BNL-108191-2015-JA

Production of 61Cu by the natZn(p,α) reaction: improved separation and specific activity determination by titration with three chelators

A. H. Asad[1,2], S. V. Smith[3], L. M. Morandeau[1], S. Chan[1], C. M. Jeffrey[1], R. I. Price[1,4]

[1]Radiopharmaceutical Production & Development (RAPID) Laboratory, Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth 6009, Western Australia
[2]Department of Imaging & Applied Physics, Curtin University, Perth 6845, Western Australia
[3]Medical Isotope Research and Production Program, Collider-Accelerator Department, Brookhaven National Laboratory, Upton, NY 11973 USA
[4]School of Physics, University of Western Australia, Nedlands 6009, Western Australia

Accepted to Journal of Radioanalytical and Nuclear Chemistry

July 2015

Collider-Accelerator Department

Brookhaven National Laboratory

U.S. Department of Energy
DOE Office of Science, DOE Office of Nuclear Physics

Notice: This manuscript has been co-authored by employees of Brookhaven Science Associates, LLC under Contract No. DE-AC02-98CH10886/DE-SC0012704 with the U.S. Department of Energy. The publisher by accepting the manuscript for publication acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or any third party's use or the results of such use of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof or its contractors or subcontractors. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Production of ^{61}Cu by the ^{nat}Zn(p,α) Reaction: Improved Separation and Specific Activity Determination by Titration with Three Chelators

Ali H. Asad[a,b,*], Suzanne V. Smith[c], Laurence M. Morandeau[a], Sun Chan[a], Charmaine M. Jeffery[a], Roger I. Price[a,e]

[a]Radiopharmaceutical Production & Development (RAPID) Laboratory, Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth 6009, Western Australia

[b]Department of Imaging & Applied Physics, Curtin University, Perth 6845, Western Australia

[c]Medical Isotope Research and Production Program, Collider-Accelerator Department, Brookhaven National Laboratory, Upton NY, USA 11973

[e]School of Physics, University of Western Australia, Nedlands 6009, Western Australia

[*]Corresponding Author

Ali H. Asad

Tel: +61 8 9346 2911; Fax: +61 8 9346 3466;

Email: ali.asad@health.wa.gov.au

Permanent address:

Medical Technology and Physics,
Hospital Avenue
Sir Charles Gairdner Hospital,
Nedlands 6009,
WESTERN AUSTRALIA

Abstract

The cyclotron-based production of positron-emitting ^{61}Cu using the (p,α) reaction at 11.7 MeV was investigated starting from natural-zinc (^{nat}Zn) and enriched ^{64}Zn-foil targets, as well as its subsequent purification. For ^{nat}Zn, a combination of three resins were assessed to separate ^{61}Cu from contaminating ^{66,67,68}Ga and ^{nat}Zn. The specific activity of the purified ^{61}Cu determined using ICP-MS analysis ranged from 143.3±14.3(SD) to 506.2±50.6 MBq/µg while the titration method using p-SCN-Bn-DOTA, p-SCN-Bn-NOTA and diamsar gave variable results (4.7±0.2 to 412.5±15.3 MBq/µg), with diamsar lying closest to the ICP-MS values. Results suggest that the p-SCN-Bn-DOTA and p-SCN-Bn-NOTA titration methods are significantly affected by the presence of trace-metal contaminants.

Key words: ^{61}Cu. ^{nat}Zn. ^{64}Zn. Specific activity. ICP-MS. Bifunctional chelators.

Introduction

Copper has four useful radioisotopes for positron emission tomography (PET) imaging, ^{60}Cu ($t_{1/2}$ = 20.7 min), ^{61}Cu ($t_{1/2}$ = 3.33 h), ^{62}Cu ($t_{1/2}$ = 9.8 min) and ^{64}Cu ($t_{1/2}$ = 12.7 h) [1]. ^{64}Cu also has a therapeutic β^- emission that can be used for radiotherapy. ATSM and PTSM have been radiolabelled with each of these radionuclides for imaging hypoxia [2] and blood perfusion, respectively [3]. While the majority of published works have used the long-lived isotope ^{64}Cu, the shorter half-life of ^{61}Cu, with its high-abundance positron emission (β^+ = 1.22 MeV, 60%; EC = 40%) and the absence of β^- make it a potentially a more attractive option for imaging hypoxia [4].

There are several reported methods for the cyclotron production of ^{61}Cu using both Ni and Zn targets [5-13]. Copper-61 can be produced from both ^{61}Ni and ^{62}Ni via (p,n) and (p,2n) reactions, respectively [5-8]. The production of ^{61}Cu via these routes is high-yielding, due to high cross sections (> 300 mb). The optimal proton energies for these nuclear reactions are 10.3 MeV for ^{61}Ni [6] and 14 MeV for ^{62}Ni [7], which are suitable for production on commercially available low energy cyclotrons. However, the high cost of the ^{61}Ni ($37 per mg) and ^{62}Ni ($14.70 per mg) can be prohibitive for routine production of ^{61}Cu when one considers the total inventory required, as well as the loss on recycling and recovery.

Proton reactions using zinc as starting material have been studied and provide a cheaper option for the production of ^{61}Cu [9-13]. For example, natural Zn (^{nat}Zn, with ^{64}Zn nat. abund. 48.6%) and enriched ^{64}Zn (>99%) using the ^{64}Zn(p,α)^{61}Cu reaction have reasonable cross sections (39 and 79 mb at 15 MeV, respectively) [12,13] for the production of ^{61}Cu. The Zn target materials are considerably cheaper (e.g. ^{64}Zn $5 per mg) than Ni targets. ^{64}Zn also presents the added advantage of being commercially available as ^{64}Zn foil. The latter

removes the need to establish an in house electroplating and recycling process as well as requalifying procedures for human use; ^{64}Zn can be used as a consumable.

However, the proton bombardment of zinc targets presents challenges such as the co-production of the Ga radionuclides {^{66}Ga ($t_{1/2}$ = 9.49 h), ^{67}Ga ($t_{1/2}$ = 3.26 d) and ^{68}Ga ($t_{1/2}$ = 68 min)} and the long half-life of co-produced ^{65}Zn ($t_{1/2}$ = 244 d) [10-12]. Figure 1 illustrates the cross sections of ^{nat}Zn(p,x) reactions for incident proton energies <18 MeV. This shows that the production of ^{61}Cu can be optimised to avoid collateral ^{65}Zn contamination by using proton energies <13.7 MeV.

Place Figure 1 about here

Separation methods have been reported in the literature for isolating Cu and Ga from Zn target materials by cation and anion exchange chromatography [14-16]. Rowshanfarzad *et al*., 2006 [14] irradiated a ^{nat}Zn target using 180 μA-h and 22 MeV proton beam energy. They isolated the ^{61}Cu using both cation and anion exchange columns, using large volumes of high concentration of HCl to separate Cu from Ga and Zn. Unfortunately, the specific activity of the isolated products was not reported.

Cyclotron facilities cannot always afford the regular use of ICP-MS as a method of determining the specific activity for the quality control of radiometals production. The other common method to determine effective specific activity (ESA) is the titration method using the chelator TETA. This method consists in incubating the isolated, purified radiocopper isotope in buffered solution in presence of increasing concentrations of the chelator. After a suitable time, the solutions are analysed by instant thin layer chromatography (ITLC) where the free Cu is separated from bound Cu and the percentage of [Cu-TETA]$^{2-}$ bound is calculated [17-19]. Thieme *et al*., 2012 [20] also reported the use of TETA for determining

the ESA of ^{64}Cu and compared the results with the non-carrier-added specific activity (NCA-SA) determined from ICP-MS data. They found that for ^{64}Cu the ESA values increased by 30% when incubating the mixtures at 80°C vs. room temperature. In all cases, the ESA values were 1-5 fold lower compared to NCA-SA determined from ICP-MS, and were dependant on the metallic backing materials used for targetry. For ^{61}Cu, the same authors also reported similar ratios between the titration method using TETA and ICP-MS when comparing ESA and NCA-SA, respectively [4]. Recently, Mastrena *et al.*, 2014 [21] compared the use of ICP-MS and ion chromatography for determining the specific activity of ^{64}Cu, with the titration method using TETA at 80°C. The ESA values obtained with the titration method were 1.5-5.0 fold lower than the ones obtained with ICP-MS.

While the polyazacarboxylate ligands show good complexation with Cu, they are not highly selective and will form complexes with other alkali, alkaline earth and transition-metal contaminating ions present in solution. Today, a wide range of commercially available polyaza, polycarboxylate and hexa-aza cages have been reported to preferentially form complexes with Cu(II) [1,22-24]. It is of interest to comparatively assess some of these ligands for determining the specific activity of no-carrier-added (NCA) ^{61}Cu.

In this work, we investigated the separation and purification of ^{61}Cu from proton-bombarded ^{nat}Zn foils and enriched ^{64}Zn foils, using commercially available resins mounted in a closed in-house column separation system. We compared the NCA specific activity of the resultant ^{61}Cu by ICP-MS and the ESA obtained by titration with each of the three ligands, p-SCN-Bn-DOTA, p-SCN-Bn-NOTA and diamsar. These were chosen for their known greater selectivity for Cu, compared with TETA [1]. Their chemical structures are shown in Fig. 2.

Place Figure 2 about here

Experimental

Materials

Enriched ^{64}Zn metal foils (^{64}Zn 99.30%, ^{66}Zn 0.45%, ^{67}Zn 0.06%, ^{68}Zn 0.17% and ^{70}Zn 0.02%) (0.05 mm thick) were purchased from Trace Sciences International Corp. (TX, USA). High-purity (>99.99%) ^{nat}Zn foils (0.05 mm thick) with composition (^{64}Zn 48.6%, ^{66}Zn 27.7%, ^{67}Zn 4.1%, ^{68}Zn 18.8% and ^{70}Zn 0.6%) were purchased from Goodfellow Metals Ltd (Cambridge, UK). Hydrochloric acid, sodium acetate and TraceSELECT water were purchased from Sigma Aldrich (NSW, Australia). Anion exchange resin AG1-X8 (200-400 mesh) and cation exchange resin AG50W-X8 (200-400 mesh) were supplied by BioRad (NSW, Australia). Cu-Resin (100-150mm) was purchased from Eichrom Technologies (IL, USA). Instant thin-layer chromatography (ITLC-SA) paper (Pacific Laboratory Products; VIC, Australia) was used to separate free ^{61}Cu(II) from complexed ^{61}Cu(II). S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA) and 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bn-DOTA) were purchased from Macrocyclics, (TX, USA). The third chelator 3, 6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine pentahydrate (diamsar) was purchased from Clarity Pharmaceuticals (NSW, Australia) (Fig. 2).

Instrumentation

The radionuclidic purity of the final product was determined by γ-spectroscopy using a high-purity germanium detector (HPGe; Canberra model GC2018) coupled to a calibrated Canberra Inspector 2000 multichannel analyser (Canberra Industries; TN, USA) and the data were processed by using Genie-2000 software (Canberra). The Automatic Wizard 2 Gamma Counter (Perkin Elmer; MA, USA) was used for counting TLC strips cut up into several pieces and to determine the % of complexed ^{61}Cu.

Irradiation of Zinc Foils

Irradiation experiments were performed using an IBA 18/18 MeV cyclotron (IBA, Belgium). A graphite degrader (1020 μm thick) was used to reduce the proton energy from 17.6 MeV to 11.70 MeV [10]. Four targets each, of ^{nat}Zn and enriched ^{64}Zn foils were irradiated at 20-40 μA for 30 min. The foils were mounted in a custom-made aluminium disc holder with a lid (25 mm diam. and thickness 0.50 mm) (see Fig. 3). The front and back of the target holder were cooled with He gas and chilled water, respectively [10].

Place Figure 3 about here

Separation & Purification of Copper-61

A. From ^{nat}Zinc Target

The separation and purification of ^{61}Cu from the irradiated ^{nat}Zn target material is shown schematically in Fig. 4(a). The columns were pre-conditioned as follows: (i) the Cu-resin column (0.5 g) with 20 mL of 0.01 M HCl :(ii) the cation exchange column (AG5W-X8) (1.0 g) with 20 mL of TraceSELECT water and (iii) the anion exchange resin (AG1-X8) (1.0 g) with 20 mL of TraceSELECT water, followed by 8 M HCl (5 mL). At end of bombardment (EOB) the target remained in the solid targetry apparatus for 1 to 3 h to allow for some decay of the annihilation gammas from ^{68}Ga ($t_{1/2}$ =68 min). The chemical separation was performed in a shielded fume hood. The irradiated ^{nat}Zn foil was dissolved in 1 mL of 8 M HCl at 150 ^{o}C and evaporated to dryness. High purity TraceSELECT water (1 mL) was added to the residue and evaporated to dryness; this process was repeated once. The residue was dissolved in 2 mL of 0.01 M HCl and an aliquot (1 μL) was taken for analysis by gamma spectroscopy. The solution was first loaded onto the Cu-resin column. The column was washed with 25 ml of 0.01 M HCl to remove the Zn target material as well as ^{66,67,68}Ga radionuclides. The ^{61}Cu

was then eluted with 1.5-2 mL of 8 M HCl which were directed to the cation exchange column connected in series with the anion exchange column. The residual gallium was retained on the cation exchange column while the ^{61}Cu and residual Zn were retained on the anion exchange column. Both columns were washed with an additional 5 mL of 8 M HCl. $^{61}CuCl_2$ was finally eluted from the anion exchange column with 2 M HCl (3.0 mL) and the solution was evaporated to dryness. The residue was dissolved in 1 mL of TraceSELECT water and evaporated to dryness three times. The final product was then dissolved in 400-800 μL of 0.01 M HCl and sampled for analysis by gamma spectroscopy. Comparison of gamma spectroscopy analysis of radionuclidic composition before and following separation is shown in Table 1.

Place Figure 4 & Table 1 about here

B. From Enriched ^{64}Zn Target

The separation and purification of the ^{61}Cu from the irradiated enriched ^{64}Zn target material is shown schematically in Fig. 4(b). The target foil was dissolved in 1 mL of 8 M HCl at 150 ^{o}C and evaporated to dryness. High purity TraceSELECT water (1 mL) was added to the residue and evaporated to dryness; the process was repeated once. The residue was dissolved in 2 mL of 0.1 M HCl. An aliquot (1 μL) was taken for γ- spectroscopic analysis. The solution was loaded onto a Cu-resin column, pre-conditioned with 20 mL of 0.01 M HCl. The Zn and Ga were eluted with 25 mL of 0.01 M HCl and kept for recovery of ^{64}Zn if required. The ^{61}Cu trapped on the Cu-resin column was then eluted off with 1.5 - 2 mL of 8 M HCl, followed by drying steps as described in the previous section. The final product was then dissolved in 400-800 μL of 0.01 M HCl and sampled for analysis by γ- spectroscopy. Comparison of gamma spectroscopy analysis of radionuclidic composition before and following separation is shown in Table 1.

In order to test the feasibility of recovery of the enriched ^{64}Zn target material, following decay of the Ga radionuclides, the 25 mL of Zn and Ga in HCl solution were evaporated to dryness. Then, the residue was redissolved in 5 mL of 8 M HCl. This eluent was transferred to a cation exchange column (pre-conditioned with 20 mL of TraceSELECT water) followed by 10 mL of 8 M HCl. The eluent was evaporated to dryness. The recovery of ^{64}Zn for potential electroplating was >95%.

Specific activity

The specific activity of ^{61}Cu (in GBq/µmol) was determined by the titration method, using purified ^{61}Cu in 0.01 M HCl with the chelating agents S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA); 2-(4-isothiocyanatobenzyl)-1,4,7, 10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bn-DOTA) and 3,6,10,13,16,19 hexaazabicyclo[6.6.6]eicosane-1,8-diamine pentahydrate (diamsar) (Fig. 2). The specific activity was converted to GBq/µg as described in the Results section.

Complexation of the ^{61}Cu was determined in a similar manner to that described by McCarthy, et al., 1997 [17]. Briefly, increasing concentrations of chelator solutions were prepared in 0.1 M sodium acetate buffer pH 6.5. Ten microliters of the ^{61}Cu in 0.01 M HCl solution were added to each chelator solution and the volume adjusted to 30 µL with 0.1 M sodium acetate buffer pH 6.5. The mixture was then incubated at room temperature for 30 min. After incubation, two-µL aliquots were withdrawn from each reaction and spotted onto ITLC–SA strips. Different mobile phases were used to separate free Cu(II) from complexed Cu(II): (i) for diamsar the mobile phase was 0.1 M NaCl : 0.1 M EDTA (9:1). R_f was <0.2 for Cu-diamsar while free Cu(II) had migrated to R_f >0.8. (ii) DOTA and NOTA Cu(II) complexes were separated using 0.1 M sodium acetate at pH 4.5 : H_2O : MeOH : ammonium

hydroxide (20:18:2:1 v/v) mobile phase. Cu-DOTA and Cu-NOTA complexes migrated at R_f > 0.8 while free Cu(II) had a R_f <0.2. The ITLC strips were cut into two-cm pieces and counted on the calibrated gamma counter using a dynamic energy window of 282 keV. The minimum ligand concentration for which 100% labelling occurred was estimated by multiplying the measured value at 50% complexation by 2. The specific activity of ^{61}Cu, in GBq/μmol of Cu (II), was then calculated by correcting for the total activity and molecular weight of the chelator and ^{61}Cu. A typical set of titration curves are shown in Fig. 7.

After complete decay of the ^{61}Cu sample, aliquots (10-20 μL) from each production run were diluted with 1% of HNO_3, adjusted to a final volume of 10 mL and sent for inductively coupled mass spectrometry (ICP-MS) analysis (Curtin University, WA, Australia).

Results and Discussion

Production of ^{61}Cu

Production of ^{61}Cu using the ^{64,nat}Zn(p,α)^{61}Cu nuclear reaction was performed under irradiation at 11.7 MeV. Two separate target materials were used, ^{nat}Zn and enriched ^{64}Zn. The activity of ^{61}Cu produced from a high-purity ^{nat}Zn foil target (99.99%, 0.05 mm thick) was lower than that of the enriched ^{64}Zn target (99.3%, 0.05 mm thick). The yield (corrected to EOB) of purified ^{61}Cu after irradiation of ^{nat}Zn foil target was approximately 9.7-19.7 MBq/μA-h, which is about 20% of the activity produced from an enriched ^{64}Zn target of the same thickness, for the same μA-h irradiation. Rowshanfarzad *et al*., 2006 [14] reported that the production yield of electroplated ^{nat}Zn with an 80 μm thickness was 445 MBq/μA-h at a proton beam energy of 22 -12 MeV. Our results are much lower than theirs, due to the energy and incident angle of the proton beam and the target size.

To ensure that no contaminating ^{65}Zn was produced we reduced the incident proton energy to 11.7 MeV [25]. Gamma spectroscopy at EOB of the digested foil prior to separation showed no evidence of ^{65}Zn. At EOB, the co-produced activities of ^{66}Ga and ^{68}Ga were significantly higher than that of ^{61}Cu, but with negligible activity of ^{67}Ga (Table 1).

The ^{61}Cu was separated from the irradiated ^{nat}Zn target using a three-step purification process, as illustrated in Fig. 4(a). The first step involved loading of the dissolved target onto a Cu-resin column to separate out the Zn and ^{66,67,68}Ga. A cation-exchange column was used to separate (by retention) any remaining trace of Ga ions from the eluted ^{61}Cu. The final step involved an anion exchange column to retain any remaining Zn ions from the eluted ^{61}Cu, which was collected as the chloride with a radionuclidic purity of >99%. The final product was eventually analysed by ICP-MS for trace metals contaminants. The results of this analysis are shown in Fig. 5.

The activity of ^{61}Cu produced from a highly enriched ^{64}Zn foil target corrected to EOB was within the range 517 to 723 MBq for an irradiation of approximately 20 μA-h. This represented a yield of 29-36 MBq/μA-h. Thieme *et al.,* (2013) [4] reported that the yield of electroplated ^{64}Zn with a thickness ranging between 244 - 597 μm was 20.7 - 48.3 MBq/μA-h with a primary proton energy of 16 MeV. We believe the higher yields obtained for our study are reflective of the higher purity (and possibly density) of the enriched ^{64}Zn foils we used, compared to electroplated ^{64}Zn layers prepared as targets. For ^{61}Cu separation and purification from the enriched ^{64}Zn target, only a single column was necessary (Cu-resin), as illustrated in Fig. 4(b), since the Ga radionuclide contamination was low. Figure 6 presents the ICP-MS derived trace metals contaminants for each ^{61}Cu separation.

Place Figures 5, 6 & 7 about here

Specific activity of ^{61}Cu

ICP-MS was used to assess both the total nonradioactive Cu content and the presence of the contaminating metal ions Co, Fe, Ni, Pb, Ga and Zn in 'cold' samples of the purified solutions containing ^{61}Cu. Figures 5 and 6 summarize the trace metals content in each target experiment derived from both types of target. The ICP-MS values for Cu were used to determine the no-carrier-added specific activity (NCA-SA) of the ^{61}Cu at EOB, and these data are compared with the effective specific activities (ESA) obtained by titration with chelators. The ESA values for the ^{61}Cu were determined using three chelators, p-SCN-Bn-DOTA, p-SCN-Bn-NOTA and diamsar. A comparison of ESA and NCA-SA values are presented in Table 2.

Put Table 2 about here

For ^{61}Cu derived from the ^{nat}Zn target irradiation, the NCA-SA values were in reasonable agreement with the calculated ESA values using the diamsar chelator (approximately 1.2 - 2.1 higher). For the ^{64}Zn target they were 1.1-1.5 higher. Thus the specific activities determined using titration with diamsar are considered to be in good agreement with specific activities calculated from ICP-MS, particularly as they were achieved in the presence of relatively high concentrations of Zn(II) and Pb(II) (Figs 5 and 6). Titration with the two other chelators produced ESA values that were significantly lower than the ICP-MS derived values (Table 2).

Thieme *et al.* [4] found that the ESA values for ^{61}Cu using the TETA chelator were 3 fold less than the ICP-MS values. In this study, we found that diamsar was the best chelator for use in determining the ESA, if the ICP-MS derived NCA-SA is considered as the 'gold

standard'. This is because it has a higher selectivity for the Cu(II) ions over other metal-ion contaminants present in the radioactive solutions. In the case of p-SCN-Bn-NOTA and p-SCN-Bn-DOTA, Fe (II/III) and Zn (II) ions were likely the main competitors of Cu (II) ions. The relatively high concentration of Zn(II) derives from residual zinc target material (Zn > 50 ppm) [26].

The complexation of the ^{61}Cu with the different chelators was analysed after incubation at room temperature for 30 mins as shown in Fig. 7. The data indicate that the complexation yield of diamsar could be achieved with μmoL concentrations of chelator, better than p-SCN-Bn-NOTA or p-SCN-Bn-DOTA under the same conditions. p-SCN-Bn-NOTA achieved 100% complexation at lower concentration than p-SCN-Bn-DOTA. Heating would likely improve the labelling efficiency with p-SCN-Bn-DOTA but also facilitate complexation of other metal ions more avidly. These results suggest that the use of diamsar as titration chelator to calculate the specific activity is more accurate than p-SCN-Bn-DOTA or p-SCN-Bn-NOTA because of the high selectivity of diamsar towards the Cu(II) ion. Furthermore, Smith [1] reported that >99.9% complexation of ^{64}Cu could be obtained in 2 min with SarAr (functionalized diamsar) at very low concentrations (1 μmolar), at room temperature using a 1:1 molar ratio Ligand:Metal and a pH range 4-9. These findings and our results support further development of the hexa-aza bicyclic cages for clinical and pre-clinical studies [27-29].

Conclusions

Copper-61 was produced from the $^{64,nat}Zn(p,\alpha)^{61}Cu$ reaction using a 11.7 MeV proton beam energy to irradiate ^{nat}Zn and ^{64}Zn disc targets. This was followed by column chromatography separation and purification (one column for ^{64}Zn, three for ^{nat}Zn), yielding ^{61}Cu as the chloride with high radionuclidic purity (>95%) from either target. Separation and purification from the ^{nat}Zn target using a combination of three columns was fast (<30 mins) and straightforward. The overall cost to produce the ^{61}Cu was considerably cheaper using either Zn target, when compared with an electroplated ^{61}Ni target; the ^{nat}Zn target being the most cost effective. Effective specific activities of the purified ^{61}Cu were measured by the titration method using the three chelators diamsar, p-SCN-Bn-NOTA and p-SCN-Bn-DOTA. These results were compared with no-carrier-added specific activities obtained using ICP-MS. While low agreement was found for p-SCN-Bn-NOTA and p-SCN-Bn-DOTA, relatively good agreement was found with diamsar.

Acknowledgments

The authors express their thanks to the Medical Technology and Physics radiochemists and engineering staff for providing scientific materials and other support.

References

1. Smith SV (2004) Molecular imaging with copper-64. J Inorg Biochem 98: 1874-1901

2. Laforest R, Dehdashti F, Lewis JS, Schwarz SW (2005) Dosimetry of 60/61/62/64Cu-ATSM: a hypoxia imaging agent for PET. Eur J Nucl Med Mol Imaging 32: 764-770

3. Wong TZ, Lacy JL, Petry NA, Hawk TC, Sporn TA, Dewhirst MW, Vlahovic G (2008) PET of Hypoxia and Perfusion with ^{62}Cu-ATSM and ^{62}Cu-PTSM Using a ^{62}Zn/^{62}Cu Generator. AJR AM J Roentgenol 190: 427-432

4. Thieme S, Walther M, Preusche S, Rajander J, Pietzsch H, Lill J, Kaden M, Solin O, Steinbach J (2013) High specific activity ^{61}Cu via ^{64}Zn(p,α)^{61}Cu reaction at low proton energies. Appl Radiat Isot 72: 169-176

5. McCarthy DW, Bass LA, Cutler PD, Shefer RE, Klinkowstein RE, Herrero P, Lewis JS, Cutler CS, Anderson CJ,Welch MJ (1999) High purity production and potential applications of copper-60 and copper-61. Nucl Med Biol 26: 351-358

6. Szelecsényi F, Blessing G, Qaim SM (1993) Excitation function of proton induced nuclear reations on enriched ^{61}Ni and ^{64}Ni: possibility of production of no-carrier-added ^{61}Cu and ^{64}Cu at a small cyclotron. Appl Radiat Isot 44: 575-580

7. Piel H, Qaim SM, Stoecklin G (1992) Excitation functions of (p,xn)-reactions on natNi and highly enriched ^{62}Ni: Possibility of production of medically important radioisotope ^{62}Cu at a small cyclotron. Radiochimica Acta 57: 1-5

8. Tanaka S, Furukawa M, Chiba M (1972) Nuclear reactions of nickel with protons up to 56 MeV. J Inorg Nucl Chem 34: 2419-2426

9. Szelecsényi F, Kovács Z, Suzuki K, Okada K, van der Walt T N, Steyn G F, Mukherjee S (2005) Production possibility of ^{61}Cu using proton induced nuclear reactions on zinc for PET studies. J Radioanal Nucl Chem 263: 539-546

10. Asad AH, Chan S, Morandeau L,Cryer D, Smith SV, Price RI (2014) Excitation functions of natZn(p,x) nuclear reactions with proton beam energy below 18 MeV. Appl Radiat Isot 94:67-71

11. Al-Saleh FS, Mungren KSA, Azzam A (2007) Excitation function measurements and integral yields estimation for ^{nat}Zn(p,x) reactions at low energies. Appl Radiat Isot 65: 1101-1107

12. Uddin MS, Khandaker MU, Kim KS, Lee YS, Kim GN (2007) Excitation functions of the proton induced nuclear reactions on ^{nat} Zn up to 40 MeV. Nucl Instr Meth B 258 : 313-320

13. Cohen BL, Newman E, Charpie RA, Handly TH (1954) *(p*, pn) and (*p*, *αn*) Excitation Functions Phys Rev 94:620

14. Rowshanfarzad P, Sabetb M, Jalilian AR, Kamalidehghan M (2006) An overview of copper radionuclides and production of ^{61}Cu by proton irradiation of ^{nat}Zn at a medical cyclotron. Appl Radiat Isot 64: 1563-1573

15. Dasgupta, AK, Mausner LF, Srivastava SC (1991) A new separation procedure for 67Cu from proton irradiation of Zn. Appl Radial Isot 42: 371-376

16. Schwarzbach R , Zimmermann K, Blauenstein P, Smith A, Schubiger PA (1995) Development of a simple and selective seprartion of ^{67}Cu from irradiated zinc for use in antibody labelling: A comparison of methods. Appl Radiat Isot 46:329-336

17. McCarthy DW, Shefer RE, Klinkowstein RE, Bass LA, Margenaeu WH, Cutler CS, Anderson CJ, Welch MJ (1997) Efficient production of high specitic activity 64Cu using a biomedical cyclotron. Nucl Med Biol 24: 35-43

18. Matarrese M, Bedeschi P, Scardaoni R, Sudati F, Savi A, Pepe A, Masiello V, Todde S, Gianolli L, Messa C, Fazio F (2010) Automated production of copper radioisotopes and prepration og high specific activity [(64)Cu]Cu-ATSM for PET studies. Appl Radiat Isot 68: 5-13

19. Avila-Rodrigueza MA, Nyeb JA, Nickles RJ (2007) Simultaneous production of specific activity ^{64}Cu and ^{61}Co with 11.4 MeV protons on enriched ^{64}Ni nuclei. Appl Radiat Isot 65 : 1115-1120

20. Thieme S, Walther M, Pietzsch H-J, Henniger J, Preusche S, Mäding P, Steinbach J (2012) Module-assisted preparation of ^{64}Cu with high specific activity. Appl Radiat Isot 70 : 602-608

21. Mastrena T, Guthrieb J, Eisenbeisd P, Vollerd T, Mebrahtud E, Robertson JD, Lapi SE (2014) Specific activity measurement of ^{64}Cu: a comparison of methods. Appl Radiat Isot 90: 117-121

22. Jarrett BR, Gustafsson B, Kukis DL, Louie AY (2008) Synthesis of 64Cu-Labeled Magnetic Nanoparticles ofr Multimodal Imaging. Bioconjug Chem 19(7): 1496-1504

23. Craft JM, De Silva RA, Lears KA, Andrews R, Liang K, Achilefu S, Rogers BE (2012) In vitro and in vivo evaluation of a 64Cu-labeled NOTA-Bn_SCN-Aoc-bombesin analogue in gastrin-releasing peptide receptor expressing prostate cancer. Nucl Med Biol 39: 609-616

24. Kong L, Mume E, Triani G, SmithSV (2013) Optimizing Radiolabeling Amine-Functionalized Silica Nanoparticles Using SarAr-NCS for Applications in Imaging and Radiotherapy. Langmuir 29: 5609-5616

25. Asad AH , Smith SV, Chan S, Jeffery CM, Morandeau L, Price RI (2012) Cyclotron Production of ^{61}Cu using Natural Zn & Enriched ^{64}Zn Targets. AIP Conf Proc 1509: 91-95. doi:10.1063/1.4773947

26. Chakravarty R, Chakraborty S, Dash A, Pillai MRA (2013) Detailed evaluation on the effect of metal ion impurities on complexation of generator eluted 68Ga with different bifunctional chelators. Nucl Med Biol 40:197-205

27. Paterson BM, Buncic G, McInnes LE, Roselt P, Cullinane C, Binns DS, Jeffery CM, Price RI, Hicks RJ, Donnelly PS (2014) Bifunctional 64Cu-labelled macrobicyclic cage amine isothiocyanates for immuno-positron emission tomography. Dalton Trans 44(11):4901-4909

28. Weia L, Yea Y, Wadasa TJ, Lewis JS, Welch MJ, Achilefu S, Anderson CJ (2009) 64Cu-Labeled CB-TE2A and diamsar-conjugated RGD peptide analogs for targeting angiogenesis: comparison of their biological activity. Nucl Med Biol 36 : 277-285

29. Liu S, Li Z, Conti P S (2014) Development of Multi-Functional Chelators Based on Sarcophagine Cages. Mol 19: 4246-4255.

Table 1. Typical radionuclidic compositions of digested foils prior to column chromatography separation, and purified ^{61}Cu product following separation. Results for both target materials are corrected to EOB and expressed as percentage of total counts attributed to the characteristic photopeak of the radionuclide, corrected for the branching fraction of the decay mode.

Target Material	Radionuclide	Prior to Separation (%)	Purified Product (%)
*^{nat}Zn	^{61}Cu	7.3	100
	^{66}Ga	12.2	Not detected
	^{67}Ga	0.2	Not detected
	^{68}Ga	80.3	Not detected
†Enriched ^{64}Zn	^{61}Cu	90.06	100
	^{66}Ga	1.67	Not detected
	^{67}Ga	Not detected	Not detected
	^{68}Ga	8.27	Not detected

*Target bombarded at 20 μA for 30 min
†Target bombarded at 40 μA for 30 min

Table 2. Comparison between no-carrier-added specific activities (NCA-SA) of purified ^{61}Cu determined from ICP-MS and effective specific activities (ESA) determined by titration with each of three Cu(II) chelators: diamsar, p-SCN-Bn-NOTA and p-SCN-Bn-DOTA. Uncertainties are ±SD, with 4 experiments for each target material. Expt numbers equate to those shown in Figs 5 and 6.

Target Material	ESA by titration with chelator (MBq/µg)			NCA-SA by ICP-MS (MBq/µg)
	diamsar	NOTA	DOTA	
^{nat}Zn				
Expt #1	185.9± 6.5	74.06± 2.7	18.6± 0.6	233.3 ± 23.3
Expt #2	92.2± 5.1	23.4± 1.4	5.9± 0.3	191.9± 19.2
Expt #3	84.7± 4.7	26.6± 1.7	4.7± 0.2	143.3± 14.3
Expt #4	137.5± 2.8	70.3± 4.1	35.9± 0.6	169.4± 16.9
Enriched ^{64}Zn				
Expt #5	379.7± 9.5	31.7± 1.1	19.0± 1.1	434.4 ± 43.4
Expt #6	326.5.5± 10.1	63.3± 3.7	30.3± 1.8	482.1± 48.2
Expt #7	412.5± 15.3	82.7± 5.4	8.3± 0.4	506.2± 50.6
Expt #8	368.9± 11.8	19.7± 1.1	16.9± 0.2	496.4± 49.6

Fig. Captions

Fig. 1 Excitation functions of $^{nat}Zn(p,\alpha)$ reactions up to 18 MeV for the production of ^{61}Cu, ^{66}Ga, ^{67}Ga, ^{65}Zn [10] and ^{68}Ga [11].

Fig. 2 Chemical structures of the Cu(II) chelators p-SCN-Bn-DOTA, p-SCN-Bn-NOTA and diamsar.

Fig. 3 Zinc foil target that is contained between an aluminium target annulus (left) and an aluminium cradle (right).

Fig. 4 Schematic representations of the column chromatography purification of ^{61}Cu from (a) irradiated ^{nat}Zn foil target and; (b) irradiated ^{64}Zn foil target.

Fig. 5 ICP-MS derived trace metals contamination in the final ^{61}Cu product obtained after separation and purification from a ^{nat}Zn foil target irradiation.

Fig. 6 ICP-MS derived trace metals contamination in the final ^{61}Cu product obtained after separation and purification from an enriched ^{64}Zn foil target irradiation.

Fig. 7 Complexation of ^{61}Cu with increasing concentrations of the Cu(II) chelators diamsar, p-SCN-Bn-NOTA and p-SCN-Bn-DOTA at room temperature for the thirty-minute time point.

Fig. 1

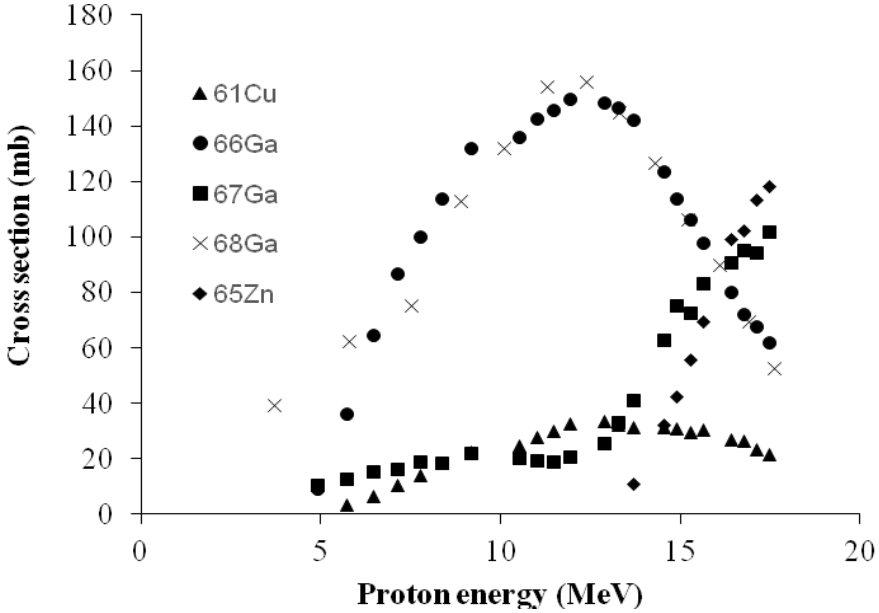
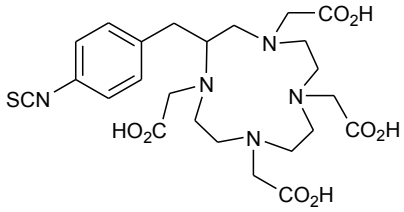
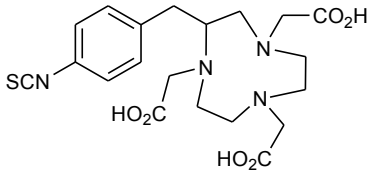

180
160
140
120
100
80
60
40
20
0
Cross section (mb)
61Cu
66Ga
67Ga
68Ga
65Zn
0
5
10
15
20
Proton energy (MeV)

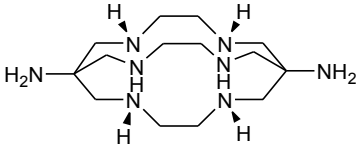
Fig. 2



p-SCN-Bn-DOTA



p-SCN-Bn-NOTA



diamsar

Fig. 3

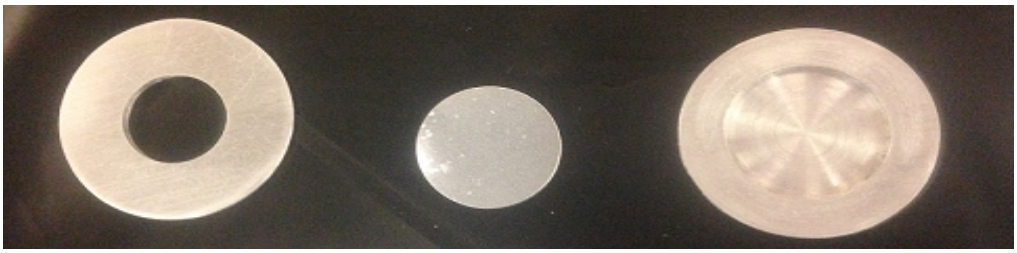
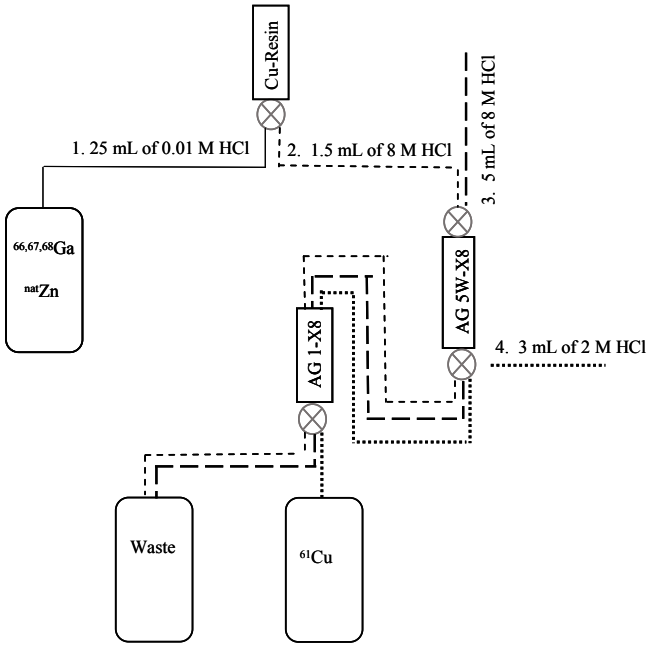
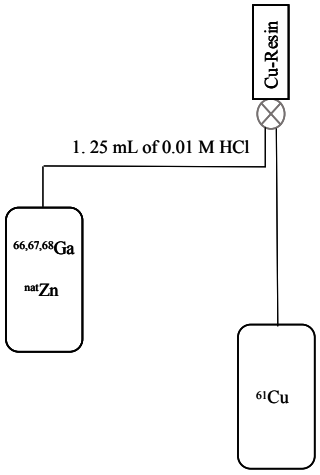


Fig. 4


Cu-Resin
1. 25 mL of 0.01 M HCl
2. 1.5 mL of 8 M HCl
3. 5 mL of 8 M HCl
66,67,68Ga
natZn
AG 5W-X8
AG 1-X8
4. 3 mL of 2 M HCl
Waste
61Cu

(a)


Cu-Resin
1. 25 mL of 0.01 M HCl
66,67,68Ga
natZn
61Cu

(b)

Fig. 5

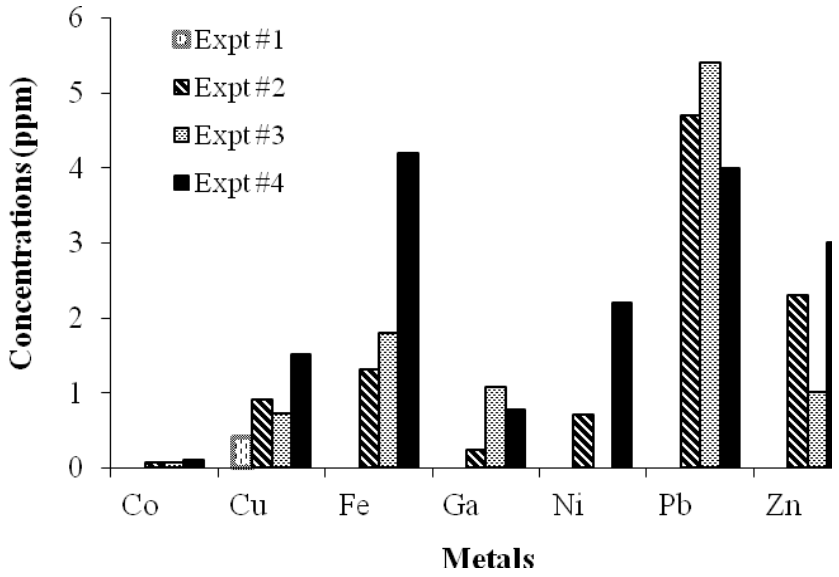

Concentrations (ppm)
6
5
4
3
2
1
0
Expt #1
Expt #2
Expt #3
Expt #4
Co
Cu
Fe
Ga
Ni
Pb
Zn
Metals

Fig. 6

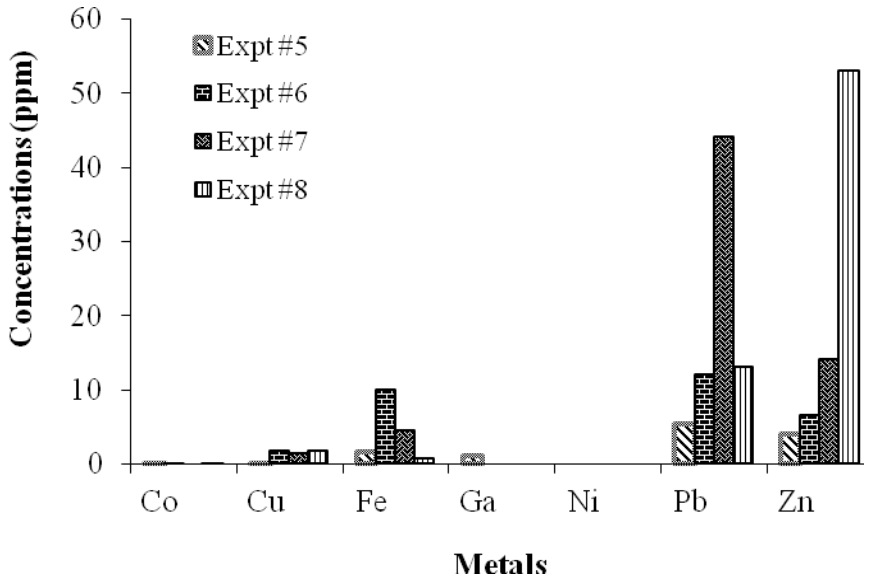
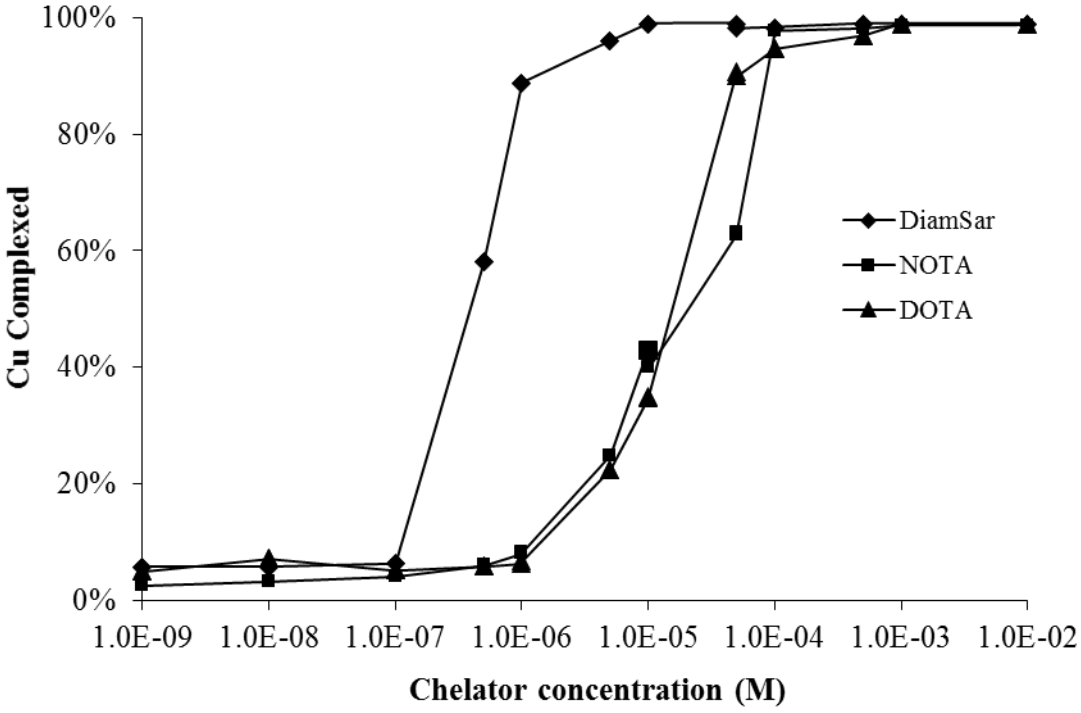

60
50
40
30
20
10
0
Concentrations (ppm)
Expt #5
Expt #6
Expt #7
Expt #8
Co
Cu
Fe
Ga
Ni
Pb
Zn
Metals

Fig. 7


100%
80%
60%
40%
20%
0%
Cu Complexed
1.0E-09
1.0E-08
1.0E-07
1.0E-06
1.0E-05
1.0E-04
1.0E-03
1.0E-02
Chelator concentration (M)
DiamSar
NOTA
DOTA