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[^{66}Ga]Oxine complex; preparation and stability as a possible PET radiopharmaceutical

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ABSTRACT

Background: Gallium-66 ($T_{1/2}$= 9.49 h) is an interesting radionuclide that has potential for positron emission tomography (PET) imaging of biological processes in intermediate to slow target tissue uptake. Oxine has been labeled with this radioisotope in the form of [^{66}Ga]gallium chloride for its possible diagnostic properties.
Materials and methods: ^{66}Ga was produced in the 30 MeV cyclotron (Cyclon-30, IBA) via the ^{66}Zn(p,n)^{66}Ga reaction.TLC was performed on polymer backed silica gel. A Gamma spectrometer HPGe detector was used for countings.
Results: After the bombardment, the production yield was 11.2 mCi/μAh. The [^{66}Ga]Oxine complex was obtained at the pH=5 in phosphate buffer medium at 37°C in 10 minutes. Radio-TLC showed an overall radiochemical yield of 97% (radiochemical purity > 98%). The chemical stability of the complex was checked *in vitro* with a specific activity of 896 mCi/ml.
Conclusion: [^{66}Ga]oxine can be used in diagnostic studies due to its suitable physico-chemical properties both *in vitro* and *in vivo*. *Iran. J. Radiat. Res., 2003; 1(3): 157 - 161*

Keywords: *Gallium-66, oxine, positron emission tomography (PET), blood cell labeling, stability.*

INTRODUCTION

The positron-emitting Ga(III) radioisotopes, $^{66}Ga^{3+}$ and $^{68}Ga^{3+}$, have been proposed for applications in positron emission tomography imaging (PET) (Loe'h *et al.* 1980, Jurisson *et al.* 1993, Daube-whiterspoon *et al.* 1997).^{66}Ga ($T_{1/2}$ = 9.49 h, E_γ: 833, 1039.5 keV; β^+: 56.5%, $E_{max}\beta^+$: 4.2 MeV; E.C: 43.5%) (Graham *et al.* 1997), is an intermediate-lived radionuclide that has potential for positron emission tomography imaging of biological processes in intermediate to slow target tissue uptake (Lewis *et al.* 2002, Goethals *et al.* 1991). 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 (Goethals *et al.* 1988, Szelecsenyi *et al.* 1991).

^{66}Ga has been used as a suitable isotope in the radiolabeling of monoclonal antibodies in the detection and staging of tumors and other lesions after dosimetric studies (Goethals *et al.* 1990), as well as the radiolabeling of blood cells (Ellis and Sharma 1999). ^{68}Ga-labeled oxine has been used in RBC labeling since 1977 (Welch *et al.* 1997). Many blood cell labeling studies have been performed using radiogallium-oxine starting from Ga-citrate at various temperatures or for the labeling of microorganisms. Some researchers have shown that *tris*(8-quinolinolato)Ga (III) complex (Ga-oxine) (figure 1) has suppressive effects on the viability of A549 human malignant lung adenocarcinoma cells (Collery *et al.* 2000).

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In continuation of our recent studies on the preparation and application of gallium radioisotopes (Tabeie *et al.* 2002), we decided to prepare ^{66}Ga, using an appropriate method and to investigate the possibility of incorporating this positron emitter isotope with a cell labeling agent, oxine, to 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 and formulation studies of [^{66}Ga]oxine complex.

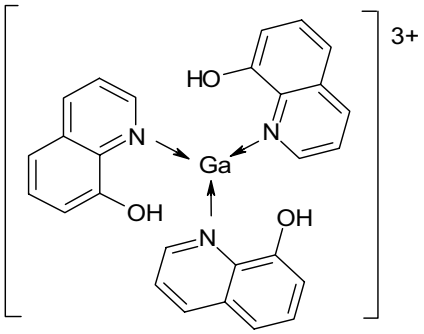

Figure 1. tris(8-quinolinolato)Ga (III) (Ga-oxine complex)

MATERIALS AND METHODS

Chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI (USA). 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) was used as an eluent. Radio-chromatography 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 1039.5 keV peak.

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 enriched electroplated 0.04 (g/cm^2) ^{66}Zn-target at the angle of 6 degrees in a 30 MeV cyclotron (Cyclone-30, IBA) based on a no-carrier-added method described previously with slight modifications (Zweit *et al.* 1998). The target was bombarded with a current intensity of 180 μA for 67 min (200 μAh). The resultant activity of ^{66}Ga was 2.23 Ci (E.O.B.) and the production yield was 11.2 mCi/μAh. After dissolution of the irradiated target by 10 N HCl (15ml, H_2O_2 added), the solution was passed through a cation exchange resin (Dowex 50 W×8, H^+ form) which had been pre-conditioned by passing 25 ml of 9 N HCl. The column was then washed by 25 ml of 9N HCl to remove copper and zinc ion contents. Finally ^{66}Ga cations were washed out by 20 ml of 4 N HCl. The 10 N HCl (20 ml) was added to the 4N (40 ml) eluent in order to obtain the optimum normality to extract ^{66}Ga ions. Disopropyl 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 0.05N HCl. The resulting high-purity ^{66}Ga chloride solution with specific activity of 896 mCi/ml was used directly for labeling. The gamma spectroscopy showed the presence of ^{66}Ga photo-peaks (figure 2).

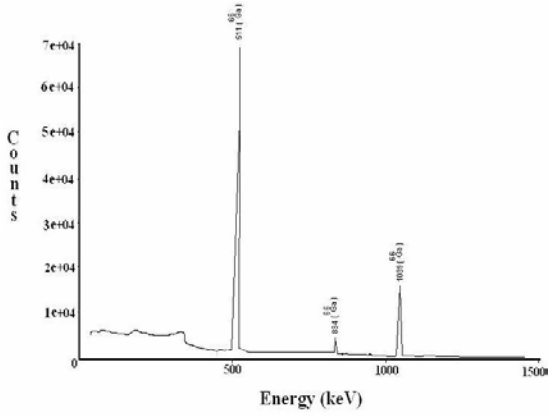

Figure 2. Gamma spectroscopy scheme of final [^{66}Ga]$GaCl_3$ solution used in the labeling step, 1ml, pH=3

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

[^{66}Ga]Gallium chloride (0.25-2.5 mCi) dissolved in acidic media obtained above (0.5-2 ml) was transferred to 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, 50, 80 and 100 °C); then it 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 chromatography using a mixture of ammonium acetate 10% and methanol 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% in each case) were also determined by RTLC method (figure 3). These analyses were carried out every one-hour after the labeling step. The final solution was then passed through a 0.22 µ filter and pH was adjusted again between 5-7 by the addition of sodium acetate (1M) buffer. The gamma spectroscopy of the final sample was carried out by a HPGe detector and showed a radio-nuclide purity higher than 97%. Pyrogen test was performed using a commercial LAL kit. Microbial-fungal tests showed a suitable pharmaceutical sterility .

Quality control of the final product

Radionuclide purity: The gamma spectroscopy of the final sample was carried out by a HPGe detector and showed a radio-nuclide purity higher than 97% showing the presence of 511, 834 and 1039 keV gamma energies, all of which are resulted from ^{66}Ga.

Chemical purity: Due to the chemical procedure used in the production of ^{66}Ga, the presence of copper ions used in the support backing of the solid target and zinc ions as target material had to be checked carefully in the final product by polarography or high resolution colorimetric assays.

Radiochemical purity: Radio thin layer chromatography showed one major and distinct radio peak at the R_f of 0.80. The radiochemical yields (>97% in each case) were also determined by RTLC method. These analyses were carried out every 30 minutes after the labeling step.

Formulation: Pyrogen test was performed using a commercial LAL kit. Microbial-fungal tests showed a suitable pharmaceutical sterility.

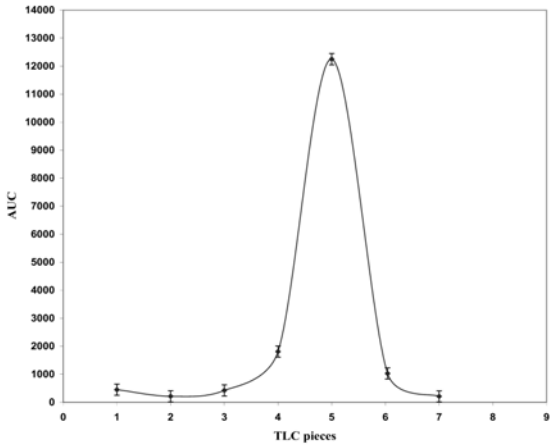

Figure 3. Radio-thin layer chromatogram of a [^{66}Ga] oxine sample at optimized conditions, n=5, SE<3%

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

A sample of [^{66}Ga] oxine (0.5 mCi) was kept at room temperature for 48 hours while being checked by RTLC at various time intervals (2, 12 and 24 hours). A micropipette sample (50 µl) was taken from the shaking mixture and the ratio of free radiogallium to [^{66}Ga] oxine was controlled by radio thin layer chromatography (eluent: 10% NH_4Oac buffer and methanol 1:1). The patterns for [^{66}Ga]$GaCl_3$ and [^{66}Ga]oxine did not change during 24 hours.

RESULTS AND DISCUSSION

Due to positron-emitting property of ^{66}Ga and the selective physical properties of this radioisotope, the strategy of incorporating such an isotope and the famous cell membrane-penetrating agent, oxine, into a moiety was of great interest.

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Labeling

Radioactive with more polar fraction (R_f =0.0) correlates to free gallium while less polar fraction [^{66}Ga] oxine was produced at a higher R_f (R_f =0.7). In all radiolabeling procedures (n=5), the area under ratio curve of the two peaks did not change (97:3).

In order to obtain the best labeling reaction conditions, the complex formation was optimized for pH, temperature, time, and the amount of oxine (less than 4). The radiochemical yield was decreasing due to the formation of N-protonated oxine molecule (figure 4). At basic conditions the radiochemical yield decreased drastically due to the degradation of oxine to less soluble compounds. At a random temperature (room temperature for instance), the best pH for the labeling step was 5, while the yield decreased at lower pHs.

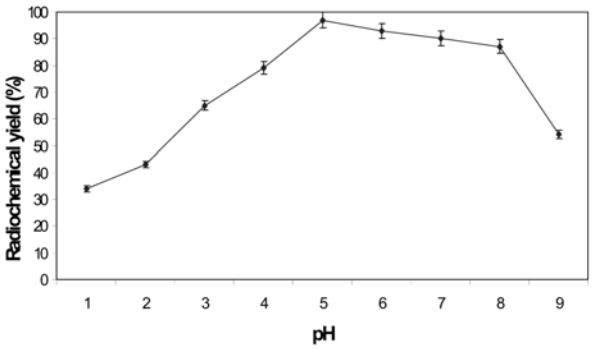

Figure 4. Effect of pH on radiochemical yield of [^{66}Ga]oxine at 25ºC, n=5, SE<3%

At the optimum reaction temperature and pH, the yield reached a maximum within 10 minutes, 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 - 3×10^{-4} mmoles (figure 5).

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

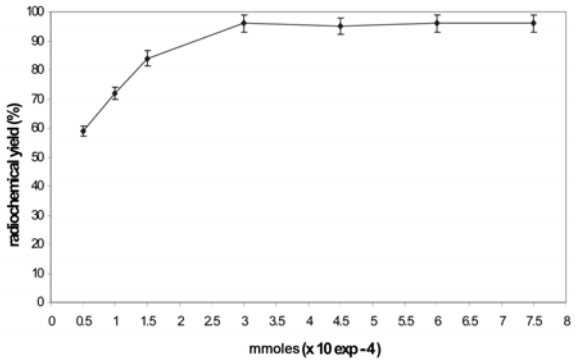

Figure 5. Effect of the amount of oxine used in the reaction on radiochemical yield of [^{66}Ga]oxine at 25ºC, n=5, SE<3%

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 3-5% free gallium on the RTLC before and after autoclaving indicated that the final product might be sterilized by this technique.

The chemical stability of [^{66}Ga] oxine was high enough to perform scanning due to the high stability of the final product in the presence of human blood serum , therefore RTLC showed no change in the amount of free gallium up to 6 hours. The presence of 3-5 % free gallium on the RTLC remained unchanged even after 6 hours.

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 activity of ^{66}Ga radioisotope due to the emission of high energy positrons (Graham *et al.* 1997), having a more stable, rather long half life labeled tracer was interesting. The stability of this radiopharmaceutical allows the preparation of the labeled compound in the laboratory, sending the ready-to-use batches to other research centers and/or clinics.

CONCLUSION

Total labeling and formulation of [^{66}Ga] oxine took about 15 minutes with a yield of 97%. A suitable specific active product was formed *via* insertion of [^{66}Ga] gallium cation. No

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unlabelled and/or labeled by-products were observed upon TLC or HPLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solutions at least for 24 hours. No significant amount of other radioactive species was detected by HPLC 24 hours after labeling. Trace amounts of [^{66}Ga] gallium chloride (≈3%) 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 896 mCi/ml.

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