Preparation and evaluation of 186/188Re-labeled antibody (A7) for radioimmunotherapy with rhenium(I) tricarbonyl core as a chelate site

Preparation and evaluation of $^{186/188}$Re-labeled antibody (A7) for radioimmunotherapy with rhenium(I) tricarbonyl core as a chelate site

*Kazuma Ogawa[1,2], Hidekazu Kawashima[3], Seigo Kinuya[4], Kazuhiro Shiba[2], Masahisa Onoguchi[4], Hiroyuki Kimura[5], Kazuyuki Hashimoto[6], Akira Odani[1], Hideo Saji[5]

[1]*Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan*

[2]*Advanced Science Research Center, Kanazawa University, Kanazawa, Japan*

[3]*Graduate School of Medicine, Kyoto University, Kyoto, Japan*

[4]*Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan*

[5]*Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan*

[6]*Japan Atomic Energy Agency, Tokai-mura, Ibaraki, Japan*

***Corresponding author**

Graduate School of Natural Science and Technology; Kanazawa University; Kakuma-machi, Kanazawa 920-1192; Japan

Telephone: 81-76-234-4460; Fax: 81-76-234-4459

E-mail: kogawa@p.kanazawa-u.ac.jp

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Abstract

Objective: Rhenium is one of the most valuable elements for internal radiotherapy because ^{186}Re and ^{188}Re have favorable physical characteristics. However, there are problems when proteins such as antibodies are used as carriers of $^{186/188}$Re. Labeling methods that use bifunctional chelating agents such as MAG3 require the conjugation of the $^{186/188}$Re complex to protein after radiolabeling with the bifunctional chelating agent. These processes are complicated. Therefore, we planned the preparation by a simple method and evaluation of a stable $^{186/188}$Re-labeled antibody. For this purpose, we selected $^{186/188}$Re(I) tricarbonyl complex as a chelating site. In this study, A7 (an IgG1 murine monoclonal antibody) was used as a model protein. $^{186/188}$Re-labeled A7 was prepared by directly reacting a $^{186/188}$Re(I) tricarbonyl precursor, $[^{186/188}Re(CO)_3(H_2O)_3]^+$, with A7. We then compared the biodistribution of $^{186/188}$Re-labeled A7 in tumor-bearing mice with ^{125}I-labeled A7.

Methods: For labeling A7, $[^{186/188}Re(CO)_3(H_2O)_3]^+$ was prepared according to a published procedure. $^{186/188}$Re-labeled A7 ($^{186/188}Re\text{-}(CO)_3$-A7) was prepared by reacting $[^{186/188}Re(CO)_3(H_2O)_3]^+$ with A7 at 43ºC for 2 hours.

Biodistribution experiments were performed by the intravenous administration of $^{186/188}$Re-(CO)$_3$-A7 solution into tumor-bearing mice.

Results: ^{186}Re-(CO)$_3$-A7 and ^{188}Re-(CO)$_3$-A7 were prepared with radiochemical yields of 23% and 28%, respectively. After purification by a PD-10 column, $^{186/188}$Re-(CO)$_3$-A7 showed a radiochemical purity of over 95%. In biodistribution experiments, 13.1% and 13.2% of the injected dose/g of ^{186}Re-(CO)$_3$-A7 and ^{188}Re-(CO)$_3$-A7, respectively, accumulated in the tumor 24 hours postinjection, and the tumor-to-blood ratios were over 2.0 at the same timepoint. Meanwhile, uptake of ^{125}I-A7 in the tumor was almost the same as those of $^{186/188}$Re-(CO)$_3$-A7 at 24 hours postinjection. Blood clearances of $^{186/188}$Re-(CO)$_3$-A7 were faster than that of ^{125}I-A7.

Conclusion: $^{186/188}$Re-labeled A7 showed high uptakes in the tumor. However, further modification of the labeling method would be necessary to improve radiochemical yields and their biodistribution.

Keywords: rhenium, radioimmunotherapy, antibody, tricarbonyl

Introduction

Radioimmunotherapy with radiolabeled monoclonal antibodies (mAb) has great potential to be a good treatment modality for cancer patients. In particular, radioimmunotherapy in B-cell non-Hodgkin's lymphoma targeting the CD20 antigen, which is found on the B-cell surface, has clearly demonstrated its efficacy [1,2]. Consequently, ^{90}Y ibritumomab tiuxetan (Zevalin) and ^{131}I tositumomab (Bexxar), both targeting the CD20 antigen, have been approved by the United States Food and Drug Administration for treatment of refractory or relapsed low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma [3,4]. Both radiopharmaceuticals have been shown to produce high response rates, but they also have some shortcomings as radionuclides. ^{131}I emits a high energy gamma ray, 364 keV, that is not ideal for imaging and exposes patients to unnecessary radiation. Meanwhile, because ^{90}Y is a pure beta emitter, imaging is difficult with ^{90}Y-mAb and dosimetry should be performed with ^{111}In-mAb before the ^{90}Y-mAb therapy. However, ^{111}In-mAb might not accurately predict the dosimetry of ^{90}Y-mAb because it has been reported that ^{111}In-mAb did not parallel the uptake of ^{86}Y-mAb in bone [5].

Rhenium has two useful radionuclides for radionuclide therapy, ^{186}Re and ^{188}Re. ^{186}Re and ^{188}Re are currently considered to be appropriate candidates for therapeutic applications due to their favorable nuclear properties [6,7]. Both rhenium radioisotopes decay with the emission of not only beta particles for therapy but also gamma rays, which are suitable for external detection with gamma cameras: ^{186}Re ($t_{1/2}$ = 3.68 d, β^{-}_{max} =1.07 MeV, γ = 137 keV) and ^{188}Re ($t_{1/2}$ = 16.98 h, β^{-}_{max} =2.12 MeV, γ = 155 keV). Additionally, in the case of ^{188}Re, a further advantage in clinical use is that ^{188}Re is conveniently produced from a transportable, in-house alumina-based $^{188}W/^{188}Re$ generator, similar to a $^{99}Mo/^{99m}Tc$ generator [8,9].

Previous reports have demonstrated the usefulness of $^{186/188}Re$ radionuclide therapy. However, there are problems when proteins such as antibodies are used as carriers of $^{186/188}Re$. A direct label method is not ideal because of the instability of labeled mAb, especially in the case of ^{186}Re [10]. The mercaptoacetylglycylglycylglycine (MAG3) ligand forms a stable ^{186}Re-MAG3 complex [11]; the usefulness of ^{186}Re-MAG3-mAb has been demonstrated in preclinical studies [12,13]. However, the labeling method using bifunctional chelating agents such as the N_3S (MAG3) and N_2S_2 (MAMA) ligand requires conjugation with the $^{186/188}$Re-complex to mAb after radiolabeling because this radiolabeling procedure requires severe conditions, such as heating and a non-neutral pH [14,15]. These complicated processes limit the clinical utility of radiolabeled mAb. Thus, we planned the preparation by a simple method and evaluation of a stable $^{186/188}$Re-labeled protein. For this purpose, we selected $^{186/188}$Re(I) tricarbonyl complex as a chelating site. In this study, A7 (an IgG1 murine mAb) was used as a model protein, and $^{186/188}$Re-labeled A7 were prepared by directly reacting a $^{186/188}$Re(I) tricarbonyl precursor, $[^{186/188}Re(CO)_3(H_2O)_3]^+$, with A7. Then, *in vitro* stability experiments and biodistribution experiments in tumor-bearing mice were performed.

Materials and Methods

Materials

^{186}Re and ^{188}W were supplied by the Japan Atomic Energy Agency (Tokai-mura, Japan) as $^{186}ReO_4^-$ and $^{188}WO_4^{2-}$ [16]. Alumina acid grade (100-200 mesh) alumina (ICN, Irvine, CA) was used as an adsorbent for the $^{188}W/^{188}Re$ generator. Silver cation exchange cartridges (Ag Plus) and anion exchange cartridges (SepPak QMA Light) were purchased from Alltech Associates, Inc. (Deerfield, IL) and Waters Corporation (Milford, MA), respectively. $^{188}ReO_4^-$ was eluted from a $^{188}W/^{188}Re$ generator using saline. The radioactive elution (5 mL) was condensed to a total of 400 μL using the method reported previously [8,9]. A7, an immunoglobulin G1 murine mAb that recognizes the 45-kDa glycoprotein in human colon cancer, was used. A7 reacts with most colorectal cancers [17]. Isolink kits for preparing $[^{186/188}Re(CO)_3(H_2O)_3]^+$ were obtained from Mallinckrodt (St Louis, MO). [^{125}I]Sodium iodide was purchased from PerkinElmer (Waltham, MA). Radiolabeling of A7 with ^{125}I was performed by the chloramine-T method [18]. Other reagents were of reagent grade and used as received.

The radiochemical purities of $^{186/188}$Re- and ^{125}I-labeled A7 were determined by thin layer chromatography (TLC) and cellulose acetate electrophoresis (CAE) (Separax-SP; Joko Co. Ltd., Tokyo, Japan). TLC analyses were performed with silica plates (Art 5553, Merck, Darmstadt, Germany) with a mixture of 99% methanol and 1% concentrated HCl as a developing solvent. CAE was run at an electrostatic field of 1.0 mA/cm for 20 minutes in veronal buffer (I = 0.06, pH 8.6).

Preparation of $^{186/188}$Re-labeled A7 ($^{186/188}$Re-(CO)$_3$-A7)

An intermediate of $^{186/188}$Re-labeled mAb, [$^{186/188}$Re(CO)$_3$ (H$_2$O)$_3$]$^+$, was prepared using an Isolink kit according to the method reported previously [19,20]. Namely, a mixture of 400 μL $^{186/188}$ReO$_4^-$ and 6 μL concentrated phosphoric acid was added to an Isolink kit to which 6 mg BH$_3$·NH$_3$ (Aldrich, Milwaukee, WI) had previously been added. The reaction mixture was heated at 65ºC for 15 minutes with a 20 mL syringe inserted to balance the pressure caused by gas production during the reaction. After the $^{186/188}$Re tricarbonyl intermediate solution was adjusted to about 7 pH, 200 μL of this solution was added to 80 μL of the A7 mAb solution (14.7 mg/mL). After 2 hours of incubation at 43ºC, this reaction mixture was purified by a PD-10 column (GE Healthcare UK Ltd., Buckinghamshire, England) with saline as the eluate.

***In Vitro* Stability**

To evaluate its stability, $^{188}Re\text{-}(CO)_3$-A7 in saline solution was incubated at 37˚C. After incubation for 24 hours, a sample was drawn and its radioactivity was analyzed by CAE and TLC. In addition, $^{188}Re\text{-}(CO)_3$-A7 solutions were diluted 10-fold with a 0.1 M solution of histidine or freshly prepared murine plasma, and the solutions were incubated at 37˚C. After 1, 3, and 24 hours incubation, the radioactivity of each sample was analyzed by TLC.

Biodistribution in tumor-bearing mice

Experiments with animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University. The animals were housed with free access to food and water at 23°C with a 12-hour alternating light/dark schedule. LS180 human colon carcinoma cells were obtained from ATCC (Manassas, VA) and grown in cell culture dishes in Eagle’s minimum essential medium with phenol red, 10% heat-inactivated fetal calf serum, 100 μg/mL glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humidified atmosphere of 95% air and 5% carbon dioxide at 37°C. They were then released from the dishes by treatment with 0.05% trypsin/EDTA. Next, to produce tumors, the mice to be inoculated were anesthetized with pentobarbital and approximately 5×10^6 cells were injected subcutaneously into the right shoulder of 4-week-old BALB/c nu/nu female mice (15-19 g). Biodistribution experiments were performed approximately 8 days postinoculation, i.e., the time required for the tumors to reach a palpable size. Groups of four or five mice were administered 100 μL $^{186}Re-(CO)_3$-A7, $^{188}Re-(CO)_3$-A7, or ^{125}I-A7 (7.4 kBq, A7:100 μg, respectively) intravenously and sacrificed at 1 and 24 hours postinjection. Tissues of interest were removed and weighed, and radioactivity counts were determined with an Auto Well Gamma System (ARC-380; Aloka, Tokyo, Japan) and corrected for background radiation and physical decay during counting.

Results

Preparation of $^{186/188}$Re-(CO)$_3$-A7

In TLC analyses, $^{186/188}Re-(CO)_3$-A7 remained at the original position (Rf = 0), while an intermediate, $[^{186/188}Re(CO)_3(H_2O)_3]^+$, and the free perrhenate ($^{186/188}ReO_4^-$) migrated to Rf = 0.2-0.4 and Rf = 0.7-0.8, respectively [20]. In CAE analyses, intact A7 migrated to the 2-2.5 cm anode from the origin, which was determined by Ponceau S dye, and $^{186/188}$Re-$(CO)_3$-A7 also migrated to the 2-2.5 cm anode (Figure 1), while colloidal $^{186/188}$Re remained at the origin. The radiolabeling yield of $[^{188}Re(CO)_3(H_2O)_3]^+$ was 41%. ^{186}Re-$(CO)_3$-A7 and ^{188}Re-$(CO)_3$-A7 were prepared with radiochemical yields of 23% and 28%, respectively. After purification using a PD-10 column, ^{186}Re-$(CO)_3$-A7 and ^{188}Re-$(CO)_3$-A7 showed a radiochemical purity of over 95%.

***In Vitro* Stability**

After incubation in saline for 24 hours, about 93% of the ^{188}Re-$(CO)_3$-A7 remained intact. In murine plasma, over 90% of radioactivity existed in a protein fraction for 24 hours, indicating that the ^{188}Re-$(CO)_3$-A7 is not degraded to $^{188}ReO_4^-$ in plasma. When challenged with an excess of histidine, part of the radioactivity dissociated from ^{188}Re-$(CO)_3$-A7 (Figure 2).

Biodistribution in tumor-bearing mice

The biodistributions of ^{186}Re-(CO)$_3$-A7, ^{188}Re-(CO)$_3$-A7, and ^{125}I-A7 in tumor-bearing mice are listed in Tables 1-3. As we expected, both radiorhenium-labeled A7 had almost identical biodistribution. ^{186}Re-(CO)$_3$-A7 and ^{188}Re-(CO)$_3$-A7 showed high uptakes in the tumors, amounting to 13.1% and 13.2% at 24 hours postinjection, respectively. Meanwhile, uptake of ^{125}I-A7 in the tumor was almost the same as those of $^{186/188}$Re-(CO)$_3$-A7 at 24 hours postinjection. Blood clearances of $^{186/188}$Re-(CO)$_3$-A7 were faster than that of ^{125}I-A7. $^{186/188}$Re-(CO)$_3$-A7 showed that the tumor/blood ratios were over 2.0 at 24 hours postinjection, but the tumor/blood ratio of ^{125}I-A7 was approximately 1.0.

Discussion

We hypothesize that $^{186/188}$Re(CO)$_3$ core binds endogenous histidine residue in an antibody when $[^{186/188}Re(CO)_3(H_2O)_3]^+$ is used to label the antibody. In our preliminary experiments, we labeled H-His-OMe with $[^{186}Re(CO)_3(H_2O)_3]^+$ at room temperature, 45˚C, or 100˚C. As a result, the radiochemical yield increased in a reaction temperature-dependent manner.

In this study, A7 was reacted with $[^{186/188}Re(CO)_3(H_2O)_3]^+$ at 43˚C because higher temperatures damage the antibody. Radiochemical yields of $^{186/188}Re$-labeled A7 were less than 30%. For clinical use, the radiochemical purity should be over 95% without purification. We suppose that some sequences, such as an oligohistidine sequence, could be inserted into the antibody to improve the radiochemical yield. Tait *et al* reported that $(His)_6$-inserted annexin V had a better radiochemical yield compared with those of $(His)_3$-inserted annexin V and wild-type annexin V when annexin V was labeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ [21]. Another cause of low radiochemical yields of $^{186/188}Re$-labeled A7 could be low yields of $[^{186/188}Re(CO)_3(H_2O)_3]^+$. In this study, the radiochemical yield of $[^{188}Re(CO)_3(H_2O)_3]^+$ was only 41%. Recently, higher yields of the preparation of the precursor, $[^{188}Re(CO)_3(H_2O)_3]^+$, were reported [22,23]. We assume that using the new method for preparing $[^{186/188}Re(CO)_3(H_2O)_3]^+$ in future studies would also improve the radiochemical yields of $^{186/188}Re$-labeled A7.

The high stability of ^{188}Re-$(CO)_3$-A7 in saline and almost no degradation to $^{188}ReO_4^-$ in plasma were shown in *in vitro* experiments.

Since accumulation in the stomach is an index of ReO_4^- in biodistribution studies [24], low radioactivity levels in the stomach after injection of $^{186/188}Re\text{-}(CO)_3$-A7 indicate little decomposition to $^{186/188}ReO_4^-$ *in vivo*. In a recent study, Chen *et al*. prepared a ^{188}Re-labeled antibody (^{188}Re(I)-trastuzumab) by a similar method [23]. ^{188}Re(I)-trastuzumab showed high stability *in vitro* and low stomach accumulation in tumor-bearing mice. These studies strongly support the validity of our results.

In other previous studies, it was reported that a radioiodine-labeled antibody, ^{88}Y-isothiocyanatobenzyl-DTPA-antibody, and ^{186}Re-MAG3-antibody showed similar blood clearances [25,26]. However, in this study, the radioactivity (%dose/g) in blood at 24 hr postinjection of $^{186}Re\text{-}(CO)_3$-A7, $^{188}Re\text{-}(CO)_3$-A7, and ^{125}I-A7 were 6.2 ±0.3, 5.9 ±0.8, and 13.9 ±3.5, respectively. That is, $^{186/188}Re\text{-}(CO)_3$-A7 showed faster blood clearance compared with that of ^{125}I-A7 in the biodistribution experiments. These results might indicate that $^{186/188}$Re detached from A7 in the blood flow. There is a possibility that the binding of the $^{186/188}Re\text{-}(CO)_3$ core to A7 is not strong, so some molecules in the blood might take the

$^{186/188}$Re-(CO)$_3$ core from $^{186/188}$Re-(CO)$_3$-A7. Actually, when ^{188}Re-(CO)$_3$-A7 was challenged with an excess of histidine, part of the radioactivity dissociated from ^{188}Re-(CO)$_3$-A7. In this experiment, the radiochemical purity of ^{188}Re-(CO)$_3$-A7 decreased to around 60% after 3 hours incubation. However, after 24 hours incubation, the radiochemical purity of ^{188}Re-(CO)$_3$-A7 was almost same as that after 3 hours incubation. These results might indicate that there are strong and weak bindings of the ^{188}Re-(CO)$_3$ core to an A7 antibody in purified ^{188}Re-(CO)$_3$-A7 because the ^{188}Re-(CO)$_3$ core does not bind to a specific site in an antibody. Recently, the biodistribution of $[^{188}Re(CO)_3(H_2O)_3]^+$ was reported [27]. The radioactivity in blood, liver, and kidney at 24 hours postinjection of $[^{188}Re(CO)_3(H_2O)_3]^+$ were 3.13 ±0.52, 9.65 ±1.40, and 9.62 ±0.09, respectively. Taking into account the biodistribution at 24 hours postinjection of $^{186/188}$Re-(CO)$_3$-A7 in this study, these results are also not inconsistent with our hypothesis.

Faster blood clearance is advantageous for fewer side effects because myelosuppression is the chief side effect associated with radioimmunotherapy [28]. However, faster blood clearance compromises the accumulation of radioactivity in tumors. In sum, although diagnostic radiopharmaceuticals should be better because they need a high tumor/blood ratio at an earlier time postinjection, therapeutic radiopharmaceuticals might be unfavorable because high accumulation and long retention in tumors is preferred for a better therapeutic effect. As mentioned above, we suppose that insertion of an oligohistidine sequence could improve radiochemical yield. Additionally, insertion could also be effective from the point of view of improving the *in vivo* stability and biodistribution of $^{186/188}$Re-labeled antibodies.

Conclusion

$^{186/188}$Re-labeled A7 showed high uptakes in tumors the same as that of ^{125}I labeled A7. However, further modifications of the labeling method would be necessary in order to improve radiochemical yields and their biodistribution.

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Figure Captions

Figure 1. Profiles of ^{188}Re-$(CO)_3$-A7 and intact A7 (Ponceau S dye) on cellulose acetate electrophoresis.

Figure 2. Stability of ^{188}Re-$(CO)_3$-A7 in L-histidine solution.

Table 1. Biodistribution of radioactivity after intravenous administration of $^{186}Re\text{-}(CO)_3$-A7 in mice.

	Time after administration	
Tissue	1 h	24 h
Blood	24.8 (1.1)	6.2 (0.3)
Tumor	5.1 (0.7)	13.1 (1.8)
Liver	18.5 (0.4)	9.7 (0.8)
Kidney	13.8 (0.4)	9.0 (1.1)
Intestine	1.5 (0.3)	2.0 (0.2)
Spleen	7.8 (1.0)	4.5 (0.9)
Pancreas	1.1 (0.1)	0.9 (0.0)
Lung	11.8 (2.5)	3.8 (0.5)
Heart	4.4 (0.6)	1.8 (0.1)
Stomach[a]	0.4 (0.0)	0.2 (0.0)
Muscle	0.9 (0.4)	0.8 (0.1)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of four or five animals.

[a] Data are expressed as % injected dose.

Table 2. Biodistribution of radioactivity after intravenous administration of $^{188}Re\text{-}(CO)_3$-A7 in mice.

	Time after administration	
Tissue	1 h	24 h
Blood	27.6 (1.8)	5.9 (0.8)
Tumor	6.0 (1.7)	13.2 (1.7)
Liver	18.9 (2.8)	9.7 (0.8)
Kidney	14.8 (1.7)	8.9 (0.4)
Intestine	1.8 (0.2)	2.0 (0.3)
Spleen	8.3 (0.8)	4.0 (0.7)
Pancreas	1.5 (0.2)	0.8 (0.0)
Lung	12.6 (2.2)	3.2 (0.4)
Heart	5.6 (0.5)	1.6 (0.3)
Stomach[a]	0.9 (0.2)	0.3 (0.1)
Muscle	0.8 (0.2)	0.5 (0.1)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of four or five animals.

[a] Data are expressed as % injected dose.

Table 3. Biodistribution of radioactivity after intravenous administration of ^{125}I-A7 in mice.

	Time after administration	
Tissue	1 h	24 h
Blood	36.7 (4.3)	13.6 (2.1)
Tumor	3.5 (0.7)	13.9 (3.5)
Liver	10.7 (4.5)	3.4 (1.0)
Kidney	8.3 (1.4)	2.9 (0.7)
Intestine	1.6 (0.3)	1.0 (0.3)
Spleen	7.5 (3.2)	2.5 (0.8)
Pancreas	1.3 (0.4)	1.2 (0.2)
Lung	19.8 (2.5)	7.8 (1.2)
Heart	6.9 (1.0)	3.1 (0.5)
Stomach[a]	1.2 (0.2)	1.0 (0.4)
Muscle	0.9 (0.2)	0.8 (0.1)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of four animals.

[a] Data are expressed as % injected dose.

Figure 1.

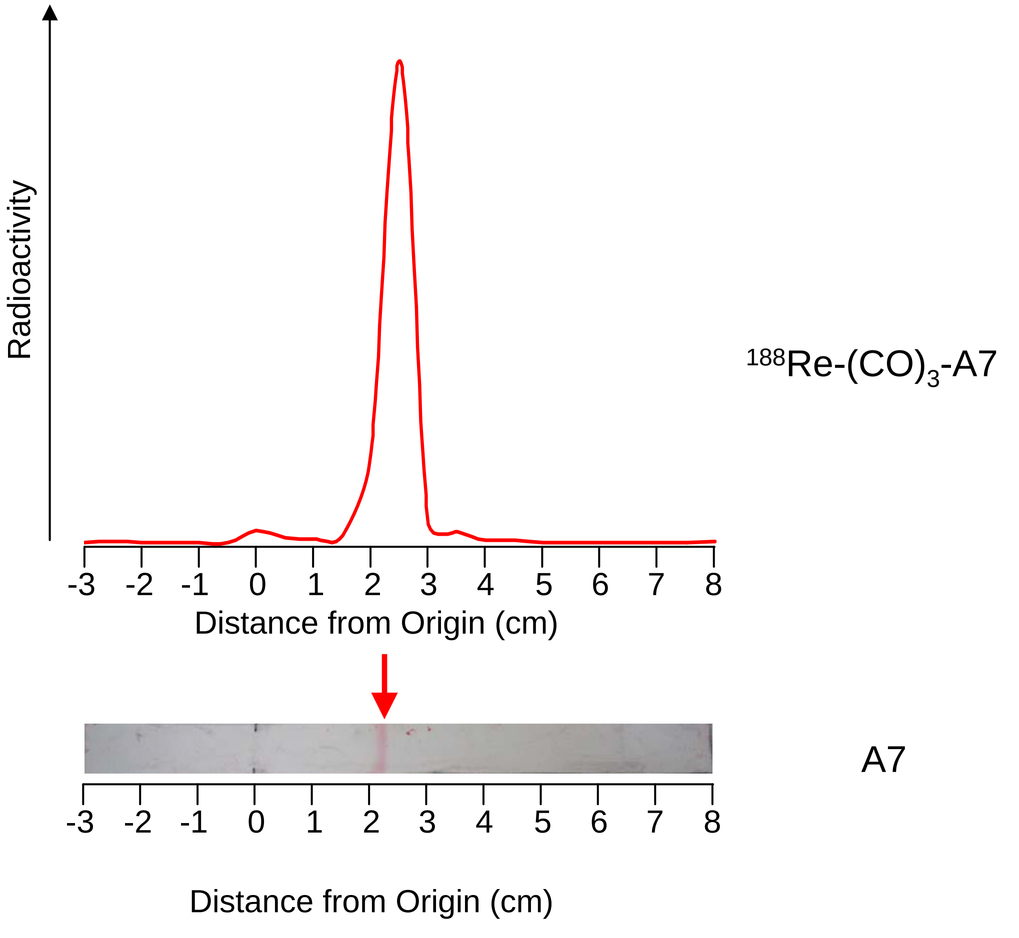
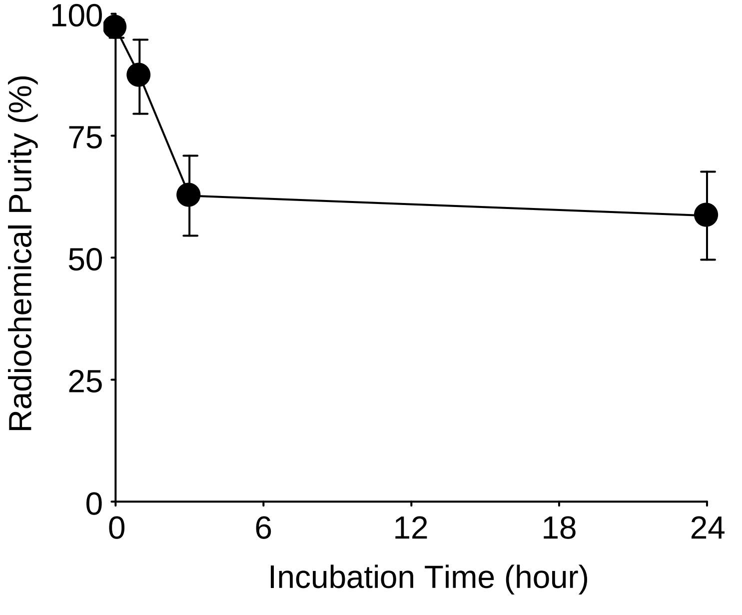

Radioactivity
-3
-2
-1
0
1
2
3
4
5
6
7
8
Distance from Origin (cm)
188Re-(CO)3-A7
A7
-3
-2
-1
0
1
2
3
4
5
6
7
8
Distance from Origin (cm)

Figure 2.


100
75
50
25
0
Radiochemical Purity (%)
0
6
12
18
24
Incubation Time (hour)