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Title: A systematic investigation of PET Radionuclide Specific Activity on Miniaturization of Radiochemistry

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Notice: There is no protected data or patentable material in this report.

Executive Summary:
The PET radionuclides, ^{18}F and ^{11}C consist of very high radiation to mass amounts and should be easily adapted to new technologies such as “chip chemistry” with nanofluidics. However, environmental contamination with nonradioactive fluorine, carbon and other trace contaminants add sufficient mass, micrograms to milligrams, to prevent adapting PET radiochemistry to the nanochip technologies. In addition, the large volumes of material required for beam irradiation make it necessary to also remove the ^{18}F and ^{11}C from their chemical matrices. These steps add contaminants. The work described in this report was a systematic investigation of sources of these contaminants and methods to reduce these contaminants and the reaction volumes for radiochemical synthesis. Several methods were found to lower the contaminants and matrices to within a factor of 2 to 100 of those needed to fully implement chip technology but further improvements are needed.

Project Objectives (taken from funded proposal):
The use of positron emission tomography (PET) is growing. Both ^{11}C and ^{18}F radio-pharmaceuticals are used for clinical research and as noninvasive probes of biochemistry in animals and humans. Until recently, high specific activity (SA) has been a requirement for only some human research probes. Now "ultra-high" specific activities for ^{18}F and ^{11}C are needed for small animal imaging and for new PET microsynthesis techniques including microfluidics and nanotechnology. These "ultra-high" SAs are not now obtained except in a very few select laboratories. Twenty years ago it was difficult to achieve "ultra-high" SAs because the state of the art in materials and the sensitivity of analytical techniques was in the ppm to high ppb range. Now supplies, chemicals and analytical techniques are achieving and detecting sub-ppb concentrations. Today rigorous, but not specialized, techniques should result in levels of mass in sub-ppb concentrations for PET radionuclide and radiopharmaceutical production. The work proposed in this application will conduct tests to bring ^{11}C and ^{18}F specific activity to ultra-high levels and to study the effect of ultra-high SA on the miniaturization of radiochemical synthesis for PET imaging and research for use in animal experimentation and human studies.

The proposed work begin with investigation of the effect of supplies and target conditions on the SA from cyclotron radionuclide production and proceed through miniaturization of chemistry to build a systematic knowledge of the causes that reduce SA. Model ^{18}F and ^{11}C compounds will be studied. These model compounds cover a range of chemical complexity to determine the effects of higher specific activity on chemistry and radiolysis in small volumes. The overall aim of this proposed work is to clearly delineate simple achievable methods to improve specific activity for ^{18}F and ^{11}C so that the masses of materials can match new radiosynthesis technologies. The ultimate objective is to define conditions so that most laboratories will be able to achieve sufficiently high SAs to develop highly sensitive probes that are at "tracer" levels, even for mice, without perturbing the biochemical system. Our goal is to increase SA by more than an order of magnitude for both ^{18}F and ^{11}C.

In summary, PET imaging and new synthesis techniques will benefit from a systematic study of the sources of carrier C and F as well as small volume geometry-dependent radiochemistry studies of radiolysis at greatly increased SA and the capacity to improve peptide reactions using microscale volumes.

Hypotheses:
- For PET the specific activity of ^{18}F and ^{11}C can be increased by decreasing the amount of carrier ^{19}F and ^{12}C by a factor >10 to 100 to match the need for small animal studies.
- With higher SA, practical and widely achievable criteria can be defined for accelerator beam quality, targets and radiosynthesis systems so that specific activities will come closer to theoretical and be more reliable.
- PET radiochemistry can be reduced in volume by a factor of 10 to 100. As a result, radiosynthesis units can be simpler, smaller, and less prone to failure than the commercial systems in use.
- Reduction in the masses of reactants and solvents for peptide and other syntheses will result from improved specific activities of ^{11}C and ^{18}F. As a result, the PET radio-pharmaceutical masses will better match small animal biochemical probes for imaging.
- Miniaturization of radiosyntheses for ^{18}F labeled peptide will lead to radiation and chemical damage that is increased when compared with larger scale reactions. Both synthesis conditions and the site of peptide labeling can be designed to maintain the biological integrity of the product in small volume reactions.

Although specific activity has been studied by many investigators, there has not been a complete study from source materials through target beams to chemical reaction conditions to account for the >1000-fold deviation from theoretical specific activity for ^{18}F and ^{11}C. This will be a systematic research effort without the goal of short-term commercialization, although it is likely that this research will eventually lead to new products. As new and substantially improved technology is developed, we will work with industry, consistent with Bayh-Dole legislation, to license our methods so they are widely available to the PET community.

Specific Aims

Three specific aims will test these hypotheses. Aim 1 will focus on the sources of carrier carbon and fluorine in target reagents, transfer lines and target material and as a function of beam quality for ^{18}F and ^{11}C. Once Aim 1 is accomplished, Aim 2 will test the effect of ultra-high SA on the ability to miniaturize volumes and radiosynthesis of model ^{18}F and ^{11}C radio-compounds. Aim 3 will test the effect of ultra-high specific activity and radiolysis effects on a model peptide, [^{18}F]-annexin V.

Aim 1. Determine experimentally the sources of carrier fluorine and carbon from targets by measuring the masses of these elements in production gases and $H_2{}^{18}O$, target transfer lines, and multiple targets with different beam alignments and focus. Other metals and impurities from target recoil chemistry will also be measured. HPLC-MS, GC-MS and ion chromatography will be used to gain analytical sensitivity for this work.

Aim 1a. Test the sources of carrier F for ^{18}F production from the ^{18}O water, purified [^{18}O]-water, transfer tubing, fittings, and target materials.

Aim 1b. Test the sources of carrier C for ^{11}C production from N_2/O_2 gas, gas purifiers, transfer tubing, fittings, and target materials.

Aim 1c. Test SA using multiple targets and beam conditions and using the materials from aims 1a and b that resulted in the least carrier for production of ^{11}C and ^{18}F. The anticipated critical variable will be in target radiochemistry.

Aim 2. Determine experimentally the minimal reaction volumes and masses of reactants needed for PET radiochemistry for several model reactions at ultra-high specific activities. The geometry for the miniaturized reaction will be correlated to radiolysis and compound stability. The conditions from Aim 1 that give the best specific activity will be used for this work.

Aim 2a. Test three methods to concentrate ^{18}F from ^{18}O water and determine the effect, if any, of these methods on ^{18}F specific activity.

Aim 2b. Evaluate the effect of high specific activity of ^{18}F on the miniaturization of the syntheses of 2-fluoro-2-deoxyglucose (FDG), fluorothymidine (FLT), fluoroestradiol (FES), and the protein annexin V (FAN), labeled by both site specific and nonspecific methods. The priority will be to minimize molar amounts of reactants and volume. Reaction conditions including temperature, time, and pressure will also be evaluated as factors in radiochemical yield, reliability and product SA.

Aim 2c. Test the effect of high specific activity for the model ^{11}C reactions; benzoic acid, thymidine, and meta-hydroxyephedrine as examples that use the precursors $^{11}CO_2$, ^{11}CN and $^{11}CH_3I$ respectively. The minimum volume for trapping of $^{11}CO_2$, ^{11}CN and $^{11}CH_3I$ will be determined. Then the minimum reaction conditions for synthesis of each product will be determined. The priority will be to minimize molar amounts of reactants and volume. Reaction conditions including solvents, temperature, time and pressure will also be investigated, again with the goal of maximizing yield, reliability and SA.

Aim 3. Evaluate the effect of radiolysis from miniaturization and ultra-high specific activity on the stability of [^{18}F]-annexin V and on biological function evaluated using a validated membrane-binding assay. While these experiments are important, they are of lower priority than Aims 1 and 2

Overview of the actual accomplishments versus the proposed accomplishments:

Details of the project are given below. Aim 1 was tested as proposed. Aim 2a was tested but none of the methods tested were optimal and further methods are under investigation using new funding. Aim 2b was modified because the work in aim 2a was found to be the limiting element of the work. We did test the labeling for FDG, and fluorobenzoic acid, the precursor for the FAN protein labeling but the chemistry for Aim 2b worked well and the limitation was the lack of a solution for concentrating ^{18}F from ^{18}O water by a method that reliably gave pure, high specific activity anhydrous product. Aim 2c was tested for several reactions including CN trapping and CH_3I and CO_2 trapping. The molecules that were tested were changed somewhat because mHED and benzoic acid are not very radiation sensitive. A radiation sensitive compound, doxorubicin, and several methyliodide compounds, notably verapamil and quinuclidinyl benzilate were tested. Thymidine is still under development but is not a molecule that requires high specific activity. We were able to test the trapping of ^{11}CN and the chemistry of the cyanide was less perturbed by the environmental contaminants than was methyliodide so we focused on the more commonly used methyl iodide. Because we were unable to find a solution for Aim 2a we were not able to proceed to Aim 3.

Summary of Progress on the specific aims

Because the aims can be divided best by separating ^{18}F work and ^{11}C work the results for ^{18}F are presented first and ^{11}C second.

Production of high specific activity F-18

Aim 1. Determine experimentally the sources of carrier fluorine

First we had to develop ultrasensitive methods for quantifying ^{19}F. We examined ion-selective electrodes for measurement of fluoride mass, colorimetric methods and ion chromatography. The greatest sensitivity was found with ion-surpressed ion chromatography. The ion chromatography also gave the advantage that we could evaluate other anions in solution that could contribute to mass that interfered with miniaturization of the radiochemistry. We started with a 4 mm ID Dionex anion exchange column and then switched to a 2 mm ID column in order to increase sensitivity by decreasing peak broadening. The methods were: Dionex brand AS12A anion exchange column 2 mm ID X 200 mm with suppressed conductivity detection. A mixed carbonate 2.7 mM sodium carbonate and 0.3 mM sodium bicarbonate mobile phase was used as recommended by the manufacturer. We were able to establish good standard curves (figure 1) that were relatively stable over time (figure 2) but for high sensitivity a

full calibration curve was run each day to maintain accuracy for measuring the solutions. We were able to measure down to approximately a 10 microliter injection of 4 microgram/mL (40 ng = 2 nmol). This is a concentration that would be consistent with micro to nanofluidics. Theoretical specific activity of ^{18}F for 500 mCi is 0.3 nmoles; our concern was unintentional carrier added from reagents used in the chemistry.

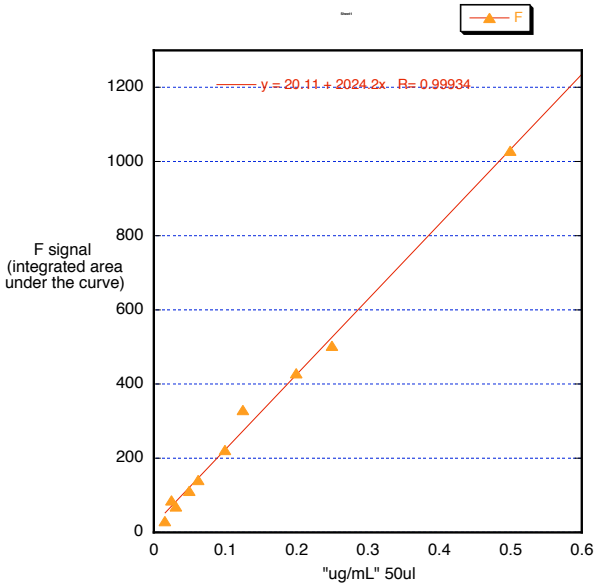

Figure 1: Carbonate-supressed ion chromatography calibration curve for fluoride.

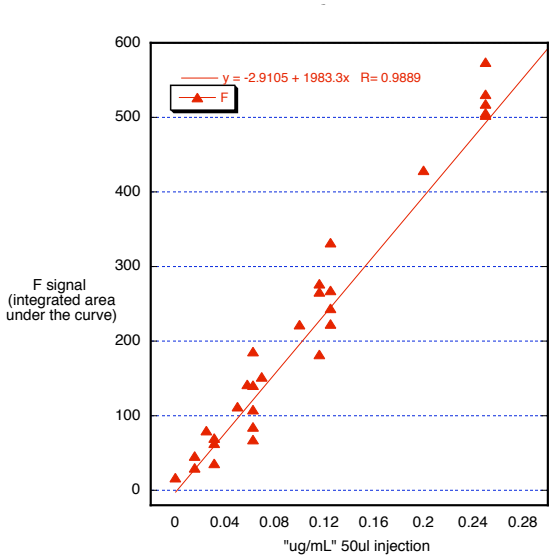

Figure 2. Calibration curves over several days of experiments showed reproducibility.

We tested for ^{19}F carrier in multiple materials and components that are needed for production of ^{18}F. The greatest source of carrier is from some types of tubing and the

chemicals used in synthesis. The contributions of carrier fluoride were the greatest for Halar and other Teflon tubing, which was not surprising (Figure 3). Stainless steel is the material of choice for minimizing carrier ions but it may affect the fluoride chemistry later in synthesis. The limiting amounts of ions for miniaturization of ^{18}F radiochemistry in the tubing were chloride and nitrate. Halar is the most radioresistant of the polymer tubes but it was the worst for introducing anion contaminants.

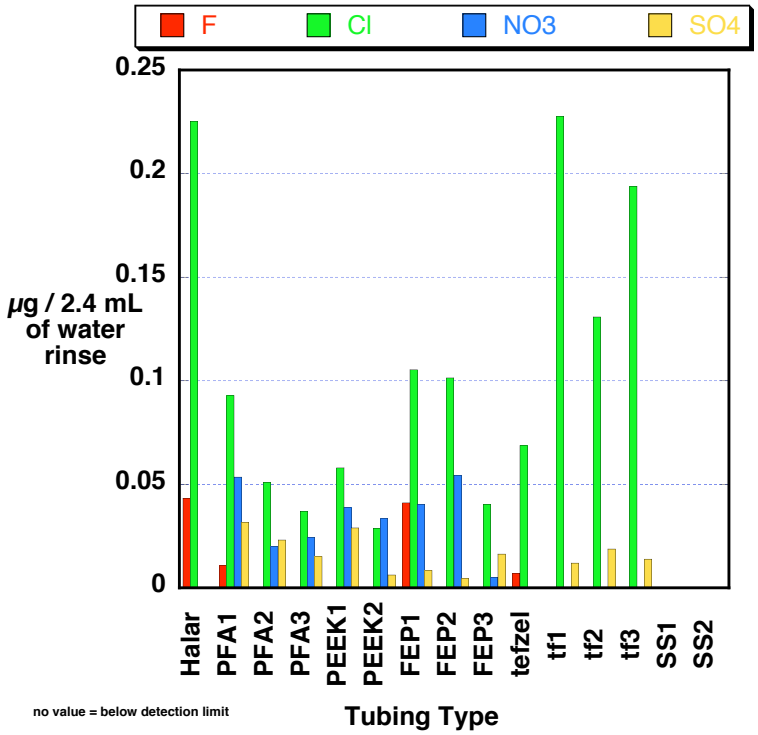

Figure 3. Measurement of ions in 2.4 mL of water (target volume) after passing the water through different types of tubing. Multiple ions were assayed. Halar is a chlorofluorocarbon. PFA, FEP, Tefzel and Teflon (tf) are all chlorofluorocarbon forms of Teflon. SS is stainless steel. Bars are colored by ion; fluoride – red, choride-green, nitrate-blue and sulfate-yellow. Stainless steel had no measurable anions and is the tubing of choice. Fittings and target materials were tested pre- and post-irradiation. In general the ^{18}O-water used for irradiation was very clean pre-irradiation and would be compatible with miniaturization. However with irradiation chloride came out of the target / tubing and valve components. Stainless steel decreases most of the chloride but the nitrate results from radiolysis of aerated water during the irradiation. While nitrate might be removable by long time inert gas sparging of the target water, it is probably best removed after irradiation (Figure 4).

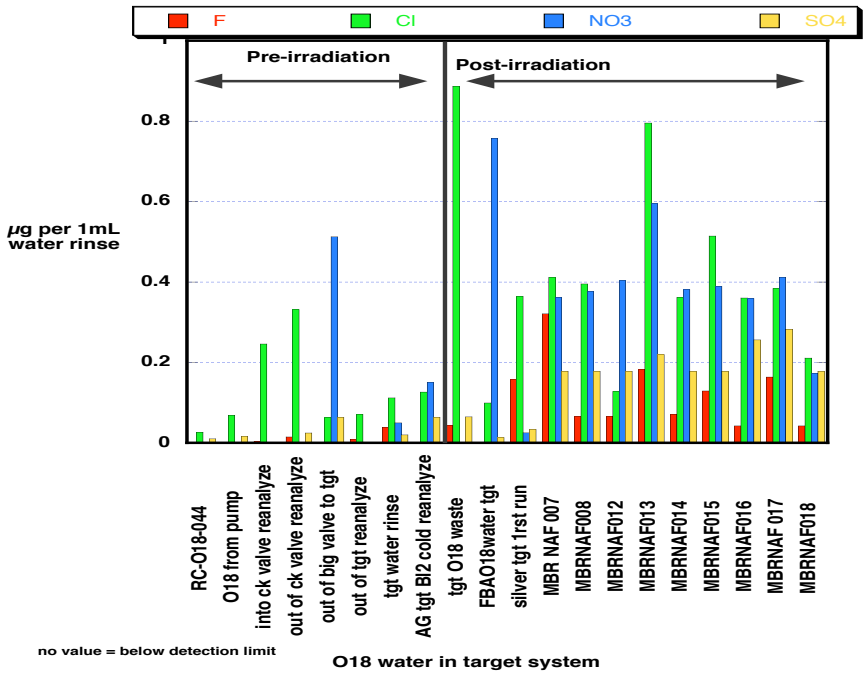

Figure 4. Ions in the target system pre- (left of black line) target and pre-irradiation and post-target / post-irradiation (right of black line). Note the high concentrations of nitrate. The nitrate pre-irradiation from the main valve was probably trapped from a previous run. O-18 denotes water. MBRNAF stands for different fluoride irradiations.

From these studies, we optimized the system to have less than 7 nmoles (130 ng of carrier F) after passing a full 2.4 mL target volume of irradiated water through PEEK tubing. However, we were unable to reduce the nitrate or chloride post-irradiation. The irradiated water contains very little fluoride but has a large amount of NO_3^- from radiolysis. The level in 2.4 mL target water that we measured is about 30 nmoles = 2 microgram. Although nitrate is not generally considered in miniaturization, this may become a limiting factor for miniaturization. Chloride was also ubiquitous in the water at about the same levels post-irradiation. We then considered the next steps in radiochemical syntheses that might allow us to remove the nitrate.

We also studied the amounts of ions in the solvents and chemicals used in typical ^{18}F nucleophilic displacement syntheses. The results are shown in figure 5. When it is possible, acetonitrile is the solvent of choice.

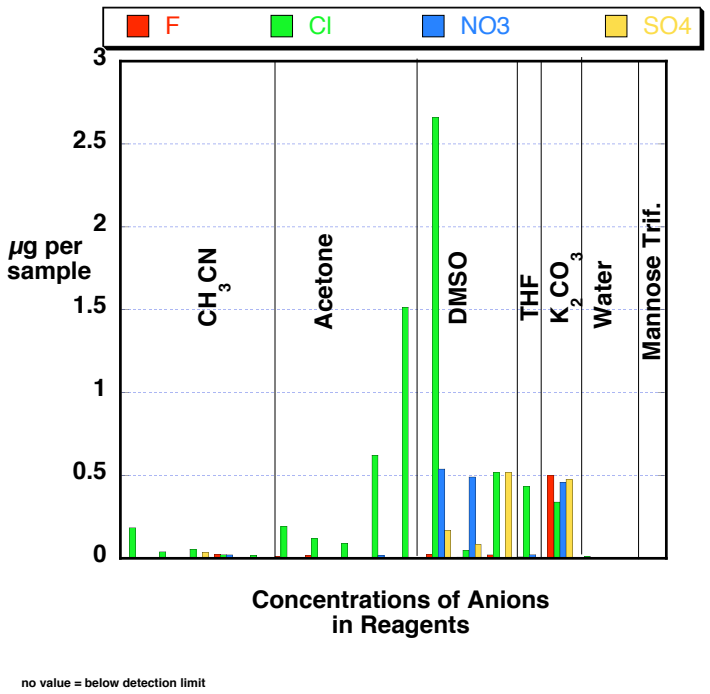

Figure 5. Concentrations of anions in reagents used in typical nucleophilic ^{18}F radiosyntheses. Potassium carbonate or bicarbonate (not shown) is used in anion exchange to concentrate ^{18}F from the O-18 water prior to making the solution anhydrous for nucleophilic substitution reactions. Several reagents were tested for anions. F was found only in K_2CO_3. Variable levels of Cl were found in different acetone samples, DMSO, THF and K_2CO_3. Nitrate and sulfate were found in K_2CO_3 and DMSO. Three mL samples of solvents were evaporated to dryness and the residual was dissolved in 1 mL of water for these assays. K_2CO_3 was 0.04 M in water. Mannose triflate was 8 mg of the commercial product added to 1 mL of water. All solvents were spectrophotometric grade or anhydrous. Potassium carbonate was 99.995+% from Aldrich dissolved in sterile water for injection. All other water was distilled / deionized.

Once we had fairly clean materials the challenge for miniaturization of ^{18}F is the removal of water. We have tried several methods to separate the fluoride from the target water for synthesis. Ion exchange adds extensive amounts of carrier fluoride even when we reduce the amount of ion exchanger. We have also tried several methods to evaporate the water leaving the fluoride.

From our results we were able to work on Aim 2 regarding methods of miniaturization. We focused on miniaturization of the radiosynthesis of fluorobenzoic acid and fluorobenzaldehyde as model reagents for labeling peptides.

We have worked on:

1. Ion exchange using different forms of dilute potassium carbonate combined with acetonitrile to minimize carrier ^{19}F, and potassium bicarbonate. This elution typically involves 0.5 mL of water/MeCN. With dilute potassium carbonate elution we can recover quantitative ^{18}F with as low as 4 micromoles of $KHCO_3$ or K_2CO_3 but that is a large amount of mass ~500 micrograms.

We have been able to reduce the precursor needed for the FBA reaction, ethyl 4-trimethylammonium benzoate trifluoromethanesulfonate salt, from the common literature recommendation of 15.5 mg to 30 micrograms which, while a factor of 500 improvement, is still a factor of 10 – 100 greater than our goal for miniaturization. The problem with this method has been that it is difficult to automate the evaporation to prevent over-drying of the Kryptofix [2.2.2] $K^{18}F$ complex. If the reaction goes completely dry, the ^{18}F becomes entrained in the salts and the reaction yield is greatly diminished. This is where the challenge remains. We need to separate the ^{18}F from the water by some type of extraction from the salts that doesn't add contaminants. The nucleophilic synthesis once we have anhydrous ^{18}F seems goes well and thus far, is not radiation sensitive.

2. Drying in a small volume without the use of ion exchange. We can empty and dry a target volume of water in 5 minutes at one atmosphere pressure pushing it out of the target. We are working to improve this with vacuum at the receiving end. We have been able to bring nucleophilic F-18 syntheses down in volume to a few microliters but we have not yet been satisfied with miniaturizing the radiosynthesis of our protein-labeling precursor, ^{18}F-FBA, at low mass amounts (high specific activity) by this method because it is too unreliable for drying. We have tried a nebulizer to speed up drying, analogous to ion spray in a mass spectrometer, but the rate was slower than bulk drying.

At the end of the DOE project, this is as far as we have progressed with miniaturization. We have come close to our goals but with unreliable results. We did not attempt to do a peptide synthesis (Aim 3) but if we can miniaturize the FBA synthesis the rest of the steps in peptide labeling should be straightforward. We have found additional funding since the end of the grant to continue this work. We have initiated other separation methods to increase the robustness of the radiochemistry to make the ^{18}F anhydrous for nucleophilic substitution. We are currently working in two areas. Rapid separation of the ^{18}F from water using electrodes and more reliable methods for evaporation of the water in acetonitrile to make the ^{18}F anhydrous.

With regard to Carbon-11 chemistry.

We have found that the sources of the CO_2 are primarily from the target gas and leaks in the gas system, including leaks in high purity regulators and in the chemical traps. These findings were not surprising. There has been one unexpected finding; even gas impermeable tubing can function as a sink / source for carrier CO_2. When we conducted our experiments to infuse dry $^{11}CO_2$ into a target and to let it sit in the target and then remove the radioactivity after different amounts of time, we found that the ^{11}C would remain not only on the target walls but also on the walls of the tubing if it was PEEK but not if it was stainless steel, such as the valve walls. This may be due to moisture and a drying trap should be kept on lines as well as CO2 traps to remove any carrier CO2 prior to entry into the target. But the reason for this finding, understanding the storage of CO_2 on tubing continues because will have a major impact on specific activity.

With flushing of stainless steel lines very carefully with dry / pure inert gas we have been able to bring our specific activity from the commercial CTI target to 60,000 Ci/mmol at saturation for a 55 uA run. This is not as high (100,000 Ci/mmol) as we have achieved on our always sealed target on our Scanidtronix cyclotron. This is an increase of a factor of about 10 over the CTI target specific activities prior to this grant work. The

improvements required only simple but carefully implementable methods of traps and stainless steel and check valves. This result translates to 10 nmoles of CO_2 in the target, a level of carrier that is acceptable for animal work. However, the systems beyond the target are also sources of carrier and we have found that the ^{11}C precursor systems, notably, $^{11}CO_2$ cold traps and a methyl iodide synthesis unit have to be flushed extensively and chemical traps heated prior to use to maintain this level of purity.

We can trap and release the entire target gas by cold trapping as CO_2, or methyl iodide on very small volume surfaces without need of a chemical trap. We can trap a complete target load of ^{11}CN in ~ 0.015 mL of liquid, depending on flow rate. For this project we performed multiple radiochemical reactions in small volumes down to 10 µL total volume.

We have also found more funding for ^{11}C and are continuing the experiments to further improve specific activity and to reduce reaction volumes.

Publications

We have no manuscripts at this time but the most appropriate place for publication of these results is the Workshop on Targetry and Target Chemistry. We intend to submit abstracts to the next meeting on the C and F specific activity work; the abstract deadline is April 30, 2012. We will provide copies of all of the manuscripts that result from this work to DOE as the foundation for all of our miniaturization was the funding from DOE.

Patents

At this time we have no patentable materials or technology transfer that resulted from this project.