
2M HCl + $Na_2S_2O_5$	99.7%	3.3%	73.2%

Along with comparing the two different PEG-resin columns, an evaluation of different column wash protocols was done. The variables evaluated were; use of 2M HCl to wash the column versus washing with 8M HCl and H_2O (with or without an added reductant). The experimental results are shown in **Table 24**.

Table 24: Isolation of ^{211}At in 8M HNO_3 solution by PEG750 and PEG350 columns (non-decay corrected values shown)

	Resin Used*	Loaded column With**	Activity Added mCi***	Washed Column with	Flow Through and Washes uCi	Activity on column after washing mCi****	Activity lost in washing	Eluted Column With	Activity on Column mCi	Activity Isolated mCi	Recovery from Column (%)*****	Recovery of original (%)
1	PEG-750	600 uL	2.5	40 mL 2M HCl	13.3 uCi	2.66	0.5%	NH4OH	1.38	1.11	45%	42%
2	PEG-350	600 uL	2.16	40 mL 2M HCl	14.2 uCi	2.22	0.6%	NH4OH	1.19	0.976	45%	44%
3	PEG-750	600 uL	1.86	20 mL 8M HCl 20 mL 2M HCl 10 mL H2O	146 uCi	1.81	7.2%	NH4OH	0.69	0.996	59%	51%
4	PEG-350	600 uL	1.37	20 mL 8M HCl 20 mL 2M HCl 10 mL H2O	462 uCi (30 uCi)	0.974	32%	NH4OH	0.636	0.306	33%	21%
5	PEG-350	600 uL	1.42	20 mL 8M HCl 20 mL 2M HCl	19.8 uCi	1.51	1.3%	NH4OH	0.776	0.646	45%	43%
6	PEG-750	600 uL	1.52	40 mL 2M HNO3 10 mL H2O	652 uCi	0.971	40%	NH4OH	0.49	0.461	49%	28%
7	PEG-750	600 uL	1.78	20 mL 8M HCl 20 mL 2M HCl 10 mL aqThiosulfate	1047 uCi (31 uCi)	0.777	57%	NH4OH	0.366	0.378	51%	20%

Values are not decay corrected
*Pre-equilibrated resin-filled column with 10 mL 2M HCl followed by 10 mL 8M HCl
**Loaded column via 1 mL syringe with At-211 solution in 10M HNO3
***Solution contained bismuth salts so quantity reported is low due to attenuation
****Activity after bismuth salts are removed (little attenuation)
*****elution of some columns done after considerable time so starting activity not representative

Column experiments #1 and #2 (Table 24) used PEG750 and PEG350 columns run under the same wash and elution conditions. In those experiments the column wash solution was 40 mL of 2M HCl. As can be seen, essentially the same results were obtained. In experiments #3 and #4, PEG750 and PEG350 columns were compared under the same wash conditions. The column wash conditions used was 29 mL of 8M HCl, 20 mL of 2M HCl and 10 mL of H_2O. Interestingly, when water was used in the wash steps, much more of the ^{211}At on the column came off (prematurely). It is not clear why that happened, but the overall recovery yield dropped from 51% to 21%, making it clear that there is a large difference in the resins under these wash conditions. To confirm that the water wash was the cause of the decreased recovery yield, another experiment, #5, was conducted with the PEG350 column without the water wash. This experiment confirmed the water wash was problematic with PEG350 columns, and puts into question the use of those columns. This is an important finding as the water wash is desired since it removes HCl from the column. Removal of HCl is desired as the eluent must be neutralized and residual HCl results in more NaCl in the final ^{211}At solution. In experiment #6, 40 mL of 2M HNO_3 was used for washing the column in the place of 2M HCl. The use of HNO_3 resulted in a large portion of the activity passing through the column rather than being captured by it. This result makes it clear that the column washes are best done with HCl. In another experiment, #7, it was evaluated whether use of a reductant in the water wash improved the amount of ^{211}At activity that could be eluted using NH_4OH. Interestingly, the aqueous reductant released a large amount of the ^{211}At activity in the wash process, so this is not a good approach.

iii. Removal of NH_4OH via distillation

(Added Task) *Evaluate removal of NH_4OH from isolated ^{211}At solution*

The fact that ^{211}At distilled from the flask during the removal of NH_4OH (NH_3) in the 1st experiment suggested that the NH_3 may have distilled early in the process. Early distillation of NH_3 allowed the solution to become neutral/acid before it went to dryness, allowing ^{211}At to distill. To test this hypothesis, two non-radioactive NH_4OH distillations were conducted. In the experiments, the pH of the solutions remaining in the distilling flask (with/without added NaOH) and the receiving flask were tested after ~1/3, 2/3 or most of the liquid was transferred. The data in **Table 24** suggest that the NH_3 distills within the first half of the original volume, as no NH_3 smell was detected in the distilling flask after the first 1/3 of the solution was transferred. Of course, when NaOH was added to the distilling flask, the pH of the solution remained very basic even after nearly all of the solution had been transferred. In contrast, without the addition of NaOH the distilling solution became acidic. The presence of acidic solution makes it easy to understand why the ^{211}At distilled (as H[^{211}At]At ?). This data suggests that a final distillation of ^{211}At could be conducted as a purification step when high purity ^{211}At is required.

(**Procedure with ^{211}At**) The ^{211}At/NH_4OH solution obtained from the PEG column was transferred to a 10 mL round bottom flask that contained *100 μL of 1N NaOH* and had been preheated to 100°C. The flask and distilling apparatus was covered and heated to 270°C to distill the HNO_3. When the distillation was done (near dryness) 1.46 mCi (9.4%) of the ^{211}At activity remained in the flask. In an attempt to recover the remaining activity, 200 uL of H_2O was added and the resulting solution was transferred to a vial. Only 0.61 mCi transferred, suggesting that the remaining ^{211}At was possibly bound to the glass surface. Interestingly, the pH of the solution was neutral. The addition of 1N NaOH at the beginning of the distillation was done to keep the pH basic during the distillation process. As in our previous studies where the

pH of the distillation became neutral (or acidic), the majority of the activity (9.78 mCi; 64.5%) was in the receiver flask. As the volume of solution in the receiver flask was much higher than

Table 24: Nonradioactive experiments to determine when the NH_3 distills from NH_4OH solutions.

Solution-1 (without NaOH in distilling flask)

	Remaining solution		Distilled solution		
	pH	NH_3 Odor	Volume	pH	NH_3 Odor
First 1/3	7.0 – 8.0	**Not detected**	0.457 mL	11	**Detected**
Second 1/3	5.5 – 5.8	Not detected	0.482 mL	9.0 - 9.5	Not detected
Last 1/3	4.0 – 4.5*	Not detected	0.6593	8.5 - 8.7	Not detected

Solution-2 (with NaOH in distilling flask)

	Remaining solution		Distilled solution		
	pH	NH_3 Odor	Volume	pH	NH_3 Odor
First 1/3	13.0	**Not detected**	0.512 mL	11 – 12	**Detected**
Second 1/3	13.0 -14.0	Not detected	0.909 mL	9.0 - 9.5	Not detected
Last 1/3	13.0 -14.0*	Not detected	0.300 mL	6.5 – 7.0	Not detected

* Residue remaining in the flask was dissolved in 200 µL H_2O

we wanted, a second distillation was conducted. To prepare for the second distillation, a *100 uL aliquot of 2N NaOH* was added to the ^{211}At solution in the receiver flask. That solution (8.78 mCi) was then transferred to another 10 mL round bottom flask, which was heated to 260°C. This time the bulk of ^{211}At (7.23 mCi; 82% recovery not considering decay) remained in the round bottom flask after all the original solution distilled because the adequate base was added to keep pH at 13-14. A 200 uL aliquot of H_2O was added and 6.80 mCi (94%) of the activity was transferred to another vial. An overall recovery of 77% was obtained in the 2nd distillation, but with the 2 distillations the overall yield was approximately 50%. Normally, only 1 distillation will be done and we might expect a recovery yield in the range of 70-80%.

Isolation of ^{211}At from NH_4OH used in the preparative runs (experiment #2) shown in **Table 17** was done by distillation in the presence of NaOH. Our previous studies (above) indicated that NaOH had to be added to keep the ^{211}At from distilling with the NH_3/H_2O. An isolation experiment was conducted where *400 µL of 0.1 N NaOH* was added to a 10 mL round bottom distilling flask containing 7.9 mCi of ^{211}At in conc. NH_4OH prior to distillation. The distillation (heated to 210°C) proceeded for 48 min to leave ~200 µL of solution remaining. After cooling for an additional 10 min (58 min total) it was found that 5.98 mCi (91%) had distilled out of the flask and 0.616 mCi remained. The pH of the remaining liquid was checked and found to be pH 5-6.

A second distillation was conducted wherein *200 µL of 1.0 N NaOH* was added to the round bottom flask containing a solution of NH_4OH and 6.28 mCi ^{211}At. The distillation was conducted (210°C) over a 42 min period. After cooling it was found that 4.4 mCi (87%) distilled out of the flask and 0.67 mCi remained in an aqueous solution with a pH ~7.

A third distillation was conducted on the solution containing ^{211}At from the first distillation (above). To that solution of ^{211}At (total 4.76 mCi) was added *200 μL of 1N NaOH*. As with the previous studies, most of the ^{211}At (3.63 mCi; 91%) distilled from the round bottom flask (0.380 mCi remained). The distilling time (210°C) was 37 min and the solution remaining in the flask was found to have a pH ~7.

A fourth distillation was conducted after combining all distilled fractions (8.50 mCi) from the other runs, then adding *200 μL of 1N NaOH*. A 50 mL round bottom flask was used so the heating temperature was increased to 280°C. The solution was heated to dryness over a 35 min period. At ~1h from the start of the distillation (distillation + cooling period) the flask contained 6.8 mCi (93%) ^{211}At and only 0.544 mCi distilled from the flask. We were interested in determining how much of the activity could be recovered after it had been taken to dryness. A 200 μL aliquot of water was added and the solution was brought to neutral (several additions of 0.5 or 2N HCl and 0.5 or 1N HCl) before removing from the distilling flask. Of the 5.97 mCi in the flask, 1.97 mCi (33%) was removed with the liquid. Addition of 500 μL water to the flask, followed by removal of the liquid provided an additional 1.62 mCi (27%). Thus, 50% of the ^{211}At could be removed. The results suggest that the ^{211}At/NaOH solution should not be taken to dryness as the radioactivity may be strongly bound with the glass surface.

These distillations demonstrated how difficult it is to make the distilling solution basic after removing the NH_3. It seems likely that this difficulty comes from the fact that mixing 2M HCl (left on the column) with conc. NH_4OH (used for elution) forms NH_4Cl salts, which can react with the NaOH that is added to neutralize all or a portion of it.

Additional studies were conducted to determine the best conditions to use in the removal of NH_4OH such that isolated ^{211}At remains in the distilling flask in a NaOH solution. An experiment was conducted to determine if washing the PEG750 column with 4 mL water after removing the bismuth with 40 mL of 2M HCl would provide the ^{211}At with a minimum of salts. In the experiment, the dissolution of bismuth target, distillation of HNO_3, and solubilization of bismuth salts in 8M HCl were conducted as usual. In brief, an irradiated target containing 21.9 mCi of ^{211}At provided 17.3 mCi (with bismuth) in 10 mL of 8M HCl after conducting the first 3 isolation steps. The 8M HCl solution was split into 2 parts (5 mL each) which were run across two PEG750 columns. Loading column A resulted in having 11.23 mCi ^{211}At retained and 0.07 mCi (0.6%) passing through. Loading column B resulted in having 10.39 mCi ^{211}At retained and 0.07 mCi (0.7%) passing through. Each column was washed with 40 mL 2M HCl. After elution of the 8M HCl; column A had 1.4% of activity in wash and column B had 1.6% of the activity in the wash. To this point everything in the study was identical, and the results were essentially the same. Column B was further washed with 4 mL of H_2O, which removed 1.9% of the ^{211}At activity. Each column was then eluted with 6 mL of conc. NH_4OH. A total of 9.40 mCi (80.5%) was eluted from column A and 8.12 mCi (77.7%) from column B. The difference in eluted activity can be accounted for by the ^{211}At lost in the H_2O wash.

The removal of NH_4OH (from the above solutions) was accomplished by distillation. Prior to distillation, a *300 uL quantity of 4N NaOH* was added to the round bottom distilling flask. Following that the NH_4OH solution containing ^{211}At was added to the flask. The ^{211}At solution eluted on column A had 7.52 mCi ^{211}At. The distillation flask was connected to the distilling head. The distillation setup was then put on a hot plate pre-heated to 160°C, which was increased in temperature to 220°C over ~15 min. Distillation of the NH_4OH took about 30 min (not completely dry). A small amount of ^{211}At (20 uCi, 0.3%) distilled with the NH_4OH, but the

bulk of ^{211}At (6.70 mCi) remained in the distilling flask. It was noted that there was very little solid in the distilling round bottom containing the ^{211}At. A 300 uL quantity of H_2O was added to the round bottom, then 6.03 mCi (90%) was removed by pipet.

The ^{211}At eluted on column B was isolated similarly. In that case (which had a H_2O wash of column prior to NH_4OH elution) the *flask was nearly dry with no visible solids*! Starting with 6.74 mCi ^{211}At added to the distilling flask, 5.99 mCi remained in the flask after distillation and only 0.02 mCi ^{211}At came over with the distillate (0.5%). [Note that the other ^{211}At losses were due to decay during the distillation process] A 300 uL quantity of H_2O was added to the distilling flask, and the liquid was transferred to a vial which had 4.99 mCi. Another 100 uL of H_2O was added to rinse the vial, and after combining the wash with the earlier transfer, the total activity isolated came to 5.65 mCi (94%).

To utilize the ^{211}At in labeling experiments, it needs to be neutralized after the NH_4OH distillation step (solution had an excess of NaOH). The ^{211}At obtained after distillation of the solution from column A was neutralized by addition of various concentrations of HCl and NaOH to reach pH of 7-7.5. Neutralization of the ^{211}At solution from column B was easier to achieve. These results suggest that the best isolation practice is to wash the mPEG750 column with water prior to elution with NH_4OH. That results in having less salt (probably NH_4Cl) and makes it much easier to neutralize.

iv. Quality Control – Antibody Labeling

(Added Task) *Evaluate ^{211}At-labeling as a Quality Control Measure*

Isolation of ^{211}At activity does not guarantee that it will be usable for labeling of biomolecules. Therefore, as part of our quality control measures we run antibody-labeling experiments to make sure the isolated ^{211}At behaves as expected in that reaction. We prepared an antibody-B10 conjugate for us in quality control labeling experiments. In general, ^{211}At-labeling of this antibody-B10 conjugate (BC8-B10) gives 80 – 90% radiolabeling yields. The BC8-B10 radiolabeling experiments were conducted by reaction of neutralized solutions of ^{211}At from our standard “wet chemistry” isolation procedure. The reactions were conducted by reaction of 500 μg antibody-B10 in aqueous PBS without an oxidant (e.g. no chloramine-T). The reactions were quenched after 1 min by addition of a sodium metabisulfite solution.

Four ^{211}At-labelings of Antibody-B10 were conducted using solutions isolated and neutralized from PEG750 columns A and B. Antibody-B10 labeling with Prep A gave 31% isolated yield. Because this value was so low, the labeling reaction was conducted a second time with added oxidant (chloramine-T). The second labeling gave no yield (0%). Antibody-B10 labeling with Prep B gave a 36% isolated yield. Labeling with Prep B in the presence of chloramine-T gave a 48% isolated yield. It seems likely that the striking difference in reactions with chloramine-T as an oxidant is due to the presence of ammonium chloride in Prep A (it was clearly visible). Once neutralized, the ammonia could react with the chloramine-T to destroy it. The overall low labeling yields may be due to a change in oxidation state of ^{211}At caused by the chloramine-T. Additional studies of ^{211}At radiolabeling after isolation from NH_4OH distillation have provided better results (70-90% labeling yields). It is not clear why the low yields were obtained here, but it seems likely that it relates to the amount of time after isolation/neutralization of the ^{211}At. The higher yielding reactions were conducted immediately after isolation of the ^{211}At.

SO2b: Evaluate noble metal adsorption/desorption approach for separation of ^{211}At from Bi (UW)

Task 5: *Evaluate adsorption/desorption of ^{125}I using a Pt metal column (UW)*

No studies were conducted on this task due to our focus on PEG column studies.

Task 6: *Evaluate adsorption/desorption of ^{211}At using a Pt metal column (UW)*

No studies were conducted on this task due to our focus on PEG column studies.

IV. Specific Objective 3: Evaluation of the use of an electrochemical flow reactor as a replacement of the distillation of ^{211}At to simplify the automated isolation method.

Task 7: *Evaluation of the reductive plating of Bi and other possible metal contaminants onto a working electrode using cyclic voltammetry (PNNL)*
Task 8: *Build and test an electrochemical flow reactor (PNNL)*
Task 9: *Evaluation of electrochemical flow reactor using ^{211}At (PNNL)*

Progress in this SO will be reported separately by Matt O'Hara, PNNL

V. Specific Objective 4: Develop an automated system for isolation of ^{211}At from irradiated bismuth targets that uses the most efficient wet chemistry separation and purification methods to provide high recovery yields from a robust automated system.

SO 4a: Select optimal separation method for automation of ^{211}At isolation (UW)'

Task 10: *Evaluate and select best separation and purification methods for automation (UW)*

This task has not been completed. Both ^{211}At isolation approaches studied, i.e. liquid/liquid extraction separation method (PNNL) and column separation method (UW), were successful. Although both are successful, they have not been directly compared so it is not clear which of the two approaches will be the best to use with an automated system. Additional studies need to be conducted to compare the two approaches to identify which is better for an automated system.

SO 4b: Obtain and test system components (Modules) for automation of ^{211}At isolation (PNNL)

Some statements about the status of module testing relative to UW are provided below. The complete information on the module testing will be provided by Matt O'Hara.

Task 11: *Obtain and program computerized control module (PNNL)*

This module is complete to the point it will run the target dissolution chamber and acid conversion modules..

Task 12: *Obtain and test automated fluidics module (PNNL)*

Some testing of this module has been carried out at UW. Results of those tests will be reported by Matt O'Hara.

Task 13: *Obtain and test real time detection module (PNNL)*

Design and manufacture of this module has been taken out of the research as the budget was cut and it was felt this was the least important module for the automated system.

Task 14: *Design, build and test bismuth target dissolution module (PNNL)*

This module has been built and tested at PNNL and UW. It has been transferred to UW for use in further studies. A program has been set up on a laptop computer to run the dissolution step. Our first attempts to utilize dissolution chamber were unsuccessful, as it leaked (causing loss of ^{211}At). Subsequently we attempted several runs using non-irradiated targets, but all attempts had leaks. We thought the leakage might be due to bad O-rings. Matt O'Hara sent some replacement O-rings, but after installing them we still had leaks. The dissolution chamber was then sent back to PNNL to determine why it was leaking. It turned out that the mechanisms used to close the dissolution chamber had become bent (or sprung) and the PNNL engineers were able to fix that problem. The dissolution chamber was sent back to UW. Several ^{211}At target dissolutions have been conducted successfully without any leaking. *We are now using it routinely for dissolution of the targets.*

Task 15: *Obtain and test acid conversion module (PNNL)*

This module has been built and tested at PNNL. It has been transferred to UW for additional testing. The module is computer controlled and is linked in to the dissolution chamber. The module works well and has been used for removing HNO_3 by distillation in several runs.

Task 16: *Incorporate best extraction module (PNNL)*
Task 17: *Incorporate ^{211}At purification module (PNNL)*
Task 18: *Mount system modules and test using [^{207}Bi]Bi (PNNL)*

SO 4c: Build and test automated system for ^{211}At isolation (UW)

Task 19: *Purchase components and build duplicate automation system (UW)*

We purchased duplicate pumps, dissolution chamber, and acid conversion equipment as those used at PNNL. Matt O'Hara has provided the software program to run these steps of the automated system. However, we found that a number of syringe pumps were required in the wet chemistry isolation process as there are no automated valves in the system. Rather than purchase additional syringe pumps, we have used a Hamilton dual syringe pump apparatus and MVP valves (also from Hamilton). A schematic representation of the automation process is shown in **Figure 3**. This setup has been assembled and tested.

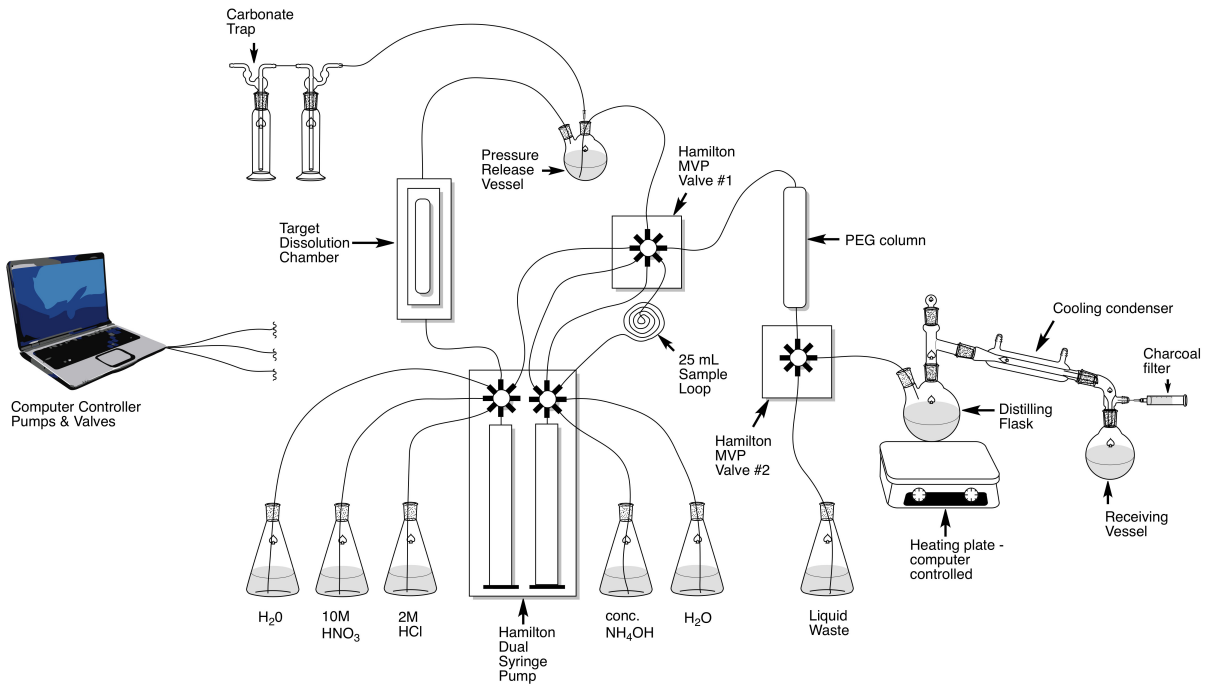


Figure 3: Schematic of automated system designed to separate ^{211}At from a bismuth target and isolate it in NaOH solution. Multi-way valves on the syringe pumps and MVP modules are used to direct the liquid flow to the desired vessels. The computer-controlled automated heating system is not shown, but is depicted by a heating plate. Other pumping and valve systems may be used in later modifications of the system.

A procedure for the isolation process using the automation setup shown in **Figure 3** is as follows. (Note that this procedure is not optimized)

(1) A 20 mL solution of 10M HNO_3 is pumped across an irradiated bismuth target in the Target Dissolution Chamber over a 20 min time period at 1 mL/min into a Pressure Release Vessel. (The Pressure Release Vessel is in-line so that NOx gases, resulting from the reaction of HNO_3 with bismuth metal, can move from the dissolution chamber into that vessel and into the Carbonate Trap such that no pressure buildup occurs on dissolution of the target).
(2) The ^{211}At/10M HNO_3 solution is withdrawn from the Pressure Release Vessel through MVP#1 into a 25 mL loop, then moved back through MVP #1 onto the PEG-resin column.
(3) A total of 40 mL of 2M HCl is pumped through MVP #1, across the PEG-resin column, through MVP #2 into a liquid waste container.
(4) A 10 mL quantity of H_2O is pumped through MVP #1, across the PEG-resin column, through MVP #2 into a liquid waste container to rid the column of acid.
(5) A 5 mL quantity of concentrated NH_4OH is pumped through MVP #1, across the PEG-resin column, through MVP #2 into the distilling flask, which is precharged with NaOH. In this step, liquid flow is very slow (e.g. 0.25 mL/min) so that there is a slow interaction with the column and ^{211}At on the column.
(6) The NH_4OH is distilled into the receiving vessel until near dryness in the distilling flask.
(7) The Na[^{211}At]At/NaOH residue in the distilling flask is diluted with H_2O (or other solvent) and removed by pipet for use in radiolabeling or shipment.

A picture of the automation setup is shown in Figure 4.

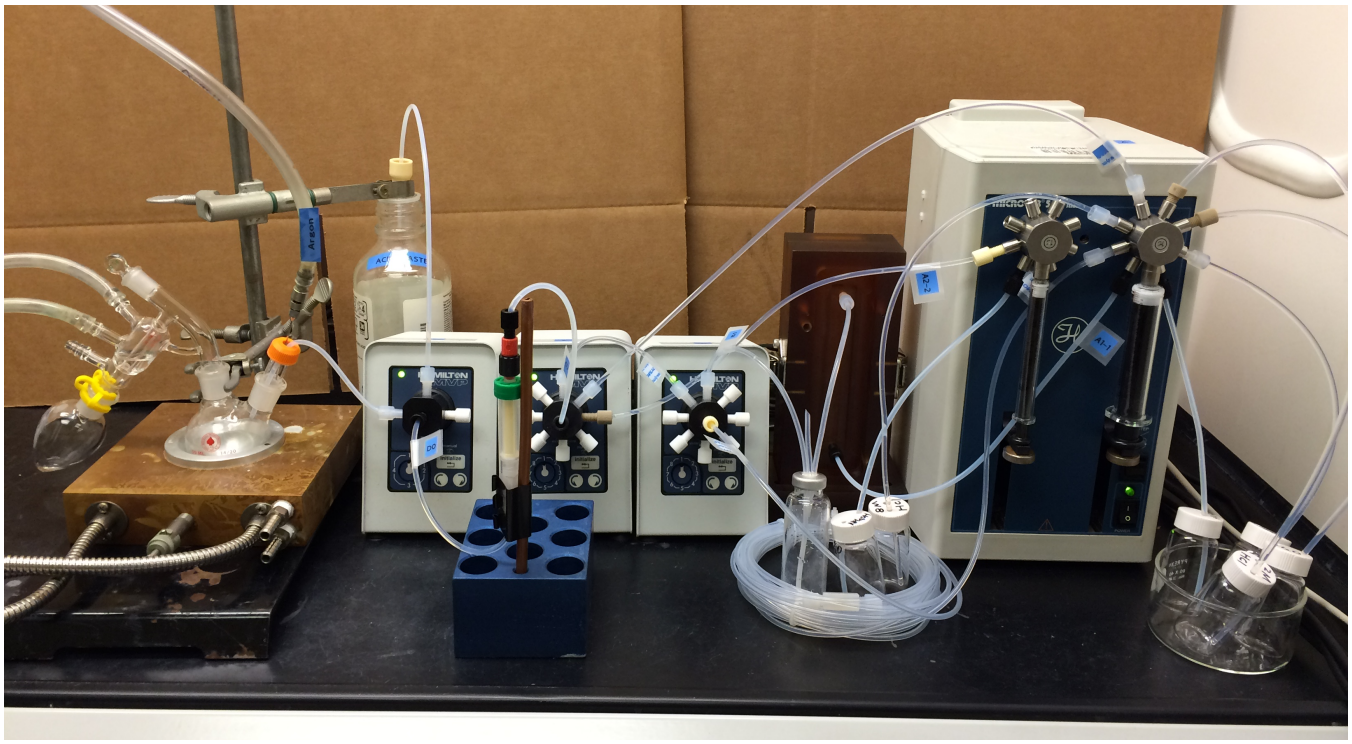


Figure 4: Photograph of automated system showing Hamilton dual syringe pump (run by computer), MVPs (3 boxes with valves in middle) and distillation setup (for removing NH_4OH from the eluted ^{211}At product)

Task 20: *Test automated ^{211}At isolation system performance*

A lot of the effort was directed at getting the modules, valves and other fluid moving parts connected and working as outlined. The Hamilton pump was used previously and apparently was not cleaned properly, as there was a problem with a multi-valve port pumping correctly. We ultimately had to order a new valve to replace the old one. Additional effort was put into programming the computer to conduct the functions needed in each fluid movement step. A period of time was spent to learn the Hamilton MICROLAB software so that the programming could be done. A method for priming, running and clean-up of the system was programmed. Another issue that had to be addressed was obtaining tubing connectors that could be used with the automated system. While this seems like a simple process, it proved to be fairly difficult to find the appropriate connectors. After these challenges were overcome, a number of pumping runs using colored water were made to make sure all of the valves and liquid transfers work properly. That was confirmed.

We felt it was important to test out the system where possible without using an irradiated target so problems could more readily be addressed, and to reduce costs. We first evaluated the speed and extent of removal of bismuth from cyclotron targets using this system. Matt O'Hara had optimized the flow in his target dissolution chamber, and determined that the fastest the bismuth could be removed was 45 min. We thought that the dissolution time might be decreased based on the fact that in the manual approach the dissolution is done in 10 min. Prior to running the automated dissolution, we evaluated the time it took for dissolution of 5g of bismuth metal in 8M, 10M, 12M and 15M HNO_3. Since we wanted to have as low a concentration of HNO_3 as possible, so that it did minimal damage to the column and the bismuth salts would be more soluble, we chose the lowest concentration that dissolved the

bismuth within 10 min. That was 10M HNO_3, which is the same concentration that Matt O'Hara used for the dissolution in his automation system.

In an initial bismuth dissolution study, 10 mL 10M HNO_3 was pumped through the dissolution chamber containing a bismuth target in 4-minutes. This appeared to remove most of the bismuth from the target. However, the resultant bismuth solution appeared cloudy, indicating that some bismuth was not fully dissolved. Therefore, a second 10 mL of 10M HNO_3 was passed through the dissolution chamber in another 4 minutes. The 2nd 10 mL of 10M HNO_3 was pumped into the previous 10M HNO_3 solution, resulting in a clear solution (i.e. no bismuth salts present). Importantly, the overall pumping time of 8 minutes was under our target time of 10 min. Subsequent automation runs included target dissolution and running the resultant bismuth solution over a PEG750 resin column. During this process, we noted some dripping from the PEG column holder during testing with water, so we purchased another column holder (see **Figure 5**) which appeared to provide much more secure and robust column housing and connections.

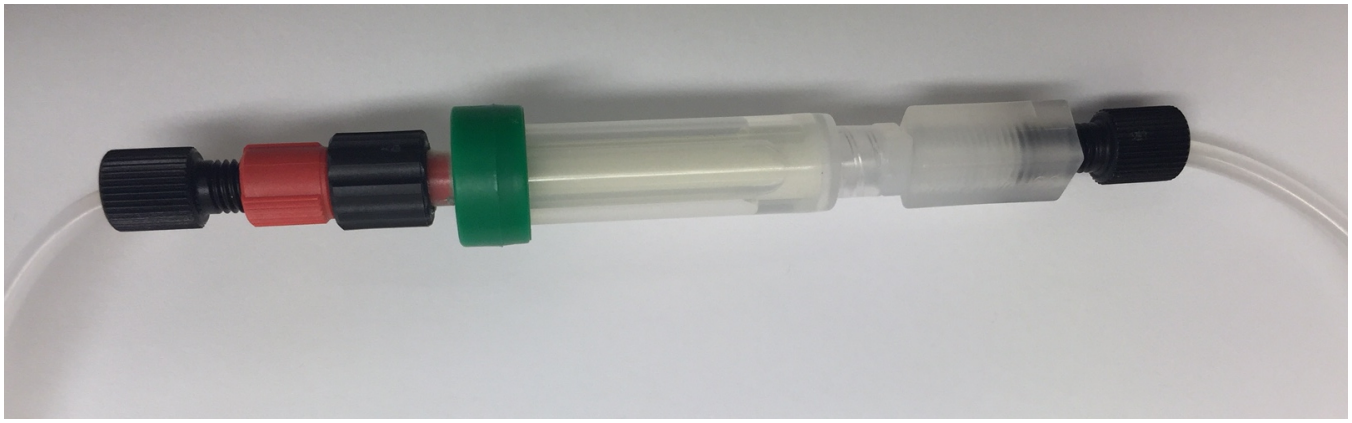


Figure 5: Photograph of Bio-Scale Mini Cartridge (obtained empty from BioRad) filled with mPEG-750 resin.

The amount of ^{211}At that can be obtained from an irradiated target using the faster flow rate (2.4 mL/min) on the Hamilton pump was evaluated. This was conducted to decrease the time it takes in the overall automated system isolation method. Thus, an irradiated target (18.3 mCi measured) was placed in the target dissolution chamber (from PNNL) and was dissolved with 2 x 10 mL of 10M HNO_3. In the first 10 mL, 12.47 mCi of ^{211}At was isolated. In the second 10 mL, 2.09 mCi was isolated, making a total of 14.56 mCi, ~80% isolated from the target (this amount is the same as recovered from a target using manual dissolution). The target was rinsed with 10 mL 2M HNO_3 and 10 mL water to give another 0.148 mCi (~1%). Measurement of the target itself showed that it had 0.07 mCi remaining (<1%). This was considered a very positive result.

A number of automation runs were conducted using non-irradiated targets to determine whether all components were functioning as desired. In one run, back-pressure was seen after loading the 20mL of 10M HNO_3 onto the PEG-resin column. It was thought that the concentration of bismuth salts was too high in the loading solvent, so higher dilution was used to decrease the back-pressure. After some initial runs were conducted (without the NH_4OH distillation step), it was decided that the overall computerized process should be modified before making a full run with an irradiated target. Thus, modifications in the programmed steps in the computer were undertaken. For example, changes in the programming was done to optimize the speed and operation time for each step on the Hamilton syringe pump. This

involved programming the computer and evaluating effects of changing flow rates and hold times. An outline of the modified isolation steps is provided below:

- Ran 2x10mL 10M HNO_3 dissolution step at 2.4 mL/min (the allowed slowest speed) to maximize the dissolution yield.
- Ran 2x10mL 10M HNO_3 solution through the PEG750 column at 2.4 mL/min (the allowed slowest speed) to prevent the build-up pressure and increase the yield of ^{211}At caught on the column.
- Ran 2x10mL 8M HCl, 2x10mL 2M HCl and 10 mL water washes at 5 mL/min to reduce the time of whole run without build-up of pressure on the column.
- Ran NH_4OH step at 0.6 mL/min (the allowed slowest speed) to increase the elution yield.
- Pause 3 min between two NH_4OH steps to allow more time for ^{211}At to equilibrate on the column with 15 M NH_4OH.

A second programming change optimized the length of tubing used to minimize residual volumes in the transfer of solvents. This involved changes where:

- Based on the sequence of a programmed step, the tubing was cut to make the shortest route between valves and the different components in the isolation process.
- Steps involving moving radioactivity into tubing were modified so that ^{211}At did not enter the syringe pump. The tubing length used was chosen by calculation of the tubing volume such that it fully contained the solution and not allow back-flow.

A third modification created a “pump prime” computer program that was run prior to actual run. An outline of the steps that were programmed include:

- Programmed steps to test the system and fill the lines before the ^{211}At target is mounted in the dissolution chamber to make sure the system is ready for the run (no leaks) and to save time.
- Programmed steps to fill the solvent lines from the solvent reservoirs.
- Programmed steps to fill the tubing connected to the mPEG750 column with 10M HNO_3 to prevent $Bi(NO_3)_3$ precipitation from clogging the tubing and column.

A fourth programming modification created a clean-up program to remove the acid and base solvents from the pumps and tubing, so that they were ready to run the next time without damage. The computer program steps in the clean-up process included:

- Passing 2M HCl through all tubing and valves connected to the target dissolution chamber to rid them of residual bismuth.
- Passing water through all tubing/valves in the system to rinse out acids and NH_4OH.
- Passing water through the Hamilton syringe pump system and MVP valves to remove acid or base and keep them from corroding.

A final computer programming modification created a heating/cooling program for the NH_4OH distillation step using the J-KEM digital controller.

- This required developing a new program on our laptop computer.
- Ramp from 20°C to 160°C for 10 min.
- Keep at 160°C for15 min.
- Cooling for 10 min.

After the modification steps were conducted, 3 runs of the full system were done using non-irradiated bismuth targets. The steps in the runs were as follows:

- Dissolve the cold target by 2x10 mL of 10M HNO_3 (2.4 mL/min)
- Pump the 10 M solution through the PEG750 column (2.4 mL/min).
- Wash the column with 2x10mL 10M HCl (5 mL/min), 2x10mL 2M HCl (5 mL/min) and 10mL water (5 mL/min).
- Elute column with 2x2 mL of 15M NH_4OH (0.6 mL/min).
- Evaporate 15M NH_4OH under 160°C for 15 min.
- Dissolve the residue of NH_4OH distillation flask in 10 mL of water.

A critical part of developing an automated system for ^{211}At isolation is to minimize the period of time it takes. To accomplish that each step was timed, including filling the syringe and moving the solvent through the tubing. The computer program's 25 steps are timed in the table below (without prime or clean-up). The steps include drawing the solvent into the syringe (shown as "in") and pushing it out of the syringe through the tubing (shown as "out"). Some steps are repeated due to using a smaller syringe than the volume required for the step. The reason for using a smaller syringe is that allows a slower rate of delivery (e.g. syringe pump has a minimum speed, so a smaller syringe delivers less volume per unit time). There are 2 syringes (A&B) that are controlled independently. Each syringe has 8 ports, so the ports from the same syringe are designated A-1 and A-8. We did not want the activity to enter the reusable glass syringes, so a holding loop was used for moving the HNO_3 from the target to the column.

Step#	Process	Syringe	Time(sec)
1	10 MHNO_3 in	A-8	25
2	10M HNO_3 out	A-1	250
3	10M HNO_3 in (#2)	A-8	25
4	10M HNO_3 out (#2)	A-1	250
5	HNO_3 into loop (#1)	A-2	25
6	HNO_3 out loop (#1)	A-2	250
7	HNO3 into loop (#2)	A-2	25
8	HNO3 out loop (#2)	A-2	250
9	8M HCl in (#1)	A-3	25
10	8M HCl out (#1)	A-4	120
11	8M HCl in (#2)	A-3	25

12	8M HCl out (#2)	A-4	120
13	2M HCl in (#1)	A-5	25
14	2M HCl out (#1)	A-4	120
15	2M HCl in (#2)	A-5	25
16	2M HCl out (#2)	A-4	120
17	H_2O in	A-6	25
18	H_2O out	A-4	120
19	NH_4OH in (#1)	B-1	25
20	NH_4OH out (#1)	B-2	250
21	pause		180
22	NH_4OH in (#1)	B-1	25
23	NH_4OH out (#1)	B-1	250
24	NH_4OH distillation		900
25	Cooling		600
			Total Time: 4055 sec, ~68 min

The overall time for running the automated system is longer than 68 min as it also includes the time it takes to get the irradiated target into the dissolution chamber and the time it takes to get the ^{211}At/NaOH residue dissolved. Thus, it is estimated that the overall process takes about 1.5 hours. While the process is not optimized, the estimated time is a significant improvement over the manual method which, depending on the person doing it, can take 2 to 3 hours.

One of the issues that we have faced with the automated wet chemistry approach using a PEG column for isolation of ^{211}At was a question of how much bismuth is present in each wash step. An evaluation was conducted dissolving a known amount of bismuth in 10M HNO_3 and using the PEG column to separate the bismuth. Below is a description of what was done.

- Dissolved 4.6g bismuth in 20 mL of 10M HNO_3. Pumped the solution through the PEG750 column (2.4 mL/min). Washed the column with 2x10mL 8M HCl (5 mL/min), 2x10mL 2M HCl (5 mL/min) and 10mL water (5 mL/min). Collected all of the column washed fractions. Neutralized the fractions with 1N NaOH solution. Filtered the white precipitate and dried it under vacuum, then weighed the dry precipitate.
- 1st 10mL 8M HCl (889 mg of precipitate)
- 2nd 10mL M HCl (9.5 mg of precipitate)
- 1st 10mL 2M HCl (0 mg of precipitate)
- 2nd 10mL 2M HCl (0 mg of precipitate)
- 10mL water (0 mg of precipitate)

This experiment clearly demonstrated that the wash steps were effective in removing virtually all of the bismuth from the column. To better assess the quantities of bismuth in the wash steps, ICP-MS was used. In that example, all of the steps in the isolation process in one of the runs with non-irradiated bismuth targets were evaluated by ICP-MS. Those included 20 mL of

10M HNO_3, 2x10mL 10M HCl, 2x10mL 2M HCl, 10mL water, 3.5 mL of NH_4OH distillation, 10 mL of NH_4OH distillation residue. The results from that study are shown in **Table 25**.

Table 25: Concentration of bismuth in each of the steps in the isolation of ^{211}At from a bismuth target using ICP-MS.

Sample Name	Bi concentration (ppb)	Volume (mL)	Bi mass (µg)
10 M HNO_3	281328207	20	5.62g
8 M HCl 1st	60790950	10	0.61g
8 M HCl 2nd	4016895	10	40 mg
2 M HCl 1st	404336	10	4 mg
2 M HCl 2nd	94322	10	943 µg
H_2O	20809	10	208 µg
NH_4OH bf	7321	4	29 µg
NH_4OH af	7789	3.5	27 µg

The data in **Table 25** clearly show that the vast majority of the bismuth is removed in the wash steps, but there is a small amount of bismuth in the final ^{211}At solution.

Additional ^{211}At isolation evaluation experiments were conducted using the Hamilton syringe-based automated system. The automated system was run as described previously. The automated setup had four vacuum traps placed in series to stop the vapors from the NH_4OH distillation being released inside of the glovebox.

In one experiment, an irradiated bismuth target having 18.5 mCi was dissolved by running 20 mL of 10 M HNO_3 over the aluminum-backed bismuth target. This removed all but ~24 uCi of the activity from the irradiated target. Thus, most of the activity was removed from the target and was moved to the “pressure release vessel” with <0.2% remaining on target. Following that, the ^{211}At was moved from the pressure release vessel into a loop of tubing so that the activity did not go into the syringe, then it was pushed onto the PEG column. This was repeated twice to get all of the 10M HNO_3 (20 mL) onto the column. The 10M HNO_3 that passed through the column was collected as the effluent in a waste bottle. To remove bismuth salts, the column was washed with 2 x 10 mL of 8M HCl, 2 x 10 mL of 2M HCl, then with 10 mL H_2O, and all of the effluents were directed into the waste bottle. Next, 4 mL of conc. NH_4OH was run on the column and collected in a distillation vessel. The collected NH_4OH solution was distilled to leave a residue. After the distillation, a second 4 mL of conc. NH_4OH was run over the column to determine if additional ^{211}At could be eluted. Activity measurements were made on liquids obtained. The results of the study are shown in **Table 26**.

We demonstrated that the target activity measured in a dose calibrator is attenuated by the bismuth metal, and a factor of 1.33x could be used to correct the readout values. Thus, a target reading of 18.5 mCi is actually closer to ~25 mCi. In the study above, we did not measure the amount of activity until after the isolation process was completed, so the ^{211}At activity was decay-corrected to a common time point, which we selected to be the middle

Table 26: Assessment of ^{211}At activity distribution using the automated system*

Activity	Activity adjustments for measured quantity		mCi	% of Total	% trapped	% Eluted
Activity reading in target	Activity adjusted for attenuation (x1.33) = 24.6 mCi		17.6	100%		
= 18.5 mCi	Activity adjusted for ~3.5 h decay (x0.7143) = 17.6 mCi					
Activity trapped on Column	Column Activity remaining after 2 NH4OH elutions		4.00	22.7%		
	Distillation Reciever		2.08	11.8%		
	Activity in 2nd NH4OH elution		0.97	5.5%		
	Activity in NH4OH traps		0.33	1.9%		
	Activity remaining in distillation flask		0.04	0.2%		
		Total	7.42	42.2%	52.5%	46.1%
Activity in setup (not column)	Activity remaining in Pressure Release Vessel		0.74	4.2%		
	Activity found in tubing rinse		1.82	10.3%		
		Total	2.56	14.5%		
Activity in Waste Bottle	5 mL = 0.336 mCi; est. 100 mL total volume		6.72	38%		
			Total =	94.9%		
				Overall Isolated Yield =		24.2%

*Values are approximate as they are based on estimates that include bismuth attenuation and decay time before measuring different components.

timeframe activity measurement. That time varied, but an estimate of ~3.5h decay time provided values that roughly added to the total activity. While the numbers in **Table 26** are not highly accurate, they do allow one to get an impression of the issues that had to be addressed in the automation procedure.

The results suggested that at least four issues needed to be addressed in subsequent experiments, including:

(1) Trapping of ^{211}At on column was not as efficient (~53%) as when using a manual operation (typically >90%)—a significant portion of activity went through the column and directly to waste.
(2) Elution of ^{211}At from the PEG column was also not as efficient (46%) as manual elution (~60-70%).
(3) The NaOH in the distilling flask was not adequate to keep the solution basic as the ^{211}At was obtained in the receiving vessel (i.e., the ^{211}At was distilled with H_2O).
(4) A lot of activity remained in the pressure relief vessel and in tubing.

It was hypothesized that the low trapping efficiency of the PEG column might be caused by having more than one species of ^{211}At present. To test this hypothesis, an experiment was conducted wherein the ^{211}At solution was mixed with a reductant ($SnCl_2$ in HCl) to assure that only astatide was present. This experiment was conducted as follows: A solution of 1g bismuth metal was dissolved in 10 mL of 10 M HNO_3 and a solution containing a small amount of ^{211}At was added to a solution of 1g $SnCl_2$ in 15 mL conc. HCl. That mixture was taken up into a syringe and the solution was passed over a PEG column (PEG750) slowly. The column was washed with 8M HCl (2 x 10 mL), then 2M HCl (2 x 10 mL). After that, 4 mL of conc. NH_4OH was passed slowly over the column. Of the ~269 μCi ^{211}At put onto the column, ~121 μCi (45%) passed through, and ~148 μCi (55%) was trapped on it. These results indicated that the reductant had not significantly altered the amount of ^{211}At that was trapped on the column. The elution with conc. ammonium hydroxide was somewhat better, as 90 μCi (61%) was recovered from the column.

Based on the shortcomings of the previous automation experiment, changes were made to the automated system and procedures used for isolation of ^{211}At based on the issues outlined above. No reductant was used in the experiment as the study above indicated that it made no difference in trapping of ^{211}At on the column. Thus, a modified automated ^{211}At experiment was conducted with an irradiated target containing 19.7 mCi ^{211}At (estimated 26 mCi corrected for Bi attenuation). The major changes were: (1) addition of a large quantity of NaOH in the NH_4OH distillation flask to prevent distillation of the ^{211}At, (2) reprogramming of the pump and valve so that the wash steps would flow through the pressure release vessel, tubing loop, and other tubing to rinse remaining ^{211}At to the column, and (3) changing back to an earlier geometry of the column format (see **Fig. 2**). The results obtained from the experiment are provided in **Table 27**. It is important to note that only ~31% of the ^{211}At was captured on the PEG column. This was again disappointing, particularly in the fact that the overall recovery was lower than in the previous ^{211}At run. However, the decreased amount of ^{211}At in the tubing and pressure release vessel, as well as increased recovery of ^{211}At from the PEG column was encouraging.

Table 27: Assessment of ^{211}At activity distribution using the automated system*

Activity	Activity adjustments for measured quantity		mCi	% of Total	% Trappe	% Eluted
Activity reading in target	Activity adjusted for attenuation (x1.33) = 26.22 mCi		21.63	100%		
= 19.72 mCi	Activity adjusted for 2h decay (0.8251) = 21.63					
Activity trapped on Colum	Column Activity remaining after 2 NH4OH elutions		2.42	11.2%		
	Redissolved At-211 in H2O (1st)		1.88	8.7%		
	Redissolved At-211 in H2O (2nd)		1.15	5.3%		
	Activity in 2nd NH4OH elution		0.58	2.7%		
	Activity in NH4OH traps		0.04	0.2%		
	Activity remaining in distillation flask		0.07	0.3%		
		Total	6.14	28.4%	30.8%	60.6%
Activity in setup (not colu	Activity remaining in Pressure Release Vessel		0.37	1.7%		
	Activity found in tubing rinse		0	0.0%		
		Total	0.372	1.7%		
Activity in Waste	10M HNO3 + 8M HCl		13.61	63%		
	8M HCl		0.15	1%		
	2M HCl		0.03	0%		
			13.79	63.8%		
			Total =	93.9%		
				Overall Isolation Yield		18.7%

*Values are approximate as they are based on estimates that include bismuth attenuation and decay time before measuring different components.

A listing of the problems that were encountered in this automated production run, and approaches taken to correct the problems, is provided as **Table 28**.

Table 28: Summary of problems and attempted solutions for the automated isolation of ^{211}At from irradiated bismuth metal targets

Problem in Previous Design	Solution in the New Experiment	Result[a]
^{211}At found in distillation receiver and subsequent NH_4OH traps (volatilized from collection vessel)	Collect the ^{211}At into a distillation vessel that contains a larger amount of NaOH	90% reduction in the receiver and traps (total)
Significant amount of ^{211}At remained on column	Speed of NH_4OH elution was slowed	58% reduction in ^{211}At remaining on column
^{211}At remained in pressure vessel	Re-routed rinse steps through the pressure relief vessel to recover remaining ^{211}At	53% reduction in ^{211}At remaining in pressure relief vessel
^{211}At remaining in tubing loop	Re-routed rinse steps through the loop to to recover remaining ^{211}At	Reduction in ^{211}At in tubing loop
^{211}At in the waste collection	Collected flow-thru, 8 M HCl, and 2 M HCl rinses separately to determine if the ^{211}At was not trapped on the column or if wash steps took it off	Discovered that ^{211}At was not binding to column and that HCl rinses were not eluting much ^{211}At. Much of the activity still did not bind to the column.
^{211}At capture on the column was low	Changed the column type back to "new column format" shown in Figure 2.	The change in column type did not increase capture of ^{211}At.

As shown in **Table 28**, we have found solutions to some of the problems with our automated system. However, the major problem of capturing all of the ^{211}At activity on the column was not solved by these two experiments. In reviewing our prior results, it appeared that this problem may arise from two possible sources brought about by changes in the procedure: (1) the column was conditioned using 10M HNO_3 prior to loading the ^{211}At (which is not usually done), or (2) the ^{211}At was put onto the PEG column in 10M HNO_3 (also not usually done as better results are obtained when the ^{211}At is in 8M HCl when put onto the column). We had no data regarding the effect of conditioning the PEG column with 10M HNO_3 versus 8M HCl. To evaluate these two variables, four manual experiments—using handheld syringes and no automation—were conducted. Because of a lack of availability of mPEG750 resin, these experiments were conducted using the mPEG2000 resin. All data obtained suggests that mPEG2000 and mPEG750 provide equivalent results, so this change was not considered a problem. The experimental procedures and results for these experiments are provided below, including percentages of the total radioactivity in each experiment. That number is based on the sum of the decay-corrected radioactivity that was measured in all elutions from the column, along with the column itself (not based on the direct measurement of the ^{211}At in the syringe that was used to load the ^{211}At onto the column).

Experiments A & B: Testing capture of ^{211}At in 8M HNO_3 when placed on columns conditioned with (A) 8M HCl or (B) 10M HNO_3

Experiment A

Procedure

1. In a 50 mL conical tube, mixed 20 mL 10 M HNO_3, ~6 g $BiCl_3$, and 81.6 uL of solution containing ^{211}At (measured radioactivity from dose calibrator = 787 uCi).
2. Prepared column with 10 mL 2 M HCl, then 10 mL 8 M HCl, then another 10 mL 8 M HCl, all at a quick rate.

3. Added 10 mL of the new solution containing ^{211}At from Step 1. Passed through at ~2 mL/min.
4. Rinsed column with 2x10 mL 8 M HCl, then 2x10 mL 2 M HCl, then 10 mL H_2O, all at ~4 mL/min.

Results

	^{211}At Quantity in uCi (% of total activity)
Flow-thru	187 (35.8%)
8 M HCl rinse	36 (6.9%)
2 M HCl rinse	0 (0.0%)
H_2O rinse	13 (2.5%)
Remaining on column	287 (54.8%)

Experiment B

Procedure

Same procedure as Experiment A; replaced the final wash in Step 2 with 10 M HNO_3. (The actual volume added from Step 1 of Experiment A was ~9.5 mL.)

Results

	^{211}At Quantity in uCi (% of total activity)
Flow-thru	187 (37.8%)
8 M HCl rinse	33 (6.7%)
2 M HCl rinse	0 (0.0%)
H_2O rinse	10 (2.0%)
Remaining on column	264 (53.5%)

Experiments C & D: Testing capture of ^{211}At in (C) 8M HCl or (D) 1:1 mixture of 10M HNO_3/8M HCl on a PEG column conditioned with 8M HCl

Experiment C

Procedure

1. In a 50 mL conical tube, mixed 10 mL 8 M HCl, ~3 g $BiCl_3$, and 40.8 uL of solution containing ^{211}At (measured radioactivity from dose calibrator = 418 uCi).
2. Prepared column following Experiment A, Step 2.
3. Added 10 mL of the new solution containing ^{211}At from Step 1. Pass through at ~2 mL/min.
4. Rinsed column following Experiment A, Step 4.

Results

	^{211}At Quantity in uCi (% of total activity)
Flow-thru	1 (0.2%)
8 M HCl rinse	0 (0.0%)
2 M HCl rinse	0 (0.0%)
H_2O rinse	0 (0.0%)
Remaining on column	615 (99.8%)

Experiment D

Procedure

1. In a 50 mL conical tube, mixed 5 mL 8 M HCl, 5 mL 10 M HNO_3, ~3 g $BiCl_3$, and 40.8 uL of solution containing ^{211}At (measured radioactivity from dose calibrator = 419 uCi).
2. Prepared the column following Experiment A, Step 2, replacing the final wash with 10 mL 1:1 (v/v) 8 M HCl:10 M HNO_3.
3. Added 10 mL of the new solution containing ^{211}At from Step 1. Pass through at ~2 mL/min.
4. Rinsed column following Experiment A, Step 4.

Results

	^{211}At Quantity in uCi (% of total activity)
Flow-thru	23 (3.8%)
8 M HCl rinse	5 (0.8%)
2 M HCl rinse	0 (0.0%)
H_2O rinse	36 (5.9%)
Remaining on column	544 (89.5%)[a]

These results provided data that clearly showed that there was no difference between the ^{211}At capture percentage if the column was conditioned (as a final step) with 8M HCl or with 10M HNO_3. The data also confirmed a very high capture percentage when the ^{211}At was loaded in an 8M HCl solution. Interestingly, the data also showed that a 1:1 mixture of 8M HCl and 10M HNO_3 could be used to greatly improve the ^{211}At capture percentage. Since HNO_3 provides adequate H^+ to the dissolved target solution, we assume that the presence of a certain minimum concentration of Cl^- ions can dramatically improve the binding of ^{211}At to a PEG column. The use of a mixture of HNO_3/HCl provides a way to alleviate the time-consuming step of distillation of HNO_3, while still obtaining reasonable ^{211}At capture on a PEG column.

VI. Specific Objective 5: Training of students and Postdoctoral Fellows in radiochemical methods involved in the production and automation of ^{211}At isolation process and in radiolabeling biomolecules with ^{211}At.

This specific objective was accomplished by providing training to graduate students and postdoctoral students in: (1) handling of radioactive materials, (2) design and testing of components of an automated system for isolation of ^{211}At, (3) preparation of bismuth targets, (4) use of analytical instruments for detection of radioactive materials, (5) methods for separation of ^{211}At, (6) methods for labeling biomolecules with ^{211}At, and (7) analytical methods for determining purity and specific activity of ^{211}At-labeled molecules

A Postdoctoral Fellow, Dr. Katie Gagnon conducted some of the early studies to determine if PEG-coated solid support columns could be used for isolation of ^{211}At from the bismuth target. A Visiting Scholar, Dr. Shigeki Watanabe, from the Japan Atomic Energy Agency, Takasaki, Japan, assisted in the studies. Also, a Postdoctoral Fellow, Dr. Ethan Balkin, assisted in the studies. Dr. Balkin and Dr. Watanabe prepared bismuth targets, conducted isolation of ^{211}At by the wet chemistry approach, and participated in the evaluation of PEG-coated solid support columns. Another Postdoctoral Fellow, A. Lake Wooten, was involved in the studies with the automated system from Jan. 2016 to June 30th.

Dr. Ethan Balkin, transitioned from being a Postdoctoral Fellow to an Assistant Professor in my department. Donald Hamlin, a Research Scientist, conducted some of the studies with Dr.

Balkin. Dr. Balkin has taken a position with the DOE in their headquarters at Germantown, Maryland.

An undergraduate student, Corey Burden, helped with making the bismuth targets and distilling the diisopropyl ether prior to isolation of ^{211}At by liquid/liquid extraction. Corey left our program to be part of the summer radiochemistry course (as an assistant) and began graduate school (Chemistry) at the University of Pennsylvania. We have hosted Matt O'Hara and his research assistant Anthony (AJ) Krazysko in collaborative studies (total of 7 studies) at UW. Mr. Krazysko has learned a lot in our collaboration and is very interested in continuing studies with ^{211}At.

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