Applied Radiation and Isotopes 121 (2017) 38–43

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Optimized procedures for manganese-52: Production, separation and radiolabeling

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ARTICLE INFO

Keywords:
Manganese
Mn-52
^{52}Mn
PET
DOTA
Serum stability
Cyclotron
Isotope production

ABSTRACT

Pressed chromium-powder cyclotron targets were irradiated with 16 MeV protons, producing ^{52}Mn with average yields of 6.2 ± 0.8 MBq/μAh. Separation by solid-phase anion exchange from ethanol-HCl mixtures recovered 94.3 ± 1.7% of ^{52}Mn and reduced the chromium content by a factor of 2.2 ± 0.4 × 10^5. An additional AG 1-X8 column was used to remove copper, iron, cobalt and zinc impurities from the prepared ^{52}Mn in 8 M HCl. The macrocyclic chelator DOTA was rapidly radiolabeled with ^{52}Mn in aq. ammonium acetate (pH 7.5 *R.T.*) with a radiochemical yield > 99% within 1 min and was stable for > 2 days in bovine serum. The improved separation and purification methodology facilitates the use of ^{52}Mn in basic science and preclinical investigations.

1. Introduction

Manganese is an element essential to living organisms in which it functions as a co-factor in a wide variety of enzymes in its divalent state (Banci, 2013). Mn^{2+} enters neurons *via* voltage gated Ca^{2+} channels and has, due to its paramagnetic properties, been used preclinically as a potent MRI contrast agent for imaging neural activity (Silva and Bock, 2008). In addition, it has been established that manganese enters active beta cells in the islets of Langerhans, thus providing the possibility of a non-invasive measure of the functional beta cell mass during the progression of diabetes (Antkowiak et al., 2013).

Manganese, however, is toxic and the maximum amount of injected MRI contrast is therefore severely limited (Crossgrove and Zheng, 2004; Koretsky and Silva, 2004; Silva et al., 2004). This fact has hindered the use of manganese enhanced MRI (MEMRI) in clinical investigations. However, the already well established methods developed in preclinical MEMRI can be readily adapted to Positron Emission Tomography (PET) by using $^{52\,g}$Mn (from here on ^{52}Mn, $t_{½}$ = 5.6 days; β^+ = 29.4%; $E_{ave\beta+}$ = 242 keV), for which sub-nM concentrations are sufficient for functional imaging, thereby completely bypassing the toxicity issues of manganese.

Currently ^{89}Zr and ^{64}Cu are the common radiometals of choice for labeling proteins with slow distribution kinetics (Severin et al., 2011; Anderson and Ferdani, 2009). However, for some preclinical and basic science applications ^{52}Mn may be a better choice. It provides aqueous chelation chemistry similar to that of ^{64}Cu, omitting the need for hard ligands like oxalate which are needed to keep ^{89}Zr chemically accessible, while having a higher β^+ branch as well as a longer half-life than both ^{64}Cu (17.6% β^+, $T_{½}$ = 12.7 h) and ^{89}Zr (22.7% β^+, $T_{½}$ = 3.3 days). In PET imaging of small animals, image resolution is critical in order to allow discrimination of different regions of interest. The primary factor limiting the resolution is the energy of the emitted positron (Moses, 2011). In principle, the lower average β^+ energy of ^{52}Mn (242 keV for ^{52}Mn *versus* 396 keV for ^{89}Zr) will result in PET resolution superior to ^{89}Zr.

Despite its facile chelation chemistry and attractive imaging properties, limited work has been reported on the separation chemistry and labeling with ^{52}Mn. This is probably a consequence of the somewhat discouraging dosimetry associated with the multiple high energy, high abundance gamma emissions of ^{52}Mn (744, 936 and 1434 keV) as seen in Fig. 1. This, however, only has significance if the purpose of the study is translational tracer design. In drug development, the dosimetry is less relevant, because the radioisotope is not intended to be part of the final drug product, but only used as a means to gather crucial information about drug kinetics, biodistribution, route of metabolization and excretion, *etc.*

Detailed work on production cross-sections of the ^{nat}Cr(p,x)^{52}Mn reaction has demonstrated that the reaction is highly capable of

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http://dx.doi.org/10.1016/j.apradiso.2016.11.021
Received 12 August 2016; Received in revised form 16 November 2016; Accepted 25 November 2016
Available online 21 December 2016

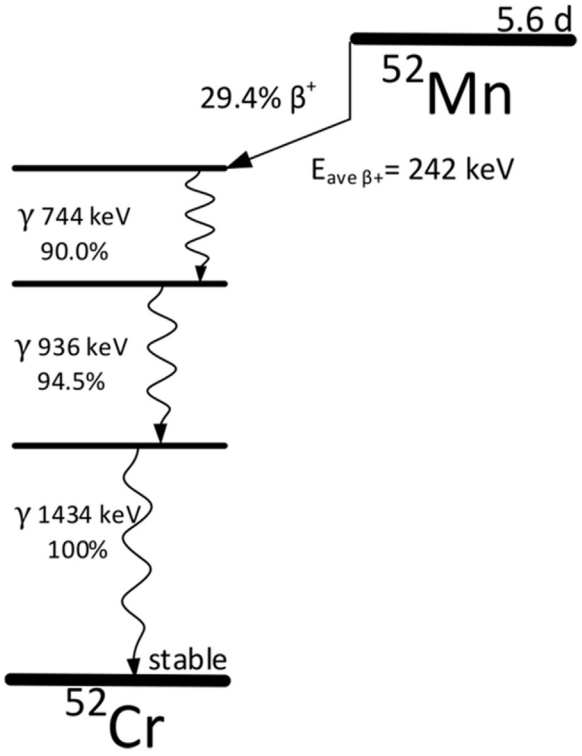

Fig. 1. A simplified decay scheme for $^{52\,g}$Mn (Dong and Junde, 2015).

providing sufficient amounts of ^{52}Mn for imaging applications (Wooten et al., 2015; Buchholz et al., 2013). Recently, Buchholz et al. reported a separation method based on solid phase extraction of manganese from acid/organic solvent mixtures (Buchholz et al., 2015). The results presented in the present study are an improvement on a conceptually similar method recently published by this group (Graves et al., 2015), demonstrating improved separation efficiency without compromising the decrease in chromium content. One major improvement on previously published work is the introduction of a remediation column, which efficiently removes non-radioactive metal-ion impurities that would otherwise hinder high specific activity labeling. The improved method is still time efficient and can be performed in less than 4 h. Additionally, optimized labeling conditions with the chelator DOTA (*1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid*) and the bio-conjugate DOTA-(Tyr^3)-octreotate (DOTA-TATE) are explored and reported. Serum stability studies of the ^{52}Mn-DOTA complex demonstrate the suitability of the chelator for *in vivo* studies. Overall, we describe tested and optimized procedures for production and purification, as well as labeling with ^{52}Mn produced on a small medical cyclotron.

2. Materials and methods

2.1. General experimental details

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich. Chromium powder (100 − 325 mesh, 99.99% purity, impurities listed on CoA in ppm: Ag 7, Cd < 4, Fe 3, Mg 4, Pb < 4) was obtained from Alfa-Aesar. Hydrochloric acid (aq. HCl ≥ 37%, TraceSelect, Fluka) was titrated and found to be 11.3 M. All other concentrations of HCl used, were prepared from this batch by dilution with TraceSELECT water (Fluka). All other water was Milli-Q grade (Sartorius Arium 611VF, σ = 0.055 μS/cm).

100 g of AG 1-X8 anion exchange resin in the formate form (8% cross-linked with divinyl benzene, 200 − 400 mesh, Bio-Rad) was converted to its chloride form by rinsing with 20 bed volumes of 2 M aq. HCl and washing with Milli-Q water until the effluent was neutral pH. Afterwards the resin was washed with ethanol (≥ 99.8%, Fluka) and dried at 20 °C.

Thin-layer chromatography (TLC) was performed on aluminum-backed silica (Merck TLC silica gel 60 F254) and eluted with 5% (w/v) ammonium acetate in a 1:1 mixture of methanol and water. In this system, the R_f values were determined to be 0.30–0.35 for Mn-DOTA and 0.4–0.5 for Mn-DOTA-TATE, while un-chelated Mn^{2+} remains at the origin. TLC plates were analyzed by autoradiography on a Cyclone Plus Storage Phosphor Scanner (PerkinElmer) and data analysis was performed using the OptiQuant software (PerkinElmer).

^{52}Mn samples in excess of 1 MBq were quantified with a Capintec CRC-55tR dose calibrator using the manufacturer's recommended calibration number "676/2″. Low activity samples of ^{52}Mn, ^{54}Mn, and all ^{51}Cr samples were quantified using either liquid scintillation counting (LSC) on a HIDEX 300 SL (fluid: Optiphase HiSafe II, Perkin Elmer), or by gamma spectroscopy a Ge detector (Princeton Gammatech LGC 5) calibrated using certified ^{133}Ba and ^{152}Eu calibration standards. The obtained gamma spectra were processed using the Genie 2000 software (Canberra).

Trace metals were quantified by ICP-OES (inductively coupled plasma optical emission spectroscopy) using a ThermoScientific iCAP 6000 Series instrument with iTeva software. The spectrometer was calibrated against standard solutions containing Cr, Mn, Co, Fe, Zn and Cu, which were prepared by dissolution and dilution of chloride salts of the tested metals in 0.3 M HCl. Samples for analysis were likewise diluted in 0.3 M HCl.

2.2. Cyclotron targetry and irradiation

The targets were prepared by pressing 90–273 mg of chromium powder onto the surface of a 1 mm thick Ø = 29 mm silver disc using a metal powder pressing mold, ensuring centering of the Ø = 13 mm pressed chromium pellets. The pressing was done using a mechanical press at 7500 kg/cm^2 of pressure (10000 kg, Ø = 13 mm) resulting in approximate target thicknesses in the range of 68–206 mg/cm^2. The disc was covered with either a 12.5 μm or 25 μm niobium front foil (99.9%, Goodfellow) and mounted on a beam port on a GE PETtrace 800 cyclotron, providing direct water cooling on the rear face of the silver disc. The target was irradiated at a beam current of 20 μA with an incident energy of 16 MeV for 80–400 min resulting in integrated currents of 28–128 μAh. ^{52}Mn was produced primarily *via* the ^{52}Cr(p,n)^{52}Mn reaction, with a small contribution from the less abundant ^{53}Cr *via* ^{53}Cr(p,2 n)^{52}Mn, by proton irradiation of naturally abundant chromium. The 2.36% abundant ^{54}Cr gave rise to co-production of small amounts of ^{54}Mn *via* the ^{54}Cr(p,n)^{54}Mn reaction.

2.3. Determination of separation conditions

In order to determine the optimal acid: ethanol ratio for selective solid phase extraction of manganese from chromium onto AG 1-X8 resin, distribution coefficients of Cr^{3+} and Mn^{2+} on AG 1-X8 were determined in mixtures of aqueous HCl (conc. range: 3.8–11.3 M) and absolute ethanol, mixed in a range of 2–7% (v/v) aq. HCl in ethanol. A total of 18 different conditions were tested by adding 100 ± 2 mg of AG 1-X8 resin to 5 mL of each of the acid: ethanol ratios, all containing 100 kBq ^{52}Mn and 250 μg ^{nat}Cr. The resulting mixtures were agitated periodically over the course of several hours at room temperature, and after settling for 72 h they were re-agitated and finally allowed to settle for a further hour. 100 μL samples of the supernatants were removed for measurement by ICP-OES to determine the Cr-content and liquid scintillation counting to determine the ^{52}Mn content. The data were analyzed by comparison to Cr and ^{52}Mn containing standards prepared without resin.