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Cyclotron production and radiochemical separation of ^{55}Co and $^{58\text{m}}\text{Co}$ from ^{54}Fe , ^{58}Ni and ^{57}Fe targets

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Abstract

This work presents the production with a cyclotron of the positron emitter ^{55}Co via the $^{54}\text{Fe}(\text{d},\text{n})$ and $^{58}\text{Ni}(\text{p},\alpha)$ reactions and the Auger electron emitter $^{58\text{m}}\text{Co}$ via the $^{57}\text{Fe}(\text{d},\text{n})$ reaction after high current (40 μA p and 60 μA d) irradiation on electroplated targets. High specific activity radionuclides (up to 55.6 GBq/ μmol ^{55}Co and 31.8 GBq/ μmol $^{58\text{m}}\text{Co}$) with high radionuclidic purity (99.995% ^{55}Co from ^{54}Fe , 98.8% ^{55}Co from ^{58}Ni , and 98.7% $^{58\text{m}}\text{Co}$ from ^{57}Fe at end of bombardment, EoB), in high activity concentration (final separated radionuclide in < 0.6 mL) and with almost quantitative overall activity separation yield (> 92%) were obtained after processing of the irradiated targets with novel radiochemical separation methods based on HCl dissolution and the resin *N,N,N',N'*-tetrakis-2-ethylhexyldiglycolamide (DGA, branched). One hour long irradiations using 38–65, 110–214 and 59–78 mg of enriched ^{54}Fe (99.93%), ^{58}Ni (99.48%) and ^{57}Fe (95.06%), respectively, electroplated over a 1.0 cm^2 surface, yielded 582 ± 66 MBq ^{55}Co , 372 ± 14 MBq ^{55}Co and 810 ± 186 MBq $^{58\text{m}}\text{Co}$, respectively, decay corrected to EoB. The separation methods allow for the recovery of the costly enriched target materials, which were reconstituted into metallic targets after novel electroplating methods, with an overall recycling efficiency of $93 \pm 4\%$ for iron. The produced radionuclides were used to radiolabel the angiogenesis marker antibody TRC105 conjugated to the chelator NOTA as a demonstration of their quality.

1. Introduction

1.1. Cobalt-55

Cobalt-55 ($t_{1/2} = 17.53$ h, 76% β^+ , $E_{\text{max}} = 1498$ keV) is an intermediate-lived positron-emitting radionuclide that is a useful radiotracer for positron emission tomography (PET) (De Reuck et al., 2004, 1999; Jansen et al., 1997a, 1997b, 1994, 1996b, 1995; Korf et al., 1998; Stevens et al., 1999) and particularly useful for labeling proteins due to its favorable complexation with established bifunctional chelators (Dam et al., 2016; Garousi et al., 2017;

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Mastren et al., 2015; Srivastava et al., 1994; Thisgaard et al., 2011b; Wallberg et al., 2010). Furthermore, when chelated in oxidation state 3+, the formed complex is considered inert (Duckworth et al., 2009; Regoeczi et al., 1995; Wegner and Spatz, 2013) and therefore it is less prone to interact with ligands in blood plasma and non-targeted organs due to trans-chelation.

Most of the early applications of ^{55}Co for PET in ischemic stroke are based on the assumption that radioactive cobalt ions behave similar to calcium ions in vivo based on *ex vivo* results in brain tissue (Gramsbergen et al., 1988). The main hypothesis was that radioactive cobalt ions would present the same influx into damaged brain cells that is observed with calcium ions. This hypothesis, however, was questioned by the work of Stevens et al. (1999), who observed that the uptake of ^{55}Co after administration of $^{55}\text{CoCl}_2$ into 16 patients with a diagnosis of stroke may be better explained by an inflammatory process. Since this latter work, the use of ^{55}Co for PET of ischemic stroke, cobalt being injected as a weakly bound complex, was abandoned.

A more promising approach for the use of this radiometal is by binding it to a chelator and potentially conjugating this complex to a targeting vector. So far, only seven studies have followed this chelator-based approach (Dam et al., 2016; Garousi et al., 2017; Goethals et al., 2000; Mastren et al., 2015; Srivastava et al., 1994; Thisgaard et al., 2011b; Wallberg et al., 2010). Goethals et al. (Goethals et al., 2000) labeled ^{55}Co to ethylene diamine tetraacetic acid (EDTA) and characterized this complex for the application in the measurement of the glomerular filtration rate in kidneys via PET, a study which is commonly performed with the perfusion tracer ^{51}Cr -EDTA (Chantler et al., 1969). Srivastava et al. (1994), Thisgaard et al. (2011b) and Mastren et al. (2015) successfully labeled ^{55}Co to bifunctional chelators (BFC) conjugated to targeting peptides or proteins, although with very low effective specific activities (ESA): 3.7, 0.21, and 2.0 GBq/ μmol , respectively. Dam et al. (2016), has reported the highest ESA of ^{55}Co at 30 GBq/ μmol by labeling a NOTA-conjugated bombesin analog under microwave heating in order to accelerate the labeling reaction.

Very interesting results were obtained by Heppeler et al. (2008) after comparing the binding affinities and internalization rates of the DOTA-conjugated somatostatin analog 4,7,10-tricarboxymethyl-1,4,7,10-tetraazacyclododecane-1-yl-acetyl-D-Phe-(Cys-Tyr-D-Trp-Lys-Thr-Cys)-threoninol (DOTATOC) radiolabeled with the long-lived ^{57}Co ($t_{1/2} = 271.8$ d; 122 keV γ , 86%; 136 keV γ , 11%) and radionuclides of gallium and yttrium. It was found that Co-DOTATOC exhibited the highest affinity towards somatostatin receptor subtype 2 (sst2) and the highest internalization rate to cells expressing this receptor. These results call for the comparison of more peptides labeled with different radiometals in order to find the tracer with the best uptake properties for more target-specific studies. Thus, by making the positron-emitter ^{55}Co available to the scientific community, it will be possible to translate these comparison studies from the in vitro to the in vivo stage with PET.

An even more interesting application of this radiometal would be as a PET label of cobalamin, also known as vitamin B₁₂, the only cobalt complex that is essential to humans. Due to its importance in the biochemical pathways that result in methylation reactions, thymidine production and mitochondrial metabolism, all of which are elevated in

proliferating cancer tissue, radiolabeled vitamin B₁₂ derivatives have been proven to be targeted agents for high-grade tumors in patients (Collins et al., 2000; Sah et al., 2014) and in small animals models (Flodh and Ullberg, 1968; Ikotun et al., 2014; Waibel et al., 2008).

Cobalt-55 can be produced with a small biomedical cyclotron using isotopically enriched target materials via the $^{58}\text{Ni}(p,\alpha)$ (Mastren et al., 2015; Spellerberg et al., 1998) or $^{54}\text{Fe}(d,n)$ (Sharma et al., 1986; Zaman and Qaim, 1996; Zaman et al., 2003) nuclear reactions. It has also been produced using more sophisticated or high-energy cyclotrons using natural iron targets via the $^{\text{nat}}\text{Fe}(^3\text{He},p+xn)$ (Neirinckx, 1977) and $^{\text{nat}}\text{Fe}(p,xn)$ (Lagunas-Solar and Jungerman, 1979) reactions. The $^{58}\text{Ni}(p,\alpha)$ route seems the most practical since isotopically enriched ^{58}Ni is relatively inexpensive (natural abundance = 68.07%) and low-energy proton accelerators are more common than dual-particle (proton and deuteron) ones. However, the high excitation functions of the $^{58}\text{Ni}(p,2p)$, and $^{58}\text{Ni}(p,p+n)$ that ultimately result in the co-production of ^{57}Co make this option less attractive when compared to the $^{54}\text{Fe}(d,n)$ route that offers the highest radionuclidically pure product (Zaman and Qaim, 1996). Nevertheless, isotopically enriched ^{54}Fe is relatively expensive due to its natural abundance of 5.84% and therefore recycling it after each separation process becomes mandatory.

Of the three publications in which isotopically enriched ^{54}Fe targets were bombarded with deuteron beams (Sharma et al., 1986; Thisgaard et al., 2011b; Zaman et al., 2003), only the one by Zaman et al. clearly reports a numerical value of the recycling efficiency of the expensive target material of 80% and the maximum current employed is merely 18 μA by Thisgaard et al.

Avriganu et al. (2014) performed cross-section measurements by irradiating thin foils of natural iron using the stacked foil technique with a 19.74 MeV deuteron beam and found that the $^{54}\text{Fe}(d,n)^{55}\text{Co}$ excitation function reaches a maximum of ~ 158 mb at 6.71 MeV. The predicted thick target production yield from this excitation function integrated over the energy on the impinging particles available from a GE PETtrace cyclotron, 8.2 MeV, as they are slowed down in the target material is 23.2 MBq/ $\mu\text{A h}$. Likewise, from the $^{58}\text{Ni}(p,\alpha)$ excitation functions that were measured by Khandaker et al. (2011) we can predict a thick target yield with our 16 MeV proton beam of 15.6 MBq/ μAh . As it will be presented in this work, the experimental target yields that were measured are between 39% and 69% of these predicted yields, due to the narrow surface area (0.9–1.2 cm $\varnothing$) of the electroplated targets as compared to the total extent of the beam transverse area, which has an elliptical shape with a long axis of ~ 1.6 cm. This narrower extent of electroplated surface was chosen purposefully in order to maximize the axial thickness using a reasonably low mass of enriched material (< 80 mg Fe and < 200 mg Ni). Taking this beam loss into account our measured yields agree with the predicted yields from the referenced excitation functions.

The radiochemical separation methods for ^{55}Co that have been published so far involve large ion exchange resin columns (> 4.0 cm $\times$ 1.0 cm $\varnothing$) that result in a final eluate of more than 5 mL volume (Mastren et al., 2015; Spellerberg et al., 1998), which necessitates for a lengthy evaporation step in a large vessel that potentially causes a variable loss of radioactivity in the vessel's surface or in the glassware of the evaporator. In order to circumvent this problem, extraction resins with higher trapping capacity compared to the traditional ion exchange

resins have proven to be more practical particularly because they allow the elution of highly concentrated radiometals in solution with high specific activity (Gagnon et al., 2012; Holland et al., 2009; Valdovinos et al., 2015).

In this work, methods for the production of high specific activity ^{55}Co using a biomedical cyclotron will be presented. The methods include: (1) electrodeposition of thick ^{54}Fe or ^{58}Ni targets; (2) irradiation at high beam current using a water jet cooled target holder; (3) ^{55}Co separation and purification involving the extraction resin N,N,N',N'-tetrakis-2-ethylhexyldiglycolamide, commercially known as DGA Branched (Eichrom) [14]; and (4) efficient recycling of the ^{54}Fe or ^{58}Ni target material. ^{54}Fe and ^{58}Ni have been successfully electroplated at thicknesses of 24–65 mg/cm² and 90–214 mg/cm², respectively, both on silver disk substrates. These targets have been bombarded with deuteron beams of up to 60 μA and proton beams of up to 40 μA , respectively. Enriched ^{54}Fe targets with thickness > 52.3 mg/cm² yield $10.3 \pm 0.8 \text{ MBq}/\mu\text{A h}$ ($n = 7$), so batches of up to 670 MBq ^{55}Co are feasible after 1 h of irradiation, with measured effective specific activities (ESA) of $27 \pm 18 \text{ GBq}/\mu\text{mol}$ of NOTA. Ni-58 targets > 90.1 mg/cm² thick yield $9.3 \pm 0.6 \text{ MBq}/\mu\text{A h}$ ($n = 3$) and 1 h-long irradiations result in 370 MBq of ^{55}Co with an ESA of $10.1 \pm 5.7 \text{ GBq}/\mu\text{mol}$ NOTA. The ESA of ^{55}Co towards the other mainstream chelators such as DOTA, HBED and TETA was also quantified.

The presented methods for the production of ^{55}Co will allow its widespread production for PET imaging at medical centers throughout the world. Moreover, the production method from iron targets can be easily adapted for the preparation of the therapeutic radionuclide $^{58\text{m}}\text{Co}$ from either proton or deuteron bombardment on isotopically enriched ^{58}Fe or ^{57}Fe , respectively.

1.2. Cobalt-58m

The internal conversion decay of $^{58\text{m}}\text{Co}$ ($t_{1/2} = 9.10 \text{ h}$, 100% IC) results in the emission of characteristic x-ray photons, conversion electrons and Auger electrons. The three main conversion electrons from the K, L and M orbitals have energies of 17.2, 24.0 and 24.8 keV, respectively, and are emitted with probabilities per decay of 73.0%, 23.5% and 3.4%, respectively (Nesaraja et al., 2010). Additionally, about three low energy Auger electrons with energies < 8 keV are emitted per decay (Chu et al., 1999). The linear energy transfer (LET) of these low-energy electrons in unit-density matter covers a range from 2 to 18 keV/ μm (Humm et al., 1994), which is at least one order of magnitude greater than the LET of energetic beta particles emitted by established therapeutic radionuclides such as ^{177}Lu and ^{90}Y .

Furthermore, the range in water of conversion and Auger electrons is less than 10 μm , at least one order of magnitude lower compared to the range > 100 μm for the main beta particle emitted by ^{177}Lu (Ziegler et al., 2010). This short path length renders Auger and conversion electrons suitable for treatment of minimal disease such as micro-metastasis or residual tumor after surgical resection of a primary lesion.

The therapeutic potential of $^{58\text{m}}\text{Co}$ has been recognized theoretically (Bernhardt et al., 2001; Thisgaard et al., 2011a; Uusijarvi et al., 2006) and experimentally (Thisgaard et al., 2014).

Experimentally, Thisgaard et al. demonstrated that ^{58m}Co -DOTATATE was significantly more efficient in cell killing per cumulated decay than ^{111}In - and ^{177}Lu -DOTATATE after exposing pancreatic tumor cells AR42J in vitro (Thisgaard et al., 2014). However, as far as we know, the therapeutic potential of ^{58m}Co has not been demonstrated in vivo.

Furthermore, ^{58m}Co decays to the positron emitter ^{58g}Co ($t_{1/2}=70.86$ d, 14.9% β^+), which carries benefits and disadvantages. The two main advantages are that ^{58g}Co can be employed to quantify the otherwise “silent” ^{58m}Co and that it can be imaged and quantified in vivo via Positron Emission Tomography (PET). This second possibility is particularly important for therapy applications because it allows the quantification of radionuclide concentration in target and non-target tissue for verification of targeting and to obtain biodistribution data that would complement previous data that has been obtained with a diagnostic analog agent labeled with ^{55}Co for dosimetry calculations. The main disadvantages are that ^{58g}Co has a long half-life and that it emits a prominent 811 keV gamma with a branching ratio of 99.5% (Nesaraja et al., 2010) that will inevitably deposit dose in normal tissue and that will have to be controlled after excretion for radiation safety reasons.

The production of ^{58m}Co can be carried out either by proton bombardment on isotopically enriched ^{58}Fe as it was done by Thisgaard et al. (2011a, 2014), or by deuteron bombardment on ^{57}Fe . From the excitation functions of the $^{58}\text{Fe}(p,n)$ and $^{57}\text{Fe}(d,n)$ reactions published by Sudar and Qaim (1996), the thick target yields of ^{58m}Co with the 16 MeV protons and 8.2 MeV deuterons available from our GE PETtrace cyclotron are 395 and 33 MBq/ μAh , respectively. However, thick targets of ^{58}Fe (440 mg/ cm^2 (Ziegler et al., 2010)) are prohibitively expensive since they would cost about \$13,300 due to the price of ^{58}Fe at US \$30/mg.¹ Much thinner targets can be used instead with acceptable production yields still attainable. For instance, Thisgaard et al. (2011a), has reported a production yield of 10.7 MBq/ μAh from the bombardment of 7.0 mg/ cm^2 ^{58}Fe with 10.1 MeV. Alternatively, thick targets of ^{57}Fe (73 mg/ cm^2 (Ziegler et al., 2010)) are economically feasible to make since ^{57}Fe costs US\$6/mg*.

In this work, the second route was followed and the production yield of ^{58m}Co from deuteron-irradiation on thick ^{57}Fe targets was measured. Furthermore, to demonstrate the high specific activity of the isolated ^{55}Co and ^{58m}Co from their respective enriched iron targets, each one of these nuclides was labeled to the anti-CD105 antibody TRC105 (TRACON Pharmaceuticals) conjugated with NOTA.

^{55}Co and ^{58m}Co represent a perfect theranostic pair since exactly the same element, in this case cobalt, can be bound to a targeting vector and act either as a diagnostic label for treatment planning and monitoring of disease through PET imaging or as a radiotoxic therapeutic.

¹Budgetary pricing offered by ISOFLEX on July 2015.

2. Materials and methods

Optima grade hydrochloric acid (32–35% HCl) comes from Aristar Ultra, VWR (West Chester, PA). Ethanol, 200 proof, and L-Ascorbic acid (TraceSELECT, > 99.9998%) and 28% ammonium hydroxide solution in H₂O (NH₄OH, 99.99% trace metals) were purchased from Sigma-Aldrich (St. Louis, MO). The chelators NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid) were purchased from Macrocyclics (Dallas, TX). The chelator HBED (N,N'-Di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid) comes from Strem Chemicals (Newburyport, MA). Isotopically enriched ⁵⁴Fe (99.93% ⁵⁴Fe, 0.06% ⁵⁶Fe, 0.005% ⁵⁷Fe, 0.005% ⁵⁸Fe), ⁵⁷Fe (95.06% ⁵⁷Fe, 0.04% ⁵⁴Fe, 3.06% ⁵⁶Fe, 1.86% ⁵⁸Fe) and ⁵⁸Ni (99.48% ⁵⁸Ni, 0.505% ⁶⁰Ni, 0.005% ⁶¹Ni, 0.005% ⁶²Ni, 0.005% ⁶⁴Ni) were purchased from ISOFLEX (San Francisco, CA). Silver disks (1.90 cm diameter × 0.56 mm thickness) were obtained from Artisan Jewelers (Sarasota, FL). Platinum wire (99.997%, 0.25 mm diameter) and ammonium oxalate monohydrate (99.0–101.0%) were purchased from Alfa Aesar (Ward Hill, MA). Radionuclidically pure ⁶⁴CuCl₂ in 5 µL 0.1 M HCl (~ 400 MBq at end of bombardment) was obtained from the weekly batch that is distributed nation-wide by the Cyclotron Group of the Medical Physics Department at the University of Wisconsin-Madison. 99% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Acros Organics. Sodium hydroxide (1 M) solution was prepared from NaOH pellets from Fisher Scientific. Trace metal grade sulfuric acid, ammonium acetate and methanol were also purchased from Fisher Scientific (Pittsburg, PA). TLC silica gel 60 plates and iron test strips were bought from EMD Chemicals (Darmstadt, Germany). Ion exchange resin (AG1-X8) was purchased from Bio-Rad (Hercules, CA). Extraction chromatography resin DGA, branched (50–100 µm) was purchased from Eichrom (Lisle, IL). A 50 ppm multi-element standard for calibration and Agilent's 4200 Microwave Plasma Atomic Emission Spectroscopy (MP-AES) system come from Agilent Technologies (Santa Clara, CA). Deionized water (> 18 MΩcm⁻¹) was obtained from a Milli-Q filter, Millipore (Billerica, MA). Phosphate buffer saline (PBS) was purchased from Thermo Scientific.

2.1. Cyclotron targetry, irradiations and target yields

The electroplating procedure for iron was an adaptation of the methodology reported by Vosburgh et al. (1948). Briefly, 62.2 ± 10.8 mg of ⁵⁴Fe (n = 13) or 81.0 ± 7.6 mg of ⁵⁷Fe (n = 6) metallic powder were dissolved in 5 mL 6 M HCl, the Fe ions were turned to oxidation state 3+ by the addition of 100 µL 30% H₂O₂ and the solution was evaporated to near dryness (< 1 mL). The residue was diluted with 15 mL of saturated ammonium oxalate (concentration ~ 44 mg/mL H₂O) followed by a pH adjustment to between 2 and 3 with either 1 M NaOH or 1 M HCl.

The electroplating procedure for nickel was very similar to the one previously reported for production of ⁶⁴Cu from ⁶⁴Ni targets (Avila-Rodriguez et al., 2007; McCarthy et al., 1997; Piel et al., 1992). Briefly, 100.0–196.8 mg of isotopically enriched metallic ⁵⁸Ni powder (n = 4) was dissolved in 6 M HNO₃, dried down and re-dissolved in 2.3 mL of 2.4 M H₂SO₄

followed by adjustment to pH ~9 with 1.5–1.8 mL of 28% NH_4OH and the addition of 270–300 mg of $(\text{NH}_4)_2\text{SO}_4$.

Each electroplating solution was quantitatively transferred to an electrolytic cell, which consists of a glass tube (Ace Glass Inc.) (I.D. 1.56 cm) with a polytetrafluoroethylene (PTFE) plugin at one of its ends with a central perforation of 0.9–1.1 cm diameter and an O-ring groove to accommodate a viton O-ring at the bottom to seal the solution. The cathode is a silver disk (0.56 mm thick, 1.90 cm diameter), which is mounted on an aluminum plate. The exposed area of the cathode to the electrolytic solution ($0.6\text{--}0.9\text{ cm}^2$) is delimited by the central perforation of the PTFE plug. A platinum wire (0.25 mm diameter) is used as the anode, with the tip bent into a spiral shape of ~ 0.5 cm diameter in order to have a homogeneous electric field across the cathode. The platinum wire remains in a constant geometry throughout each electrodeposition procedure by fixing it inside a 13 cm long, 0.016" diameter, teflon tubing with the tip at 1 cm from the silver anode. A power supply provides the DC voltage. Fig. 1(a) shows a schematic of this setup. To prepare the iron deposits, the cell was operated at 7.0 V with an average current at the beginning of the process of 157 mA for 22 ± 3 h. Nickel was electroplated applying 3.0–3.6 V and 30–116 mA across the electrodes for 2–3 days. The electroplating was ended when the electrolyte solutions were fully clear and the colorimetric nickel or iron strips indicated that 0–0.05 mg of metal remained in solution.

During irradiation, the back of the disks is in direct contact with a water jet stream for cooling, as shown in Fig. 1(b). Irradiations were performed on the UW-Madison PETtrace cyclotron using 8.2 MeV deuterons and 16 MeV protons with maximum currents of 60 μA and 40 μA on the iron and nickel targets, respectively. After irradiation, the targets were dismounted from the cyclotron and weighted with a balance in order to verify that no mass was lost during irradiation.

Co-55 activities for separation yield quantification were measured with a Capintec CRC-Dual PET (Capintec, Ramsey NJ) dose calibrator using the calibration setting 481 suggested by the manufacturer. However, the actual activity from ^{55}Co and other radionuclidic impurities was measured from the gamma intensities of 10 μL samples placed at distances with known efficiency calibration from a 60 cm^3 high purity germanium (HPGe) detector (Canberra C1519) (FWHM = 2.7 keV @ 1333 keV). The efficiency calibration at fixed distances was obtained using radioactive sources of ^{152}Eu , ^{137}Cs and ^{60}Co traceable to the National Institute of Standards and Technology (NIST). Gamma-ray spectrum analysis software package, Maestro-32 MCA Emulator (Ortec, Oak Ridge TN), was used to collect and analyze the gamma-ray spectra. The gamma lines used to determine yields are listed in Table 1. The dead time was always kept below 10% and the acquisition time was set so that the statistical uncertainty from the number of counts per peak was kept below 1%. From the accurate activity value of ^{55}Co it was observed that the Capintec measurement with the calibration setting 481 was overestimating the actual ^{55}Co activity from the HPGe measurement by $93 \pm 11\%$ ($n = 9$) when the assayed ^{55}Co had a radionuclidic purity > 99.99%, after it was already isolated from the co-produced ^{52}Mn or ^{57}Ni from $^{54}\text{Fe}(\text{d},\alpha)$ and $^{58}\text{Ni}(\text{p,pn})$, respectively. Hence, the Capintec reading had to be multiplied by 0.52 throughout our studies, in order to have accurate activity values.

Likewise, production yields of ^{58m}Co and ^{58g}Co were quantified via gamma spectroscopy with efficiency-calibrated HPGe detectors using the gamma emissions listed in Table 1. A low energy (2–65 keV) HPGe detector with a beryllium window (Canberra GL0110P) was used for ^{58m}Co and a broad energy range (30–2280 keV) HPGe detector (Canberra C1519) was used for ^{58g}Co . The efficiency calibration at fixed distances for the low energy HPGe detector was obtained using a ^{241}Am source that is traceable to NIST. The sample employed for the yield quantification was a 10 μL aliquot drawn from the 3.1 mL target dissolution.

2.2. Radiochemical separations

The radiochemical separation of radio-cobalt from iron was performed in aqueous solution using the partition properties of the involved elements between HCl, HCl-Ethanol, the anion exchange resin AG1-x8 (Bio-Rad) and the extraction resin *N,N,N',N'*-tetrakis-2-ethyl-hexyldiglycolamide (DGA, branched) (Kraus and Nelson, 1955; Pietrzyk, 1960; Pourmand and Dauphas, 2010). First, the electroplated surface was exposed to 3 mL of 6 M HCl, heating the back side of the disk with a heater set to 90 °C. Only the front face of the electroplated target was exposed to HCl by use of a teflon mask with an inserted O-ring that seals against the surface of the substrate disk. After the iron was completely dissolved in approximately 20 min, 100 μL of 30% H_2O_2 was added to turn the iron ions to the 3+ oxidation state. At this point, a 10 μL sample from this solution was collected for gamma spectroscopy in order to quantify the radioactivity of every radionuclide that was generated.

Afterwards, the iron solution was diluted with 57 mL of ethanol (EtOH) (200 proof, Sigma-Aldrich) to a concentration of ~ 0.3 M HCl and 95% EtOH. This new target solution was then transferred to a 60 mL syringe connected to a peristaltic-pump driven automated module similar to the one described in a previous publication (Valdovinos et al., 2015), which sent the new solution in steps of 20 mL into an anion exchange column (AG1-x8, 1.5 cm diameter, 6.3 cm long), pre-equilibrated with 20 mL of 0.3 M HCl/95% EtOH solution, with the flow rate being induced by gravity. The stable iron target material and the cobalt and manganese radionuclides were quantitatively retained in the column as expected from the distribution coefficients $> 10^3$ for these elements in ethanolic HCl and anion exchange media reported by Pietrzyk (1960). The radioactive cobalt and manganese nuclides were quantitatively eluted in 10 mL of 4 M HCl, while the expensive target material was quantitatively retained in the column, a behavior that is explained by the retention properties of the chloro-complexes in anion exchange media reported by Kraus and Nelson (1955). Next, the 10 mL solution of 4 M HCl containing the radionuclides of interest was diluted to a concentration of ~8 M HCl by adding 10 mL of concentrated (12.1 M) HCl. This solution was then transferred to the reservoir of a second peristaltic-driven automated module to which a 300 mg DGA column loaded in a 0.5 cm diameter column cartridge, pre-equilibrated with 3 mL of 8 M HCl, was connected. In this case the flow rate at 1.1 mL/min was dictated by the peristaltic pump. In this chromatography setting, the radioactive cobalt was retained in the resin while radioactive manganese was eluted (Pourmand and Dauphas, 2010). In order to quantitatively separate the manganese from the trapped cobalt, the resin was washed with 10 mL of 8 M HCl. Subsequently, the radioactive cobalt was eluted in fractions of 200 μL of 2 M HCl pushed by the peristaltic pump. The two fractions with the largest amount of radioactivity were mixed together and dried down in ~ 15 min at 100 °C

under a stream of argon. The residue was re-dissolved in 100 μL of 0.1 M HCl, which constituted the separated stock of $^{55}\text{CoCl}_2$ ready for radiolabeling. A 2–5 μL aliquot from the separated stock was assayed with the HPGe detector to accurately quantify the separation yield. The isotopically enriched ^{54}Fe was eluted from the AG1-x8 column in 20 mL of 0.1 M HCl. This solution was dried to ~ 1 mL at 125 $^\circ\text{C}$ under a flow of N_2 and the residue was reconstituted into the 15 mL saturated ammonium oxalate electrolyte for re-electrodeposition. The electroplated iron mass was compared to the original target mass before irradiation in order to quantify the iron recycling yield. Fig. 2(a) shows a schematic of this separation procedure.

Similarly, the irradiated ^{58}Ni was dissolved off the silver disk in 7 mL of concentrated HCl at 90 $^\circ\text{C}$ in 0.5–1 h, followed by a dilution with 2 mL of deionized H_2O to a concentration of ~ 9 M HCl and then by a transfer to a 0.5 cm diameter column filled with 300 mg DGA branched resin at 1.0 mL/min with the peristaltic pump-driven module. Co-55 is trapped in the resin and the isotopically enriched ^{58}Ni that flows through is collected for recycling. A rinse of 18 mL of 9 M HCl is applied to the resin to remove the remaining bulk nickel target material as well as traces of other metals, such as copper. The elution of ^{55}Co was carried out by pumping 200 μL fractions of 3 M HCl through the loaded resin. The two or three most concentrated fractions were combined in a 1.5 mL centrifuge vial, evaporated to dryness at 100 $^\circ\text{C}$ under argon flow, and the residue was re-dissolved in 100 μL of 0.1 M HCl, which constituted the separated stock of $^{55}\text{CoCl}_2$. The elution profile for the main elements involved in the separation, i.e. nickel, cobalt and copper, was obtained by collecting fractions from a separation run with volumes of 3 mL in the loading (9 mL) and washing steps (18 mL), followed by fractions of 200 μL in the elution step. 64 MBq of radionuclidically pure ^{64}Cu in 5 μL of 0.1 M HCl was mixed with the target solution to serve as a tracer for copper. The fractions were then analyzed by gamma ray spectrometry using ^{55}Co and ^{57}Ni (from ^{58}Ni (p,pn)) as the other radiotracers. The recovered isotopically-enriched nickel solution was evaporated to dryness, re-dissolved in 3 mL of 6 M HNO_3 in order to digest possible organic material from the extraction resin, evaporated to dryness again, and the electroplating solution was reconstituted and transferred to an electroplating cell as described before. Fig. 2(b) shows the schematic of this separation procedure.

Trace metal analysis was performed on samples from four and three ^{55}Co separation runs using ^{54}Fe and ^{58}Ni targets, respectively. Ten μL samples from the target solutions and 2–20 μL from the ^{55}Co separated stock were analyzed for trace metal quantification with Agilent's Microwave Plasma Atomic Emission Spectroscopy system (4200 MP-AES).

2.3. Effective specific activity of ^{55}Co and $^{58\text{m}}\text{Co}$ with mainstream chelators

The effective specific activity of ^{55}Co from ^{54}Fe was evaluated by titrating equal amounts of $^{55}\text{CoCl}_2$ solution (0.25 μL from the separated stock) with increasing amounts 0–300 ng in 100 μL H_2O) of the chelators NOTA, DOTA, TETA and HBED at pH 7.5 in 0.25 M HEPES buffer at room temperature for 30 min, except for DOTA which was complexed at 85 $^\circ\text{C}$. In the case of $^{58\text{m}}\text{Co}$ and ^{55}Co from ^{58}Ni the reactivity was assessed similarly by titration but only with NOTA. The total volume in each labeling reaction was 200 μL . The labeling yield in each reaction vial was assessed by thin layer chromatography (TLC) using silica gel

plates (60 F254, EMD Chemicals) and 1:1 MeOH:10%NH₄AOc mobile phase, with the activity distribution in the plates quantified by autoradiography using a Packard Cyclone Phosphor-Plate imaging system (Perkin Elmer). The reactivity is defined as the ratio of activity decay-corrected to EoB to the amount of chelator in moles that results in 50% labeling yield, multiplied by two.

2.4. Preparation of ⁵⁵Co- and ^{58m}Co-NOTA-TRC105

NOTA conjugation to TRC105 was carried out by mixing the NOTA derivative para-isothiocyanato-benzyl-NOTA (p-SCN-Bn-NOTA) (Macrocyclics) dissolved in dimethyl sulfoxide (DMSO) to a solution containing TRC105 at pH 8.5–9.0 with a 25:1, chelator to antibody molar ratio. After 2 h of reaction time at room temperature, the conjugate NOTA-TRC105 was purified by size exclusion chromatography with a PD-10 column (GE Healthcare) using phosphate-buffered saline (PBS) as the mobile phase. The concentration of the conjugated protein was measured by analyzing a 1 µL sample with a UV-Vis spectrophotometer Nanodrop One (Thermo Scientific).

For radiolabeling with ⁵⁵Co (from ⁵⁴Fe) or ^{58m}Co, 20 and 90 µL of each respective separated stock with ⁵⁵CoCl₂ was buffered to pH ~ 7.5 by adding 1.0 mL of 0.25 M HEPES and then ~ 190 or ~ 860 µg, respectively, of TRC105-NOTA from the 3.8 µg/µL antibody stock that was eluted from the PD-10 column. One to two hours later, each of these solutions was purified by size exclusion chromatography with a PD-10 column and PBS mobile phase collecting five fractions of 400 µL. Each fraction and the PD-10 column were assayed with the dose calibrator in order to quantify the radiolabeling yield per fraction.

3. Results and discussion

3.1. Cyclotron targetry, irradiations and target yields

The iron electroplating efficiency was $93 \pm 4\%$ ($n = 18$). The average ⁵⁴Fe mass lost after each irradiation was 0.6 ± 0.5 mg ($n = 13$), that is, $< 1\%$ of the initial iron target material. This demonstrates that the presented iron electroplating method allows the fabrication of high quality targets for high power irradiations of up to 492 W (8.2 MeV $\times$ 60 µA, since there was total degradation of the deuteron beam in the target).

The ⁵⁸Ni electroplating efficiency was $94 \pm 1\%$ ($n = 3$). The ⁵⁸Ni mass loss after each irradiation was negligible, that is, within the 0.1 mg error offered by the weighting balance. Hence, the obtained nickel targets electroplated over silver allow for high power irradiations of up to 640 W (16 MeV $\times$ 40 µA, although only ~ 2–5 MeV are degraded in the electroplated ⁵⁸Ni and the rest in the silver backing). With both methods, high production yields are attainable after short irradiation times using high deuteron or proton currents.

Table 2 lists the experimental activity yields at end of bombardment (EoB) involved in the production of ⁵⁵Co and ^{58m}Co.

Clearly, the ⁵⁵Co yields from thick ⁵⁴Fe and ⁵⁸Ni targets are comparable. The average and standard deviation of the ⁵⁵Co yields from all the irradiations on ⁵⁴Fe, except for the one on a thin 24.3 mg/cm² target, are 9.7 ± 1.1 MBq/µA h; and from the three irradiations on ⁵⁸Ni

the ^{55}Co yields are $9.3 \pm 0.6 \text{ MBq}/\mu\text{A h}$. Thus, using currents of $60 \mu\text{A}$ deuterons or $40 \mu\text{A}$ protons in a typical 1 h long irradiation would generate batches of 582 and 372 MBq ^{55}Co , respectively.

Co-58m and ^{58g}Co yields at EoB were 13.5 ± 3.1 and $0.13 \pm 0.02 \text{ MBq}/\mu\text{A h}$, respectively ($n = 4$). Therefore, a typical irradiation with $60 \mu\text{A}$ for 2 h resulted in a ^{58m}Co yield of 1.6 GBq. Table 2 contains the irradiation settings and resulting yields from each production run. The main radionuclidic impurity in the separated product was ^{57}Co from $^{56}\text{Fe}(\text{d},\text{n})$, which accounted for a production yield of $0.04 \pm 0.01 \text{ MBq}/\mu\text{A h}$.

Experimental yields are in close agreement ($> 70\%$) of predicted yields calculated from the referenced experimental excitation functions if only the beams' transverse area that irradiates the electroplated surface is taken into account. The narrow extent of electroplated surface was chosen purposefully in order to maximize the axial thickness using a reasonably low mass of enriched material ($< 80 \text{ mg Fe}$ and $< 200 \text{ mg Ni}$).

3.2. Radiochemical separation

The radio-cobalt separation efficiency from iron was $95 \pm 3\%$ ($n = 11$) in $50 \mu\text{L}$ of 0.1 M HCl . The whole separation process from EoB to the re-dissolution of the separated cobalt took 3 h 40 min. The ^{54}Fe and ^{57}Fe recycling yields after twelve and four production runs on the same target material were $94 \pm 4\%$ and $94 \pm 3\%$, respectively. This renders the production of ^{55}Co and ^{58m}Co economical since the replenishment of relatively expensive target material will only be required at an affordable rate.

The ^{55}Co separation efficiency from ^{58}Ni was $92 \pm 5\%$ ($n = 3$) in $100 \mu\text{L}$ of 0.1 M HCl . The whole separation process took 2 h. The ^{58}Ni recycling yield after three production runs on the same target was $94 \pm 1\%$.

Both production routes depend on the extraction properties of DGA resin in HCl solution, which were published by Pourmand and Dauphas (2010). Cobalt in HCl solution is expected to be trapped in the DGA resin when the mobile phase solution has a concentration $\sim 8 \text{ M HCl}$, at which the distribution coefficient ($\log K_d$) for cobalt is > 10 , while the $\log K_d$ for nickel and manganese is $< 10^{-1}$. Cobalt is expected to release from the column in HCl at a concentration $\sim 3 \text{ M}$, at which the distribution coefficient towards the resin for this element is ~ 1 . This separation method was successfully verified when the amount of DGA resin packed in a 0.5 cm diameter column is $\sim 300 \text{ mg}$. Lower amounts of resin resulted in lower trapping efficiencies (data not shown). Fig. 3(a) and (b) show the ^{55}Co elution profiles from DGA in terms of the percentage of the total radioactivity produced at EoB eluted in each $200 \mu\text{L}$ fraction.

Fig. 3(c) and (d) show the complete elution profiles of the main elements involved in the separation of radio-cobalt from ^{58}Ni using a 300 mg DGA branched column in percentage per fraction and in cumulative percentage of the total radioactivity produced at EoB.

The γ -spectra, with a broad energy range of $28\text{--}2000 \text{ keV}$, of ^{55}Co and ^{58m}Co from isotopically enriched iron targets before and after the purification process are shown in the first two rows of Fig. 4. The un-purified ^{55}Co and ^{58m}Co target samples contained

significant levels of non-cobalt radionuclidic impurities, such as radio-manganese (^{52}Mn (744 keV) and ^{56}Mn (847 keV), respectively) and ^{24}Na (1369 keV), which could be completely removed after the purification process (Fig. 4(a) and (b), second column), resulting in ^{55}Co and $^{58\text{m}}\text{Co}$ with radionuclidic purities at EoB of $99.995 \pm 0.001\%$ ($n = 10$) and $98.7 \pm 0.1\%$ ($n = 4$), respectively. Likewise, the γ -spectra of ^{55}Co from ^{58}Ni targets before and after the separation procedure is shown in the last row of Fig. 4. In this case the main radionuclidic impurity that was removed in the final product was ^{57}Ni (1378 keV), and final radionuclidic purity at EoB was $98.8 \pm 0.3\%$ ($n = 3$). Table 3 presents the details of the radionuclidic components of each separated product.

Na-24 is generated by deuteron activation of traces of stable ^{23}Na that is incorporated in the electroplated iron targets. The reason why relatively high quantities of ^{24}Na are generated from trace amounts of target ^{23}Na is because the $^{23}\text{Na(d,p)}$ reaction has a high excitation function that remains between 0.4 and 0.5 barns (Hirsh et al., 2015) in the energy range that traverses the targets in this work (< 8.2 MeV).

Ten μL samples from the target solutions as well as 2–20 μL samples from the separated stocks were analyzed for trace metal quantification with Agilent's 4200 MP-AES system. The main metal impurities found in the separated stock of ^{55}Co from ^{54}Fe targets are: cobalt (268 μM), copper (275 μM), nickel (150 μM), silver (95 μM), iron (55 μM) and zinc (48 μM). The main metal impurities in the separated stock of ^{55}Co from ^{58}Ni targets are: cobalt (2.76 mM), nickel (2.05 mM), silver (148 μM), zinc (125 μM), iron (87 μM) and copper (87 μM). These results are summarized in Table 4, along with the corresponding separation factor for each element.

As it was mentioned in Section 3.1, a typical 60 μAh irradiation on a 37 mg/cm^2 thick ^{54}Fe target generates ~ 582 MBq ^{55}Co . 95% of this activity is separated, dried down and re-dissolved in 100 μL of 0.1 M HCl, which represents ~ 553 MBq ^{55}Co with an activity concentration of 5530 GBq/L, decay corrected to EoB. Therefore, the effective specific activity (ESA) of the separated ^{55}Co from such representative irradiation would be 5530 GBq/L divided by the total molar concentration of metal impurities shown in Table 4, that is, 891 μM , which equals 6.2 GBq/ μmol of metal impurities. Likewise, a typical 40 $\mu\text{A h}$ irradiation on a 110 mg/cm^2 thick ^{58}Ni target would result in a separated stock of ^{55}Co with a concentration of 3420 GBq/L, and the division of this over the total molar concentration of metal impurities, 5248 μM , results in a ESA of 0.65 GBq/ μmol of metal impurities. Both of these ESA are between 2 and 10 times lower than the ESA that are obtained from the titration experiments with mainstream chelators, as it will be presented in the following Section 3.3 and the reason is because not all metal impurities have the same affinity towards the chelator. In other words, not all metal impurities compete for the coordination by the chelator with the same strength.

3.3. Effective specific activity of ^{55}Co and $^{58\text{m}}\text{Co}$ with mainstream chelators

The retention factors (R_f) of unlabeled ^{5x}Co , $^{5x}\text{Co-NOTA}$, $^{5x}\text{Co-DOTA}$, $^{5x}\text{Co-HBED}$ and $^{5x}\text{Co-TETA}$ in the plates after the TLC method were 0.2, 0.5, 0.3, 0.8 and 0.7, respectively. The effective specific activities of ^{55}Co from ^{54}Fe targets with NOTA, DOTA, HBED and TETA, decay-corrected at EoB, were 27 ± 18 , 9 ± 5 , 17 ± 10 and 14 ± 7 GBq/

μmol , respectively. The ESA of ^{55}Co from ^{58}Ni and $^{58\text{m}}\text{Co}$ from ^{57}Fe with NOTA are 10 ± 6 and 25 ± 6 GBq/ μmol , respectively.

The thermodynamic stability constants ($\log K_{ML}$) between metals and ligands, such as the chelators employed in this work, provide a convenient gauge of the ligand's relative affinity for a specific metal (Wadas et al., 2010). However, the differences between the $\log K_{ML}$ values of cobalt with the four chelators used to characterize ^{55}Co from ^{54}Fe do not explain by themselves the differences in the measured ESA. For instance, since the stability constants of cobalt chelated with NOTA, DOTA, TETA and HBED are 17.8, 19.3, 16.6 and 19.4, respectively (Martell et al.), it would be expected that the highest ESA should have been measured with HBED followed by DOTA, NOTA and TETA. However, this was not the case because the amount of other non-radioactive metallic impurities different from cobalt and the affinity of these impurities towards the chelators (inferred from their $\log K_{ML}$ values) also need to be taken into account, since both of these factors define how strong is the competition of the impurities for the chelator against ^{55}Co . The main non-radioactive metal contaminants in the final product are cobalt and copper and the latter has thermodynamic stability constants when chelated with NOTA, DOTA, TETA and HBED of 20.1, 22.7, 21.7 and 22.9, respectively (Martell et al.). Thus, copper is a weaker competitor for the chelation with NOTA compared to the other three chelators and a relatively strong competitor for the chelation with DOTA. This, in part, explains the order in ESA that was measured in our separated product.

An important consideration in the chelation of ^{55}Co and $^{58\text{m}}\text{Co}$ is that both radionuclides decay to long-lived radioactive daughter nuclides, namely ^{55}Fe ($t_{1/2} = 2.7$ y, 100% EC) and $^{58\text{g}}\text{Co}$ ($t_{1/2} = 70.9$ d), and therefore it is imperative to determine whether they remain chelated, and hence bound to the targeting vector. Theoretically, the ^{55}Fe that results from the decay of ^{55}Co is released from the chelating ligand. The maximum recoil energy of the daughter ^{55}Fe , after positron emission from ^{55}Co with maximum energy of 1.498 MeV, is 36.9 eV or 851 kcal/mol, which is more than 6 times larger than the typical binding energies of the metal(III)-oxygen bonds (116 kcal/mol) and the metal(III)-nitrogen bonds (134 kcal/mol) (Basolo and Pearson, 1967; Mirzadeh et al., 1993) that are involved in the coordination of the chelating ligands that were employed in this work. Perhaps some of the released ^{55}Fe will be re-coordinated to the original chelating ligand due to the high affinity between them (the thermodynamic stability constants of the iron-chelator complexes in $\log K_{ML}$ are 28.0, 19.8, 13.3 and 39.0, for NOTA, DOTA, TETA and HBED, respectively (Martell et al., 2004), but most likely it will circulate as a weakly-bound ion in the subject's bloodstream until it is coordinated by one of many iron-binding proteins in circulation such as transferrin ($\log K_{ML} = 21.4$) (Sun et al., 1999), which has a relatively high concentration in the blood of 25 μM (Jackson and Byrne, 1996). In this case, and assuming that the ^{55}Fe is not physiologically excreted from the subject's body, this long-lived radionuclide will deposit an absorbed dose for a very long time. However, such dose will be very low and will not significantly affect tissue as long as the radionuclide is not accumulated in close proximity to cell nuclei (Rao et al., 1985). The absorbed dose from ^{55}Fe will be low because the amount of ^{55}Fe activity that is generated from complete decay of ^{55}Co is 7.3×10^{-4} times the activity of ^{55}Co that is injected, which is the ratio of the parent-daughter half-lives. For instance, if 74 MBq of ^{55}Co -labeled agent are injected and there is no excretion of radioactive material, then the

subject will contain 54 kBq ^{55}Fe in its system and, according to ICRP 60 (ICRP, 1991), this will result in a radiation dose of 0.31 mSv, which represents 0.6% of the recommended annual dose constraint (FDA, 2017).

In the case of $^{58\text{m}}\text{Co}$, its daughter nuclide $^{58\text{g}}\text{Co}$ will not be ejected from the chelating ligand due to the low recoil energy from the electron capture decay. Theoretically, the maximum recoil energy on the $^{58\text{g}}\text{Co}$ atom is caused by the 24.8 keV conversion electron with a value of 0.24 eV or 5.5 kcal/mol, which is two orders of magnitude lower than the binding energy of the covalent bonds between metal and chelating ligand. Experimentally, the stabilities of the ^{55}Co -NOTA, DOTA, TETA and HBED complexes in mouse serum at 37 °C for 46 h were quantified via TLC analysis as 99.2%, 99.7%, 96.7% and 98.7%, respectively.

3.4. Preparation of ^{55}Co - and $^{58\text{m}}\text{Co}$ -NOTA-TRC105

After mixing 20 and 90 μL from each separated stock (0.18 and 1.42 GBq of ^{55}Co and $^{58\text{m}}\text{Co}$ at EoB, respectively) with ~ 190 and ~ 860 μg NOTA-TRC105, respectively, in 230 μL PBS and waiting for 1–2 h, the purification of ^{55}Co - and $^{58\text{m}}\text{Co}$ -NOTA-TRC105 resulted in a total labeling yield of 82% and $90 \pm 2\%$ ($n = 3$), respectively, in 4 fractions of 400 μL of PBS. This corresponds to effective specific activities of 118 and 204 ± 18 GBq/ μmol for the antibody labeled with ^{55}Co and $^{58\text{m}}\text{Co}$, respectively, assuming that the antibody conjugate has a molar mass of ~ 150.4 kDa. These ESA are 4–8 times higher than the ones measured with the unconjugated chelators most likely because each antibody is conjugated to more than a single chelator, which effectively increases the ESA per mole of protein. The lower radiolabeling yield for ^{55}Co was caused by a 5% loss of activity in the reaction vial that was not transferred to the size-exclusion column. In the radiolabeling of $^{58\text{m}}\text{Co}$ the loss in the reaction vial was lowered to $< 3\%$ by having a mixer shaking the reaction vial. Taking these losses into account, the radiolabeling yield with both nuclides is very similar and reproducible at $89 \pm 2\%$ ($n = 4$).

3.5. Comparison of published separation methods and clinical perspective

Comparing the separation chemistry methods of this work with previously reported methods (Mastren et al., 2015; Sharma et al., 1986; Spellerberg et al., 1998; Thisgaard et al., 2011a, 2011b, 2014; Zaman et al., 2003) there are many improvements. First, more than 99.99% of the co-produced ^{52}Mn from $^{54}\text{Fe}(\text{d},\text{a})$ was removed from the separated ^{55}Co ; second, more than 95% of the produced ^{55}Co , decay corrected to EoB, in either ^{58}Ni or ^{54}Fe targets was isolated at a very high concentration in 600 μL , which is volume that can be easily evaporated for reconstitution in any desired volume of any desired acid or buffered solution; and third, the effective specific activities of ^{55}Co and $^{58\text{m}}\text{Co}$ toward established chelators are comparable to the highest ones that have been reported in the literature. All of these improvements are due to the chromatographic method that has been presented using a small column filled with DGA branched resin. Table 5 compares the present work with the results from previous publications.

The first highlight of the three production methods reported in this work compared to the other ones presented in Table 5, is that we have used the highest bombardment currents without any compromise in target integrity. In terms of radiochemical separation, the

presented method to isolate ^{55}Co from ^{58}Ni is clearly superior to any of the reported methods shown in Table 5, especially with regards to the volume in which ^{55}Co is separated while maintaining a sufficiently high effective specific activity that is comparable to the highest reported values in the literature. The presented method separates ^{55}Co in 400 μL , a volume that can be quickly evaporated even in the original 1.5 mL vial where the separated fractions are collected, which avoids the use of a large vessel ($> 10\text{ mL}$) connected to a roto-evaporator in which a variable loss of radioactivity may be lost in the large surface area of the glassware as it is difficult to re-dissolve all the activity off the walls of the vessel with a small volume ($< 1\text{ mL}$) of diluted HCl acid. Similarly, in the presented separation method of radio-cobalt from bulk iron, the use of the DGA branched extraction resin in the second part of the separation allows for: 1) the almost complete removal of radio-manganese impurities; and 2) a separated radio-cobalt that is concentrated in a small volume (0.4–0.6 mL) and has an effective specific activity with NOTA that is comparable to the highest one that has been reported using a NOTA-conjugated peptide (Dam et al., 2016). However, regarding the radionuclidic purity of ^{55}Co , the $^{58}\text{Ni}(\text{p},\alpha)$ route is worse than the $^{54}\text{Fe}(\text{d},\text{n})$ one due to the unavoidable presence of MBq amounts of ^{57}Co from $^{58}\text{Ni}(\text{p},\text{pn})^{57}\text{Ni} \rightarrow ^{57}\text{Co}$ and from the $^{60}\text{Ni}(\text{p},\alpha)$ reaction on the 0.505% impurity of ^{60}Ni in the target material. However, in terms of adsorbed dose to a patient, assuming that up to 74 MBq ^{55}Co are injected, the impurity levels of ^{57}Co will be $< 1\text{ MBq}$, which may be acceptable depending on internal dosimetry estimates that will be contingent on the pharmacokinetics, particularly on the biological half-life, of the particular radiolabeled agent that is administered.

Furthermore, this is the first time that the effective specific activity of ^{55}Co with HBED has been reported, which has recently become very relevant in the field of nuclear medicine since the HBED-PSMA molecule labeled with ^{68}Ga is increasingly becoming the workhorse for detection and monitoring of prostate cancer (Afshar-Oromieh et al., 2015). Hence, this discovery opens the possibility of introducing the Auger and conversion electron emitter $^{58\text{m}}\text{Co}$ for therapy of prostate cancer with the HBED-CC-PSMA targeting vehicle.

The main contribution of the presented production method for $^{58\text{m}}\text{Co}$ is that it demonstrates that there is another practical alternative to the proton irradiation of expensive ^{58}Fe (~ US \$30/mg). In this work, isotopically enriched ^{57}Fe (~ US\$6/mg) was irradiated with 8.2 MeV deuterons and, as it is shown in Table 5, the measured production yields from thick targets is higher than the production yield obtained by Thisgaard et al. (2011a), after irradiation of 10.1 MeV protons on a thin ^{58}Fe target (7.0 mg/cm²). The reason ^{57}Fe is much cheaper than ^{58}Fe , even though they both have similar natural abundances of 2.119% and 0.282%, respectively, is because ^{57}Fe already has a steady demand in the market of Mössbauer spectroscopy (Martinho and Münck, 2010). Thus, the availability of the ^{57}Fe target material is less subject to variations in supply and price, although deuteron accelerators are less common than their proton counterparts. Another drawback of the $^{57}\text{Fe}(\text{d},\text{n})^{58\text{m}}\text{Co}$ route compared to the $^{58}\text{Fe}(\text{p},\text{n})^{58\text{m}}\text{Co}$ alternative is that there will always be a higher amount of ^{57}Co impurity in the separated $^{58\text{m}}\text{Co}$, which is originated from the $^{57}\text{Fe}(\text{d},2\text{n})$ reaction, with a threshold energy of 4.0 MeV, and from the $^{56}\text{Fe}(\text{d},\text{n})$ reaction on the 3.06% ^{56}Fe that is present in the target material. Such high percentage of ^{56}Fe impurity is acceptable for Mössbauer spectroscopy applications and, depending on dosimetry estimates and radiation safety policies, it may not be adequate for production of $^{58\text{m}}\text{Co}$ for clinical applications. In

such case, a target material that is closer to 100% enrichment of ^{57}Fe would be necessary to significantly reduce the presence of ^{57}Co in the separated product. The $^{58}\text{Fe}(\text{p},\text{n})^{58\text{m}}\text{Co}$ route completely avoids the production of ^{57}Co by irradiating with protons with less than 11.9 MeV, the threshold energy of the $^{58}\text{Fe}(\text{p},2\text{n})^{57}\text{Co}$ reaction, and therefore makes this a more attractive option in terms of radionuclidic purity.

From a clinical perspective, the ^{55}Co production yields that were obtained in this work would be able to supply activities for several patient studies. Co-55 has been administered to human subjects for PET imaging with injected activities that range from 19 to 74 MBq (De Reuck et al., 1999; Jansen et al., 1997a, 1997b, 1996a, 1996b, 1994, 1995; Stevens et al., 1999). These administered activities depend on the positron branching ratio of the radionuclide (77% for ^{55}Co), the pharmacokinetics of the radiolabeled tracer and the annual dose constraint of 50 mSv (FDA, 2017) for adult research subjects. This means that 1 h-long irradiations on either ^{54}Fe or ^{58}Ni targets with the specifications and separation yields that were presented in Sections 2.1 and 3.2, could provide sufficient ^{55}Co activity for between 5 and 32 patients, or one half of these, between 2 and 16 patients, if the activity needs to be transported overnight, assuming that one half-life has elapsed from EoB.

4. Conclusions

Efficient methods for the production and isolation of GBq amounts of the positron emitter ^{55}Co and its therapeutic pair $^{58\text{m}}\text{Co}$ have been presented, which result in separated radionuclides with high specific activities at EoB that allow for quantitative radiolabeling of chelator-conjugated targeting vectors, such as antibodies, in ratios 45 MBq ^{55}Co and 31 MBq $^{58\text{m}}\text{Co}$ per nmol ($\sim 150\text{ }\mu\text{g}$) of NOTA-conjugated protein.

A novel iron electroplating method was presented that allows for the elaboration of thick targets that can sustain high power irradiations of up to 492 W and that allows for the recovery of expensive isotopically enriched iron after an aqueous radiochemical separation process.

Compared to previously published methods, the introduction of the extraction resin DGA branched greatly simplified the radiochemical separation of radio-cobalt from both iron- or nickel-based targets, because it allows the elution of the separated nuclide in $< 0.6\text{ mL}$ instead of tens of milliliters.

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HIGHLIGHTS

- ^{55}Co is produced via $^{54}\text{Fe}(\text{d},\text{n})$ and $^{58}\text{Ni}(\text{p},\alpha)$ with maximum power depositions 492 W.
- The Auger-emitter $^{58\text{m}}\text{Co}$ is produced via $^{57}\text{Fe}(\text{d},\text{n})$ with 60 μA and 8.2 MeV.
- The electroplating method for Fe allows the efficient recovery of target material.
- > 92 % of the activity at EoB is eluted in 0.6 mL of HCl.
- The effective specific activities with NOTA are 10 GBq/ μmol .

Fig. 1.

(a) Schematic of the electrolytic cell. (b) Schematic of how the electroplated iron or nickel targets are mounted into one of the ports of the cyclotron.

Fig. 2.

(a) Schematic of the radiochemical separation of ^{5x}Co from ^{5x}Fe . (b) Schematic of the radiochemical separation of ^{5x}Co from ^{58}Ni .

Fig. 3.

Elution profiles of ^{55}Co separated from ^{54}Fe (a) or ^{58}Ni (b) dissolved in HCl using a 300 mg DGA branched resin packed into a 0.5 cm ID column with a flow rate of 1.1 mL/min. Complete elution profile of the main elements involved in the separation of ^{55}Co from ^{58}Ni dissolved in HCl in terms of percentage per volume (c) and cumulative percentage per total volume (d).

Fig. 4.

Gamma spectra of the target solutions before separation (first column) and of the separated stocks (second column). The separated product in rows (a) and (b) show the absence of gamma peaks from the impurities ^{52}Mn and ^{56}Mn from $^{54}\text{Fe}(\text{d}, \alpha)$ and $^{58}\text{Fe}(\text{d}, \alpha)$ respectively, and ^{24}Na from $^{23}\text{Na}(\text{d}, \text{p})$. Row (c) shows the absence of the gamma peaks from ^{57}Ni from $^{58}\text{Ni}(\text{p}, \text{pn})$. The gamma spectrum at low energies is shown in (d).

Table 1

Gamma emissions used for production yield and separation efficiency quantifications.

Nuclide	Reaction	Gamma energies [keV]	Branching ratio
^{55}Co	$^{54}\text{Fe}(\text{d},\text{n})$	477 ^a	0.202
	$^{58}\text{Ni}(\text{p},\alpha)$	931	0.75
^{57}Co	$^{56}\text{Fe}(\text{d},\text{n})$	122	0.8560
	$^{60}\text{Ni}(\text{p},\alpha)$		
$^{58\text{g}}\text{Co}$	$^{57}\text{Fe}(\text{d},\text{n})$	811	0.9945
$^{58\text{m}}\text{Co}$	$^{57}\text{Fe}(\text{d},\text{n})$	24.9	0.000397
^{57}Ni	$^{58}\text{Ni}(\text{p},\text{pn})$	1378	0.817
^{52}Mn	$^{54}\text{Fe}(\text{d},\alpha)$	744	0.900
^{56}Mn	$^{58}\text{Fe}(\text{d},\alpha)$	847	0.9885
^{24}Na	$^{23}\text{Na}(\text{d},\text{n})$	1369	0.9999

^aThe 477 keV gamma peak was used instead of the 931 keV gamma because there is partial overlap with the 936 keV gamma from ^{52}Mn , which has a branching ratio of 94.5%.

Table 2

Activity yields related to the production of ^{55}Co and ^{58}mCo .

Target	Q [$\mu\text{A h}$]	x [mg/cm^2]	^{55}Co Yield [$\text{MBq}/\mu\text{A h}$]	^{52}Mn Yield [$\text{kBq}/\mu\text{A h}$]	^{57}Ni Yield [$\text{MBq}/\mu\text{Ah}$]	^{58}mCo Yield [$\text{MBq}/\mu\text{A h}$]	^{58}Co Yield [$\text{kBq}/\mu\text{A h}$]	^{57}Co Yield [$\text{kBq}/\mu\text{A h}$]
^{54}Fe	60.0	24.3	5.5	63	–	–	–	–
	80.0	46.1	8.9	74	–	–	–	–
	50.0	49.0	8.1	71	–	–	–	–
	120.0	47.1	9.9	80	–	–	–	–
	70.0	37.6	7.7	71	–	–	–	–
	120.0	46.3	9.4	87	–	–	–	–
	70.0	45.3	9.5	74	–	–	–	–
	120.1	65.4	11.3	87	–	–	–	–
	60.0	59.2	11.2	83	–	–	–	–
	120.0	58.1	10.6	79	–	–	–	–
^{58}Ni	44.1	52.3	10.0	84	–	–	–	–
	120.0	63.9	8.8	68	–	–	–	–
	87.5	61.2	9.9	78	–	–	–	–
	45.0	58.6	10.4	78	–	–	–	–
	10.0	110.2	8.6	–	1.1	–	–	–
	25.0	214.2	9.5	–	1.3	–	–	–
^{57}Fe	20.0	201.5	9.9	–	1.0	–	–	–
	124.0	78.2	–	–	–	11.0	135	38
	90.0	70.6	–	–	–	11.5	115	35
	120.0	71.6	–	–	–	13.5	118	37
	120.0	58.7	–	–	–	17.8	158	52

The irradiation times in hours are equal to Q divided by 60 or 30 μA for iron and nickel targets, respectively.

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Table 3

Longed-lived radionuclidic components in the separated radio-cobalt.

Desired product	Target	% abundance	Reaction	Product	% in separated stock at EoB
⁵⁵ Co	⁵⁴ Fe	99.93%	⁵⁴ Fe(d,n)	⁵⁵ Co	99.995 ± 0.001%
			⁵⁴ Fe(d,α)	⁵² Mn	0.0011 ± 0.0003%
	⁵⁶ Fe	0.06%	⁵⁶ Fe(d,n)	⁵⁷ Co	0.0025 ± 0.0004%
			⁵⁶ Fe(d,α)	⁵⁴ Mn	Unobserved
⁵⁵ Co	⁵⁷ Fe	0.005%	⁵⁷ Fe(d,n)	^{58g} Co	Unobserved
	⁵⁸ Ni	99.48%	⁵⁸ Ni(p,α)	⁵⁵ Co	98.8 ± 0.3%
			⁵⁸ Ni(p,pn)	⁵⁷ Ni	Unobserved
	⁶⁰ Ni	0.505%	⁶⁰ Ni(p,α)	⁵⁷ Co	1.2 ± 0.3%
^{58 m} Co	⁶¹ Ni	0.005%	⁶¹ Ni(p,α)	^{58g} Co	0.003%
	⁵⁷ Fe	95.06%	⁵⁷ Fe(d,n)	^{58m} Co	98.7 ± 0.1%
				^{58g} Co	0.93 ± 0.03%
	⁵⁶ Fe	3.04%	⁵⁶ Fe(d,n)	⁵⁷ Co	0.29 ± 0.01%
	⁵⁴ Fe	0.04%	⁵⁴ Fe(d,n)	⁵⁵ Co	0.19%

Table 4

Results from trace metal analysis on the separated stock of ^{55}Co used for labeling experiments.

Target	Element	Mass [μg]	Molarity [μM]	Separation Factor ^a
^{54}Fe	Cobalt	1.6 ± 0.2	268 ± 38	–
	Copper	1.7 ± 1.0	275 ± 153	3.6×10^3
	Nickel	0.9 ± 0.8	150 ± 140	5.2×10^3
	Silver	1.0 ± 0.3	95 ± 23	4.2×10^3
	Iron	0.3 ± 0.2	55 ± 41	4.8×10^5
	Zinc	0.3 ± 0.1	48 ± 23	6.4×10^3
^{58}Ni	Cobalt	16.2 ± 9.1	2755 ± 1545	–
	Nickel	12.0 ± 10.0	2046 ± 1705	2.0×10^4
	Silver	1.6 ± 0.9	148 ± 84	8.3×10^2
	Zinc	0.8 ± 0.3	125 ± 40	3.8×10^3
	Iron	0.5 ± 0.3	87 ± 58	1.2×10^3
	Copper	0.6 ± 0.4	87 ± 55	1.2×10^3

^aCalculated by dividing the mass in the target solution over the mass in the separated stock.

Table 5

Comparison of production methods of radio-cobalt for biomedical applications.

Sep. Method	Desired product	Target	p ⁺ or d ⁺ E [MeV]	i [μA]	A Yield [MBq/μA h]	⁵⁵ Co Sep. yield [%]	Vol. of eluted ⁵⁵ Co [mL]	Nuclidic impurities at EoB	ESA [GBq/μmol] (chelator)	Target recycling efficiency [%]	Ref.
AEC	⁵⁵ Co	Plated ⁵⁸ Ni (99.87%)	15–9	10	9.6	Not reported	15	0.5% ⁵⁷ Co	Not reported	Not reported	Spellerberg et al. (1998)
AEC	⁵⁵ Co	Plated ⁵⁸ Ni (99.48%)	15	30	6 ± 1	92 ± 3	10	0.2% ⁵⁷ Co	1.96 (DOTA)	95 ± 3	Mastren et al. (2015)
SPEC (DGA)	⁵⁵ Co	⁵⁸ Ni (99.48%)	16–11	40	9.3 ± 0.6	92 ± 5	0.4	1.2 ± 0.3% ⁵⁷ Co	10 ± 6 (NOTA)	94 ± 1	This work
Solvent extraction + AEC	⁵⁵ Co	Pressed ⁵⁴ Fe ₂ O ₃ /Al powder (91.6%)	12.6–5	4	13	Not reported	40	0.06% ⁵⁷ Co < 0.01% ⁵² Mn < 0.02% ⁵⁶ Co 0.016% ⁵⁷ Co	Not reported	> 80	Zaman et al. (2003)
AEC	⁵⁵ Co	Pressed ⁵⁴ Fe (97.08%)	12	3	62.9	90	40	Not reported	Not reported	Not reported	Sharma et al. (1986)
AEC	⁵⁵ Co	Plated ⁵⁴ Fe (99.84%)	8.5	18	1.4	> 97	8–10	Not reported	> 0.21 (DOTA) 30 (NOTA)	Not reported	Thisgaard et al. (2011b) & Dam et al. (2016)
AEC	^{58m} Co	Plated ⁵⁸ Fe (99.86%)	10.1	18	10.7	> 97	8–10	0.85% ⁵⁸ Co	> 4 (DOTA)	Not reported	Thisgaard et al. (2011a) & Thisgaard et al. (2011b)
AEC + Chromafix 30-PS-HCO ₃	^{58m} Co	“	“	“	“	“	0.2	“	38–84 (DOTA)	“	Thisgaard et al. (2014) & Thisgaard et al., 2017
	⁵⁵ Co	Plated ⁵⁴ Fe (99.84%)	8.5	18	1.4	“	“	Not reported	7.05 (DOTA)	“	Garousi et al. (2017)
AEC + SPEC (DGA)	⁵⁵ Co	⁵⁴ Fe (99.93%)	8.2	60	10.3 ± 0.8	94 ± 3	0.4–0.6	0.002% ⁵⁷ Co 0.001% ⁵² Mn	27 ± 18 (NOTA) 118 (NOTA-Ab) 9 ± 5 (DOTA) 17 ± 10 (HBED) 15 ± 7 (TETA)	94 ± 4	This work

Sep. Method	Desired product	Target	p ⁺ or d ⁺ E [MeV]	i [μA]	A Yield [MBq/μA h]	⁵⁸ Co Sep. yield [%]	Vol. of eluted ⁵⁸ Co [mL]	Nuclidic impurities at EoB	ESA [GBq/μmol] (chelator)	Target recycling efficiency [%]	Ref.
"	⁵⁸ mCo	⁵⁷ Fe (95,06%)	8.2	"	13 ± 3	96 ± 2	"	0.93% ⁵⁸ Co 0.29% ⁵⁷ Co	25 ± 6 (NOTA) 204 ± 18 (NOTA-Ab)	94 ± 3	This work

AEC: Anion Exchange Chromatography.
SPEC (DGA): Solid Phase Extraction Chromatography with DGA resin.
Ab: Antibody, in this work it is TRC105.