

Preparation of [^{64}Cu] Pyruvaldehyde-bis (N^4 -methylthiosemicarbazone) complex as a PET and/or therapeutic radiopharmaceutical

A.R. Jalilian*, P. Rowshanfarzad, J. Moafian, M. Kamali-dehghan, M. Mirzaii

Cyclotron & Nuclear Medicine Department, Nuclear Research Center for Agriculture and Medicine, Karaj, Iran

ABSTRACT

Background: Copper-64 ($T_{1/2}=12.7$ h) is an important radionuclide used both in PET imaging and therapy. [^{64}Cu]-pyruvaldehyde-bis (N^4 -methylthiosemicarbazone) ([^{64}Cu]-PTSM) is one of the most famous copper radiopharmaceuticals with unique specifications (suitable half life, stability, etc.). The wide range of ^{64}Cu applications arouse great interest for its production.

Materials and Methods: Cu-64 was produced via the $^{68}\text{Zn} (p,\alpha) ^{64}\text{Cu}$ nuclear reaction and isolated from the irradiated target by a two-step chemical method. [^{64}Cu]-PTSM was prepared using in-house made PTSM ligand and [^{64}Cu] cuprous acetate. The complex formation parameters (time, temperature, concentration and elution methods) were determined carefully.

Results: Copper-64 was prepared in chloride form (≈ 200 mCi, $>95\%$ chemical yield at $180 \mu\text{A}$ for 1.1 h irradiation, radionuclidic purity $>96\%$, copper-67 as impurity). The solution of ^{64}Cu -PTSM was prepared in $>80\%$ radiochemical yield and more than 98% radiochemical purity. Quality controls and stability tests were performed for the final solution.

Conclusion: [^{64}Cu]-PTSM was prepared at the radiopharmaceutical scales with high quality and potential to be used in therapeutic/imaging centers. *Iran. J. Radiat. Res., 2004; 2 (3): 107-115*

Keywords: Copper-64, PTSM, targeted therapy, hypoxia imaging, cyclotron, PET.

INTRODUCTION

Copper offers a unique selection of radioisotopes (^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , and ^{67}Cu) with half-lives ranging from 9.8 min to 61.9 h, suitable for imaging and/or radiotherapy (Blower *et al.* 1996). Copper-64 (half-life= 12.7 h; β^+ 655 keV [19%]; β^- 573 keV [40%]; E.C. [41%]) is an attractive radionuclide for PET imaging and targeted therapy of cancer (Cutler *et al.* 1999). Copper-64 has been widely used in the labeling of peptides like octreotide (Maa *et*

al. 2002, Anderson *et al.* 1995), bombesin analogs (Rogers *et al.* 2004), integrin (Chen *et al.* 2004), VIP (Hou *et al.* 2002). Some of these compounds have already been used in PET imaging (Mathias *et al.* 1990, Mathias *et al.* 1991, Shelton *et al.* 1989, Green *et al.* 1988). [^{64}Cu]- diethylenetriaminepentaacetic acid ([^{64}Cu]-DTPA) has been used for the differential investigation of disorders in cerebrospinal fluid (CSF) transit and absorption (Maziere *et al.* 1983).

[^{64}Cu]-pyruvaldehyde-bis (N^4 -methyl thiosemicarbazone ([^{64}Cu]-PTSM) was prepared to be used in internal radiation therapy and imaging of hypoxic tissues in late 1980's (Green 1987, Kostyniak *et al.* 1990). Since then, this complex has been applied in the determination of regional blood flow and renal perfusion (Shelton *et al.* 1990, Shelton *et al.* 1989, Young

* Corresponding author:

Dr. A.R. Jalilian, Cyclotron & Nuclear Medicine Department, Nuclear Research Center for Agriculture and Medicine: Atomic Energy Organization of Iran, Karaj, Iran.
P.O.Box:31585-4395, Fax: +98 261 4436397
E-mail: ajalilian@nrcam.org

et al. 1994a, Young et al. 1994b), tumor blood flow (Mathias et al. 1991), ischemia (Wada et al. 1994, Young et al. 1994b) and finally radiotherapy of tumors (Jason et al. 2001, Adonai et al. 2002). Figure 1 shows the chemical structure of PTSM.

This radionuclide is mainly produced via ^{64}Ni (p,n) ^{64}Cu reaction at medical cyclotrons (Zweit et al. 1990, Szelecsényi et al. 1992, Hou et al. 2002), although it can be prepared in lower yields by ^{68}Zn (p, α) ^{64}Cu reaction (Hilgers et al. 2003, Boothe et al. 1991).

Based on the interesting therapeutic/imaging properties of ^{64}Cu -PTSM and possibility of copper-64 production via ^{68}Zn (p, α) ^{64}Cu reaction as a by-product at our 30MeV cyclotron, we had become interested in production and yield optimization of [^{64}Cu]-PTSM as a possible PET tracer/therapeutic agent in hypoxia imaging.

MATERIALS AND METHODS

Chemicals were purchased from Aldrich Chemical Company (Milwaukee, U.S.A.). Thin layer chromatography (TLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20×20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Ethyl acetate and normal saline used for labeling were of high purity. $^1\text{H-NMR}$ spectra were obtained on a FT-80 (80

MHz) Varian instrument with tetramethylsilane as the internal standard. Infrared spectra were taken on a Perkin-Elmer 781 instrument (KBr disc). Production of ^{64}Cu was performed in the NRCAM 30 MeV cyclotron (IBA, Cyclone-30). Enriched Zn-68 with a purity of more than 98% was provided by the Ion Beam Application Department, NRCAM, Karaj, Iran. Radiochromatography was fulfilled by counting 5 mm-slices of polymer-backed silica gel paper using a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL). Radionuclide purity was checked by the same detector. All calculations and TLC counting were based on 1346 keV peak.

Targetry of zinc-68

An electroplated ^{68}Zn target on a gold-coated copper backing plate was irradiated at an angle of 6 degrees by the proton beam in order to achieve higher production yield. The target was cooled by a flow of 18°C distilled water, with a rate of 50 Lit/min. while the optimum energy for the production of ^{64}Cu via ^{68}Zn (p, α) ^{64}Cu reaction is usually 35-20 MeV (Hilgers et al. 2003), the highest available proton energy was 30 MeV. On the other hand, since the threshold energy of the ^{68}Zn (p, α) ^{64}Cu reaction is 8 MeV (Hilgers et al. 2003), the target had to be thick enough to reduce the energy of the incident protons from 30 MeV to about 20

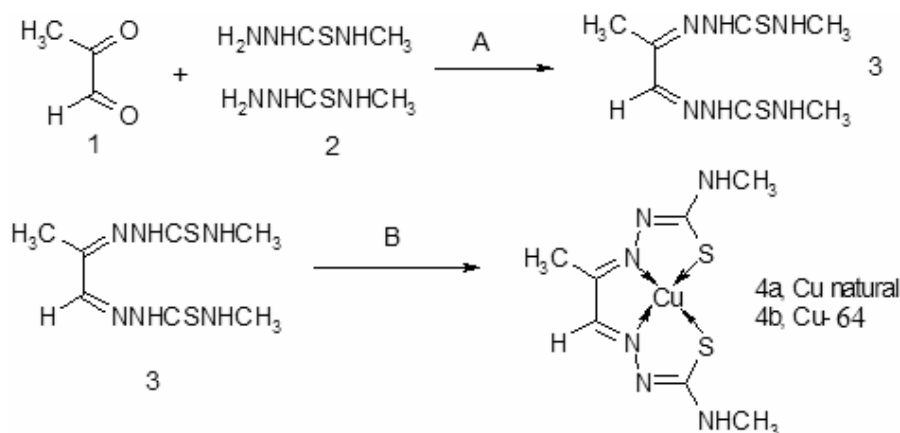


Figure 1. Schematic diagram of the preparation method for PTSM (3) and [^{64}Cu] PTSM (4b), A; ethanol, 50°C, B; [^{64}Cu] CuOAc, C₁₈ Sep-Pak.

MeV. SRIM nuclear program (Ziegler *et al.* 2000) was run in order to determine the best target thickness in the above energy range. The results of SRIM program showed that the best target thickness was 984 mm, but the target angle of 6° reduced the required target thickness by 10 folds. Thus we only needed to electroplate about 100 μm of the target material on the copper backing. To do so, ^{68}ZnO was dissolved in 0.05 N HCl to prepare a zinc cation-containing solution. The mass of zinc ions in the cell had to be twice of the electrodeposited layer. Hydrazine dihydrochloride (2 ml) was added as the reducing agent. Electrodeposition was performed at pH=2.5-3, with a cell volume of 480 ml and accurate density of 35 mA/cm². Platinum was used as the anode material which resulted in a 100 μm zinc layer on the gold-coated copper backing after 3.5 hours.

Separation of copper-64 from radiogallium and zinc

Ion exchange chromatography was employed in the separation process. After the target bombardment process, chemical separation was carried out in no-carrier-added form. The irradiated target was dissolved by 10 N HCl (15 ml, 20 ml of H₂O₂ added) and the solution was passed through a cation exchange resin (AG 50 WX8, H⁺ form; mesh 200-400) (h:10 cm, \O :1.3 cm) which had been preconditioned by passing 25 ml of 9 N HCl. The column was then washed by 25 ml of 9 N HCl with a rate of 1 ml/min to elute copper and zinc ion contents. To the latter elute was added 30 ml of DDH₂O.

The mixture, then, was passed through another cation exchange resin (Dowex 1X8, Cl⁻ form; mesh: 100-200) (h: 25 cm; \O :1.7 cm), which was preconditioned with 100 ml of 6N HCl. In order to elute copper-64 ions, the column was eluted by 50 ml of 2 N HCl. For the recovery of precious zinc-68 contents, the column was finally eluted by 0.05 N HCl (150 ml). The whole chemical separation process took about 105 min. The resulting high-purity [^{64}Cu] CuCl₂ solution was used directly in the labeling step.

Radionuclide purity

The gamma spectroscopy of the final sample was carried out by an HPGe detector. The peaks were observed and the area under the curve was counted for 1000 seconds.

Preparation of pyruvaldehyde-bis (N^4 -methylthiosemicarbazone)

PTSM was prepared according to the reported method for the production of thiosemicarbazones (Gingras *et al.* 1965). Schematic diagram of the preparation method for PTSM and [^{64}Cu] PTSM are shown in figure 1.

Ethanol was added to a stirring mixture of N^4 -methylthiosemicarbazide (210 mg, 2 mmol) in absolute anhydrous drop wise pyruvaldehyde (115 mg, 1 mmol) during 5 min. The mixture was stirred for 10 min at 50°C. The reaction mixture was cooled in ice bath and the precipitate was filtered. The filtered mass was crystallized by hot ethanol to give a light yellow powder (60%) m.p. 241-243°C. ¹H NMR (D₆-DMSO) δ (ppm) 11.74 (s, 1H, NH-N₂), 10.33 (s, 1H, NH-N₂), 9.43 (m, 2H, NH-N₄), 7.68 (s, 1H, H-C=N), 3.31 (s, 3H, CH₃-C=N). IR (CHCl₃) ν max 3208, 3132 (N-H), 1429 (C=N), 1111 (C=S). Mass (electrospray) 246.1 (14%) M⁺, 215 (7), 172 (4), 157.1 (76), 130 (65), 115.8 (98), 73.8 (100), 56.9 (68).

Preparation of [^{64}Cu] pyruvaldehyde-bis (N^4 -methylthiosemicarbazone)

The obtained [^{64}Cu] CuCl₂ (3 mCi) dissolved in acidic medium (about 2 ml) was transferred to a 5 ml-vial containing 3M (4 ml) sodium acetate to prepare a [^{64}Cu] copper acetate solution. A mixture of pyruvaldehyde-bis (N^4 -methyl thiosemicarbazone) (3 μg) in absolute ethanol (0.1 ml) (Mathias *et al.* 1991) was added to the copper acetate solution and vortexed at 50°C for 3-5 min. The mixture (about 5 ml) was then cooled in an ice bath, and rapidly injected into a C₁₈ Sep-Pak column pretreated with 5 ml of ethanol, and 2 ml of water. The column was washed with water (4 ml) and purged with a stream of dry N₂. The labeled compound was

finally eluted using 0.2 ml-portions of absolute ethanol and the fractions were counted in HPGe detector (figure 2). The vial containing the maximum radioactivity was diluted to a 5% solution by addition of normal saline. The active solution was checked for radiochemical purity by

polymer-backed silica gel layer chromatography using dry ethyl acetate as mobile phase. The final solution was then passed through a 0.22 μm filter while the pH was adjusted to 5-7 by the addition of 3 M sodium acetate buffer.

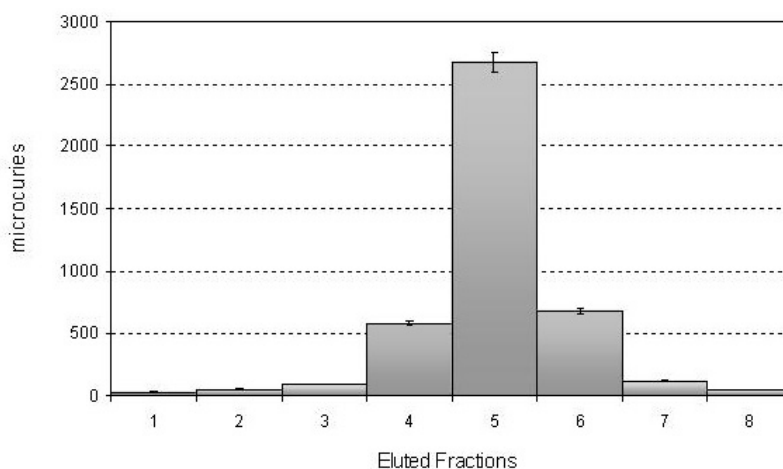


Figure 2. Radioactivity of eluted ethanol fractions from C_{18} .

Chemical purity

The formation of colored dithizone-zinc complex was measured using visible spectroscopic assay to determine Zn cation concentrations according to the literature (Marczenko 1976) using dithizone organic reagent (0.002% in CCl_4). Briefly, the presence of pinkish color of zinc-dithizone complex was checked for the test samples, 1, 5, 10 ppm standards and finally a blank solution (1 ml each). The color of the test tube had been less than that of the standard.

Radiochemical purity

Radio thin layer chromatography was performed using a mixture of dry ethyl acetate as the mobile phase for both pre-column and post-column fractions (figures 3 and 4). The radiochromatogram showed a major and distinct radio peak at the R_f of 0.90, using an in-house made radiochromatogram scanner equipped with a HPGe detector. The step motor was installed to count 0.4 cm-piece each 30 second through a slot of a shielded chamber. Uncomplexed ^{64}Cu eluted at R_f of 0.0. Thus, the radio-

chemical yields (more than 98% in each case, $n=9$) were determined by comparison of uncomplexed ^{64}Cu and the major radio peak at $R_f=0.90$.

Stability of [^{64}Cu] PTSM complex in the final product

Stability studies were based on the previous studies performed for radiolabeled copper complexes. A sample of [^{64}Cu] PTSM (0.5 mCi) was kept at room temperature for 5 hrs while checked by RTLC every half an hour. A micropipet sample (5 μl) was taken from the shaking mixture and the ratio of free radiocopper to [^{64}Cu] PTSM was checked by radio thin layer chromatography (eluent: dry ethyl acetate).

Stability of [^{64}Cu] PTSM complex in presence of serum

A mixture of 5 parts of serum and one part of radiopharmaceutical (0.2 mCi) was shaken in a 37-degree incubator under nitrogen atmosphere. A micropipette sample (5 μl) was taken from the shaking mixture every 30 minutes. The

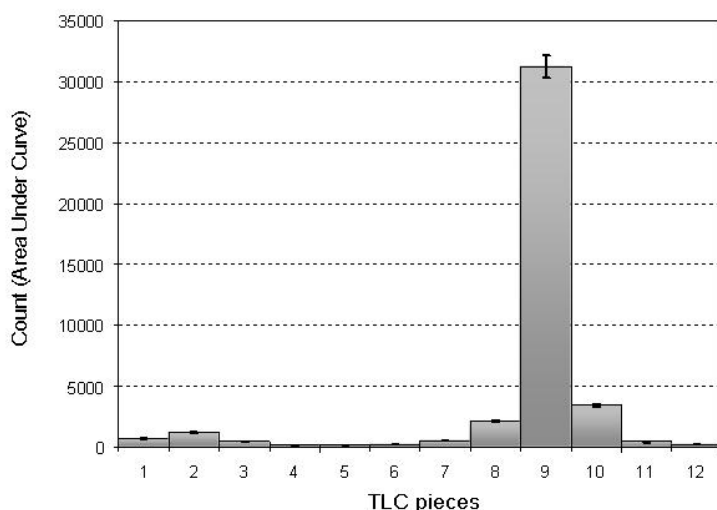


Figure 3. RTLC of the radiolabeling vial before injection into C₁₈ Sep-Pak.

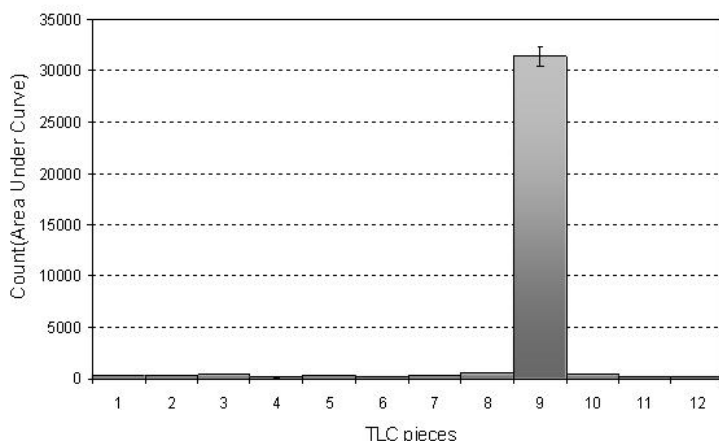


Figure 4. RTLC of final fraction after injection into C₁₈ Sep-Pak.

ratio of free radiocopper ($R_f=0$) to [^{64}Cu] PTSM ($R_f=0.8$) was checked by radio thin layer chromatography (eluent: dry ethyl acetate).

RESULTS AND DISCUSSION

Targetry & irradiation: Various nuclear reactions used for the production of ^{64}Cu were suggested. Since we had used a proton accelerator in the energy range of 15-30 MeV with a maximum current intensity of 220 microamperes, the only available reactions were $^{64}\text{Ni}(p,n)^{64}\text{Cu}$ and $^{68}\text{Zn}(p,\alpha n)^{64}\text{Cu}$. Among the mentioned reac-

tions, $^{68}\text{Zn}(p,\alpha n)^{64}\text{Cu}$ was selected according to the availability of enriched zinc-68 in our institute. The problem with the use of copper backing for the bombardment was that it could have been dissolved in the target dissolution process, introducing carrier copper into ^{64}Cu solution. For the very same, a gold layered (thickness $\sim 50\ \mu$) copper backing was used as the target substrate. Radioisotope impurities such as zinc and gallium were easily separated by chemical processes.

Many research groups have reported that the proton energy range between 35-20 MeV is the best for the production of ^{64}Cu with a minimum amount of radioactive impurities (Hilgers *et al.* 2003, Boothe *et al.* 1991); however, 20-30 MeV proton energy was chosen to achieve the maximum possible production yield, according to our available energies.

[^{64}Cu] CuCl_2 was prepared by 30 MeV proton bombardments of an electroplated enriched $0.0714\ \text{g}/\text{cm}^2$, ^{68}Zn -target at an angle of 6° in our 30 MeV cyclotron (Cyclone-30, IBA). The target was bombarded with a current intensity of 180 mA for about 1.1 h (200 μAh).

The chemical separation process was based on a no-carrier-added method. The resulting activity of ^{64}Cu was 202 mCi and the production yield was 1.01 mCi/mAh by the end of bombardment (E.O.B.).

Preparation and structure confirmation of the ligand

In order to prepare pyruvaldehyde-bis (N^4 -methylthiosemicarbazone) which was not commercially available, we tried the general thiosemicarbazone preparation procedure (Gingras *et al.* 1965). The reaction was performed in absolute ethanol containing N^4 -methyl thiosemicarbazide.

In mass spectroscopy, the molecular weight peak was observed, yet it was not significant at 246. That was not surprising; because, thiosemicarbazones are not very stable against temperature. The mass spectrometer ion source produced rather high temperatures. The mass spectrum of pyruvaldehyde-bis (N⁴- methylthiosemicarbazone) is shown in figure 5.

¹H NMR spectrum of the above mentioned compound (figure 6) was performed in DMSO at 25°C. The chemical shifts of N-CH₃ groups were very close but not exactly the same (2.96 and 3.04 ppm). The torsion of the molecule made a loss of complete symmetry for the methyl groups, so that different chemical shifts could have been observed. The imino methyl group (CH₃-C=N) had a separate chemical shift around 3.4 ppm. Broad singlet peaks were observed at various chemical shifts representing N-H protons (7.6, 10.3 and 11.7). The vinylic proton was branched by the β-CH₃ substituent into a quartet at 8.4 ppm. A multiple broad peak, at 2.5 ppm, corresponded to the NMR solvent, i.e. DMSO.

Radionuclidic purity

Gamma spectroscopy of the final product showed a radionuclidic purity higher than 96 % showing the presence of 511, 1346 keV gamma energies, all of which were resulted from ⁶⁴Cu (figure 7).

Chemical purity

In order to check the chemical purity, the concentration of Zn (from target material) was determined using colorimetric assay. The presence of zinc cations was checked by visible colorimetric assays. Even at 1 ppm of standard zinc concentration, the pinkish complex was visible by naked eye, while the test sample remained similar to the blank. The colorimetric assay demonstrated that the zinc cation concentration was far below the maximum permitted levels, i.e. 5 ppm (less than 1.5 ppm zinc).

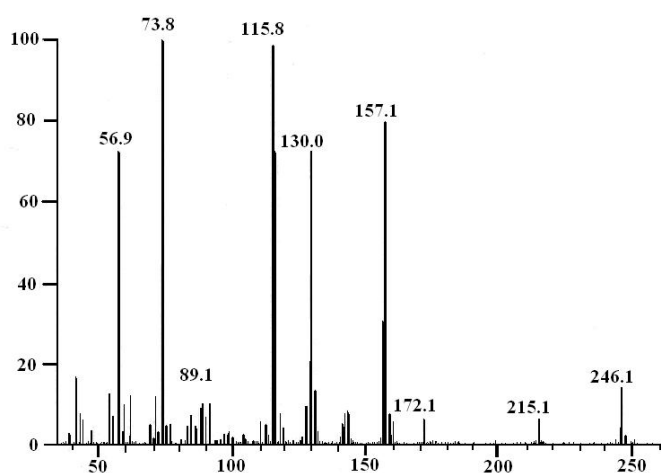


Figure 5. Mass spectrum of pure PTSM used in the labeling procedure.

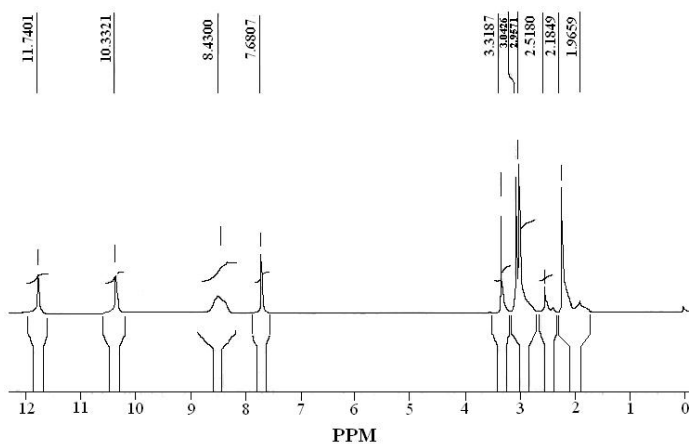


Figure 6. ¹H-NMR spectrum of pure PTSM used in the labeling procedure.

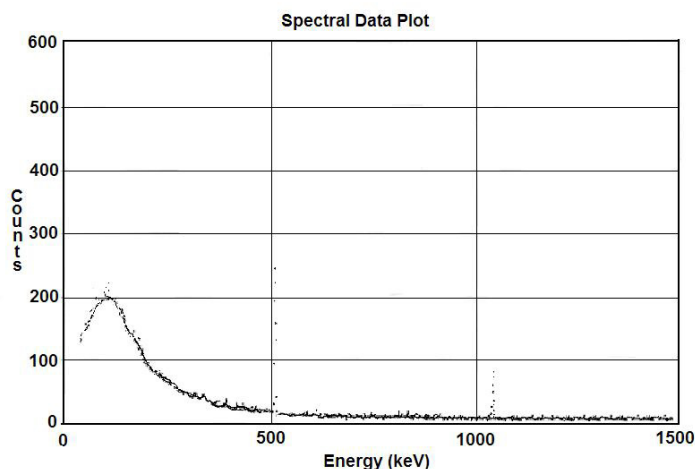


Figure 7. Gamma spectroscopic diagram of the final purified [⁶⁴Cu] CuCl₂ solution.

Radiolabeling of pyruvaldehyde-bis (N^4 -methylthiosemicarbazone)

The freshly eluted copper-64 chloride solution was changed into copper acetate using a 3M sodium acetate solution, keeping a suitable pH between 5-7 for the complex formation. The ligand 3, dissolved in absolute ethanol, was then added to the buffered solution, so that a final 2% concentration of ethanol was obtained. This procedure was superior to the former labeling procedure using DMSO as the ligand solvent (Green *et al.* 1988). The mixture was then vortexed in a tube shaker and left at room temperature for 5-10 minutes. RTLC of the compound showed that the most of the radio-copper was complexed into PTSM ligand at this stage (figure 3).

The solid phase separation of the ^{64}Cu -PTSM from free copper cations was performed to obtain a higher purity and then the lipophilic complex was eluted using 0.2-ml absolute ethanol fractions (figure 2).

Because of the engagement of several polar functional groups in its structure, labeling of PTSM with copper cation greatly affects its chromatographic properties and the final complex is, usually, highly lipophilic. Thus, free copper, the labeled and unlabeled PTSM, could easily be separated using solid phase C_{18} Sep-Pak column. In the TLC studies, the more polar un-complexed PTSM and free copper fractions, correlate to smaller R_f s ($R_f = 0.1-0.2$), while the complexed PTSM migrates at the higher R_f ($R_f = 0.8-0.9$). Since it has been shown that Cu-PTSM complex possesses hypoxia-seeking properties, its radiolabeled forms can be either used in hypoxic tumor cell diagnosis, or cerebral and myocardial infarctions. In all radiolabeling runs ($n=5$), after solid phase extraction of the labeled mixture, the integral ratio of the two peaks were constant (98:2), showing the high radiochemical purity (figure 4).

In order to obtain the best labeling reaction conditions, the complex formation was studied for temperature. Heating the reaction mixture to 50°C did not change the yield, while some

degradation products were obtained *via* TLC and RTLC. So we continued the labeling procedure at ambient temperature.

The final radiolabeled complex, diluted in normal saline, was then passed through a 0.22 mm filter (Millipore) when filtration was used to sterilize the product. Due to its thermal instability, [^{64}Cu] PTSM preparation could totally be degraded and left detectable amounts of free copper after autoclaving. The chemical stability of [^{64}Cu] PTSM was high enough to perform further studies. The final product RTLC showed no change in stability, and the patterns for trace [^{64}Cu] CuOAc and [^{64}Cu] PTSM were not changed during 5 hrs.

CONCLUSION

Total labeling and formulation of [^{64}Cu] PTSM took about 10 min, with a yield of 97-98%. A suitable specific activity product was formed *via* insertion of [^{64}Cu] copper cation. No unlabelled and/or labeled by-products were observed upon RTLC analysis of the final preparations after solid phase extraction (SPE) purification. The radio-labeled complex was stable in aqueous solutions for at least 5 hours and no significant amount of other radioactive species were detected by RTLC 12 hours after labeling. Trace amounts of [^{64}Cu] copper acetate (<2%) were detected by RTLC. The radio-chemical purity of the [^{64}Cu] PTSM was higher than 98%. [^{64}Cu] PTSM is a therapeutic/PET radiotracer with an intermediate half life, and the high chemical stability of this radiopharmaceutical makes it a very suitable diagnostic/therapeutic agent.

REFERENCES

- Adonai N., Nguyen K.N., Walsh J., Iyer M., Toyokuni T., Phelps M.E., McCarthy T., McCarthy D. W., Gambhir S. S. (2002). *Ex vivo* cell labeling with ^{64}Cu -pyruvaldehyde-bis (N^4 -methylthiosemicarbazone) for imaging

- cell trafficking in mice with positron-emission tomography. *Proc. Natl. Acad. Sci., USA*, **99**: 3030–3035.
- Anderson C.J., Pajeau T.S., Edwards W.B., Sherman E.L.C., Rogers B.E., Welch M.J. (1995). *In vitro* and *in vivo* evaluation of copper-64-octreotide conjugates. *J. Nucl. Med.*, **36**: 2315-2325.
- Anderson C.J., Connett J.M., Schwarz S.W., Rocque P.A., Guo L.W., Philpott G.W., Zinn K.R., Meares C.F., Welch M.J. (1992). Copper-64-labeled antibodies for PET imaging. *J. Nucl. Med.*, **33**: 1685-1691.
- Blower P. J., Lewis J. S., Zweit J. (1996). Copper radionuclides and radiopharmaceuticals in nuclear medicine. *J. Nucl. Med. Biol.*, **23**: 957-980.
- Boothe T.E., Tavano E., Munoz J., Carroll S. (1991). Coproduction of Copper-64 and Copper-67 using protons on Zinc-68. *J. Label. Comp. Radiopharm.*, **30**: 108.
- Cutler C.S., Lewis J.S., Anderson C.J. (1999). Utilization of metabolic, transport and receptor-mediated processes to deliver agents for cancer diagnosis. *Advanc. Drug Deliver. Rev.*, **37**: 189-211.
- Chen X., Liu S., Hou Y., Tohme M., Park R., Bading J.R., Conti P.S. (2004). Micro PET imaging of breast cancer alpha V-integrin expression with ⁶⁴Cu-labeled dimeric RGD peptides. *Mol. Imaging Biol.*, **6**: 350-359.
- Gingras B. A., Suprunchuk T., Bayley C. H. (1962). The preparation of some thiosemicarbazones and their copper complexes. Part III. *Can. J. Chem.*, **40**:1053-1057.
- Green M.A., Klippenstein D.L., Tennison J.R. (1988). Copper (II) Bis (thiosemicarbazone) complexes as potential tracers for evaluation of cerebral and myocardial blood flow with PET. *J. Nucl. Med.*, **29**: 1549-1557.
- Green M.A. (1987). A potential copper radiopharmaceutical for imaging the heart and brain: copper-labeled pyruvaldehyde bis (^N⁴-methylthiosemicarbazone). *Nucl. Med. Biol.*, **14**: 59-61.
- Hilgers K., Stoll T., Skakun Y., Coenen H.H., Qaim S.M. (2003). Cross-section measurements of the nuclear reactions ^{nat}Zn(d,x) ⁶⁴Cu, ⁶⁶Zn (d,α) ⁶⁴Cu and ⁶⁸Zn (p,αn) ⁶⁴Cu for production of ⁶⁴Cu and technical developments for small-scale production of ⁶⁷Cu via the ⁷⁰Zn (p,α) ⁶⁷Cu process. *Appl. Radiat. Isot.*, **59**: 343-351.
- Hou X., Jacobsen U., Jorgensen J.C. (2002). Separation of no-carrier-added ⁶⁴Cu from a proton irradiated ⁶⁴Ni enriched nickel target. *Appl. Radiat. Isot.*, **57**: 773-777.
- Jason S.L., Laforest R., Buettner T.L., Song S.K., Fujibayashi Y., Connett J.M., Welch M.J. (2001). Copper-64-diacetyl-bis (^N⁴-methylthiosemicarbazone): An agent for radiotherapy. *Proc. Natl. Acad. Sci., USA*, **98**: 1206–1211.
- Kostyniak P.J., Nakeeb S.M., Schopp E.M., Maccubbin A.E., John E.K., Green M.A., Kung H.F. (1990). Acute toxicity and mutagenicity of the copper complex of pyruvaldehyde-bis (N-4-methylthiosemicarbazone), Cu-PTSM. *J. Appl Toxicol.*, **10**: 417-421.
- Maa D., Lua F., Overstreet T., Milenica D.E., Brechbiela M.W. (2002). Novel chelating agents for potential clinical applications of copper. *J. Nucl. Med. Biol.*, **29**: 91-105.
- Marczenko Z. (1976). Spectrophotometric determination of elements, Ellis Horwood Ltd., 4th Ed. Warsaw, Poland. pp. 601-603.
- Mathias C.J., Welch M.J., Perry D.J., McGuire A.H., Zhu X., Connett J.M., Green M.A. (1991). Investigation of copper-PTSM as a PET tracer for tumor blood flow. *Int. J. Rad. Appl. Instrum. [B]*, **18**: 807-811.
- Mathias C.J., Welch M.J., Raichle M.E., Mintun M.A., Lich L.L., McGuire A.H., Zinn K.R., John E.K., Green M.A. (1990). Evaluation of a potential generator-produced PET tracer for cerebral perfusion imaging: Single-Pass cerebral extraction measurements and imaging with radiolabeled Cu-PTSM. *J. Nucl. Med.*, **31**: 351-359.
- Mathias C.J., Welch M.J., Perry D.J., McGuire

- A.H., Zhu X., Connett J.M., Green M.A. (1991). Investigation of copper-PTSM as a PET tracer for tumor blood flow. *Nucl. Med. Biol.*, **18**: 807-811.
- Maziere B., Stulzaft O., Verret J.M., Comar D., Syrota A. (1983). (^{55}Co)- and (^{64}Cu) DTPA: New radiopharmaceuticals for quantitative tomocisternography. *Appl. Radiat. Isot.*, **34**: 595-601.
- McCarthy D.W., Shefer R.E., Klinkowstein R.E., Bass L.A., Margeneau W.H., Cutler C.S., Anderson C.J., Welch M.J. (1997). Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl. Med. Biol.*, **24**: 35-43.
- Rogers B.E., Manna D.D., Safavy A. (2004). *In vitro* and *in vivo* evaluation of a ^{64}Cu -labeled polyethylene glycol-bombesin conjugate. *Cancer Biother. Radiopharm.*, **19**: 25-34.
- Shelton M.E., Green M.A., Mathias C.J., Welch M. J., Bergmann S. R. (1989). Kinetics of copper-PTSM in isolated hearts: A Novel Tracer for Measuring Blood Flow with Positron Emission Tomography. *J. Nucl. Med.*, **30**: 1843-1847.
- Shelton M.E., Green M.A., Mathias C.J., Welch M.J., Bergmann S.R. (1990). Assessment of regional myocardial and renal blood flow with copper-PTSM and positron emission tomography. *Circulation*, **82**: 990-997.
- Szelecsényi F., Blessing G., Qaim S.M. (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.
- Wada K., Fujibayashi Y., Taniuchi H., Tajima N., Tamaki N., Konishi J., Yokoyama A. (1994). Effects of ischemia-reperfusion injury on myocardial single pass extraction and retention of Cu-PTSM in perfused rat hearts: comparison with ^{201}Tl and ^{14}C -iodoantipyrine. *Nucl. Med. Biol.* **21**: 613-617.
- Young H., Carnochan P., Zweit J., Babich J., Cherry S., Ott R. (1994a). Evaluation of copper (II)-pyruvaldehyde bis (N -4-methylthiosemicarbazone) for tissue blood flow measurement using a trapped tracer model. *Eur. J. Nucl. Med.*, **21**: 336-341.
- Young H., Carnochan P., Zweit J., Babich J., Cherry S., Ott R. (1994b). Tissue blood flow estimation with copper (II)-pyruvaldehyde bis (N -4-methylthiosemicarbazone) and PET. *J. Nucl. Biol. Med.*, **38**: 89-91.
- Ziegler J.F., Biersack J.P., Littmark U. (2000). The stopping and range of ions in matter (SRIM Code). *Version 2000 XX*.
- Zweit J., Smith A.M., Downey S., Sharma H. L. (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.