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Microwave-Assisted Rapid Synthesis of ^{166}Ho-1,4,7,10-Tetraazacyclododecane-*N,N′,N″,N‴*-Tetraacetic Acid: A Bifunctional Chelating Agent for Radiopharmaceuticals

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A rapid procedure for the preparation of ^{166}Ho-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA), a bifunctional chelating agent for therapeutic radiopharmaceuticals, was established. The [^{166}Ho(DOTA)]$^-$ chelate was prepared in very short reaction times (0.5–1 min) with good yields (99%) under microwave irradiation (300 W), while it was prepared at elevated temperatures over 3 h in the conventional method. The microwave irradiation offers a rapid and efficient methodology for the formation of the ^{166}Ho-complex.

Key Words: [^{166}Ho(DOTA)]$^-$ chelate, Bifunctional chelating agent.

Non carrier-free ^{166}Ho is currently obtained by the neutron irradiation of ^{165}Ho [^{165}Ho(n, γ)^{166}Ho] and the carrier-free ^{166}Ho is currently obtained by the separation from neutron irradiated $^{164}Dy_2O_3$ target from a nuclear reactor[1]. Due to its excellent physical properties such as 26.8 h half life and decays with the emission of high-energy β particles with energies of 1.78 MeV (49%) and 1.86 MeV (51%) corresponding to a maximum soft tissue penetration of 8.5 mm and with the emission of one gamma photon with an energy of 80.6 keV suitable for gamma imaging, ^{166}Ho has received much scientific attention for its therapeutic applications[2–5].

1,4,7,10-Tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA, Fig. 1) and its derivatives[6–10] have been widely applied in the field of radioisotope-labelled biologically active molecules for pharmaceutical purposes. These bifunc-

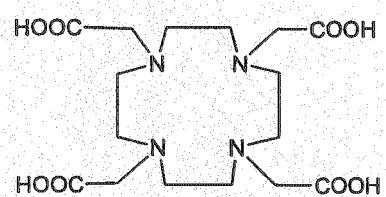


Fig. 1. 1,4,7,10-Tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid

tional chelating agents (BFCs) enable the indirect labelling of the biomolecules. In addition, they afford their complexes with radioactive metals for applications in diagnostic (^{64}Cu, ^{111}In, ^{67}Ga)[11] and therapeutic (^{90}Y, $^{186/188}$Re)[12] medicine and with paramagnetic ions for magnetic resonance imaging (Gd^{3+})[13]. Further, it is noted that tetraazamacrocycles have most recently been used in applications as anti-tumour and anti-HIV agents[14, 15].

Labelling of monoclonal antibodies (mAbs) with radioactive metals for cancer diagnosis and therapy has usually been accomplished by using bifunctional chelating agents[15], which contain both a reactive functional group for covalent attachment to proteins and a strong metal-binding group. One class of the chelating agents that has shown great promise for monoclonal antibody applications is the polyazamacrocyclic polycarboxylate ligands[13, 14], one of which compounds, DOTA, has gained widespread interest because of its ability of forming extremely stable complexes with a wide variety of metals[6, 9, 10]. However, the radiolabelling of DOTA-conjugated mAbs has drawbacks such as the slow rate of formation of metal ion-DOTA complexes and the presence of trace metals which can compete effectively for the ligand. The radiolabelling procedures which have been established for mAbs conjugated to other chelating agents results often in low radiochemical yields when applied to mAb-DOTA conjugates[16]. In order to utilize carrier-free radiometal solutions and minimize radiation damage to the protein, the formation of the radiolabelling should occur rapidly and efficiently[16, 17].

Herein a rapid and facile labelling method for the preparation of high specific activity [^{166}Ho(DOTA)]$^-$ chelate under microwave irradiation (MWI) is reported, which would be suitable for radiolabelling of biologically active molecules such as peptides and proteins for the preparation of therapeutic radiopharmaceuticals. The very rapid rise of temperature of reactants *via* microwave irradiation favours some reaction pathways over others and thus leads to selectivity and hence cleaner products[18].

EXPERIMENTAL

^{166}Ho was produced at the Hanaro research reactor (30 MW) installed at the Korea Atomic Energy Research Institute by the neutron irradiation of a double capsulated $^{165}Ho(NO_3)_3$ target for 72 h at a neutron flux (max. 5×10^{14} n/cm^2 s)-(period). And it was cooled for 24 h and dissolved in 3 mL of HCl solution (pH 3). The radionuclidic purity of ^{166}Ho was estimated by gamma-ray spectrometry (GEM-10175 HPGe Detector coupled to multi-channel analyzer, Ortec, Oak Ridge, TN, USA). A typical gamma-ray spectrum obtained from the HPGe detector is shown in Fig. 2. Photopeaks due to gamma-rays of ^{166}Ho were observed, and the 80.6 and 1379.4 keV photopeaks were used for the assay[2].

The initial specific activity of ^{166}Ho was 11.7 GBq/mg. The radioactivity was measured by using an ionizing chamber (Capintec 15R, Biodex Atomlab 200, NY, USA) by setting the calibration value for ^{166}Ho that was corrected and calibrated by the manufacturer.

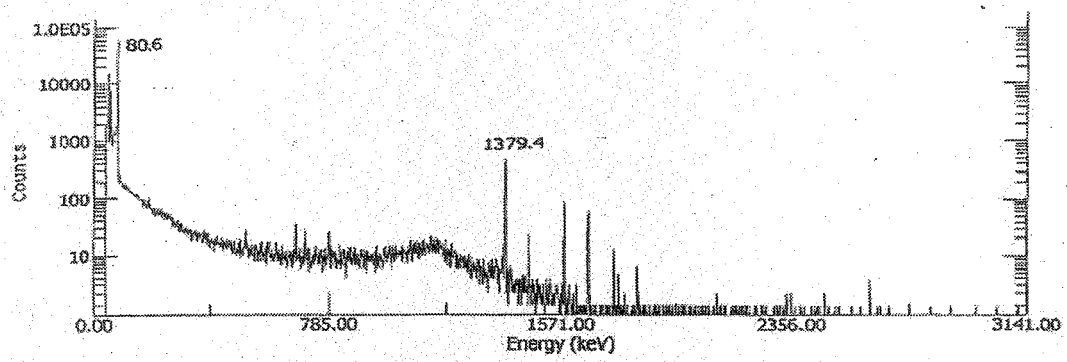
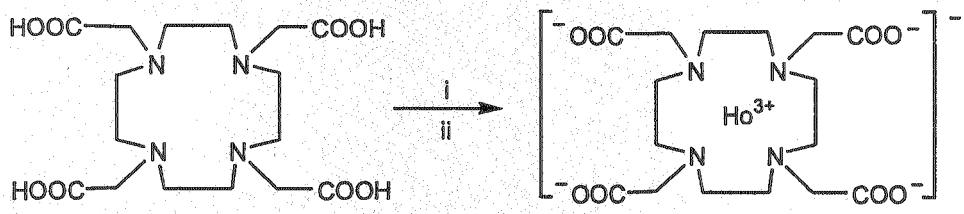


Fig. 2. Gamma-ray spectrum of ^{166}Ho

DOTA was purchased from Fluka Chemie GmbH (Buchs, Switzerland) or prepared by the previously reported procedures[8, 9, 10, 13]. Radiolabelling the DOTA with ^{166}Ho, the formation of [^{166}Ho(DOTA)]- chelate, was achieved by the new method as follows: A solution of 0.1 mL of $^{166}Ho(NO_3)_3$ (370 MBq) and 0.4 mg of DOTA in 0.9 mL of physiological saline was added into a 50 mL glass vessel. The reaction vessel was capped with a TFM teflon cover and placed in a rotor in a microwave reactor. After irradiating the solution for 0.5–1 min at the power of 300 W, it was cooled to room temperature. And it was filtered by a membrane filter (0.22 μm) **(Scheme-1).**



Scheme-1. Microwave assisted synthesis of [^{166}Ho(DOTA)]$^-$ chelate. Reagent and condition: (i) $^{166}Ho(NO_3)_3$, MWI, 0.5–1 min, 130 W; (ii) $^{166}Ho(NO_3)_3$, 80°C, 3 h.

Conventional radiolabelling of the DOTA with ^{166}Ho was achieved according to the reported methods[7, 8, 17]. A solution of 0.1 mL of $^{166}Ho(NO_3)_3$ (370 MBq) and 0.4 mg of DOTA in 0.9 mL of physiological saline was added into a 50 mL three-necked round bottom flask equipped with a condenser. The reaction was heated under reflux condition for 3 h. The progress of the reaction was monitored. It was cooled to room temperature and it was filtered by a membrane filter (0.22 μm) **(Scheme-1)**.

RESULTS AND DISCUSSION

The assay for the formation and structure of the [^{166}Ho(DOTA)]$^-$ chelate and $^{166}Ho(NO_3)_3$ can be achieved by investigating their positions using an instant thin-layer chromatography (ITLC) and reverse-phase HPLC. Table-1 shows the results of the thin-layer chromatography for [^{166}Ho(DOTA)]$^-$ by performing ITLC (EG&G Berthold linear analyzer, Bad Wildbad, Germany) on silica gel impregnated glass fibre sheets (Gelman Science Inc., Ann Arbor, MI, USA) using 75% methanol as a developing solvent.

TABLE-1
ITLC ANALYSIS OF $[^{166}Ho(DOTA)]^-$ PRODUCED BY THE NEW METHOD

Chromatographic system		^{166}Ho species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	75% Methanol	0% of $^{166}Ho^{3+}$	100% of $[^{166}Ho(DOTA)]^-$

As apparent in Table-1, showing the result of the ITLC of $[^{166}Ho(DOTA)]^-$, there was no observation of a peak of $^{166}Ho(NO_3)_3$ at the origin. Radiochemical purity of $[^{166}Ho(DOTA)]^-$ was determined by HPLC (2695 separations module, Waters, Milford, MA, USA) equipped with a radiometric detector (γ-RAM, IN/US Systems Inc., FL, USA) using a reverse-phase μBondapak C-18 column (3.9 × 300 mm, Waters, USA) for separation. HPLC solvents consisted of H_2O (0.1% TFA)/acetonitrile (0.1% TFA), while maintaining a flow rate of 1 mL/min. As shown in Fig. 3, only one peak was seen at a retention time of 3.3 min due to the compound of interest, indicating a formation of $[^{166}Ho(DOTA)]^-$ with more than a 99% radiochemical purity. These results confirm that $[^{166}Ho(DOTA)]^-$ having more than a 99% labelling efficiency was formed. In order to estimate the stability of the radiolabelled compound $[^{166}Ho(DOTA)]^-$, it was stored in closed vials at room temperature and the labelling efficiency was determined at 1, 2, 4, 6 h, respectively. It was found to be over 96% till 6 h.

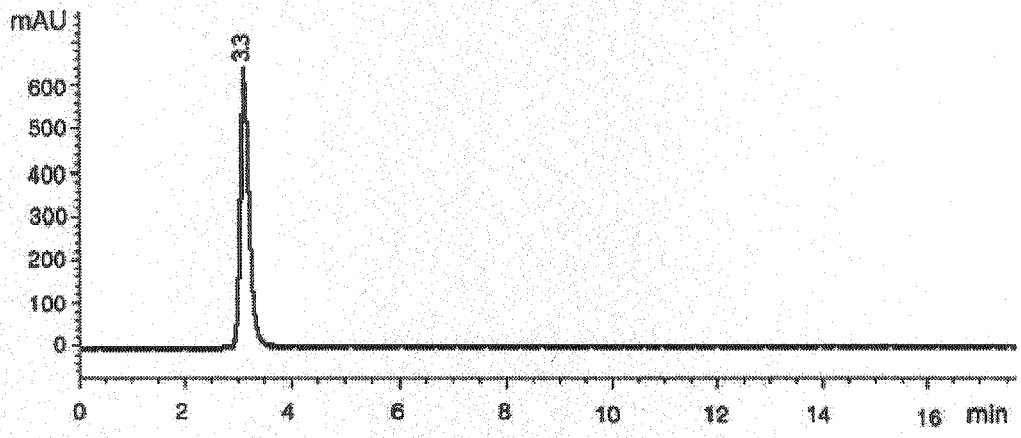


Fig. 3. HPLC Elution profile of $[^{166}Ho(DOTA)]^-$ chelate

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