A purification system for $^{64}\text{Cu}$ produced by a biomedical cyclotron for antibody PET imaging

Teruaki Toyota · Tadashi Hanafusa · Takashi Oda · Iwane Koumura · Takanori Sasaki · Eiji Matsuura · Hiromi Kumon · Tsuneo Yano · Toshiro Ono

Received: 15 November 2012 / Published online: 6 December 2012 © The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract

Ion exchange is a simple and efficient method for separating no-carrier-added $^{64}\text{Cu}$ from an irradiated Ni target. We developed a semi-automated two-round $^{64}\text{Cu}$ separation system equipped with a strong-base anion exchange resin column. We first verified the efficiency of the system using a non-radioactive substitute consisting of 25 mg of Ni and 127 ng of Cu, and confirmed that Cu was completely eluted at the second round of the separation step. After the bombardment, separation of $^{64}\text{Cu}$ from the Ni target was achieved with high radiochemical purity. $^{64}\text{Cu}$ produced and separated in this study had an extremely low level of Ni impurity. It could be used for labeling monoclonal antibodies for antibody positron emission tomography imaging and synthesizing radiopharmaceuticals.

Keywords

$^{64}\text{Cu}$ · Cyclotron · Anion exchange chromatography · Positron emission tomography (PET) · Atomic absorption spectrometry

Introduction

$^{64}\text{Cu}$ is a useful radionuclide for positron emission tomography (PET) [1, 2] as well as a potential radiation therapeutic reagent [3, 4], due to its intermediate half-life of 12.7 h and emission characteristics of both $\beta^-$ (40 %) and $\beta^+$ (19 %). $^{64}\text{Cu}$ is normally produced from highly enriched $^{64}\text{Ni}$ via the reaction of $^{64}\text{Ni}$ (p, n) $^{64}\text{Cu}$ by a cyclotron [5, 6]. For the separation of $^{64}\text{Cu}$ from a $^{64}\text{Ni}$ target and other trace amounts of byproducts, several methods can be used, such as precipitation, solvent extraction, electroplating, and ion exchange [7–9]. Among them, an ion exchange methodology using strong-base anion exchange resin is the most effective for the separation and purification of $^{64}\text{Cu}$ [5, 10–12]. However, it is difficult to completely separate a tiny fraction of the cyclotron-produced $^{64}\text{Cu}$ from the extremely large amount of the $^{64}\text{Ni}$ target. For example, the ratio of $^{64}\text{Ni}$ target to $^{64}\text{Cu}$ is in the order of millions.

In the case of handling cyclotron-produced radioactive nuclides, we must avoid any manual performance, as this involves very high irradiation doses to the operators. Obata et al. [13] developed a remote-controlled $^{64}\text{Cu}$-separation apparatus equipped with a strong-base anion exchange resin column. In this study, we developed a semi-automated $^{64}\text{Cu}$-separation system, which is placed in the hot cell. It enabled the separation of high-quality and no-carrier-added $^{64}\text{Cu}$ suitable for labeling monoclonal antibodies for antibody PET imaging.

Materials and methods

Reagent

Isotopically enriched $^{64}\text{Ni}$ (99 %) was purchased from Isoflex Co. (San Francisco, CA, USA). Ultra-grade HCl
and HNO₃ were purchased from Sigma Aldrich (Tokyo, Japan). Cu and Ni standard solution (1 mg/ml) for atomic absorption spectrometry were obtained from Wako Pure Chemical Industries (Tokyo, Japan). Ultra-pure water was also from Wako Pure Chemical Industries.

Preparation of Ni target and ⁶⁴Cu production

The Ni target was prepared by the electrodeposition of enriched ⁶⁴Ni on a 31-mm-diameter Au disk (Sumitomo Heavy Industries, Ltd., Tokyo, Japan). The Au disk, with the plated ⁶⁴Ni (0.5 cm²), was mounted on a water-cooled target holder and irradiated with 12 MeV protons using a biomedical cyclotron (Cypris HM-12S, Sumitomo Heavy Industries, Ltd.). The production of ⁶⁴Cu was performed at currents of 15–20 µA.

Separation of ⁶⁴Cu

After bombardment, ⁶⁴Cu was separated from the Ni target in a single step on a strong-base anion exchange resin column using a prototype semi-automated separation apparatus (Sumitomo Heavy Industries, Ltd.). All of the solution was pumped and supplied to the column by N₂ gas. The irradiated ⁶⁴Ni was dissolved off the Au disk in 10 ml of 6 M HCl at 200 °C for 40 min and evaporated to dryness. The residue was dissolved in 10 ml of 6 M HCl and transferred onto a 0.8 × 4-cm AG1-X8 anion exchange column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) equilibrated with 6 M HCl. The column was washed twice with 8 and 5 ml of 6 M HCl, and we collected ⁶⁴Ni effluent for recycling. After switching the eluent to 10 ml of 0.1 M HCl, ⁶⁴Cu was eluted and collected. ⁶⁴Cu radioactivity of each eluate was measured in a dose calibrator (CRC-25PET, Capintec, Inc., Ramsey, NJ, USA).

Non-radioisotope substitute for target-dissolved solution

The solution consisting of non-radioactive Ni and Cu was prepared to substitute for radioactive target-dissolved solution. A 101 mg of nickel chloride hexahydrate (25 mg of Ni) and 341 ng of copper chloride dihydrate (127 ng of Cu) were dissolved in 10 ml of ultra-grade nitric acid. The amount of Ni and Cu was described in a previous report [14].

Atomic absorption spectrometry

A flame atomic absorption spectrometer (Z-9000, Hitachi, Ltd., Tokyo, Japan) equipped with a hollow cathode lamp was used for the determination of Ni and Cu. The wavelengths were 232.0 and 324.8 nm for Ni and Cu, respectively. Analytical working solution containing 100, 200, 400, and 800 ng of Ni and 12.5, 25, 50, and 100 ng of Cu were prepared by the appropriate dilution of the 1 mg/ml standard solution with ultra-grade nitric acid, respectively. The absorbance of blank, analytical solutions, and sample solutions was measured successively at the optimized operating conditions.

Determination of radionuclide purity

The determination of radionuclide purity of separated ⁶⁴Cu was performed by γ-spectrometry with a Ge semiconductor detector (GMX15P4-70, SEIKO EG&G, Tokyo, Japan). Data analysis was performed using Gamma Studio software (SEIKO EG&G).

Results and discussion

Table 1 shows the results of two independent production of ⁶⁴Cu. The separation of ⁶⁴Cu from the Ni target was performed in a single-round anion exchange resin column using a prototype semi-automated apparatus. Nearly 50 % of ⁶⁴Cu was eluted and separated. However, a significant amount of the ⁶⁴Cu radioisotope went to Ni waste (column flow-through and effluent) and remained on the resin column. The results showed the poor reproducibility of the single-round separation methodology.

In order to investigate the separation efficiency of the single-round methodology, a non-radioisotope substitute consisting of 25 mg of Ni and 127 ng of Cu was applied to the prototype apparatus. A 1-ml fraction was collected at each step for Ni or Cu determination using the flame atomic absorption spectrometer. As shown in Fig. 1, Ni was not completely washed out even in the last washing step with 6 M HCl. A large amount of Ni remained on the

| Table 1 ⁶⁴Cu production and separation results by the prototype separation apparatus |
|-----------------|---|---|
|  | 1  | 2  |
| Bombardment    |   |   |
| Current (µA)   | 15 | 20 |
| Time (min)     | 70 | 150 |
| Charge (mC)    | 60.3 | 174.1 |
| Yield (MBq/µA h) | 33.5 | 28.6 |
| Predicted yield (MBq/µA h) | 36.8 | 35.3 |
| Radioactivity (MBq) |   |   |
| Cu eluate      | 31.0 (47.3 %) | 277 (52.8 %) |
| Ni effluent    | 9.4 (14.3 %) | 244 (46.0 %) |
| Remaining in column | 25.2 (38.4 %) | 5.1 (1.1 %) |

a Radioactivity was decay-corrected at the measurement after loading of the target-resolved solution onto the column according to the half-life of ⁶⁴Cu (12.7 h)
column. Ni was continuously stripped from the column and mixed in the Cu eluate portion. Cu was efficiently eluted with 1 M HCl compared with 0.1 M HCl. However, twice the amount of Ni (ab. 200 ng) was contaminated even in the second 1-ml portion of the Cu eluate (ab. 100 ng).

Because of the insufficient separation performance of the single-round methodology, we developed the two-round separation methodology and constructed a new semi-automated separation apparatus (Figs. 2, 3). After the first round of column treatment, eluted and collected Cu in 1 M HCl solution was readjusted to 6 M HCl solution by adding an appropriate volume of 12 M HCl. It was further purified through the second round of column treatment (Fig. 2). All the solution was transferred to the column reservoir by N₂ gas. Then, the solution was passed through the resin column from the reservoir at a normal atmospheric pressure (Fig. 3). We analyzed the Cu separation performance by this apparatus using a non-radioisotope substitute. A significant amount of Ni was still co-eluted with Cu at the first round of separation. However, by the second round of
separation, a negligible amount of Ni was found in the Cu eluate (Fig. 4). Cu was completely eluted in the second to fourth fractions.

We performed three independent 64Cu separations from a Ni target using the two-round semi-automated separation system. As shown in Table 2, 64–67 % of 64Cu was eluted by the first round of the separation step. In the subsequent second round of separation, 62–66 % of 64Cu was eluted and separated. This revealed that nearly 95 % of 64Cu eluted in the first round of separation was recovered in the second round of separation. A small amount of 64Cu was found in the column flow-through solution and effluent. A negligible amount of 64Cu remained on the resin column. With the use of the Ge detector, the radiochemical purity of the separated 64Cu was confirmed (Fig. 5).

Ion exchange is a simple and efficient method for separating metals. It is based on the formation of anionic metal chloro complexes in a highly concentrated HCl solution and on the difference of their distribution coefficients in a strong-base anion exchange resin [15]. Ni cannot form a chloro complex in HCl solution and is not retained by the anion exchange resin. On the other hand, Cu can form a stable chloro complex in a concentrated HCl solution and is retained by the resin. After Ni is stripped from the resin column with a concentrated HCl solution, Cu can be successfully eluted from the resin column by an appropriate concentration of HCl. 64Cu is produced from highly enriched 64Ni via reactions of 64Ni (p, n) 64Cu by a cyclotron. Because the produced 64Cu is a very tiny fraction of the irradiation Ni target, it is usually difficult to obtain a high yield of 64Cu with extremely low Ni impurity. The remaining Ni will interfere with the antibody-labeling process of 64Cu. It may also be harmful to human health [16, 17].

Taken together, our semi-automated system enabled the separation of high-quality 64Cu suitable for labeling monoclonal antibodies for antibody PET imaging. In the current system, however, the target recovery at the end of bombardment and transfer to the hot cell has to be

---

**Fig. 3** Two-round 64Cu separation system. (1) Vessel for target dissolving, (2) 6 M HCl washing solution vessel, (3) 1 M HCl washing solution vessel, (4) strong-base anion exchange resin column, (5) reservoir, (6) Ni collection vial (column flow-through), (7) vial for effluent, (8) vial for 64Cu eluate 1 (first round), (9) vial for 64Cu eluate 2 (second round)

---

**Fig. 4** Separation and purification of Cu from Ni in a non-radioactive substitute. Non-radioactive substitute solution was applied to the two-round semi-automated separation system. A 1-ml fraction was collected at each step, and the Ni and Cu concentration was determined by the atomic absorption spectrometer. **a** First round of separation, **b** second (final) round of separation
A full-automated target handling system is currently being constructed to reduce exposure doses to the operators to as low as possible.

Conclusion

In this study, we developed a semi-automated two-round \(^{64}\)Cu separation system. It is equipped with a strong-base anion exchange resin column. We first verified the efficient performance of the system, and confirmed that Cu was completely eluted at the second round of the separation step. There was a negligible amount of Ni in the Cu eluate. After the bombardment, separation of \(^{64}\)Cu from the Ni target was successfully achieved with high radiochemical purity. The semi-automated system enabled the separation of cyclotron-produced \(^{64}\)Cu suitable for labeling monoclonal antibodies for antibody PET imaging.

Acknowledgments

We thank Mr. H. Okamoto and Mr. S. Iwasa (Central Research Laboratory, Okayama University Medical School) for atomic absorption spectrometry. We also thank Dr. T. Nagamatsu (Advanced Science Research Center, Okayama University) for excellent technical assistance and Mrs. T. Terada for preparation of the manuscript. This work was supported in part by Japan Science and Technology Agency.

Open Access

This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Matarrese M, Bedeschi P, Scardaoni R, Sudati F, Savi A, Pepe A, Masiello V, Todde S, Gianelli L, Messa C, Fazio F (2010) Automated production of copper radioisotopes and preparation of high specific activity \(^{64}\)Cu-ATSM for PET studies. Appl Radiat Isot 68:5–13
2. Achmad A, Hanaoka H, Yoshioka H, Yamamoto S, Tominaga H, Araki T, Ohshima Y, Oriuchi N, Endo K (2012) Predicting cetuximab accumulation in KRAS wild-type and KRAS mutant colorectal cancer using \(^{64}\)Cu-labeled cetuximab positron emission tomography. Cancer Sci 103:600–605
3. Connett JM, Anderson CJ, Guo LW, Schwarz SW, Zinn KR, Rogers BE, Siegel BA, Philpott GW, Welch MJ (1996) Radioimmunotherapy with a \(^{64}\)Cu-labeled monoclonal antibody: a comparison with \(^{67}\)Cu. Proc Natl Acad Sci USA 93:6814–6818
4. Nguyen K, Parry JJ, Rogers BE, Anderson CJ (2011) Evaluation of copper-64-labeled somatostatin agonists and antagonist in SST2-transfected cell lines that are positive and negative for p53: implications for cancer therapy. Nucl Med Biol 39:187–197
5. Zweit J, Smith AM, Downey S, Sharma HL (1991) Excitation functions for deuteron induced reactions in natural nickel: production of no-carrier-added \(^{64}\)Cu from enriched \(^{64}\)Ni targets for positron emission tomography. Appl Radiat Isot 42:193–197
6. Szelecényi F, Blessing G, Qaim SM (1993) Excitation functions of proton induced nuclear reactions 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. Wolmachev V, Lundqvist H, Einarsson L (1998) Production of \(^{61}\)Cu from a natural nickel target. Appl Radiat Isot 49:78–81
8. Shaw MJ, Haddad PR (2004) The determination of trace metal pollutants in environmental matrices using ion chromatography. Environ Int 30:403–431
9. Fan X, Parker DJ, Smith MD, Ingram A, Yang S, Seville JP (2006) A simple and selective method for the separation of Cu radioisotopes from nickel. Nucl Med Biol 33:938–944
10. McCarthy DW, Shefer RE, Klinkowstein RE, Bass LA, Margeneau WH, Cutler CS, Anderson CJ, Welch MJ (1997) Efficient production of high specific activity \(^{64}\)Cu using a biomedical cyclotron. Nucl Med Biol 24:35–42
11. Hou X, Jacobsen U, Jørgensen JC (2002) Separation of no-carrier-added \(^{64}\)Cu from a proton irradiated \(^{64}\)Ni enriched nickel target. Appl Radiat Isot 54:772–777
12. Avila-Rodríguez MA, Nye JA, Nickles RJ (2007) Simultaneous production of high specific activity \(^{64}\)Cu and \(^{61}\)Ni at a small cyclotron. Appl Radiat Isot 65:1115–1120
13. Obata A, Kasamatsu S, McCarthy DW, Welch MJ, Saji H, Yonekura Y, Fujibayashi Y (2003) Production of therapeutic quantities of \(^{64}\)Cu using a 12 MeV cyclotron. Nucl Med Biol 30:535–539

Table 2 \(^{64}\)Cu separation results by the semi-automated two-round separation system

|                      | 1 (%) | 2 (%) | 3 (%) |
|----------------------|-------|-------|-------|
| First round          |       |       |       |
| Column flow-through 1| 0.3a  | 0.4   | 0.1   |
| Effluent 1-1         | 8.1   | 8.0   | 6.7   |
| Effluent 1-2         | 16.2  | 14.8  | 13.9  |
| Eluate 1             | 65.2  | 67.1  | 69.3  |
| Second round         |       |       |       |
| Column through (equilibration) | 0.8   | 0.7   | 0.6   |
| Column flow-through 2| 0     | 0.1   | 0     |
| Effluent 2           | 1.3   | 2.4   | 1.6   |
| Eluate 2             | 62.3  | 62.4  | 65.8  |
| Remaining in column  | 0.9   | 0.8   | 0.4   |
| Remaining in tube line | 9.6   | 10.4  | 11.0  |

a They were decay-corrected at EOB according to the half-life of \(^{64}\)Cu (12.7 h), and expressed as a percentage

Fig. 5 The γ-ray spectrum of the separated \(^{64}\)Cu fraction analyzed by a Ge semiconductor detector
14. Tazawa S, Hasegawa K, Takahashi K, Yano T, Watanabe Y (2011) Current practice of producing $^{64}$Cu-DOTA-Trastuzumab injections RIKEN CMIS according to GMP for investigational products (in Japanese). Pharm Technol Jpn 27:431–440

15. Michaelis C, Tarlano NS, Clune J, Yolles R (1962) A complete separation of a mixture of iron(III), cobalt(II), molybdenum(VI), aluminum(III), and nickel(II) by ion exchange chromatography. Anal Chem 34:1425–1426

16. Oller AR, Costa M, Oberdörster G (1997) Carcinogenicity assessment of selected nickel compounds. Toxicol Appl Pharmacol 143:152–166

17. Zoroddu MA, Schinocca L, Kowalik-Jankowska T, Kozłowski H, Salnikow K, Costa M (2002) Molecular mechanisms in nickel carcinogenesis: modeling Ni(II) binding site in histone H4. Environ Health Perspect 110(Suppl 5):719–723