Preparation and Evaluation of [^{55}Co](II)DTPA for Blood Cell Labeling

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Abstract: Co-55 ($T_{1/2}$=17.53 h) was produced by 150 μA irradiation of a natural nickel target by 15 MeV protons and was separated from the irradiated target material by two ion exchange chromatography steps and was used for the preparation of [^{55}Co]diethylenetriaminepentacetate ([^{55}Co]DTPA). Optimization studies were performed using Co-57 due to its longer half-life. Cobalt-57 ($T_{1/2}$=271.79 d) was produced by irradiation of a natural nickel target with 150 μA current of 22 MeV protons. The ^{57}Co was separated from the irradiated target material using a no carrier added method with a radiochemical yield of >97%. The ^{55}Co was separated from the irradiated target material using a two step method with a radiochemical yield of >95%. Both products were controlled for radionuclide and chemical purity. The solutions of [^{55}Co]complex were prepared (radiochemical yield>80%) starting with ^{55}CoOAc ligand at room temperature after 30 min. RTLC showed the radiochemical purity of more than 99%. Specific activity was obtained about 9.1 TBq/mmol. The tracer showed to be stable in the final product and in the presence of human serum at 37°C up to 15 h. [^{55}Co]DTPA was successively used in the radiolabeling of red blood cells for PET[1] diagnostic studies.

Keywords: Cobalt-55**,** DTPA, Cell labeling, PET, radiopharmaceuticals.

INTRODUCTION

Cobalt offers a unique selection of radionuclides suitable for imaging and/or radiotherapy [1]. The most medically used cobalt radionuclides, ^{55}Co and ^{57}Co, provide very good physical properties for diagnostic purposes using PET and SPECT[2] imaging respectively (Table **1**).

Many ^{57}Co-labeled compounds have been prepared and used in the detection of head and neck cancers [2], pernicious anemia [3], renographic studies [4] and transferrin mediated tumors [5]. On the other hand, series of studies using ^{55}Co-$CoCl_2$ have been reported in the detection of caratid artery disease [6], vascular dementia [7], renographic studies [8] and stroke [9] using PET scanners.

Thus, preparation of Co(II)DTPA$^-$ complex may afford stable complexes with potential low biodegradation in presence of serum components, resulting in a lipophillic complex to cross membranes. In many biological studies rate of Co incorporation into microorganisms have been calculated in various concentrations of chelator as well as cobalt cation [12].

The idea of incorporation of a PET radioisotope into suitable chelates used in cisternographic and blood cell labeling, came to our interest based on our recent background on the production and use of non-conventional PET tracers [13, 14].

Interesting imaging properties of cobalt-55 and the possibility of its production *via* ^{58}Ni(p,α)^{55}Co and/or ^{nat}Ni(p, x)^{55}Co reaction using natural nickel provided a suitable source of this radionuclide for its ultimate use in radiolabeling of DTPA.

Table 1. Physical Characteristics of Co-55 and Co-57 [4]

Gamma Photon (keV) Intensities (%)	Decay Mode	Half-Life	Radionuclide
1408(16.8%), 1369(2.9%), 1316(7.0%), 931(75%), 803(1.8%), 477(20.2%) 411(1.0%), 91(1.1%) and 511(154%)	β^+(60%), E.C.(40%)	17.53 h	^{55}Co
136(10.6%), 122(85.6), 14(9.1%)	E.C.(100%)	271.9 d	^{57}Co

There have been reports in the literature concerning the preparation and use of radiometal-DTPA complexes for cisternography in early 1980's [10]. Co-DTPA complex is a stable complex among polyaminecarboxylate complexes (K_{NTA}10.38, K_{EDTA} 16.45 and K_{DTPA}=19.15) [11].

EXPERIMENTAL

Production of cobalt radionuclides (^{55}Co and ^{57}Co) was performed at the AMIRS[3] 30 MeV cyclotron (Cyclone-30, IBA). All chemicals were of analytical grade and were purchased from Merck Chemical Company (Darmstadt, Germany). The ion-exchange resins were provided commercially (Bio-Rad Laboratories, Canada). Radio-thin-layer-chromatography (RTLC) was performed on polymer-backed

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[1]Positron Emission Tomography.

[2]Single Photon Emission Computed Tomography.

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silica gel (F 1500/LS 254, 20×20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Normal saline and sodium acetate used for labeling were of high purity and had been filtered through 0.22 μm Cativex filters. Radiochromatography was performed by counting 5-mm portions of the strip using an in-house made scanner equipped with a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL) or counting each 5mm-strip after cutting it into pieces in a CRC-15R Capintec activity meter (NJ, USA). Radionuclide purities were checked using the same detector. All calculations and RTLC counting were based on the 477.2 keV peak.

Production of ^{57}Co

The high current natural nickel target was electroplated on a gold-plated copper backing in order to avoid the contamination with troublesome copper removal chemistry. In IBA-Cyclone 30 solid target systems, the extracted proton beam falls at an angle of 6 degrees, therefore, the required target thickness is reduced by 10 times and a 65 μm layer of nickel was enough for our purpose. Electrodeposition was carried out using a special cell which gave a surface area of about 1×11 cm^2. The target was irradiated by a 150 μA current of 22 MeV protons (22-10 MeV on the target). Radiochemical separation was carried out in no-carrier-added form [15, 16] two weeks later, in order to provide enough time for the decay of ^{57}Ni to ^{57}Co and also for elimination of short lived radionuclide impurities. The irradiated target was dissolved in refluxing warm 7 M HNO_3 (30 ml) for 30 min. The solution was heated almost to dryness and the latter process was repeated after the addition of 20 ml distilled water. The residue was reconstituted by 9 M HCl (20 ml) and the solution was passed through an anion exchange resin (AG 1X8, Cl^- form, 100-200 mesh, h: 8 cm, Ø: 1 cm) preconditioned with 25 ml of 9 M HCl. The column was then washed by passage of 50 ml of 9 M HCl at a rate of 1 ml/min to remove nickel ions. Finally, the cobalt-57 was eluted as ^{57}Co-$CoCl_2$ using 4 M HCl (50 ml); the whole process took about 3 hours.

Production of ^{55}Co

The procedure used for the targetry and bombardment leading to the production of ^{55}Co was quite similar to that of ^{57}Co with the exception of using a 30 μm layer of nickel as the target material and 15 MeV protons for target irradiation (15-8 MeV on the target). The radiochemical separation process including the recovery of cobalt from nickel and copper was carried out immediately after the target bombardment. The recovery of cobalt and copper from nickel was carried out exactly in the same way as explained above for radiochemical separation of ^{57}Co. The only differences were: using 20 ml of refluxing warm 7 M HNO_3 in the first step and passage of 20 ml of 4 M HCl for the recovery of radiocobalt and radiocopper ions.

The eluent was evaporated to dryness and dissolved in 25 ml of 0.3 M HCl-94% ethanol and was then injected to an anion exchange resin (AG 1X8, Cl^- form, 100-200 mesh, h: 5 cm, Ø: 1 cm) pre-equilibrated with 25 ml of 0.3 M HCl-82% ethanol. More than 95% of cobalt ions were recovered as ^{55}Co-$CoCl_2$ by passage of 25 ml of 0.3 M HCl-72% ethanol through the resin [17]. The whole process took about 4 hours.

Quality control of the product

Radionuclide Purity

Radionuclide purity of the products were controlled by gamma spectroscopy of the final samples using a HPGe detector coupled with a Canberra™ multi-channel analyzer. The peaks were observed for 3 h in case of ^{57}Co and 1 h for ^{55}Co.

Chemical Purity

Since the production was based on the irradiation of natural nickel over a gold backing, the presence of nickel cation was detected using visible colorimetric assays. The most important photometric reagents for determining nickel are dioximes, which give specific and fairly sensitive methods. Dimethylglyoxime reacts with nickel ions in a neutral or ammoniacal medium to form a pink, flocculent precipitate. Even at 2 mg.kg^{-1} of standard nickel concentration, the colored Ni-dimethylglyoxime complex is visible to naked eye [18]. The amount of gold cation was controlled in the final solution using color formation with acidic rhodamine B reagent based on a previously reported colorimetric method [19].

Preparation of Fresh Cyclic DTPA Dianhydride For Radiolabeling

This compound was prepared according to the methods previously given in the literature with slight modifications [20]. Briefly, DTPA in acidic form (0.1 mol) was heated with a 4-fold molar excess of acetic anhydride (0.4 mol), dissolved in 50 ml of pyridine and heated at 65°C for 24 h. The resulting anhydride was insoluble in pyridine and was collected by filtration, purified by repeated washing with acetic anhydride, and finally with anhydrous ether. Drying in an oven at 50-60°C removed the last traces of solvent. The melting point was 178-180°C. ^{1}H NMR and IR spectra were consistent with the spectra reported in the literature.

Preparation of [^{55or57}Co]-diethylenetriaminepentacetate

[^{55or57}Co]$CoCl_2$ (111 MBq) dissolved in acidic media obtained above (2.5-3 ml) was transferred to a 5 ml-vial and evaporated to dryness using a flow of N_2 gas at 50-60°C. Then, 3M sodium acetate (0.5 ml) was added to the residue to prepare a [^{55or57}Co]cobalt acetate solution. A portion of diethylenetriaminepentacetic acid dianhydride (0.1 mg, 280 nmol) was added to the cobalt acetate solution and stirred at 25°C for 3-5 min. The mixture was then left at room temperature for 30 min. The vial mixture was diluted by the addition of 0.9% saline. pH was adjusted to 5.5-7 and the final solution was then passed through a 0.22 μm filter.

Radiochemical purity of [^{55or57}Co]DTPA

Radio thin layer chromatography was performed on polymer-backed silica gel layer chromatography sheets using anhydrous ethanol as the mobile phase. The step motor was installed to count 0.4 cm-strip for 30 seconds through the slot of a shielded chamber. Thus, the radiochemical yields

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were determined by comparison of uncomplexed ^{55}Co and the major peak (Figs. **1-3**).

Stability of [^{55or57}Co]DTPA in Aqueous Solution

Stability tests were based on previous studies performed for radiolabeled metal complexes [21]. Sample of [^{55or57}Co]DTPA (185 MBq) in the aqueous solution were kept at room temperature for 3 hours while checked by RTLC every half an hour. Micro-samples (5 µl) taken from the shaking mixture, were transferred on the TLC papers and the ratio of free radiocobalt to [^{55}Co]DTPA was checked (eluent: anhydrous ethanol or acetate buffer pH.7).

Stability Studies in Serum

To 36.11 MBq of [^{55or57}Co]DTPA (100 µl), 500 µl of freshly prepared human serum was added separately. The resulting mixture was incubated at 37°C for 5 h, and 1.5-µl aliquots were analyzed by RTLC after 0, 0.25, 0.5, 1, 2 and 15 h of incubation to determine the complex stability, TLC results were not statistically meaningful after 15h due to radioactivity decay.

Determination of Partition coefficient

Partition coefficient (log *P*) of [^{55or57}Co]DTPA was calculated followed by the determination of *P* (*P*= the ratio of specific activities of the organic and aqueous phases). A mixture of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH=7) containing approximately 3.7 MBq of the radiolabeled copper complex at 37°C was vortexed 1 min and left 5 min. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well-type counter. A 500 µl sample of the octanol phase from this experiment was shaken again two to three times with fresh buffer samples to ensure that traces of hydrophilic ^{55}Co impurities did not alter the calculated *P* values. The reported log *P* values are the average of the second and third extractions from three to four independent measurements.

Cell Labeling

In a typical run, healthy male volunteer blood samples (3 ml) were collected in sterile anticoagulant added polymer tubes. The samples centrifuged at 3000 rpm for 5 minutes then the serum was discarded. The cell pellets were reconstituted in 1 ml of PBS followed by the addition of the ^{55}Co-DTPA final solution (3 MBq). The samples were kept at 4, 25 and 37℃ bath up to 3 hours. One -ml samples were taken at various time intervals (30, 60, 120 and 240 min) and centrifuged at the 3000 rpm for 5 min. The cell pellets were carefully washed with PBS and the washing solution was discarded. The activity of the cell pellet and the supernatants were counted in a dose calibrator and the ratio of cell/supernatants were determined (n=5).

RESULTS AND DISCUSSION

Production and Quality Control

Cobalt-57

Cobalt-57 was produced by bombardment of a 65 µm thick natural nickel target using a 150 µA current of 22-10 MeV protons. The production yield was 33 µCi/µAh two weeks after the bombardment. The radiochemical separation process was based on a no-carrier-added method with a yield of 97%. The radionuclide purity was 97.5% two weeks after the bombardment. The rest of activity was attributed to ^{56}Co. In the chemical control process, no nickel ions were detected with a detection limit of 2 p.p.m. The test sample remained similar to the blank after the addition of 1% dimethylglyoxime to the alkaline sample previously treated with ammonia [22]. Gold concentration confirmed to be less than 0.9 mg.kg^{-1}.

Cobalt-55

Cobalt-55 was produced by bombardment of a 30 µm thick natural nickel target using a 150 µA current of 15-8 MeV protons. The production yield was 270.2 µCi/µAh at the end of bombardment. The radiochemical separation process was based on a two step no-carrier-added method with a yield of 95%. The radionuclide purity was more than 99.3%. The rest of activity was attributed to ^{57}Co. In the chemical control process, no nickel ions were detected with a detection limit of 2 p.p.m. Gold concentration confirmed to be less than 0.9 mg.kg^{-1}.

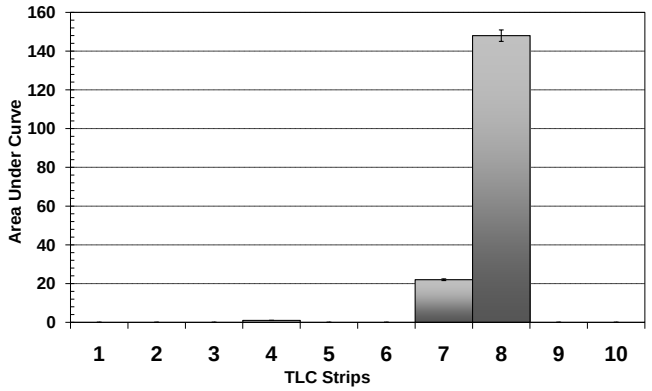

Fig. (1). RTLC of the [^{55}Co]chloride (n=5) eluted by ethanol as the mobile phase (counting were based on area under curve of the 473 keV peak) on Si paper.

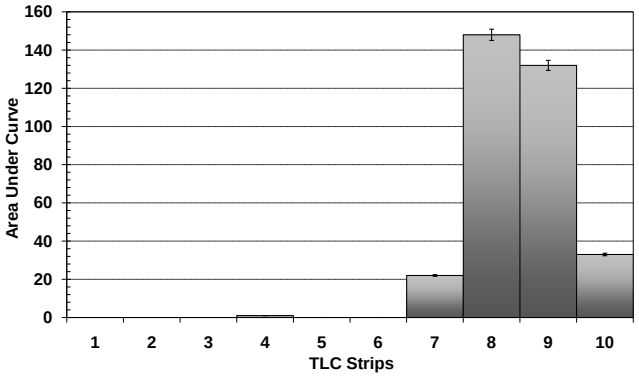

Fig. (2). RTLC of final [^{55}Co]$CoCl_2$ solution using pH.7 acetate buffer pH.7 as the eluent (counting were based on area under curve of the 473 keV peak) on Si paper.

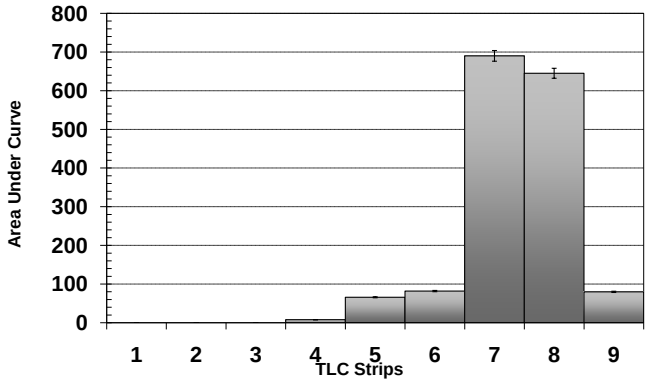

Fig. (3). RTLC of final [^{55}Co]$CoCl_2$ solution in pH.7 normal saline as the eluent (counting were based on area under curve of the 477 keV peak) on SiO_2.

Radiolabeling

In TLC studies, the more polar cobalt DTPA fraction, correspond to smaller R_fs (R_f =0.1-0.2) (Fig. **4**), while the free cobalt migrate at the higher R_f (R_f=0.8) (Fig. **5**).

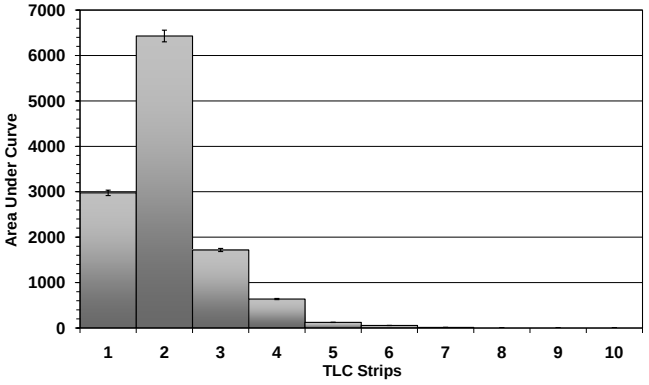

Fig. (4). RTLC of final [^{55}Co]DTPA solution in ethanol as the eluent (counting were based on area under curve of the 477 keV peak).

Since it has been shown that the Co-DTPA and Co-EDTA complexes have still lipophillic properties, their radiolabeled forms can be used either in blood cell labeling if added directly to human blood cell fractions, or (if injected into subarachnoid space) in the imaging of cerebrospinal flow for the detection of tumors and/or pathological abnormalities in CNS.

In all radiolabeling runs (n=9), the area under curve ratio of the radiotracer peak was constant (variation<1%), showing the high radiochemical purity of the labeling method. Since the other labeling methods already reported used acidic media and the final product was finally adjusted to the pH of interest using NaOAc solution, we tried another approach using alkaline media. Interestingly the labeling was fast in this media and no unlabeled cobalt species was found by RTLC, showing the radiochemical purity higher than 99%.

Although cobalt can exist in the +2 and +3 oxidation states, Co(II) is the stable valence state in water under most conditions. Under oxidizing and moderately reducing conditions, the uncomplexed ion Co^{2+} is the dominant cobalt aqueous species at pH values less than 9.5. Complexation of cobalt by organic ligands, such as DTPA, also significantly reduces cobalt adsorption at near neutral and basic pH values. Cobalt(II) is initially complexed to form the anionic complex Co(II)DTPA$^-$ [23]. According to these information a possible tracer structure can be suggested (Fig. **5**).

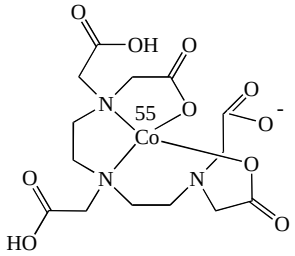

Fig. (5). Possible ^{55}Co(II)DTPA$^-$ complex structure at pH 5.5-7.

The final radiolabeled complex diluted with normal saline was then passed through a 0.22 micron (Cativex) filter (filtration was used to sterilize the product). Due to possible thermal instability at the autoclave temperatures, [^{55}Co]DTPA preparation could be totally degraded giving detectable amounts of the free cobalt ions after autoclaving especially due to oxidation to Co(III). The chemical stability of [^{55}Co]DTPA was high enough to perform further studies. RTLC of the final product showed no change in stability and the pattern for [^{55}Co]DTPA in aqueous solutions at the pH=5.5-7 was not changed during 15 hours at room temperature.

Serum Stability Studies

[^{55}Co]DTPA was incubated in freshly prepared human serum for 15 h at 37°C. The aliquots of the resulting mixtures were analyzed to determine the kinetic stability of the radiolabeled conjugate. No decomposition of ^{55}Co from the complex was observed during the course of the studies, and the radiochemical purity of the complex remained >99% for 15 h under physiological conditions.

Partition Coefficient of the [^{55}Co]DTPA

As expected, the lipophilicity of the [^{55}Co]DTPA compound is rather high. The measured octanol/water partition coefficient, *P*, for the ^{55}Co-complex was found to depend on the pH of the solution. At the pH.7 the logP ≈ 2.

Cell Labeling

Since it has been shown that the Co-DTPA complex has lipophillic properties, it can easily pass through bi-layer phospholipids of cell membrane. In many cases the complexes remain intact in the intracellular space, resulting in easy leakage back to the outer cell space. Degradation of Co-DTPA complex in the intracellular space is possible due to pH changes as well as reaction with cytoplasmic thiol-containing molecules. This can be an advantage of many radiometal labeled DTPA complexes as cell labeling agent including Co-DTPA, since the tracer is trapped in the cytoplasmic space. In any case of free or thiol-comlexed forms of cobalt the leakage to outer space has not been reported. Thus, ^{55}Co-DTPA can be used either in blood cell labeling if

added directly to human blood cell fractions, or in the imaging of cerebrospinal flow for the detection of tumors and/or pathological abnormalities in CNS (if injected into subarachnoid space). Three various temperatures were chosen for the cell labeling. In our experiences, room temperature and 4℃ were suitable since the labeling capacity (cell pellet count: supernatant count ratio) in the course of time was not linear and/or the data were not reproducible. At 37℃ reproducible and linear data obtained with the course of time. This might be explained by the fact that at this temperature natural existing enzymes or mechanism in cells are working properly so that the trapping of the tracer in sell occurs more efficiently. While at 25℃ for instance, the leak of the tracer 2 hours after labeling was observed (data not shown). The linearity of the plot also shows that the penetration of the tracer into the cells is performed by simple diffusion mechanism and no active mechanism involved.

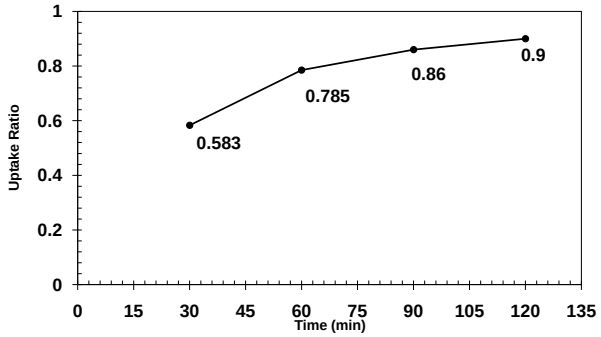

Fig. (6). The rate of red blood cell labeling using [^{55}Co]DTPA at 37℃; n=5.

DISCUSSION

The method used in this research for the production and chemical separation of ^{55}Co was quite simple and cost effective. Total labeling and formation of [^{55}Co]DTPA took about 40 minutes, with a yield higher than 99%. A significant specific activity (about 9.1 TBq/mmol) was obtained *via* insertion of [^{55}Co]cobalt(II) cation. No unlabelled and/or labeled by-products were observed upon RTLC analysis of the final preparations. The radiolabeled complex was stable in aqueous solutions as well as in human serum at 37°C for at least 15 hours and no significant amount of other radioactive species were detected by RTLC. No traceable amounts of other [^{55}Co]cobalt species were detected by RTLC which showed that radiochemical purity of the [^{55}Co]DTPA was higher than 99%. In contrast to other labeled DTPA complexes, [^{55}Co]DTPA can be a PET radiotracer with an intermediate half life. The high chemical stability of this radiopharmaceutical makes it a possible candidate for cisternographic and other diagnostic applications.

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