Production and isolation of ^{72}As from proton irradiation of enriched $^{72}GeO_2$ for the development of targeted PET/MRI agents

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Introduction

Two current major research topics in nuclear medicine are in the development of long-lived positron-emitting nuclides for imaging tracers with long biological half-lives and in theranostics, imaging nuclides which have a chemically analogous therapy isotope. As shown in TABLE 1, the radioisotopes of arsenic (As) are well suited for both of these tasks with several imaging and therapy isotopes of a variety of biologically relevant half-lives accessible through the use of small medical cyclotrons. The five naturally abundant isotopes of germanium are both a boon and challenge for the medical nuclear chemist. They are beneficial in that they facilitate a wide array of producible radioarsenic isotopes. They are a challenge as monoisotopic radioarsenic production requires isotopically-enriched targets that are expensive and of limited availability. This makes it highly desirable that the germanium target material is reclaimed from arsenic isolation chemistry.

	$t_{1/2}$	Production reaction (% nat. abun.)	Application
^{70}As	53 min	$^{70}Ge(p,n)$ (20.4 %)	PET
^{71}As	65.3 h	$^{70}Ge(d,n)$ (20.4 %)	PET/SPECT
^{72}As	26.0 h	$^{72}Ge(p,n)$ (27.3 %)	PET
^{73}As	80.3 d	$^{73}Ge(p,n)$ (7.8 %)	Auger therapy
^{74}As	17.8 d	$^{74}Ge(p,n)$ (36.7 %)	PET
^{76}As	26.4 h	$^{76}Ge(p,n)$ (7.8 %)	β^- therapy

TABLE 1. Properties of radioarsenic isotopes

One major factor which has limited the development of radioarsenic has been difficulties in its incorporation into biologically relevant targeting vectors. Previous studies have labeled antibodies and polymers through covalent bonding of arsenite (As(III)) with the sulfydryl group[1,2,3]. Recent work in our group has shown the facile synthesis and utility of superparamagnetic iron oxide nanoparticle- (SPION-)bound radioarsenic as a dual modality positron emission tomography (PET)/magnetic resonance imaging (MRI) agent[4].

Presently, we have built upon previous studies producing, isolating, and labeling untargeted SPION with radioarsenic[4,5]. We have incorporated the use of isotopically-enriched $^{72}GeO_2$ for the production of radioisotopically pure ^{72}As. The bulk of the $^{72}GeO_2$ target material was reclaimed from the arsenic isolation chemical procedure for reuse in future irradiations. The ^{72}As was used for ongoing development toward the synthesis of targeted, As-SPION-based, dual-modality PET/MRI agents.

Material and Methods

Targets of ~100 mg of isotopically-enriched $^{72}GeO_2$ (96.6% ^{72}Ge, 2.86% ^{73}Ge, 0.35% ^{70}Ge, 0.2% ^{74}Ge, 0.01% ^{76}Ge, Isoflex USA) were pressed into a niobium beam stop at 225 MPa, covered with a 25 µm HAVAR containment foil, attached to a water-cooling target port, and irradiated with 3 µA of 16.1 MeV protons for 2–3 hours using a GE PETtrace cyclotron. After irradiation, the target and beam stop were assembled into a PTFE dissolution apparatus, where the $^{72}GeO_2$ target material was dissolved with the addition of 2 mL of 4 M NaOH and subsequent stirring. After dissolution was completed, the clear, colorless solution was transferred to a fritted glass column and the bulk $^{72}GeO_2$ was reprecipitated by neutralizing the solution with the addition of 630 µL $[HCl]_{conc}$, filtered, and rinsed with 1 mL $[HCl]_{conc}$. To the combined ^{72}As-containing filtrates, 100 µL 30% H_2O_2 was added to ensure that ^{72}As was in the nonvolatile As(V) oxidation state. The ~3 mL solution was then evaporated at 115 ˚C while the vessel was purged with argon, followed by a second addition of 100 µL H_2O_2 after the volume was reduced to 1 mL. When the filtrate volume was ~0.3 mL, the vessel was removed from heat, allowed to cool with argon flow, and the arsenic reconstituted in 1 mL $[HCl]_{conc}$ and loaded onto a 1.5 mL bed volume Bio-Rad AG 1×8, 200–400 mesh anion exchange column preconditioned with 10 M HCl. The radioarsenic was eluted in 10 M HCl in the next ~10 mL, with 90% of the activity eluting in a 4 mL fraction. The column was then eluted with 5 mL 1 M HCl. The ^{72}As-rich

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10 M HCl fraction was reduced to As(III) with the addition of ~100 mg CuCl, and heating to 60 ˚C for 1 hour. The resulting $AsCl_3$ was then extracted twice into 4 mL cyclohexane, which were combined and back extracted into 500 µL of water as $As(OH)_3$.

This solution of ^{72}As in H_2O was then used directly to label SPION and for subsequent experiments conjugating ^{72}As-SPION with TRC105, an angiogenesis-marking monoclonal antibody (MAb) targeting endoglin/CD105. Several methods were initially attempted involving directly conjugating the surface-modified SPION to the MAb through a polyethylene glycol (PEG) linker. More recent studies have investigated the radioarsenic labeling of SPION encapsulated in hollow mesoporous silica nanoparticles (SPION@HMSN) and its subsequent conjugation to TRC105.

Results and Conclusion

Irradiation of pressed, isotopically-enriched $^{72}GeO_2$ resulted in a production yield for ^{72}As of 17 ± 2 mCi/(µA·hr·g) and for ^{71}As of 0.37 ± 0.04 mCi/(µA·hr·g), which are 64 % and 33 %, of those predicted from literature[6], respectively. However, these production yields are in agreement with those scaled from observed production yields using analagous $^{nat}GeO_2$ targets. The end-of-bombardment ^{72}As radionuclidic purity can be improved by minimizing the $^{72}Ge(p,2n)^{71}As$ reaction by degrading the beam energy. A 125 µm Nb containment foil would degrade impinging protons to 14.1 MeV and is predicted to reduce ^{71}As yield by a factor of three, while only reducing ^{72}As yield by 1 %[6], improving end-of-bombardment radionuclidic purity from 98 % to greater than 99 %.

Overall decay-corrected radiochemical yield of the ^{72}As isolation procedure from $^{72}GeO_2$ were 51 ± 2 % (n = 3) in agreement with those observed with $^{nat}GeO_2$ 57 ± 7 % (n = 14). The beam current was limited to 3 µA as higher currents 4–5 µA exhibited inconsistent dissolution and reprecipitation steps, resulting in an overall yield of 44 ± 21 % (n = 6). Dissolution time also played an important role in overall yield with at least one hour necessary to minimize losses in these first two steps. The separation procedure effectively removed all radiochemical contaminants and resulted in $^{72}As(OH)_3$ isolated in a small volume, pH~4.5 water solution. Over the course of minutes to hours after back extraction, rapid auto-oxidation to $^{72}AsO_4H_3$ was observed. The bulk $^{72}GeO_2$ target material, which was reclaimed from the isolation procedure, is being collected for future use.

The synthesis of a targeted PET/MRI agent based on the functionalization of ^{72}As-SPION has proved to be a difficult task. Experiments conjugating ^{72}As-SPION to TRC105 through a PEG linker were unsuccessful, despite the investigation of a variety bioconjugation procedures. Current work is investigating the use of SPION@HMSN, which have a similar affinity for ^{72}As as unencapsulated SPION. This new class of ^{72}As-labeled SPION@HMSN has a hollow cavity for potential anti-cancer drug loading, as well as the mesoporous silica surface, which may facilitate the efficient conjugation of TRC105 using a well-developed bioconjugation technique.

In summary, radioarsenic holds potential in the field of diagnostic and theraputic nuclear medicine. However, this potential remains locked behind challenges related to its production and useful *in vivo* targeting. The present work strives to address several of these challenges through the use of enriched $^{72}GeO_2$ target material, a chemical isolation procedure that reclaims the bulk of the target material, and the investigation of new targeted nanoparticle-based PET/MRI agents.

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