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ORIGINAL PAPER

Development of [^{66}Ga]oxine complex; a possible PET tracer

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Abstract The aim of this work is development of a possible blood cell labeling agent for ultimate use in PET. Gallium-66 ($T_{1/2}$ = 9.49 h) is an interesting radionuclide that has a potential for positron emission tomography (PET) imaging of biological processes with intermediate to slow target tissue uptake. Oxine has been labeled with this radionuclide in the form of [^{66}Ga]gallium chloride for its possible diagnostic properties. In this study, ^{66}Ga was produced at a 30 MeV cyclotron (IBA-Cyclone 30) via the ^{66}Zn(p,n)^{66}Ga reaction. The production yield was 445.5 MBq/μAh. The [^{66}Ga]oxine complex was obtained at pH = 5 in phosphate buffer medium at 37°C in 10 min. Radio-TLC showed a radiochemical purity of more than 98 ± 2%. The chemical stability of the complex was checked *in vitro* with a specific activity of 1113 GBq/mmol. The serum stability and log *P* of the complex were calculated. The produced [^{66}Ga]oxine can be used for diagnostic studies, due to its desirable physico-chemical properties both *in vitro* and *in vivo* according to internationally accepted limits.

Key words gallium-66 • oxine • positron emission tomography • blood cell labeling • stability

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Introduction

The positron-emitting Ga(III) radionuclides, ^{66}Ga and ^{68}Ga, have been proposed for applications in PET [2, 9, 13]. ^{66}Ga ($T_{1/2}$ = 9.49 h, E_γ: 833.5, 1039.3 keV; β^+: 56.5%, $E_{max}\beta^+$: 4.153 MeV; EC: 43.5%) [4, 8] is an intermediate-lived radionuclide that is potentially suitable for positron emission tomography imaging of biological processes with intermediate to slow target tissue uptake [7, 13]. Various nuclear reactions have been used for the production of this PET radionuclide such as ^{63}Cu(^{4}He,n)^{66}Ga and ^{66}Zn(p,n)^{66}Ga [6, 18]. Although there are some better physical properties for ^{68}Ga over ^{66}Ga for imaging studies (Table 1) like a higher ^{66}Ga positron energy and a lower positron decay, unavailability of Ga target systems for ^{68}Ge productions to prepare the ^{68}Ge/^{68}Ga generator [14] as well as our limitations in buying the generator from external vendors, made us to produce ^{66}Ga for our preliminary studies. Due to the availability of ^{66}Zn in our Center prepared by zinc isotopes separation for our routine ^{67}Ga-citrate productions [16], ^{66}Zn(p,n)^{66}Ga was chosen as the best route of production of this nuclide. ^{66}Ga has been used as a suitable nuclide for radiolabeling of monoclonal antibodies in the detection and staging of tumors and other lesions after dosimetric studies [5, 19], as well as the radiolabeling of red blood cells [3]. ^{68}Ga-labeled oxine has been used in RBC labeling since 1977 [20]. Many blood cell labeling studies have been performed using radiogallium-oxine starting from Ga-citrate at various temperatures or for the labeling of micro-

Table 1. Physical properties of two PET gallium radioisotopes

Properties	^{68}Ga	^{66}Ga
Gamma energies [keV]	511(β^+)	511(β^+) 834 1039 2752
Positron energy	1900(β^+)	4153(β^+)
Mode of decay	10% EC to ^{68}Zn 90% β^+	43% EC to ^{66}Zn 57% β^+
Half-life	68 min	9.5 h
Route of production	^{68}Ge daughter, ^{66}Zn(α,2n)^{68}Ge	^{66}Zn(p,n)^{66}Ga
Possible impurity	^{68}Ge	^{65}Zn
Proton energy [MeV]	12–22	6–15

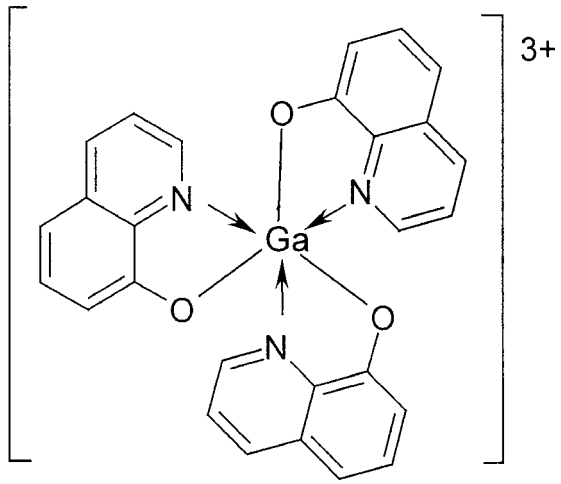

Fig. 1. *Tris*(8-quinolinolato)Ga(III) (Ga-oxine complex).

organisms [10–12]. Some researchers have shown that *tris*(8-quinolinolato)Ga(III) complex (Ga-oxine) has suppressive effects on the viability of A549 human malignant lung adenocarcinoma cells [1]. In continuation of our recent studies on the preparation and application of gallium-66 labeled compounds [9], we decided to produce ^{66}Ga using an appropriate method and to investigate the possibility of incorporating this positron emitter nuclide with a cell labeling agent – oxine – for use in blood cell diagnostic studies. Due to the interesting properties and increasing importance of positron emission tomography, we optimized ^{66}Ga complex formation conditions with oxine, in order to develop [^{66}Ga]oxine. We hereby report the preparation, optimization, stability, formulation studies of [^{66}Ga]oxine complex (Fig. 1).

Experimental

Materials

Chemicals were purchased from Aldrich Chemical Company (Germany). Thin-layer chromatography (TLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Methanol and normal saline used for labeling were of high purity. A mixture of ammonium acetate and 10% methanol (1:1, v/v) was used as eluent. Radiochromatography was performed by counting different 5 mm slices of polymer-backed silica gel paper using a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL). All calculations and TLC counting were based on the 1039.3 keV peak.

Methods

Preparation of [^{66}Ga]gallium chloride from enriched zinc-66 solid target

[^{66}Ga]gallium chloride was prepared by 15 MeV proton bombardment of an electroplated enriched 0.04 (g/cm^2) ^{66}Zn-target at an angle of 6 degrees in a 30 MeV cyclotron (IBA-Cyclone 30). The target was bombarded with a current intensity of 180 μA for 67 min (200 μAh). The resultant activity of ^{66}Ga was 89.17 GBq (EOB) and the production yield was 445.5 MBq/μAh. Radiochemical separation was based on a no-carrier-added method described previously, with slight modifications [17, 18, 21]. After dissolution of the irradiated target in 10 M HCl (15 ml), the solution was passed through a cation exchange resin (BioRad AG 50W, 200–400 mesh, H^+ form) which had been pre-conditioned by passing 25 ml of 9 M HCl. The column was then washed with 25 ml of 9 M HCl to remove copper and zinc ion contents. Finally, ^{66}Ga cation was washed out by 20 ml of 4 M HCl. Then, 10 M HCl (20 ml) was added to the 20 ml of 4 M eluent in order to obtain a 7 M mixture to extract ^{66}Ga ions. Diisopropyl ether was used to extract ^{66}Ga from the aqueous phase (2 times). The mixed organic layers were back-extracted using 12.5 ml of 0.05 M HCl. The resulting high-purity ^{66}Ga chloride solution with 7.13 GBq/ml activity concentration was used for labeling after quality control.

Labeling of oxine with [^{66}Ga]gallium chloride

[^{66}Ga]gallium chloride (9.25–92.5 MBq) dissolved in acidic media obtained as mentioned above was transferred into a 2 ml-vial. The mixture was evaporated by slight warming under a nitrogen flow followed by reconstitution with phosphate buffer solution (pH = 5, 0.4 ml). A volume of ethanolic oxine solution (300 μl, 0.14 mg/ml) was then added to the residue and kept at different temperatures (25°C, 50°C, 80°C and 100°C) and was cooled in an ice bath and rapidly sent for use. The active solution was checked for radiochemical purity by polymer-backed silica gel layer chromato-

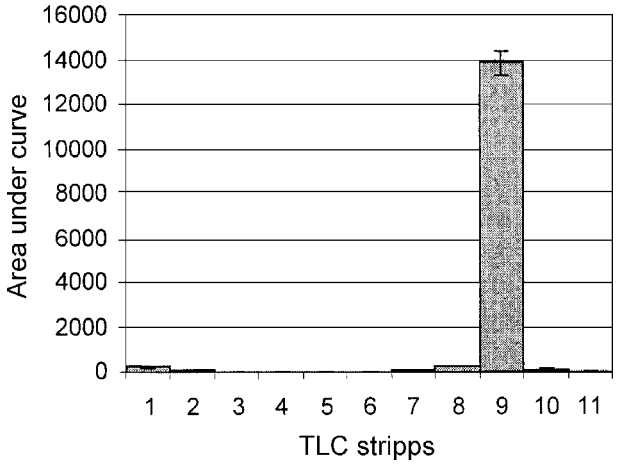

Fig. 2. Radio thin-layer chromatogram of a [^{66}Ga]oxine sample at pH.5 at room temperature after 10 min, n = 5, SE < 3%.

graphy using a mixture of 10% ammonium acetate and methanol (1:1, v/v) as the mobile phase. Radio thin-layer chromatography showed a major and distinct radio peak at the R_f of 0.8. The radiochemical yields (> 97 ± 2% in each case) were also determined by the RTLC method (Fig. 2). These analyses were carried out every one hour after the labeling step up to 9 h. The final solution was then passed through a 0.22 μm filter and pH was adjusted again between 5–7, by addition of sodium acetate (1 M) solution.

Quality control of the final product

1. Radionuclide purity control. Gamma-ray spectroscopy of the final sample was carried out by an HPGe detector coupled with a Canberra™ multi-channel analyzer, to control the radionuclide purity of the product. The counting time was 1000 s.
2. Chemical purity control. The [^{66}Ga]$GaCl_3$ solution was controlled for the presence of ions before labeling. The presence of zinc and copper cations were checked by visible colorimetric assays. Even at 5 ppm of standard zinc concentration, the pinkish complex is visible to the naked eye, while the test sample remains similar to the blank [15]. The serial diluted copper standard solutions were checked up to our polarography apparatus limit of detection. For colorimetric assay, our limit of detection was 0.5 ppm. Standard copper concentrations were complexed by dithizone forming a pinkish complex [15].
3. Radiochemical purity control. The RTLC was performed using polymer-backed silica gel layer chromatography using 10% ammonium acetate:methanol (1:1, v/v) as the mobile phase. After developing the TLC, it was cut into 0.5 cm pieces rapidly before drying and TLC pieces were counted in an activity meter.

Stability of [^{66}Ga]oxine complex in the final product

A sample of [^{66}Ga]oxine (18.5 MBq) was kept at room temperature for 24 h while checked by RTLC at various time intervals (2, 12 and 24 h). A micropipette sample (5 μl) was taken from the shaken mixture and the ratio of free radiogallium to [^{66}Ga]oxine was checked by radio thin-layer chromatography (eluent: 10% NH_4OAc solution and methanol 1:1, v/v).

Stability studies in serum

To 36.11 MBq of [^{66}Ga]oxine (100 μl) 500 μl of freshly prepared human serum was added. 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 3 h of incubation to determine the complex stability.

Determination of partition coefficient

Partition coefficient of [^{66}Ga]oxine was measured as the ratio of specific activities of the organic and aqueous phases, following 1 min vigorous vortex mixing of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH = 7) with approximately 3.7 MBq of the radiolabeled gallium complex at 37°C. Following centrifugation at >2500 rpm 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 ^{66}Ga 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.

Results

Due to positron-emitting property of ^{66}Ga and its selective physical properties, the strategy of incorporating such a radionuclide with the famous cell membrane-penetrating agent – oxine – into one moiety was of great interest. A solvent extraction route led to the production of high-purity gallium-66. Results of radionuclide purity control is shown in Fig. 3.

The formation of colored dithizone metal complexes demonstrated that the zinc and copper cation concentrations were far below the internationally accepted limits (less than 1.5 ppm of zinc and 0.75 ppm of copper

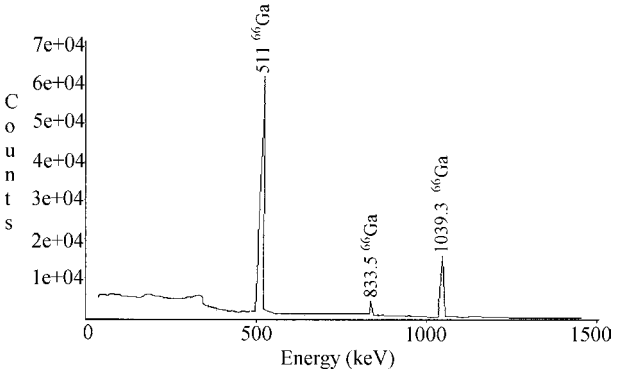

Fig. 3. Gamma spectrum of final [^{66}Ga]$GaCl_3$ solution used in the labeling step, 1 ml, pH = 3.

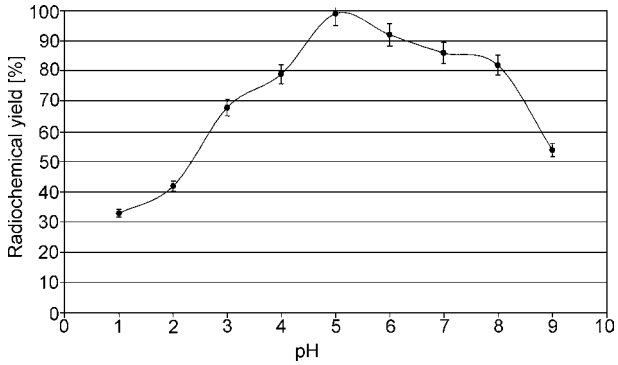

Fig. 4. Effect of pH on radiochemical yield of [^{66}Ga]oxine at 25°C, $n = 5$, SE < 3%.

cations in our assay compared to the USP limits: 5 ppm for each).

Radiochromatography showed a major and distinct radio peak at the R_f of 0.8. Uncomplexed ^{66}Ga stayed at the origin ($R_f = 0.0$).

Labeling

The more polar radioactive fraction ($R_f = 0.0$) correlates with free gallium while the other less polar fraction [^{66}Ga]oxine comes at higher R_f ($R_f = 0.8$). In all radiolabeling procedures ($n = 5$), the area under curve ratio of the two peaks (97:3) did not change.

In order to obtain the best labeling reaction conditions, the complex formation was optimized for pH, temperature, time, and amount of oxine. The radiochemical yield decreased due to the formation of *N*-protonated oxine molecule (Fig. 4).

In basic conditions, the radiochemical yield decreased drastically due to hydrolysis of the Ga cation in basic conditions. At random temperature (room temperature for instance), the best pH for labeling step was 5, while the yield decreased at lower pHs.

At optimum reaction temperature and pH, the yield reached a maximum within 10 min, and stayed constant for longer reaction times. Increasing the ratio of oxine to radioactivity increased the labeling yield, presumably due to more available chelate in solution up to $2.5–3 \times 10^{-4}$ mmoles (Fig. 5).

Heating the reaction mixture to 50°C did not increase the yield which remained constant. Further heating reduced the radiochemical yield due to the decomposition of oxine and/or the product.

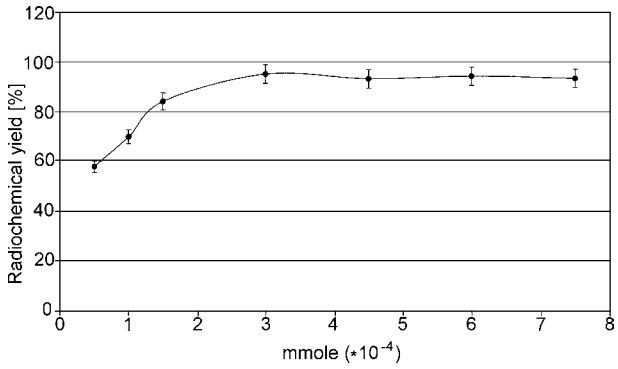

Fig. 5. Effect of the amount of oxine, used in the reaction, on radiochemical yield of [^{66}Ga]oxine at 25°C, $n = 5$, SE < 3%.

The stability of the complex was checked every hour after labeling up to 24 h in order to check the stability of the final labeled preparations due to time needed in order to do blood cell labeling which can take up to hours. In this way, each batch can be used in several blood cell samples.

The thermal stability of [^{66}Ga]oxine was so excellent, that autoclaving a [^{66}Ga]oxine preparation showed no change in the amount of free gallium present. The presence of 3 ± 1% free gallium on the RTLC before and after autoclaving indicated that the final product might be sterilized by this technique.

Stability in the final solution

The chemical stability of [^{66}Ga]oxine was high enough to perform scanning due to the high stability of the final product. Therefore, RTLC showed no change in the amount of free gallium up to 24 h. The presence of 3 ± 1% free gallium on the RTLC remained unchanged even after 24 h. The patterns for [^{66}Ga]$GaCl_3$ and [^{66}Ga]oxine did not change during 24 h (Fig. 6).

Serum stability studies

[^{66}Ga]oxine was incubated in freshly prepared human serum for 3 h at 37°C. The aliquots of the resulting mixtures were analyzed to determine kinetic stability of the radiolabeled conjugate. No loss of ^{66}Ga from the complex was observed during the course of the studies, and the radiochemical purity of the complex remained > 98 ± 2% for 3 h under physiological conditions.

Partition coefficient of the [^{66}Ga]oxine

As expected, the lipophilicity of the [^{66}Ga]oxine compound was rather high. The measured octanol/

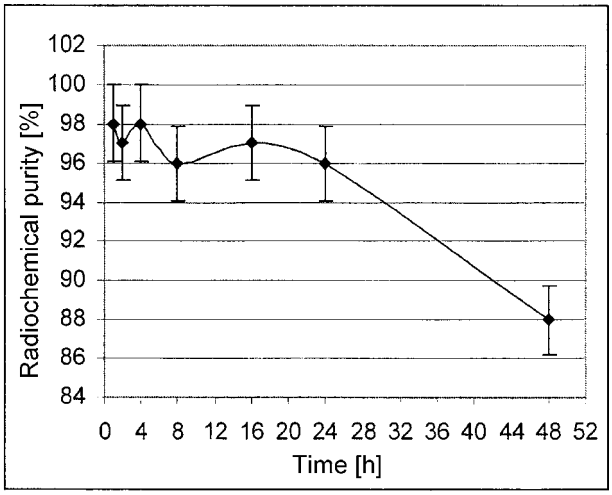

Fig. 6. Effect of time on the radiochemical purity of [^{66}Ga]oxine complex at 25°C in the final preparations, $n = 5$, SE < 3%.

water partition coefficient, *P*, for the [^{66}Ga]complex was found to depend somewhat on the pH of the solution. Log *P* was higer than 2 at pH = 7.

Discussion

In this report, [^{66}Ga]oxine chemical stability was studied by chromatographic methods for longer times after labeling. Based on recent reports on possible therapeutic application of ^{66}Ga radionuclide due to the emission of high energy positrons, having a more stable, rather long half-life labeled tracer was interesting. The stability of this radiopharmaceutical allows for the preparation of labeled compound in the laboratory, followed by sending the ready-to-use batches to other research centers and/or clinics.

Total labeling and formulation of [^{66}Ga]oxine took about 15 min, with a yield of 97 ± 2%. A suitable specific activity product was formed via insertion of [^{66}Ga]gallium cation. No unlabeled and/or labeled by-products were observed upon TLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solutions at least for 24 h. No significant amount of other radioactive species was detected by RTLC 24 h after labeling. Trace amounts of [^{66}Ga]gallium chloride (≈ 3 ± 2%) were detected by paper chromatography. HPLC and TLC showed that radiochemical purity of the ^{66}Ga-labeled components was higher than 95% with a specific activity of 33.152 GBq/ml. The high chemical stability of this radiopharmaceutical makes it a very suitable candidate for diagnostic applications.

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