Production scale purification of Ge-68 and Zn-65 from irradiated gallium metal

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Abstract: Germanium-68 (Ge-68) is produced by proton irradiation of a gallium metal target, purified by organic extraction and used in a medical isotope generator to produce Gallium-68 PET imaging agents. The purpose of this work was to implement a production scale separation of Ge-68 and Zn-65 that does not use organic solvents and uses a limited number of columns. The current separation approach was modified to use AG1 resin and/or Sephadex[®] G25 with zinc spikes to purify Ge-68 with near quantitative recovery. The purified Ge-68 meets DOE specifications. Methods utilizing zinc spikes resulted in the purist Ge-68 produced at Brookhaven National Lab with no other impurities by ICP-OES. During process optimization approximately 2.5 Ci of Ge-68 was purified utilizing the different processing methods, and the material was sold to the Nuclear Medicine community between 2012-2013.

Key words: Gallium-68 / ⁶⁸Ga / Germanium-68 / ⁶⁸Ge / Radiochemical separation

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Introduction

Germanium-68 (^{68}Ge) has been used in the development and marketing of the ^{68}Ge / gallium-68 (^{68}Ga) medical isotope generator.^{1,2} ^{68}Ge has a long half-life ($T_{1/2}=271$ days) and decays by electron capture to ^{68}Ga ($T_{1/2}=68.1$ min). There are no ^{68}Ga imaging agents approved for routine clinical use for PET imaging in the United States. However, ^{68}Ga -DOTATOC recently received FDA orphan drug designation for neuroendocrine tumor management, and the imaging agent will be fast tracked for approval.³ Due to the short half-life of ^{68}Ga , the synthesis of ^{68}Ga imaging agents utilize automated systems. In June 2013 Applied Radiation and Isotopes dedicated a special issue to ^{68}Ga that covered clinical applications of ^{68}Ga ,⁴ new ^{68}Ga imaging agents,⁵ $^{68}\text{Ge}/^{68}\text{Ga}$ generators,² automated synthesis utilizing synthesis modules,⁶ and dosimetry of ^{68}Ga -labeled compounds.⁷

At Brookhaven National Laboratory (BNL) ^{68}Ge is produced at the Brookhaven Linac Isotope producer (BLIP) by irradiating ~50-100 grams of natural Ga targets with a ~25 MeV protons beam.⁸ At Los Alamos National Laboratory ^{68}Ge is produced as a spallation product after proton irradiation of molybdenum or rubidium.⁹ The primary long lived contaminant is ^{65}Zn with a half-life of 244 days. The ^{65}Zn is coproduced in a radioactivity ratio of ~4/1 $^{68}\text{Ge}/^{65}\text{Zn}$ at the end of bombardment (EOB). The large gallium mass in the target, radioactive zinc and cobalt species must be removed from the ^{68}Ge in order to produce ^{68}Ge suitable for a ^{68}Ge / ^{68}Ga generator for human use.

Various prior attempts have been made for the separation of ^{68}Ge from irradiated natural gallium and they focus on three separation approaches: liquid/liquid extraction, liquid/gas distillation, and liquid resin separation.¹⁰ Germanium and gallium tetrachloride are both extremely volatile,¹⁰ and the volatile species have been used in distillation approaches for the separation of ^{68}Ge from the other metals. This approach can lead to losses of ^{68}Ge if the distillation fittings leak and can cause contamination problems. One approach used at BNL to isolate ^{68}Ge from the target material starts with leaching the ^{68}Ge from the gallium with 4 M HCl and H_2O_2 .⁸ The leaching solutions are pooled and liquid/liquid solvent extractions with either toluene or carbon tetrachloride/HCl are used to produce ^{68}Ge in the final form. The solvent extraction approach recovery yields are 85% with greater than 99% radiopurity, and the average ^{68}Ge radioactivity concentrations was 92.96 mCi/ml.¹¹ This method works well to produce ^{68}Ge for PET calibration sources. However, the use of the organic solvents such as carbon tetrachloride or toluene is classified by the FDA as Q3C – class 1 and 2 solvents in its Guidance for Industry documentation.¹² This FDA classification places the solvents in a classification that should not be employed in manufacturing of drugs due to unacceptable toxicity. To obtain FDA approval of the $^{68}\text{Ge}/^{68}\text{Ga}$ generators the initial purification of ^{68}Ge should be performed using non-toxic and non-carcinogenic solvents and meet the properties outlined by both the FDA and the DOE.

According to DOE specifications, the ideal purification of ^{68}Ge would remove all the zinc and cobalt and leave part per million levels of natural Ga.¹³

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4 The separation process should recover over 85% of the ^{68}Ge . The purified ^{68}Ge
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6 should have a radiochemical concentration greater than 50 mCi/ml, and the
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8 product should be in ~0.1 M hydrochloric acid solution. These properties make
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10 the final product ideal for use in making the ^{68}Ge / ^{68}Ga medical isotope
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12 generator. As mentioned previously, Ge and Ga tetrachloride are both extremely
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14 volatile,¹⁰ so volume reduction of the Ge species from dilute HCl solution to meet
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16 the activity concentration requirement would result in contamination issues or
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18 loss of product. These requirements for the ^{68}Ge product make separations of
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23 ^{68}Ge from gallium difficult.

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26 The challenges associated with the purification of ^{68}Ge were initially
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28 examined utilizing a Sephadex[®] G25 column approach using alkaline solutions
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30 without a complexing agent. The studies indicated very high germanium
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32 concentrations could be obtained, but cobalt retention on Sephadex[®] G25 was
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34 identical to germanium.¹⁴ This approach would require 4-5 columns to purify ^{68}Ge
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36 that meet specifications. Subsequent modification of the method with citrate
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38 buffer at pH12.5 produced ^{68}Ge that meet both FDA and DOE specification for
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40 product purity and the method used only one column.¹⁵ However; when new
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42 Sephadex[®] G25 was purchased from Sigma-Aldrich the amount of ^{65}Zn in the
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44 purified Ge-68 was outside DOE specifications. As a result different processing
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46 methods were developed to produce Ge-68 within DOE specifications, and
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48 optimize the time of the separation. We report here the optimization of production
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50 scale separation approaches of ^{68}Ge utilizing AG1 and/or Sephadex[®] G25 resin.
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Methods

Materials

All solutions were prepared with 18MΩ Millipore water, a Hanna Instruments disposable pH Tester was purchased from Fisher Scientific. Fine Sephadex[®] G25 was purchased from Sigma Aldrich. Econo-Pac 10 ml disposable columns, analytical grade AG1X8 resin were purchased from BIO-RAD, and 5 ml disposable centrifuge columns were purchased from Pierce. ICP standards for gallium (Ga), germanium (Ge), cobalt (Co), zinc (Zn) and a 19 element mixed standard were purchased from SPEX CertiPrep (Metuchen, NJ) and used to prepare a set of ICP calibration and QC standards. Gallium metal and other chemicals were purchased from Sigma Aldrich.

Production Scale Separations

Figure 1 outlines the different processing methods used for the production and purification of ⁶⁸Ge at BNL from February 2012 to September 2013. Processing methods 2-7 used Sephadex[®] G25 column geometries and bed volumes that are summarized in Table 1 which correspond to the recommended conditions.¹⁵

Processing method 1: Leaching of the irradiated gallium metal, preparation of the Sephadex[®] G25 column and processing method 1 were described previously.¹⁵

Processing Method 2: Processing method 1¹⁵ was used with the exception that a 4 ml BV of fine Sephadex[®] G25 (Sigma) column was used in the separation and the 0.1 M hydrochloric acid elution was fractionated.

Processing Method 3: The eluted HCl product fraction 2 from method 2 was passed through a 1 ml column of AG1X8 medium mesh and rinsed with 1 ml of 0.1 M HCl to produce the final ^{68}Ge product.

Processing Method 4: The pooled leached ^{68}Ge solution was diluted to 1.5 M HCl and passed through a 5 ml column of AG1X8 (fine mesh, 200-400 mesh). The column was rinsed with 5 ml of 1.5 M HCl and the eluted HCl was pooled and added to 125 ml of 1 M sodium citrate, pH adjusted to 12.5 with 10 M NaOH. The Sephadex[®] G25 separation described for processing method 1¹⁵ was used with a 1 ml BV of fine Sephadex[®] G25 (Sigma) to produce the final product.

Processing Method 5: Processing method 1¹⁵ was used with the exception that a 2 ml BV of fine Sephadex[®] G-25 (Sigma) was prepared with zinc at 2.5 ppm concentration, and the column was left overnight. The load solution (^{68}Ge and 1 M sodium citrate) was modified with 500 μl of 1,000 ppm zinc ICP standard prior to pH adjusting the solution to 12.5 and performing the separation.

Processing Method 6: The load, washings and HCl elutions from processing method 5 were combined, pH adjusted to 12.5 then the ^{68}Ge was purified according to processing method 1¹⁵ with a 2 ml BV of fine Sephadex[®] G25 (Sigma).

Processing Method 7: The pooled leached ^{68}Ge solution was processed according to method 4 with the exception that 100 μl of 1,000 ppm zinc ICP standard was added prior to pH adjusting the solution to 12.5. The solution was loaded onto a 2 ml BV of fine Sephadex[®] G25. For the recovery of ^{65}Zn the AG1X8 column that retained the ^{65}Zn was rinsed with 1.5 M HCl, and the

concentration of gallium was tracked by ICP-OES. When the concentration of gallium in the eluted 1.5 M HCl was below 20 ppm the ^{65}Zn was eluted with 0.01 M HCl. The solution was concentrated; ^{65}Zn was redissolved in 0.1 M HCl with a concentration of 10 mCi/ml and characterized by gamma spectroscopy and ICP-OES.

2.3 Analysis

Radioactive analysis was performed with a high purity germanium detector, and metal analysis was performed on a Perkin Elmer ICP-MS ELAN DRC or an ICP-OES Optima 7300 DV. For both ICP instruments a five point calibration curve was used and checked by three QC samples at the low, middle and high points on the curve. The ICP-MS instrument was tuned to pass the manufacturer's specifications with a Perkin Elmer "smart tune" solution.

Results

Figure 1, Table 1 & Table 2 summarize the processing methods, data and results for the seven processing methods. In all separations the concentration of Ga was below the amount from the organic extraction method. In processing methods 1, 3, 4, 6 and 7 the products meet DOE specifications for purity, radioactive concentration and recoveries of ^{68}Ge were 85-100%. The radioactive amount of ^{65}Zn in fractions 1 and 2 of processing method 2 were 0.93 mCi and 1.3 mCi compared to 0.05 mCi and a non-detectable of similar fractions from processing method 1. The ^{65}Zn in the samples from processing method 2 resulted in the batch not meeting specifications. The sample was passed through an AG1X8 column (processing method 3), and the eluted solution meet DOE

specifications. In processing method 5 the Sephadex[®] G25 was prepared with zinc, and this resulted in a prolonged elution time and minimal retention of ⁶⁸Ge. Processing methods 6 and 7 utilize a cold spike of zinc metal to the ⁶⁸Ge citrate solution prior to loading on the Sephadex[®] G25 column. The cold zinc spike competed with ⁶⁵Zn retention on the Sephadex[®] G25 column; the result is the reduction of ⁶⁵Zn in the product. The purified ⁶⁸Ge from both methods contained no visual peaks for gamma rays from ⁶⁵Zn, and the amount of ⁶⁵Zn was below the detection limits (0.003 µCi) of the HPGE radiation detector. The corresponding zinc levels are below the lower limit of quantification (LLOQ) by ICP-OES (0.05 ppm). ICP-OES analysis of processing methods 1, 3, and 4 indicated the purified ⁶⁸Ge contained 4-40 ppm gallium. In processing methods 6 & 7 gallium was below the LLOQ (0.01 ppm) of the ICP-OES, which may be caused by the zinc spike in both methods. In processing methods 6 and 7 germanium was the only element detected. ⁶⁵Zn purification: In processing method 7 ⁶⁵Zn was quantitatively recovered, and the concentration of zinc and gallium was 112.6 and 42 ppm. The specific activity of ⁶⁵Zn was 82 mCi/mg, and the radiochemical concentration was 9.19 mCi/ml. The characteristics of the purified ⁶⁵Zn were within the DOE specifications of >1mCi/ml and a radiopurity >99%.

Discussion

This paper optimizes the purification of ⁶⁸Ge and ⁶⁵Zn using a leaching step followed by purification of the leached germanium solution by an AG1X8 and/or Sephadex[®] G25 resin. Processing method 1 was initially successful and produced ⁶⁸Ge that meet DOE specifications.¹⁵ However, switching

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4 manufacturers of Sephadex[®] G25 from Pharmacia to Sigma resulted in a
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6 noticeable change in the amount of ⁶⁵Zn in the purified ⁶⁸Ge and caused the
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8 product to fail DOE specifications. Subsequent processing methods were
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10 performed to eliminate ⁶⁵Zn from the product, reduce the processing time or allow
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12 a purification of both ⁶⁵Zn and ⁶⁸Ge. Processing methods 3, 4, 6 and 7 could be
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14 used to produce ⁶⁸Ge that meet DOE specifications. Processing method 3 is not
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16 recommended as performing the AG1X8 column after the Sephadex[®] G25
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18 column adds 1-1.5 hours to the processing time. The method required a small
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20 AG1X8 column, and the potential to dilute the purified ⁶⁸Ge below 50 mCi/ml and
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22 out of DOE specifications. When the AG1X8 column was performed before the
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24 Sephadex[®] G25 column a larger AG1X8 column was used and only 15 minutes
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26 were added to the separation time (processing methods 4 and 7). This approach
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28 allows the recovery and purification of ⁶⁵Zn from ⁶⁸Ge according to traditional
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30 methods.¹⁶ If ⁶⁵Zn is a waste product then processing method 6 is the best
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32 method and only a Sephadex[®] G25 column would be needed to purify the
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34 leached ⁶⁸Ge.
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43 Specific optimized column geometries and processing methods allowed
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45 very high ⁶⁸Ge activity concentrations where germanium was the only detected
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47 element. Ideally the purification should be performed with greater than 600 mCi,
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49 so a 4 ml BV of Sephadex[®] 25 can be used and the column separation time
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51 would be reduced to 2-3 hours. A 4 ml BV of Sephadex[®] G25 was not used with
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53 less than 600 mCi as more fractionation is needed of the ⁶⁸Ge eluted in 0.1 M
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HCl. Each fraction would require a dose measurement to identify ^{68}Ge to pool, which would lead to higher radiation doses to personnel.

Conclusions

This paper focuses on the development of a production scale separation of ^{68}Ge for use in a $^{68}\text{Ge}/^{68}\text{Ga}$ generator. The aqueous chemistry approaches with the resins was able to produce purified ^{68}Ge that meets both FDA and DOE specifications and was implemented in production of ^{68}Ge . The processing methods were used to process 2.5 Ci of ^{68}Ge at BNL in 2012-2013. The implementation of the processing methods were used to produced ^{68}Ge at the highest radioactive concentrations (>250 mCi/ml) and with the highest purity compared to any other approach. Processing method 7 has been implemented for the purification of both ^{68}Ge and ^{65}Zn and will be used for future purifications.

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Table 1. Summary of methods/results/DATA for ^{68}Ge production runs at BNL from 02/2012 – 09/2013.

Processing Method	1 ¹⁵	2	3	4	5	6	7
Leached							
⁶⁸ Ge (mCi)	300	610	Used HCl product from method 2	257	440	Load and HCl solutions from method 5	410
⁶⁵ Zn (mCi)	70	202		62	125		118
Method							
Sequence [Sephadex Column: BV-Col.-Mesh]	Sephadex ^a [1-10-F] (Phamacia)	Sephadex [4-30-F]	Sephadex, AG1	AG1, Sephadex [1-10-F]	Zn, Zn preloaded Sephadex [2-5-F]	Zinc, Sephadex [2-5-F]	AG1, Zinc, Sephadex [2-5-F]
Processing Method Comments		Sigma different retention of Zinc compared to Pharmacia Sephadex	AG1 adds ~1-1.5 hr smaller column, slow flow	AG1 adds ~ 30 min	Poor retention of Ge, Low Ge recovery	Easiest method	Zn-65 recovered from AG1, ~4hrs for Sephadex load, ~ 1 hr for rinse & elute
Load Solution after column							
⁶⁸ Ge (mCi)	<1mCi	17	Same as method 2	NA	~400	40	NA
⁶⁵ Zn (mCi)	NA	200	Same as method 2	NA	NA	NA	NA

a-fine Sephadex G25 was from pharmacia. Processing methods 2-7 used fine Sephadex G25 from Sigma. Column: 1-10 is 1 ml bed volume in a Bio-Rad 10 ml disposable column. 2-5 is 2 ml bed volume in a 5 ml disposable centrifuge columns (Pierce). 4-30 is a 4 ml bed volume in a Bio-Rad 30 ml disposable column. Mesh: F fine. NA Not analyzed.

Table 2. Product data for ^{68}Ge production runs at BNL from 02/2012 – 09/2013.

Processing Method	DOE specs.	Organic extraction ¹¹	1 ¹⁵	2	3	4	5	6	7
Sequence		Reference 3	Sephadex ^a (Phamaceia)	Sephadex (Sigma)	Sephadex, AG1	AG1, Sephadex	Zn, Zn preloaded Sephadex	Zinc, Sephadex	AG1, Zinc, Sephadex
% Ge recovery in HCl form	>80	85	1.6/86.6/20	97	99	99	10%	90%	99%
Radiopurity Ge (%)	99	100%	100	NA	99.7	100	NA	100	100
Ge activity Fr1/2/3 mCi	---	130-2917	5/260/60	128/465 ^c / ^{<} 5	465 ^c	257	40	140/274/ ^{<} 5	408
Conc Ga Fr 1/2/3 ppm	---	Ave 63	NA/42/40	NA	9.56	4.00	NA	31.7/ND/NA	ND
Activity ⁶⁵ Zn Fr 1/2/3 (mCi)	---	ND	0.05/ND/ND	0.93/1.3	ND	ND	NA	ND	ND
Total Vol or mass Fr 1/2/3 or product	---	2.32-29.2 ml	1/1/1 (ml)	4/5/NA (ml)	6.116 (g)	3.25 (g)	NA	5.795 (g)	2/2/2 mls
Ge-68 Conc. (mCi/ml or mCi/g)	>10 mCi/ml		5/260/60	ND	76	79	ND	71.44	204
Specific activity (mCi/mg)	----	1337-2012 ^a	3818	NA	1454	3755	NA	1909 ^a	1622 ^a
Out of specs - problem		Organic solvent	Fr 1 Ge-68 conc. to low	High Zn-65 activity	-----	-----	Ge-68 conc too low	----	Fr1,3 ^{<} 5 mCi
Within spec		---	Fr 2 & 3	none	AG1 elution	Fr 2 & 3	none	Fr 1 & 2	Fr 2

ND-not detected, NA-no analyzed performed, a-Analysis by ICP-OES, other analysis performed by ICP-MS.

Target Material: Ga metal in a niobium capsule. Nuclear Production Reaction: $^{69}\text{Ga}(p,2n)^{68}\text{Ge}$; $^{71}\text{Ga}(p,4n)^{68}\text{Ge}$

Figure 1. Flow chart for processing methods 1-7 used in Ge-68 production. (M)= processing method