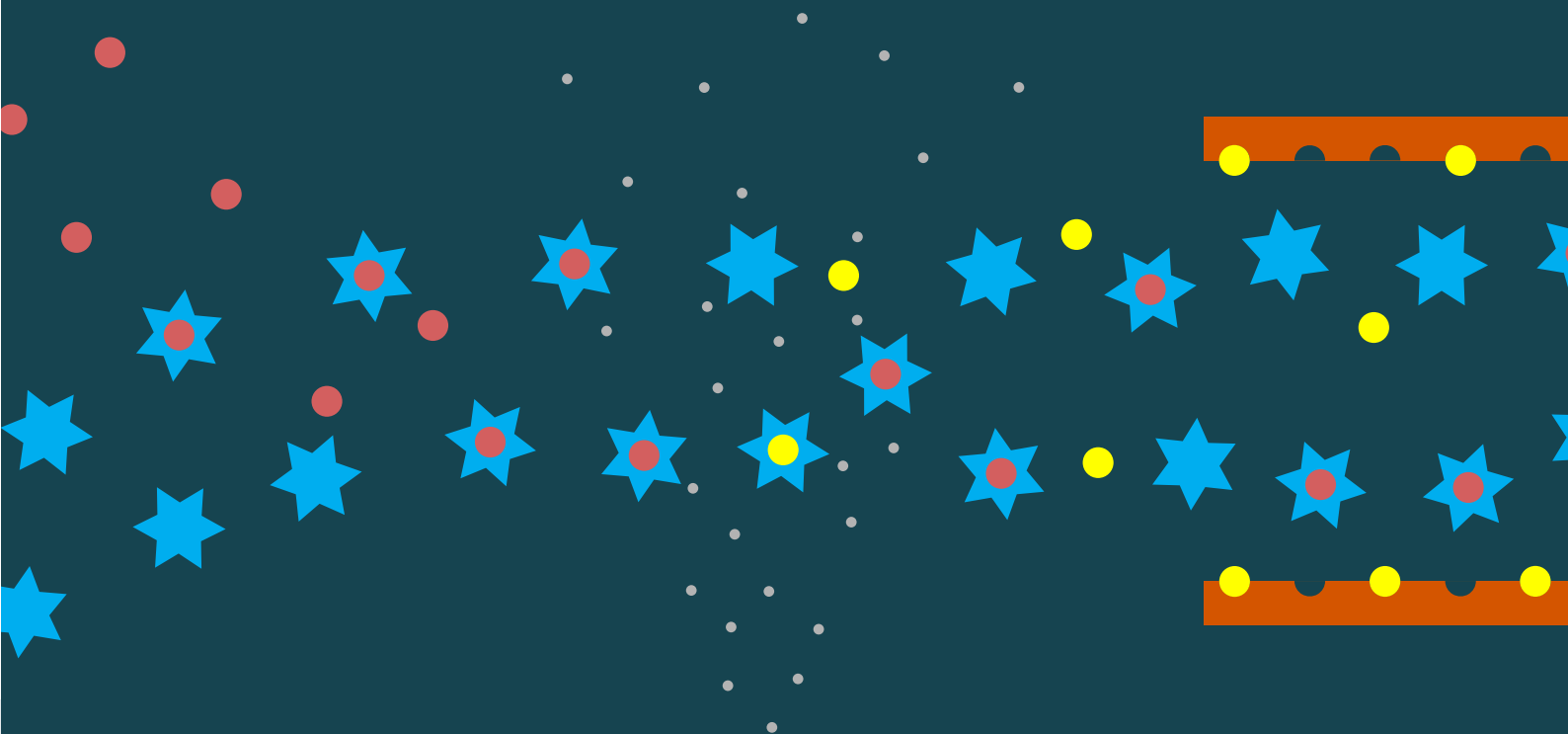


Investigation of the processes expected in a continuous flow Szilard-Chalmers system for production of high specific activity radionuclides

Wout Schenk



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By

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Abstract

^{166}Ho is a promising radionuclide for targeted radionuclide therapy, because of its beneficial decay characteristics and the possibility to track its dose delivery in a patient. However, a problem for the use of ^{166}Ho in these treatments is its limited maximally produced specific activity in neutron irradiation of a pure ^{165}Ho target, since the produced ^{166}Ho is chemically inseparable from the ^{165}Ho target. The Szilard-Chalmers method is known to be able to increase the achieved specific activity. It does so by irradiating the target isotope while it is incorporated in a compound. As the target isotope undergoes (n, γ) neutron capture it forms the product isotope and escapes the compound due to recoil from prompt gamma emissions. Then by separating the product isotopes from the compound, which still contains the target isotopes, a high specific activity can be achieved. However, batch irradiations using the Szilard-Chalmers method for production of high specific activity gives low production yields. In order to increase these production yields, a continuous flow Szilard-Chalmers system is proposed. The objective of this research is to investigate the main processes at play in a continuous flow system using the Szilard-Chalmers method on $^{165}\text{HoDOTA}^-$ for the production of high specific activity ^{166}Ho . Investigations on the labelling of Ho^{3+} to DOTA^{4-} have shown that 96 ± 3 % labelling efficiency is achievable. Experiments on relabelling and isotope-exchange have shown that less than 8 % of $^{166}\text{Ho}^{3+}$ undergoes relabelling with DOTA^{4-} or isotope-exchange with $^{165}\text{HoDOTA}^-$. Also, a cation exchange separation method for separation of Ho^{3+} from DOTA^{4-} and HoDOTA^- was developed. Moreover, investigations on Szilard-Chalmers separation of a dried $^{165}\text{HoDOTA}^-$ sample have shown that with a 4-minute irradiation it was possible to achieve a specific activity of 2.8 ± 0.2 MBq mg^{-1} . This corresponds to an enrichment factor of 1.62 ± 0.03 compared to an irradiation without Szilard-Chalmers separation. Decreased radiolysis by shielding and slowed neutrons, and increased initial labelling are expected to be able to increase the achieved enrichment factor even more. The continuous flow Szilard-Chalmers system could in potential prove very useful in high specific activity radionuclide production, but further improvements on its subprocesses are necessary before practical implication is worthwhile.

Table of contents

1. Introduction	1
2. Theoretical background	5
2.1 Szilard-Chalmers method	5
2.2 HoDOTA ⁻	6
2.3 Continuous flow Szilard-Chalmers system	8
2.4 Subprocesses	10
2.4.1 Labelling	
2.4.2 Irradiation	
2.4.3 Transport	
2.4.4 Separation	
2.5 Thin layer chromatography	15
2.6 Measurement devices	16
2.6.1 Phosphor imager	
2.6.2 Wallac gamma counter	
2.6.3 ICP-OES	
2.6.4 Germanium detector	
3. Materials & methods	19
3.1 Materials	19
3.2 Devices	19
3.3 Instant thin layer chromatography	20
3.4 Determining the ¹⁶⁶ Ho-DOTA labelling efficiency at different incubation times	20
3.5 Determining the amount of isotope-exchange and labelling by ¹⁶⁶ Ho ³⁺ in a solution with near 100% of the DOTA labelled to Ho	21
3.6 Design of a cation exchange chromatography method for the separation of Ho ³⁺ from DOTA ⁴⁻ and HoDOTA ⁻	22
3.7 Determining the specific activity achieved by the Szilard-Chalmers method in irradiation of a dried HoDOTA ⁻ sample	23
4. Results & discussion	27
4.1 Determining the ¹⁶⁶ Ho-DOTA labelling efficiency at different incubation times	27
4.2 Determining the amount of isotope-exchange and labelling by ¹⁶⁶ Ho ³⁺ in a solution with near 100% of the DOTA labelled to Ho	29

4.3 Design of a cation exchange chromatography method for the separation of Ho^{3+} from DOTA^{4-} and HoDOTA^-	30
4.4 Determining the specific activity achieved by the Szilard-Chalmers method in irradiation of a dried HoDOTA^- sample	32
5. Conclusion	37
5.1 Conclusion	37
5.2 Future research recommendations	37
Literature	41
Appendix	47

List of figures

- 1.1** Schematic representation of the composition of a radiopharmaceutical used in targeted radionuclide therapy.
- 1.2** Scheme of the decay of ^{166}Ho to ^{166}Er showing the most important emissions.
- 1.3** Schematic representation of the Szilard-Chalmers separation method.
- 2.1** Schematic representation of the direct ^{166}Ho production route and the indirect ^{166}Ho production route.
- 2.2** Depiction of the chemical structure of the fully protonated H_4DOTA molecule.
- 2.3** Depiction of the chemical structure of the complex resulting from chelation of DOTA with a trivalent lanthanoid.
- 2.4** Graph showing the prompt gamma emission in neutron capture by ^{165}Ho .
- 2.5** Schematic representation of a continuous flow Szilard-Chalmers system for the production of ^{166}Ho .
- 2.6** Graphs showing the labelling efficiency of ^{166}Ho and ^{90}Y to DOTA after 1 hour of labelling for varying pH and DOTA:radionuclide ratios.
- 2.7** Scheme showing the deexcitations possible from the 40 lowest energy levels of ^{166}Ho .
- 2.8** Graph showing the stability of the LuDOTA^- complex in gamma radiation.
- 2.9** Schematic representation of a cation exchange separation.
- 2.10** Schematic representation of thin layer chromatography.
- 4.1** Error bar plots of labelling efficiencies against incubation time at 25, 40 and 80 °C.
- 4.2** Plots of the percentage of ^{166}Ho bound to DOTA due to relabelling and isotope-exchange after different labelling times.
- 4.3** Plot on the ^{166}Ho labelling efficiency in the controls checking whether near 100% of the DOTA was labelled with Ho before the start of the isotope-exchange and labelling reactions.
- 4.4** Elution diagram of the various investigated eluents on free Ho^{3+} and HoDOTA^- samples.
- 4.5** Elution diagram of the three iterations of the HoDOTA^- irradiation.
- A.1** Radioactivity distribution map of the iTLC strips resulting from the first and second iteration of the measurement on the ^{166}Ho -DOTA labelling at 40 °C.
- A.2** Radioactivity distribution map showing the continued labelling of ^{166}Ho to DOTA after application on an iTLC strip.

1. Introduction

In the year 2018, almost 2 million people died from cancer in Europe alone and together with the fact that cancer is the most important cause of death for people under the age of 65 in the EU,^{1,2} it is no surprise that the treatment of cancer has been among the top priorities of medical research for a long time. Research has already significantly improved cancer survival rates.³ However, the still large remaining number of people who do not survive cancer, shows that there is without a doubt a lot more to win in treatment improvements.

Various types of cancer treatments are available and their effectiveness varies from one cancer case to another.⁴ Some treatment methods are based on irradiation. In these kinds of treatment the cancer is irradiated with ionising radiation, which causes cancer cells to stop growing and dividing or even to die.⁵ Although cancer cells are more vulnerable to radiation,⁶ healthy non-cancer cells can be affected too.

Radiation treatment can be done by external radiation, where beams of ionising radiation, emitted from a source outside of the patient's body, are aimed on cancer tissue. This type of irradiation is useful when a cancer is very localised, because the beams can be aimed on the cancer very precisely.⁷ However, after a cancer grows and spreads through the body, so after reaching metastasis, external irradiation is less suitable. The reason is that irradiation of delocalised cancer tissue would require too much irradiation, and thereby damaging, of healthy tissue. When cancer is delocalised, a form of internal irradiation using targeted therapy can be a solution.⁸

In this targeted radionuclide therapy, a radioactive nuclide is delivered specifically to a cancer cell, and less to healthy cells, because if the radionuclides decay close to the cancer cells, the radiation emitted by the decay can kill the cells.⁹ Cancer cells can be targeted by targeting certain proteins on the surface of the cells that are not or less present on healthy cells or have mutations in them that healthy cells do not have. By binding the radioactive atoms to a molecule that only binds to these unusual surface proteins, the radionuclides will be present specifically near the cancer cells.⁶ Figure 1.1 shows the typical composition of a radiopharmaceutical used in targeted radionuclide therapy.¹⁰ A linker links the radionuclide to a molecule that targets only the proteins characteristic for the cancer cells.

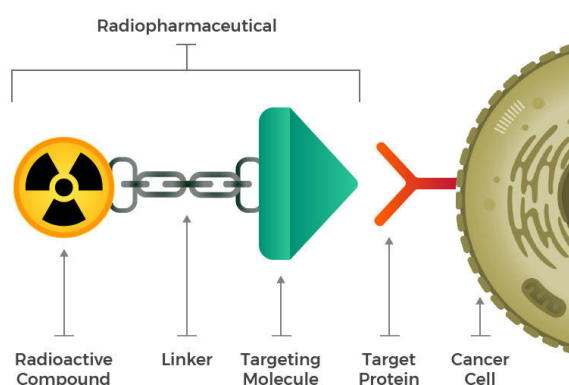


Figure 1.1. Schematic representation of the composition of a radiopharmaceutical used in targeted radionuclide therapy. The radionuclide is bound to a cell with a target protein matching the targeting molecule. The linker links the targeting molecule to the radioactive nucleus.¹⁰

Targeted radionuclide therapy can prove very useful in cancer treatment, but it requires a radionuclide with a specific set of properties for the best therapy results. Important properties of a radionuclide to be considered in developing a treatment plan for specific cancer cases are its decay energy, half-life and imaging possibilities.^{11,12,13} Moreover, on certain tumour types the amount of targeting proteins can be limited. In order to still deliver enough radiation dose from this limited amount of targeting sites, the available sites need to be used with high efficiency. This means that a radionuclide should be used with a high specific activity, which is the activity of a certain isotope of an element per unit weight of that element.^{8, 11, 14}

Holmium-166, or ^{166}Ho , is a good candidate for targeted radionuclide therapies, due to its beneficial decay and imaging properties. Figure 1.2 shows the decay scheme of ^{166}Ho .

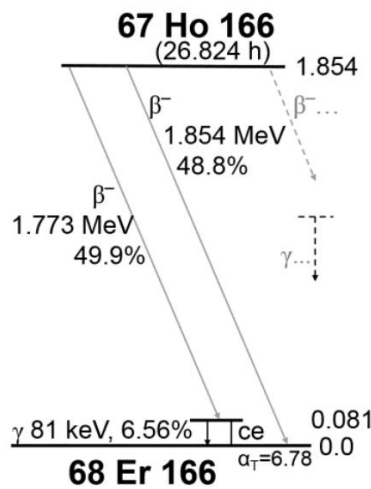


Figure 1.2. Scheme of the decay of ^{166}Ho to ^{166}Er showing the most important emissions.¹⁵

^{166}Ho can be made by a $^{165}\text{Ho}(n, \gamma)^{166}\text{Ho}$ reaction. The general equation for such a (n, γ) neutron capture reaction is shown in equation 1.1.¹³



In this equation a nuclide of element X with atomic number z and mass number y captures a neutron and thereby forms an isotope of X with mass number y+1 and emits a gamma photon. The specific activity of a radioactive isotope produced by a (n, γ) reaction reaches its maximum when the amount of produced radioactive isotopes is such that its activity matches its production rate. This production rate is linearly dependent on the (n, γ) neutron capture cross-section, which is a measure of the chance that a nucleus will undergo a (n, γ) reaction.¹⁶ A large problem for the use of ^{166}Ho in targeted radionuclide therapy, is that the highest achievable specific activity of ^{166}Ho possibly produced by (n, γ) neutron capture in a nuclear reactor is limited, compared to other currently used radioisotopes, because of its lower (n, γ) neutron capture cross-section.^{17, 18}

Because isotopes of the same element share their chemical properties, it is not possible to increase the specific activity of an isotope by conventional chemical separation methods. However, a separation method that enables exceeding the limited specific activity achieved by (n, γ) neutron capture in a nuclear reactor, is the Szilard-Chalmers method. This method was first described by L. Szilard and T. A. Chalmers in 1934.¹⁹ In the Szilard-Chalmers method, the isotope intended to capture a neutron, the target isotope, is incorporated in a chemical compound, the target compound. Upon neutron capture, the desired radioactive isotope is formed. After neutron capture, the created radioactive isotope is in excited state, but the radioactive isotope immediately returns to its ground

state by emission of gamma photons. These gammas are called prompt gammas. Since photons have a momentum, emission of the gammas will give the radioactive isotope a momentum too. When the momentum of the gamma radiation is high enough, it can even cause the radioactive isotope to break from the target compound it is incorporated in. Since the target isotopes are still in the target compound then and the radioactive isotopes are not, the different isotopes can be chemically separated. In this way a high specific activity of the radioactive isotope can be created that exceeds the specific activity achieved by neutron capture of the target isotope without use of the Szilard-Chalmers method.¹⁹ Because the prompt gammas emitted in neutron capture by ^{165}Ho are able to cause an escape of the formed ^{166}Ho from certain target compounds, the Szilard-Chalmers method can help increase the produced specific activity of ^{166}Ho .^{20, 21} Figure 1.3 shows a schematic representation of the Szilard-Chalmers method.

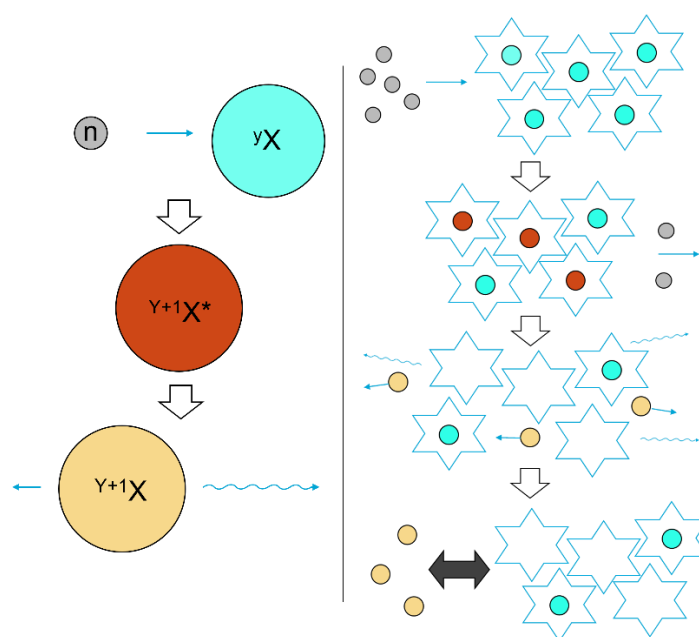


Figure 1.3. Schematic representation of the Szilard-Chalmers separation method. On the left the (n, γ) neutron capture reaction is depicted and on the right it is depicted how that reaction is used in the Szilard-Chalmers method.

A large problem in using the Szilard-Chalmers method is that its efficiency decreases quickly over irradiation time.²¹ This is caused by processes such as recombination of the escaped radioactive isotope with the chemical compound it escaped from or such as radiolysis, in which the radiation coming from the nuclear reactor breaks down the target compound that contains the target isotope.²¹ For a high specific activity of produced ^{166}Ho to be achieved with the Szilard-Chalmers method, a quick separation of the escaped radioactive isotopes and the target isotopes remaining in the target compound is required.²¹ However, the short irradiation time required for quick separation results in low activity production yield per irradiation.

In order to achieve both quick separation as well as high production yields, a continuous flow Szilard-Chalmers system could be used for the production of radioactive isotopes.²² In such a system, a solution that contains the target compound which contains the target isotope would be passed by the reactor core for a short time span. During the time close to the reactor core, the target compound containing the target isotope would be irradiated in order for the Szilard-Chalmers reactions to occur, in which neutron capture causes escape of the produced isotope from the target compound. After irradiation, the solution would be passed by a continuous flow separation system that separates the produced free radioactive isotopes from the target isotopes that are still incorporated in the target compound. Due to the continuous production of radioisotopes, a high

production yield is expected to be able to be achieved with the continuous flow system. Moreover, due to the implementation of the Szilard-Chalmers method with a short time between the neutron capture reactions and the separation, a high specific activity of the produced radionuclide is expected to be able to be achieved, as was shown in a concept experiment on a continuous flow Szilard-Chalmers system by Safavi-Tehrani.²²

In order to optimize the specific activity that could be achieved in ^{166}Ho production using a continuous flow Szilard-Chalmers system, as is necessary for improvements in targeted radionuclide therapy,¹¹ it needs to be understood to what extent the various processes occurring in the system influence the produced specific activity. In this thesis, the influence of the most important processes expected to occur in the continuous flow Szilard-Chalmers system on the produced specific activity will be investigated. To this end, the efficiency of the incorporation of the target isotope in the target compound, the amount of radioactive isotopes that recombine with the target compound after a Szilard-Chalmers reaction, the possibility of efficient separation of the produced radioactive isotopes from the target compounds and the efficiency of the Szilard-Chalmers reactions will be investigated.

This report presents the results found in this thesis on the influence of the most important processes expected to occur in the continuous flow Szilard-Chalmers system on the produced specific activity. The chapter "Theoretical background" will provide a more detailed theoretical background on the subject. Then the chapter "Materials & methods" will discuss what materials were used in the done experiments and how these experiments were carried out. Then the chapter "Results & discussion" will present the results found in these experiments. Moreover, this chapter will discuss the considerations in interpreting the found results and in the assessment of their quality. Lastly, the report will end with the chapter "Conclusion", which will conclude what the most important outcomes of this thesis are and which will discuss recommendations for further research.

2. Theoretical background

This chapter explains some subjects necessary for understanding the experiments done in this work and the results following from them. The chapter starts with explaining the Szilard-Chalmers separation method, after which a promising metal-chelator pair for production of ^{166}Ho using the Szilard-Chalmers method is presented. After that, the continuous flow Szilard-Chalmers system and the most important processes expected to be at play in such a system are explained. Lastly, the theory behind instant thin layer chromatography is discussed, since this is an important analysis method in the experiments done in this work.

2.1 Szilard-Chalmers method

It is possible to change an isotope into another of the same element by neutron irradiation, since it can cause neutron capture as in equation 1.1. The maximally achievable specific activity of this newly formed isotope is limited by the available neutron flux.²³ Since isotopes have the same chemical properties, it is difficult to increase the specific activity any further by chemical separation techniques.²⁴ However, a method available that helps increase the specific activity of an isotope produced by neutron irradiation, is the Szilard-Chalmers method.¹⁹

When a nucleus captures a neutron, the nucleus will be in an excited state. Immediately after capture, usually within 10^{-9} s,²² the nucleus returns to its ground state by emitting prompt gamma photons.^{25, 26} These prompt gammas can have very high energies even reaching 10 MeV.²⁷ The momentum of these photons is given by equation 2.1.²⁸

$$p = \frac{E_\gamma}{c} \quad (2.1)$$

Here p is the photon momentum in kg m s^{-1} , E_γ is the gamma photon energy in J and c is the speed of light in m s^{-1} . Conservation of momentum requires that upon emission of a prompt gamma, with its momentum, the emitting nucleus also has a change in momentum and therefore in kinetic energy. The kinetic energy obtained by a nucleus in emission of a prompt gamma is given by equation 2.2.²⁸

$$E_{\text{kinetic}} = \frac{p^2}{2m} \quad (2.2)$$

Here E_{kinetic} is the kinetic energy of the nucleus in J and m is the atomic mass of the nucleus in kg. Equation 2.1 and 2.2 can be combined to equation 2.3.

$$E_{\text{kinetic}} = \frac{E_\gamma^2}{2mc^2} \quad (2.3)$$

After conversion to more convenient units, equation 2.3 changes to equation 2.4.²⁸

$$E_{\text{kinetic}} = 5.368 * 10^{-4} * \frac{E_\gamma^2}{m} \quad (2.4)$$

In equation 2.4 the kinetic energy of the nucleus and the gamma photon energy are given in MeV and the mass of the nucleus is given in u. This equation shows the kinetic energy a nucleus can obtain from an emitted photon with a certain energy. As an example, with the emission of a 1 MeV gamma, a 100 u nucleus obtains a kinetic energy of 5 eV. Since chemical bonds generally have a bond

dissociation energy in the order of eV,²⁹ this gives the important result that the recoil energy of a nucleus obtained by emission of the prompt gamma can be enough to break chemical bonds.

The Szilard-Chalmers method is based on this breaking of chemical bonds upon neutron capture. It works as follows: First atoms of a certain isotope (neutron number N) are bound to a certain compound before irradiation. Then the compound-isotope complex is irradiated to produce the isotope of interest (N+1) by neutron capture. Then recoil energy from the prompt gamma emission at the neutron capture can break the bond between the compound and the newly created isotope of interest. The result is a mixture of the unbound newly created isotopes (N+1) and the still bound non-changed isotopes (N). Since the two different isotopes are now in different chemical states, namely bound and free, it is possible to separate them using conventional chemical separation methods.²³ In figure 1.3 a schematic representation of the Szilard-Chalmers method is shown.

2.2 HoDOTA

A nuclide that fits many of the requirements for targeted radionuclide therapy, as discussed in the introduction, is ^{166}Ho . ^{166}Ho decays through β^- emission with an energy of 1.8 (50 % of decays) or 1.9 (49 % of decays) MeV.^{18, 30} Therefore, 90% of the radiation dose delivered by ^{166}Ho in tissue is delivered within 2.1 mm from the decay site. ^{166}Ho also emits γ radiation of 81 keV in 7 % of the decays and is paramagnetic, allowing imaging of its location in a patient both by gamma imaging and magnetic resonance imaging.¹⁷ ^{166}Ho has a half-life of 26.824 hours,^{18, 30} which means that ^{166}Ho allows some time for logistics, while after 8 days still more than 99 % has decayed. ^{166}Ho decays to the stable erbium-166, or ^{166}Er , and can be produced in two ways. Figure 2.1 shows these two ways and the decay of ^{166}Ho .¹⁷

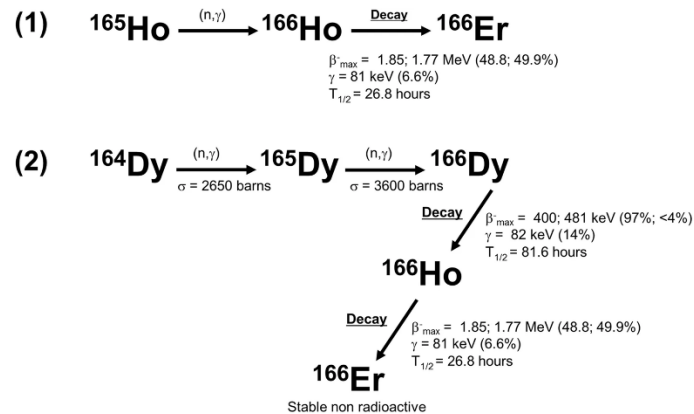


Figure 2.1. Schematic representation of the direct ^{166}Ho production route (1) and the indirect ^{166}Ho production route (2). Adapted from Klaassen et al.¹⁷

In the direct route (figure 2.1 - (1)), ^{166}Ho is irradiated with neutrons, causing (n, γ) neutron capture reactions that will create ^{166}Ho . In the indirect route (figure 2.1 - (2)), dysprosium-164, or ^{164}Dy , is irradiated with neutrons, causing (n, γ) neutron capture reactions that create ^{165}Dy , which in their turn can capture neutrons in a (n, γ) reaction, creating ^{166}Dy . This ^{166}Dy then decays to ^{166}Ho by β^- decay. Due to the 100% natural abundancy of ^{165}Ho , the direct production route, (n, γ) can produce ^{166}Ho with a limited amount of by-products, of which the mostly produced is $^{166\text{m}}\text{Ho}$.³⁰ However, the neutron capture cross-section of ^{165}Ho for creation of ^{166}Ho is 58 barn, while the neutron capture cross-section for creation of $^{166\text{m}}\text{Ho}$ is 3.1 barn.³⁰ The produced activity of $^{166\text{m}}\text{Ho}$ is even $7 \cdot 10^6$ times lower than the produced activity of ^{166}Ho at the start of irradiation, due to the 1133 year half-life of $^{166\text{m}}\text{Ho}$.³⁰

^{166}Ho is a good candidate for targeted radionuclide therapy. However, a large problem is that the specific activity of ^{166}Ho possibly produced in a nuclear reactor via the direct production route is limited, since after some time the rate of decaying ^{166}Ho in a reactor will equal the ^{166}Ho production rate.¹¹

Chelators, which are molecules able to bind metal ions, are useful target compounds in the Szilard-Chalmers method. A chelator expected to be suitable for the Szilard-Chalmers separation of ^{166}Ho and ^{165}Ho in a continuous flow Szilard-Chalmers system is DOTA, also called tetraacetan or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid.^{21, 31} With all carboxyl groups fully protonated, the H_4DOTA molecule looks as shown in figure 2.2.³²

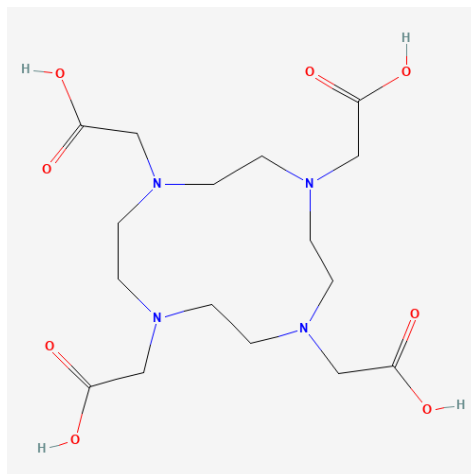
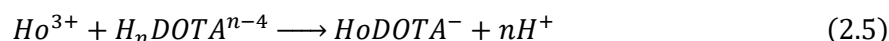


Figure 2.2. Depiction of the chemical structure of the fully protonated H_4DOTA molecule.³²

DOTA is known to make strong and stable complexes with trivalent lanthanoids, such as holmium.^{29, 33} The chemical reaction of the chelation, or labelling, of Ho and DOTA is given in equation 2.5.



The resulting complex of a trivalent lanthanoid bound to DOTA^{4-} is shown in figure 2.3.³⁴

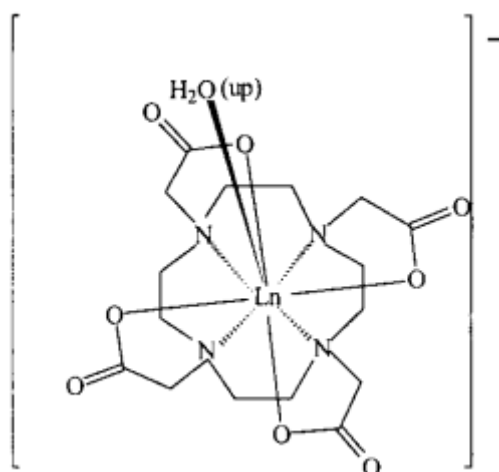


Figure 2.3. Depiction of the chemical structure of the complex resulting from chelation of DOTA with a trivalent lanthanoid.³⁴

The formations of most of these lanthanoid-DOTA complexes have high equilibrium constants with $\log(K)$ between 20 and 25.^{33, 35} From these equilibrium constants, the Gibbs free energy of these bonds can be calculated using equation 2.6.²⁸

$$\Delta G = R * T * \log(K) * \ln(10) \quad (2.6)$$

Here ΔG is the change in Gibbs free energy in J mol^{-1} , R is the ideal gas constant in $\text{J K}^{-1} \text{mol}^{-1}$ and the K is the equilibrium constant, which is unitless. With $R=8.3 \text{ J K}^{-1} \text{mol}^{-1}$, $T=293 \text{ K}$ and $\log(K)=24$, we find a Gibbs free energy change of $1.3 \cdot 10^5 \text{ J mol}^{-1}$ or 1.4 eV per complex.²⁸ From equation 2.4 it follows that the recoil energy needed to break a chemical bond of this strength, can be given by a single prompt gamma photon of 0.65 MeV . In figure 2.4 the prompt gamma emission spectrum from (n, γ) neutron capture by ^{165}Ho is shown.³⁶

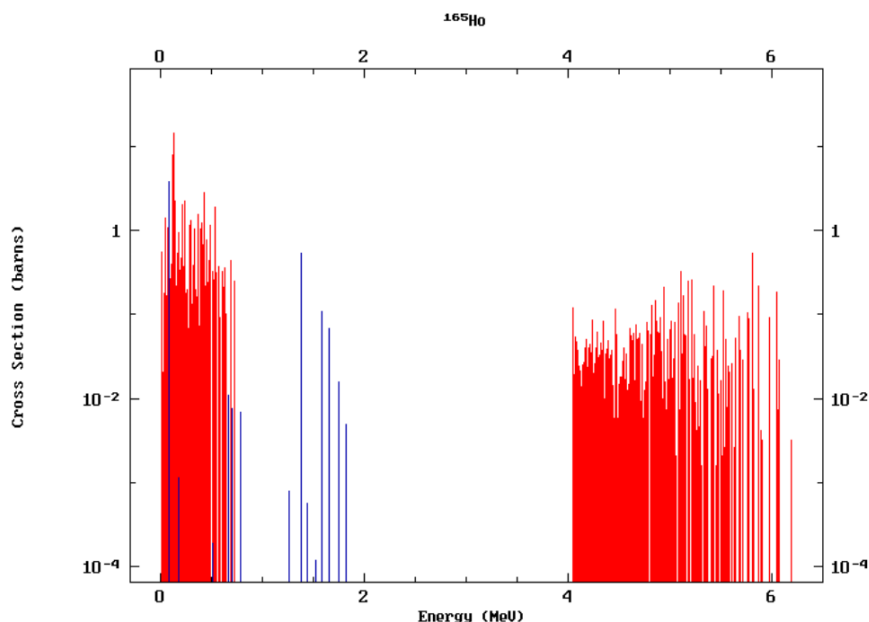


Figure 2.4. Graph showing the prompt gamma emission in neutron capture by ^{165}Ho . The x-axis shows the photon energy in MeV and the y-axis shows the cross-section of the emission in barn in neutron irradiation. (Red lines indicate emissions from p-levels and blue lines indicate emissions from d-levels.)³⁶

Figure 2.4 shows that ^{166}Ho does emit prompt gammas that have enough energy to break the bond with DOTA. Therefore the HoDOTA^- complex could be used for the Szilard-Chalmers method. Important to take into account is that due to multiple simultaneous prompt gamma emissions, recoil energy gained from these photons can add up or cancel out.

Another property of HoDOTA^- that is very useful in Szilard-Chalmers separation is the slow kinetics of the complex, since this will also give slow relabelling after a Szilard-Chalmers reaction.¹⁴ The importance of this slow relabelling will be explained more elaborately in the subchapter “subprocesses”. Lastly, since DOTA contains only C, N, H and O atoms, which have low neutron capture cross-sections, HoDOTA^- irradiation gives few radioactive by-products.³⁰

2.3 Continuous flow Szilard-Chalmers system

The achieved specific activity of the Szilard-Chalmers separation decreases with irradiation time.²¹ This is due to processes like radiolysis, relabelling and isotope-exchange during irradiation,²¹ which will be explained more elaborately in subsection 2.4. A short irradiation time is therefore desired for high specific activity.²¹ However, in bulk production, where a radionuclide is inserted into the core and taken out after a certain amount of time, a short irradiation time also means a low production yield. A continuous flow Szilard-Chalmers system could be a solution to this problem. By continuously irradiating target isotopes and separating produced radioactive isotopes in a continuous flow system,

a high production yield can be achieved. Moreover, the time between formation and separation of the radioactive isotopes can still be short and therefore the processes that reduce the achieved specific activity can occur for a shorter amount of time. Figure 2.5 shows a schematic representation of a possible continuous flow Szilard-Chalmers system for production of ^{166}Ho using DOTA as a target compound.

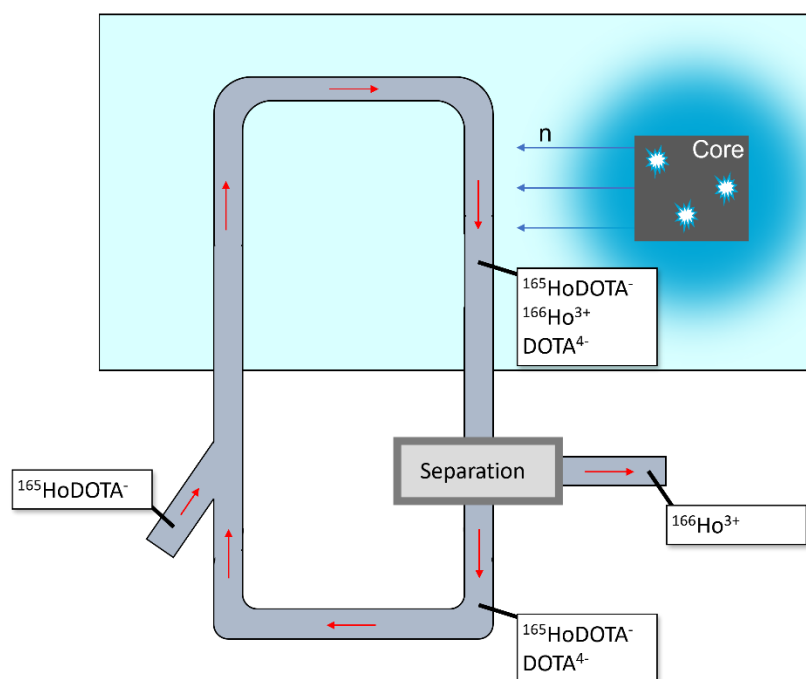


Figure 2.5. Schematic representation of a possible continuous flow Szilard-Chalmers system for the production of ^{166}Ho from ^{165}Ho with the Szilard-Chalmers method using DOTA as a target compound.

This example system shown in figure 2.5 starts out with a solution containing $^{165}\text{Ho}^{3+}$ ions bound to the DOTA^{4-} target compound. Then this solution is passed by the reactor core, where the Szilard-Chalmers reactions can take place. In these reactions some $^{165}\text{Ho}^{3+}$ ions in the $^{165}\text{HoDOTA}^-$ complexes capture a neutron, emit a prompt gamma and break their bond to the DOTA molecule. After some time near the core, the resulting mixture of $^{166}\text{Ho}^{3+}$, DOTA^{4-} and $^{165}\text{HoDOTA}^-$ is chemically separated by a continuous flow separation method. One of the resulting separated solutions is a mixture of $^{165}\text{HoDOTA}^-$ and DOTA^{4-} . The other is a solution of the desired high specific activity $^{166}\text{Ho}^{3+}$.

Continuous flow Szilard-Chalmers systems can in potential be used to increase the specific activity for many radioactive nuclides that are produced by (n, γ) reactions, as long as a target compound can be found:

- To which the target isotope is stably bound
- From which the formed radioactive nuclide can break free upon its formation
- With which the formed radioactive nuclide does not recombine quickly after the Szilard-Chalmers reaction.
- For which a continuous flow separation method is available that separates the bound target nuclide from the unbound radioactive product isotope.^{22, 37}

The amount of free target isotopes in a continuous flow Szilard-Chalmers system after formation of the target compound is given by equation 2.7.

$$F_{tar} = (1 - B) * N \quad (2.7)$$

Here F_{tar} is the amount of free target isotopes, B is the fraction of target isotopes incorporated in the target compound and N is the total amount of target isotopes. Then the amounts of free target isotopes and free product isotopes in the system after irradiation are given by equation 2.8 and 2.9 respectively. Here the amount of target isotopes is assumed to be much larger than that of the product isotopes.

$$F_{tar} = (1 - B) * N + \mu_{rad} * B * N = (1 - B + \mu_{rad} * B) * N \quad (2.8)$$

$$F_{pro} = (1 - B + \mu_{rad} * B) * N * \mu_{cap} + (N - (1 - B + \mu_{rad} * B) * N) * \mu_{Sz} \quad (2.9)$$

Here F_{pro} is the amount of free product isotopes, μ_{rad} is the fraction of radiolysed target compounds, μ_{cap} is the chance for a target isotope to undergo an (n, γ) reaction and μ_{Sz} is the chance for a target isotope incorporated in the target compound to undergo a successful Szilard-Chalmers reaction, in which the complexed target isotope undergoes a (n, γ) neutron capture, due to which the product isotope escapes the complex. The amounts of free target isotopes and free product isotopes after relabelling and isotope exchange are given by equation 2.10 and 2.11 respectively.

$$F_{tar} = (1 - B + \mu_{rad} * B) * N * \mu_{non-rel} \quad (2.10)$$

$$F_{pro} = \left((1 - B + \mu_{rad} * B) * N * \mu_{cap} + (N - (1 - B + \mu_{rad} * B) * N) * \mu_{Sz} \right) * \mu_{non-rel} * \mu_{non-iso} \quad (2.11)$$

Here $\mu_{non-rel}$ is the chance for a product nuclide to not undergo relabelling and $\mu_{non-iso}$ is the chance for a product nuclide to not undergo isotope-exchange. The ratio between free target and free product isotopes, which is proportional to the specific activity of the free product isotope, is then given by equation 2.12.

$$\frac{F_{pro}}{F_{tar}} = \mu_{cap} * \mu_{non-iso} + \frac{B - \mu_{rad} * B}{1 - B + \mu_{rad} * B} * \mu_{Sz} * \mu_{non-iso} \quad (2.12)$$

Since isotopes have the same chemical properties, chemical separation of free isotopes from the target compound does not alter the achieved specific activity.

2.4 Subprocesses

This subchapter will explain in more detail the subprocesses expected to be involved in the production of high specific activity radioactive isotopes using a continuous flow Szilard-Chalmers system.

2.4.1 Labelling

Before Szilard-Chalmers reactions are possible, the target isotope is bound to a target compound. This reaction is called labelling. High target isotope labelling efficiency, which is the percentage of the target isotope that is labelled to the target compound, is important in order for the Szilard-Chalmers method to yield a high specific activity. When a target isotope is not bound to a target compound, it will remain a free isotope. These remaining free target isotopes will mix with the radioactive free isotopes coming from the Szilard-Chalmers reactions during irradiation. As separation of the free isotopes is not possible, these remaining free target isotopes decrease the ratio between radioactive and target isotopes and thus the specific activity of the produced radioactive isotope.

When using ^{165}Ho as target isotope and DOTA as target compound, the labelling reaction is given by equation 2.5. The efficiency of the labelling reaction is dependent on multiple factors such as reaction time, temperature and pH.^{31, 38} In Figure 2.6a, the achieved labelling efficiency of Ho in a 1:3 Ho:DOTA molar ratio is shown as a function of the pH at which the labelling occurs.³⁹

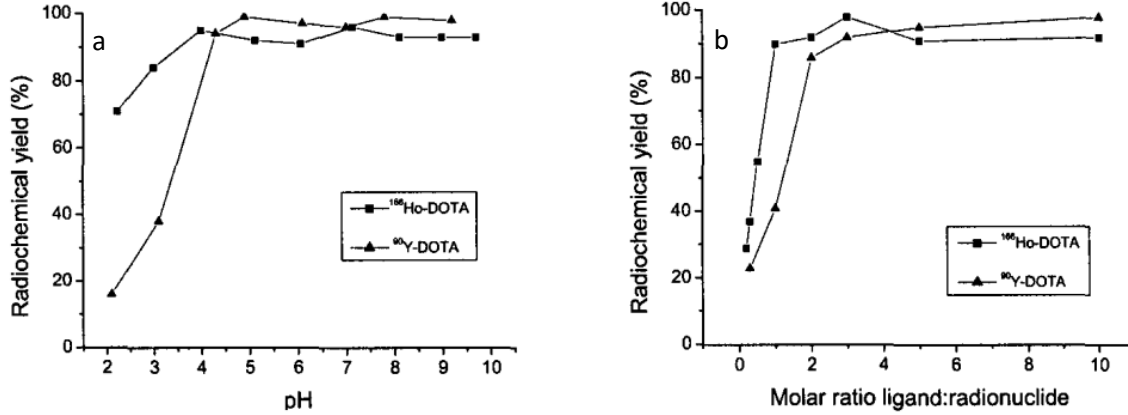


Figure 2.6. Graphs showing the labelling efficiency of ^{166}Ho and ^{90}Y to DOTA after 1 hour of labelling for various pH at a 3:1 DOTA:radionuclide ratio (a) and for various DOTA:radionuclide ratios at a pH of 5.5 ± 0.2 (b).³⁹

The figure shows that for a pH of 4 and above, more than 90% of the ^{166}Ho is labelled and below a pH of 4 this labelling percentage drops. Therefore labelling Ho to DOTA is done best at a pH of 4 or higher. Figure 2.6b shows that Ho-DOTA labelling with more than 90% of the Ho labelled at pH 5.5 is achieved, when the molar Ho:DOTA ratio is 1:1 or lower.³⁹ In order to minimize relabelling of Ho to the target compound, the amount of unlabelled DOTA should be minimised.

2.4.2 Irradiation

During irradiation of the target isotopes incorporated in the target compound, two main processes occur: Neutron capture, potentially resulting in Szilard-Chalmers reactions, and radiolysis. In (n, γ) neutron capture, an isotope, the target isotope, will capture neutrons from neutron irradiation, changing the nucleus into an isotope with one more neutron in the nucleus, the product nuclide. The specific activity of the product nuclide is given by equation 2.13.^{16, 40}

$$SA = \frac{N_a * \sigma * \phi}{M} * (1 - e^{-\lambda t}) \quad (2.13)$$

In this equation SA is the specific activity in Bq g⁻¹, N_a is Avogadro's constant in atoms mol⁻¹, σ is the (n, γ) neutron capture cross-section in cm², ϕ is the neutron flux in neutrons cm⁻² s⁻¹, M is the molar mass of the target nuclide in g mol⁻¹, λ is the decay constant in s⁻¹ and t is the irradiation time in s. For equation 2.13 the assumptions are made that the target isotope comprises 100% of the nuclides of that element present at $t=0$ and that the depletion of the target nuclide is negligible. For $t \rightarrow \infty$, equation 2.13 reduces to equation 2.14.¹⁶

$$SA = \frac{N_a * \sigma * \phi}{M} \quad (2.14)$$

Equation 2.14 shows that the maximum produced specific activity is limited by the neutron capture cross-section, molar mass, decay constant and neutron flux. The former three are constant for the used isotope and the latter is limited by the used production reactor.

After neutron capture, the Szilard-Chalmers reactions take place as explained in the subchapter “Szilard-Chalmers method”. Important to notice is that not every neutron capture will result in a successful Szilard-Chalmers reaction, in which the radioactive nuclide breaks its bond to the target compound. The reason for this is the many different ways in which prompt gamma emissions can take place. For example some prompt gamma emissions do not give enough recoil energy for a bond rupture or some opposite prompt gamma emissions cancel each other’s delivered recoil energy.¹³ A produced radioactive isotope that remains bound to the target compound is not separable from the target isotopes. Therefore such unsuccessful Szilard-Chalmers reactions decrease both the achieved specific activity and the production yield. Figure 2.7 shows the many possible deexcitation emission of the lowest 40 energy levels of the ^{166}Ho nucleus as an illustration on the many ways of prompt gamma emissions via which a nucleus can deexcite.¹⁸

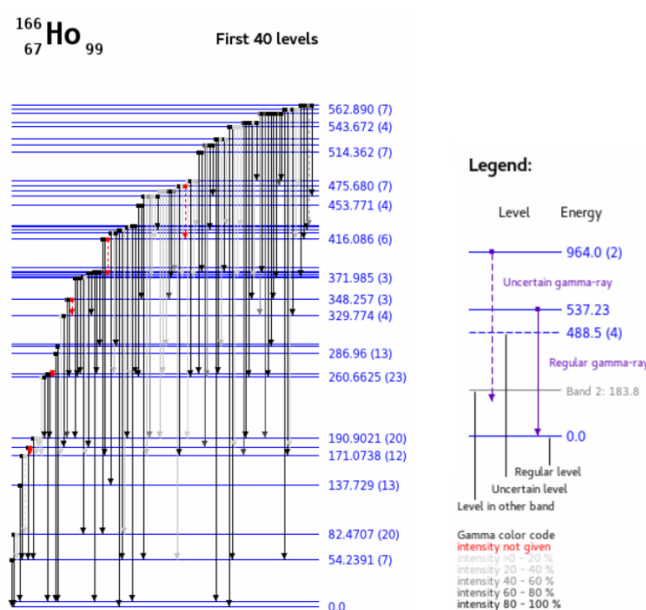


Figure 2.7. Scheme showing the deexcitations possible from the 40 lowest energy levels of ^{166}Ho . Energies are shown in keV.¹⁸

At the same time during irradiation as the neutron capture, radiolysis of the target compound can occur.⁴¹ In this process both gamma and neutron radiation coming from the fission reactions in the reactor core can cause the chemical bonds that constitute the target isotope-compound complex to break. This can be the bonds between the isotope and the compound, as well as the bonds within the compound. When these bonds are broken, the target isotope can be separated from the compound. This means that, next to the Szilard-Chalmers reactions, there is another way that isotopes can escape the target compound during irradiation. However, in the escape caused by radiolysis, the isotope has not captured a neutron and therefore a target isotope is freed from the target compound. The addition of these free target isotopes to the product isotopes freed by the Szilard-Chalmers reactions, lowers the produced specific activity of the radioactive isotope.^{22, 42}

Neutrons will have little interaction with the target isotope and compound directly, besides the neutron capture, as they have little interaction with charged particles. However, the interactions neutrons do have, even in solvent, can create charged particles that can more easily interact with the target isotope and compound. Therefore also neutron irradiation can cause radiolysis.⁴¹

Assuming the neutron flux, the delivered gamma dose and the radiolysis rate all scale linearly to each other, equations 2.15 gives the radiolysis rate of an irradiation target.

$$-\frac{dN}{dt} = k_r * N * \phi \quad (2.15)$$

Here N is the amount of the target, k_r is a radiolysis constant in cm^2 and ϕ is the neutron flux in $\text{cm}^{-2} \text{s}^{-1}$. From equation 2.15, equation 2.16 can be found. Because of the assumption of a linearly scaling neutron flux and gamma irradiation dose, both effects can be accounted for in the radiolysis constant. The radiolysis constant given by equation 2.16 is therefore a neutron cross-section for radiolysis by gamma and neutron radiation combined.

$$k_r = \frac{\ln(F)}{\phi * t} \quad (2.16)$$

Here F is the fraction of the original amount of compound that is not radiolysed after irradiation and t is the irradiation time in s.

Figure 2.8 shows how much radioactive lutetium, Lu, is freed up from the DOTA chelator by radiolysis after a certain gamma irradiation dose.³¹ As Lu and Ho are both trivalent lanthanoids with similar atomic radius, it is expected that for the HoDOTA⁻ complex a similar behaviour of complex radiolysis after these radiation doses occurs.⁴³

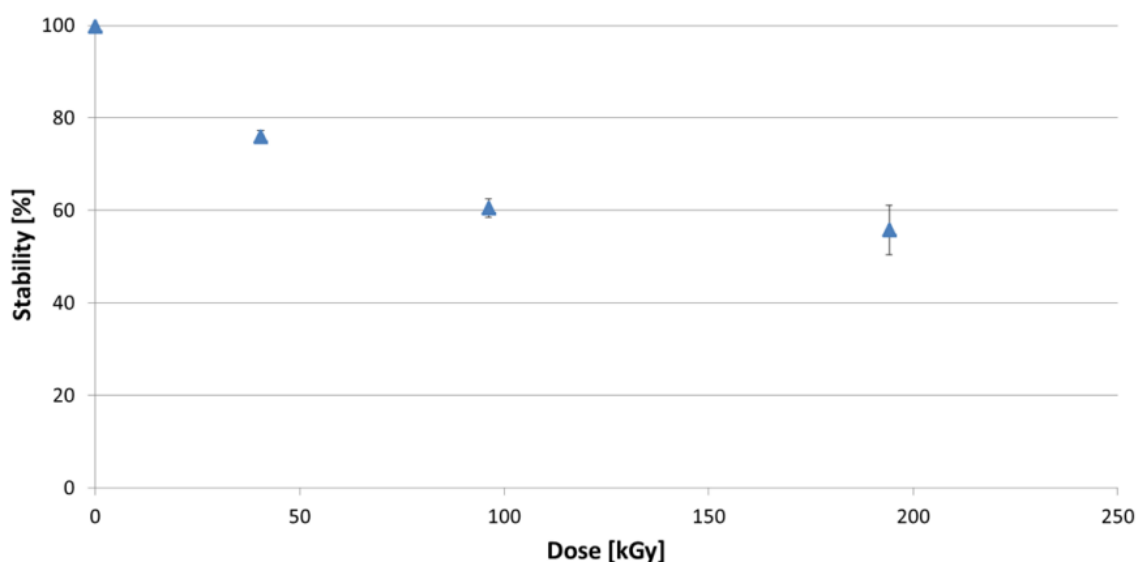


Figure 2.8. Graph showing the stability of the LuDOTA⁻ complex by showing the percentage of LuDOTA⁻ that was not radiolysed after a certain gamma radiation dose shown in kGy.³¹

2.4.3 Transport

In the time between irradiation and separation, two important reactions are expected to occur that influence the radionuclide production yield and specific activity. The first reaction is relabelling.^{20, 21} In relabelling a reaction occurs where freed isotopes, both radioactive and stable, bind to a free target compound again.²² This is again the reaction of equation 2.5. After rebinding to the target compound, the isotope will not end up with the free isotopes after separation. As isotopes share their chemical properties, both the radioactive and stable isotopes will undergo relabelling in the same relative amount. Therefore this process would decrease the amount of free ions yielded by a continuous flow Szilard-Chalmers system, but it does not influence the achieved specific activity.

The second reaction that would influence the production of radioactive isotopes is isotope-exchange.²¹ In isotope-exchange a complexed isotope switches place with a different free isotope as in reaction 2.17.⁴⁴



As ^{165}Ho and ^{166}Ho are isotopes and therefore have the same chemical properties, thermodynamics will predict that the $^{166}\text{Ho}/^{165}\text{Ho}$ ratio will be the same in both labelled and free form after equilibration.²⁸ This gives the important result that when free ^{166}Ho is created after irradiation, i.e. the $^{166}\text{Ho}^{3+}/^{165}\text{Ho}^{3+}$ ratio is increased, the isotope-exchange reaction will decrease the specific activity of the free radioactive isotope until a new equilibrium is reached.²⁸

2.4.4 Separation

The separation of free isotopes from the target compound and the target isotope-compound complexes can be done via ion exchange chromatography. In this separation method a solution containing ions or charged molecules is run through an ion exchange resin and the contents of the solution are separated based on their attraction to the resin. The ion exchange resin is built up from a structure that contains certain functional groups that attract ions or charged molecules. Depending on the resin, it could attract negative or positive charge.⁴⁵ Figure 2.9 shows a schematic representation of an ion exchange separation. When a solution containing neutral, negatively charged and positively charged ions or molecules is sank through a resin that attracts positive charge, a cation exchange resin, the resin will bind the positively charged ions or molecules. Meanwhile the neutral and negatively charged ions or molecules sink completely through the resin with the solvent. This way the positively charged ions and molecules are separated from the neutral and negatively charged ones. In order to obtain the positively charged ions or molecules from the resin, a solution, the eluent, is run through the resin containing a substance that binds stronger to the resin than the desired positively charged ions and molecules. This could for example be a solution with a high concentration of positively charged ions, such as a concentrated acid. The stronger binding substance takes over the binding to the functional group of the resin that the positively charged ions and molecules have and the positively charged ions and molecules are therefore flushed, or eluted, from the resin.⁴⁶ The solution exiting the column is called the eluate. Resins attracting negative charge, anion exchange resins, work the same, but separate negatively charged ions and molecules from neutral and positively charged ones.⁴⁷

Inefficient extraction of free radioactive isotopes from a continuous flow Szilard-Chalmers system will leave the radioactive isotopes in the system. This means high separation efficiency is important for a high production yield.²¹ Extraction of labelled target compound with the extraction of free isotopes causes the achieved specific activity to be lowered, since the specific activity of the labelled target compound is expected to be lower than that of the free isotopes.

As DOTA^{4-} and HoDOTA^- have a negative charge and free Ho^{3+} ions have a positive charge, the positive attracting cation exchange resins can be used to separate the free ions from the DOTA^{4-} and HoDOTA^- .³⁴

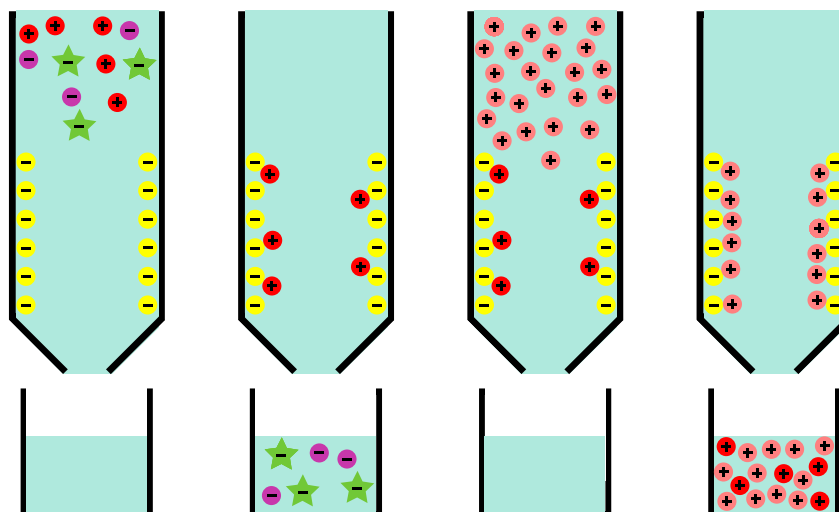


Figure 2.9. Schematic representation of a cation exchange separation. From left to right there is sample loading, elution of the components of the sample that do not bind to the resin, addition of a strong eluent and elution of the components of the sample that do bind to the resin.

2.5 Thin layer chromatography

Another method for separation of free isotopes from target compounds and target isotope-compound complexes is through thin layer chromatography (TLC). Figure 2.10 shows a schematic representation of the TLC process. In thin layer chromatography substances mixed in a solution are separated by their differences in affinity to certain materials and solvents. Use is made of a stationary phase that is a thin layer of absorbent material and a mobile phase that is a solvent. First, the solution containing the desired material is applied close to the bottom of a piece of the stationary phase. Then the stationary phase is placed vertically in the mobile phase solvent, which is called the eluent. It is placed in such a way that the applied solution is at the bottom of the stationary phase, but above the eluent. Then the eluent will move up on the stationary phase by capillary forces and drag the different components in the sample solution with it. The distance a component is displaced upwards from the point of application by the eluent depends on the affinity of the component for the mobile and the stationary phase. Just before the mobile phase reaches the top of the stationary phase, the stationary phase is taken out of the eluent and different components with various affinities will have travelled a different distance on the stationary phase and are in that way separated.⁴⁸ Thin layer chromatography is very useful both in qualitative and quantitative analysis of the contents of a solution.

A special kind of thin layer chromatography is instant thin layer chromatography (iTLC). Instant thin layer chromatography makes use of a silica gel impregnated microfiber stationary phase instead of aluminium, polymer or glass with an absorbent coating, as used in conventional TLC. This allows for higher resolution, easier cutting of the stationary phase and quicker movement of the mobile phase, which reduces the time it takes to perform the separation.^{49, 50}

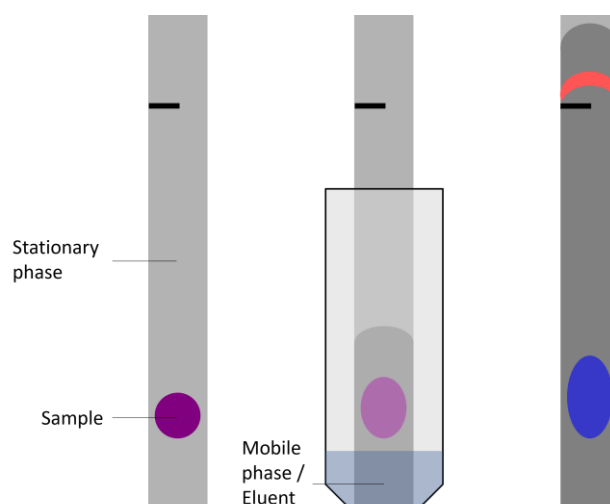


Figure 2.10. Schematic representation of thin layer chromatography.

2.6 Measurement devices

2.6.1 Phosphor imager

A phosphor imager performs phosphor imaging in order to map radiation intensities on a surface. This is done by pressing a radioactive sample surface against a phosphor imaging plate for a certain amount of time. During this time, phosphor atoms in the plate, which are irradiated by the sample surface, are excited. Then the sample surface is removed from the plate and the phosphor plate is measured by a scanner. By stimulated emission of the energy stored in the phosphor plate, the scanner can count the amount of phosphor atoms that have been excited on all locations on the surface. The resulting image is a map of the radioactivity distribution of the sample surface.⁵¹ Even though the created map gives a clear picture of the radiation distribution on the strip, quantitative analysis using the phosphor imager is difficult due to incoherent background signal.

2.6.2 Wallac gamma counter

A gamma counter measures the amount of gammas of a certain energy range coming from a sample. The Wallac gamma counter does so by use of a NaI:TI scintillation detector. The Wallac will have different detection efficiencies for different emitted gamma energies. As a result, only measurements of the same isotope can be compared to each other. The position of samples in a well detector such as the Wallac gamma counter is of slight influence on the counting results, therefore the position of the measured samples need to be the same for the best sample comparisons.⁵² Due to high detection efficiency and easy background measurement, by measuring an empty sample, a Wallac measurement is well suited for quantitative analysis. Especially at low emitted photon energies self-absorption should be accounted for when measuring with the Wallac gamma counter.⁵³ Self-absorption is the absorption of radiation in a sample, which was emitted by the sample itself.

2.6.3 ICP-OES

An ICP-OES performs inductively coupled plasma optical emission spectroscopy. In this measurement method a plasma is created that excites the atoms in a sample. These atoms will then emit photons with wavelengths characteristic for a specific element. When the intensities of the photons of these characteristic wavelengths are measured and compared to calibration standards, the contents of the sample can be determined quantitatively.⁵⁴

2.6.4 Germanium detector

A germanium detector can perform activity measurements. When ionizing radiation interacts with the semiconductor in the detector, electron-hole pairs are created, which are measured by the detector as a pulse with a magnitude proportional to the energy of the radiation. As nuclides have known characteristic energies and intensities of decay radiation, the amount of measured pulses at these characteristic energies can be used to calculate the activity of a sample.⁵⁵

3. Materials & methods

This chapter first explains what materials were used in the performed experiments. Then it describes how instant thin layer chromatography was performed, since it was used in the same way in many of the performed experiments. Then this chapter explains the experiments on the subprocesses expected to play part in the continuous flow Szilard-Chalmers system.

3.1 Materials

The materials used in this work are shown in table 3.1.

Table 3.1. List of the materials used in the experiments of this thesis.

Used material	Product name	Supplier
iTLC paper	- iTLC-SG	Agilent
MQ	- Ultrapure water	Merck Milli-Q Advantage A10
5 % acetonitrile (v/v %)	- Acetonitrile CHROMATOSOLV, for HPLC, for UV, >99.9% - Ultrapure water	Honeywell Riedel-de Haën Merck Milli-Q Advantage A10
Acetic acid buffer	- Acetic acid, Puriss. p.a., ACS Reagent, Reag. ISO, Reag. Ph. Eur., ≥99.8% - Sodium Acetate Anhydrous >99%, FCC, FG - Ultrapure water	Honeywell Fluka Sigma-Aldrich Merck Milli-Q Advantage A10
HoCl ₃	- Holmium chloride hexahydrate 99.9% trace metals basis	Sigma-Aldrich
HCl solution	- Hydrochloric acid. app. 30%, techn. - Ultrapure water	Boom Merck Milli-Q Advantage A10
DOTA	- 2,2',2'',2'''-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl) tetraacetic acid	CheMatech
DOWEX cation exchange resin	- Dowex 50W X8 hydrogen form, strongly acidic, 200-400 mesh	Supelco.
pH paper	pH indicator paper, 1 to 14	VWR Chemicals
NaOH	- Sodium hydroxide puriss. p.a., ACS reagent, reag. Ph. Eur., K ≤0.02%, ≥98%, pellets	Sigma-Aldrich

3.2 Devices

The devices used in this thesis are shown in table 3.2.

Table 3.2. List of the devices used in the experiments of this thesis.

Used device	Product name	Manufacturer
Phosphor imager	Typhoon Trio Variable Mode Imager	GE Healthcare
Phosphor plate	BAS Imaging Plate MS	Fujifilms
Eraser	Amersham Eraser	GE Healthcare
Wallac	2480 Wizard Automatic Gamma Counter	Perkin Elmer
Thermo shaker	PHMT PSC24N Thermo Shaker	Grant Instruments
Glass column	Glass Econo-Column (0.7x15cm)	Bio-Rad Laboratories
ICP-OES	Optima 8000 ICP-OES	Perkin Elmer
Germanium detector	Well-type Ge(Li) Detector	Canberra Industries

3.3 Instant thin layer chromatography

In order to perform the instant thin layer chromatography separation in the done experiments, first a sheet of silica gel iTLC paper was cut in pieces of 15x0.8 cm. Then 5 μL of the solution that was analysed, was applied 2 cm above the bottom of the resulting strip. The applied sample liquid was left to dry for a time span between 20 and 60 minutes. After this drying time, the strip was put vertically in a 5 % acetonitrile eluent (v/v %),^{31, 56} where the volume of the eluent was such that it did not submerge the strip more than 1 cm. After 9 minutes placed in the eluent, the eluent would be near the top of the strip. At this moment the strip was taken out of the eluent. After the strip was taken out of the eluent, it was left to dry for 30 minutes. Separation of radioactive isotopes on the strip was then analysed using the phosphor imager and the Wallac. For phosphor imaging, first the phosphor plate was erased for at least 10 minutes using the eraser. Then the strips were pressed against the erased phosphor plate for at least 15 minutes, after which the plate was scanned in the phosphor imager. The resulting matrix of count data was then analysed using Matlab R2021b to form a radioactivity distribution map. For the Wallac measurement the strips were cut in two pieces at a height of 11 cm. Both this top and bottom parts were then put in a separate scintillation vial and measured. As the HoDOTA^- would have moved to the top of the strip and the Ho^{3+} would have stayed at the bottom, the percentage of ^{166}Ho labelling could be calculated by equation 3.1.

$$L = \frac{C_{top}}{C_{bottom} + C_{top}} * 100 \quad (3.1)$$

Here L is the percentage of ^{166}Ho that is labelled to DOTA. C_{top} and C_{bottom} are the amount of counts measured from the top and the bottom of the strip respectively.³¹

3.4 Determining the ^{166}Ho -DOTA labelling efficiency at different incubation times

Approximately 25 μL of a $^{165}\text{HoCl}_3$ 10 mM in pH2 HCl solution containing 15-30 kBq ^{166}Ho was added to an Eppendorf vial containing 120 μL acetic acid buffer 1 M solution at pH4.2. Then the resulting solution was left on the thermo shaker for 5 minutes at 300 rpm at either 25, 40 or 80 $^{\circ}\text{C}$, depending on what reaction temperature was investigated. After these 5 minutes, 25 μL of DOTA 10 mM in pH2 HCl was added to the Eppendorf vial, so the $\text{Ho}:\text{DOTA}$ molar ratio in the Eppendorf was 1:1. After addition of the DOTA, the Eppendorf was immediately put back in the thermo shaker at 300 rpm and the same temperature as before. For the 40 and 80 $^{\circ}\text{C}$ experiments, 1, 2, 4, 8 and 16 minutes after

addition of the DOTA to the Eppendorf, 5 μL of the Eppendorf was taken and analysed using the iTLC procedure as explained in subsection 3.3. For the 25 °C reaction this was done after 1, 4, 16, 64 and 180 minutes.

The 25 and 40 °C measurements were done in quadruplo and the 80 °C measurement was done in triplo. The standard deviations of the means were calculated using equation 3.2.⁵⁷

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (3.2)$$

Here s is the standard deviation, n is the number of data points, x_i is the i^{th} data point and $\bar{x}$ is the average of the data set of length n .

3.5 Determining the amount of isotope-exchange and labelling by $^{166}\text{Ho}^{3+}$ in a solution with near 100% of the DOTA labelled to Ho

First 35 μL DOTA 10 mM in a pH2 HCl solution and 15 μL HoCl_3 in a pH2 HCl solution were added to an Eppendorf vial containing 120 μL acetic acid buffer 1 M at a pH of 4.2. The concentration of the added HoCl_3 was such that the added amount of Ho resulted in a molar Ho:DOTA ratio of either 6:5 or 2:1 after addition of the $^{166}\text{HoCl}_3$ containing solution, which was done later. This vial was then put in a thermo shaker for 20 minutes at 60 °C and 300 rpm. After these 20 minutes, the vials were either heated to 80 °C or left to cool to 40 °C in the thermo shaker. When the vials had reached the desired temperature, 5 μL of a solution containing radioactive $^{166}\text{HoCl}_3$ in pH2 HCl was added to the vial. The amount of added Ho in this 5 μL was such that the added activity was more than 15 kBq. Immediately after addition of the $^{166}\text{HoCl}_3$, the created sample vial was put back in the thermo shaker at 300 rpm and the same temperature as before the addition of the $^{166}\text{HoCl}_3$. After 1, 4 and 16 minutes in the thermo shaker, 5 μL was taken from the vial and each was pipetted on a different iTLC strip. These strips were then measured as explained in subsection 3.3.

As a check on the results, a control was added to the experiment to check whether a near 100% DOTA labelling efficiency was achieved by the 20 minutes at 60 °C. This control contained a Ho:DOTA ratio higher than 1:1. The contents of this vial were 120 μL acetic acid buffer 1 M at a pH of 4.2, 35 μL DOTA 10 mM in pH2 HCl, 10 μL HoCl_3 in pH2 HCl and 5 μL of the same $^{166}\text{HoCl}_3$ containing solution as used for the sample. The concentration of the added 10 μL HoCl_3 solution was chosen such that the total of the Ho added to this control was equal to the amount of Ho present in the sample before addition of the $^{166}\text{HoCl}_3$ containing solution. Only after 16 minutes in the thermo shaker, 5 μL was taken from the control and put on an iTLC strip. The percentage of ^{166}Ho labelled to DOTA was then measured as explained in subsection 3.3. The percentage of DOTA labelled to Ho was then calculated by equation 3.3.

$$L_{\text{DOTA}} = L_{^{166}\text{Ho}} * \frac{N_{\text{Ho}}}{N_{\text{DOTA}}} \quad (3.3)$$

Here L_{DOTA} is the percentage of DOTA labelled to Ho, $L_{^{166}\text{Ho}}$ is the measured percentage of ^{166}Ho labelled to DOTA, N_{Ho} is the amount of Ho added and N_{DOTA} is the amount of DOTA added.

The measurements on each temperature-ratio combination were done in quadruplo. The standard deviations of the means were again calculated using equation 3.2.⁵⁷

3.6 Design of a cation exchange chromatography method for the separation of Ho^{3+} from DOTA^{4-} and HoDOTA^-

In order to perform separation of free Ho^{3+} from HoDOTA^- and DOTA^{4-} using ion exchange chromatography, first the separation column was prepared. This preparation started with activating the DOWEX ion exchange resin. For this an amount of MQ was mixed with the dry resin of more than three times the volume of the resin. The resulting mix was left overnight and then turned upside down several times for mixing. After the resin had sunk to the bottom again, 0.5 mL of the DOWEX cation exchange resin was added to a glass column with a filter which most liquids could pass, but the resin could not. Any abundant water on top of the resin was drained through the column. Then 3 mL of a 3 M HCl solution was passed through the resin.⁴⁶ Next a pH4.2 HCl solution was passed through the resin. The pH4.2 HCl elution was stopped when the solution eluate remained at pH4.2. This was verified by measurement with a pH paper. Usually 4 mL of pH4.2 HCl sufficed. After this column preparation, the sample solution could be loaded in the resin.⁵⁸ The solution of a new step was added as soon as the solution of the previous step had sunk into the resin entirely.⁵⁹

Three different eluents were tested for Ho^{3+} or HoDOTA^- elution, namely an acetic acid 1 M pH4.2, a pH4.2 HCl and a 6 M HCl solution. The contents of the samples used in the experiments with the various eluents are shown in table 3.3. The sample vials containing DOTA were put on the thermo shaker for at least an hour at 80 °C before loading the samples in the resin. The buffer eluent was not tested for a HoDOTA^- sample.

Table 3.3. The sample contents for the measurements on various sample-eluent combinations. No measurement was done on the HoDOTA^- -Acetic acid 1M pH4.2 combination.

Sample\Eluent	Acetic acid 1 M pH4.2	pH4.2 HCl	6 M HCl
Free Ho^{3+}	1.0 μmol HoCl_3 30-50 kBq $^{166}\text{Ho}^{3+}$ 0.30 mL 1 M acetic acid pH4.2 0.22 mL pH4.5 HCl	1.0 μmol HoCl_3 30-50 kBq $^{166}\text{Ho}^{3+}$ 0.42 mL pH4.3 HCl	1.0 μmol HoCl_3 30-50 kBq $^{166}\text{Ho}^{3+}$ 0.30 mL 1M acetic acid pH4.2 0.22 mL pH4.5 HCl
HoDOTA^-	-	1.0 μmol HoCl_3 30-50 kBq $^{166}\text{Ho}^{3+}$ 2.0 μmol DOTA 0.42 mL pH4.2 HCl	1.0 μmol HoCl_3 30-50 kBq $^{166}\text{Ho}^{3+}$ 2.0 μmol DOTA 0.42 mL pH4.2 HCl

From the samples given in table 3.3, 20 μL was taken out. After preparation of the column as described before, the rest of the sample was added to the column. The sample was passed through the resin until it had completely sunk into the resin. A scintillation vial collected all of the solution that was exiting the column during this passing. Then 0.5 mL of the tested eluent was added to the column and as soon as this 0.5 mL had entirely sunk into the resin, another 0.5 mL was added. This was repeated until 0.5 mL had been added 12 times. Moreover, every time a new 0.5 mL of eluent was added to the column, the vial collecting the eluate was changed. Then the resin was washed out of the glass column with MQ and collected in another scintillation vial. Then vials collected after 6 M HCl elution were diluted by addition of 3 mL MQ. Lastly, all collected vials were measured using the Wallac gamma counter, where gamma emissions in the range of 65-95 keV were measured for 2 minutes. Normalisation of the number of counts measured in 1 minute was done by dividing the measured counts per minute values in the 14 collected vials of a separation by the sum of the counts per minute values of those 14 vials.

3.7 Determining the specific activity achieved by the Szilard-Chalmers method in irradiation of a dried HoDOTA⁻ sample

First 20 μL of NaOH 10.0 mM was added to an Eppendorf vial containing 100 μL HoCl₃ 10 mM in a pH4.2 HCl solution. Then 100 μL DOTA 10.0 mM in a pH4.2 HCl solution was added to the vial and the closed vial was put in a thermo shaker for 30 minutes at 300 rpm and 80 °C. After these 30 minutes, the cap was removed from the vial and the vial was left on the thermo shaker at 300 rpm and 80 °C for 17 to 18 hours. The resulting dried HoDOTA⁻ and by-product mix was then put in an irradiation cup and weighed. The cup and sample were then irradiated for 4 minutes in the BP3 irradiation facility of the HOR (Hoger Onderwijs Reactor) at the Delft University of Technology with a thermal neutron flux of $5 \pm 1 \cdot 10^{12} \text{ s}^{-1} \text{ m}^{-2}$ and a gamma dose rate of 265 kGy h⁻¹.⁶⁰ Unpacking of the irradiated sample was done 2 hours after irradiation had ended. After opening the cup, it was emerged in 1.5 mL of pH4.2 HCl in a scintillation vial and shaken for homogenization. Then 2 times 5 μL was taken from the vial and each was put on an iTLC strip. The strips were measured as described in subsection 3.3. The remaining sample solution was then added to an ion exchange column, which was prepared in the same way as the column was prepared in the ion exchange separation experiment. The solution exiting the column while passing the sample through the resin was collected in a scintillation vial. As soon as the sample solution had entirely sunk into the resin, 2 mL of a pH4.2 HCl solution was added to the column. As soon as this 2 mL had sunk entirely into the resin, another 2 mL of the pH4.2 HCl solution was added. This was repeated two more times and as soon as the fourth 2 mL had sunk into the resin, 2 mL of a 6 M HCl solution was added to the column. As this 6 M HCl solution had sunk into the resin entirely, another 2 mL 6 M HCl solution was added and this was also repeated two more times. After every addition of 2 mL pH4.2 or 6 M HCl solution, the eluate-collecting vial was renewed.

Then the remaining resin was washed out of the glass column with MQ and collected in another vial. Then MQ was added to every vial, until all contained 12 mL of solution. After dilution, the samples were measured in the Wallac in the 65-95 keV energy range for 2 minutes. The Ho concentrations in the 1st, 2nd and 6th vial, the vial of the sample loading, the first pH4.2 HCl elution and the first 6 M HCl elution respectively, were measured using the ICP-OES. This was done by measuring the photon emissions at wavelengths of 339.9, 345.6 and 347.4 nm.⁶¹ After that, the emission intensities were compared to a calibration line between 0.0 and 8.0 mg Ho per litre, in order to find the Ho concentrations in the measured vials.

As a control on the labelling efficiency before and after drying, 1 μmol of HoCl₃ in 101 $\pm$ 2 μL of a pH4.2 HCl solution containing 30-50 kBq of ¹⁶⁶Ho was added to an Eppendorf vial. Then 20 μL NaOH 10.0 mM was added. Next 100 μL of DOTA 10.0 mM in a pH4.2 HCl solution was added to the control vial and the control vial was put in the thermo shaker for 30 minutes at 300 rpm and 80 °C next to the sample vial. After these 30 minutes, 2 times 5 μL was taken from the control vial and each was put on an iTLC strip. The strips were then measured as described in subsection 3.3. The control vial was then put back on the thermo shaker without a cap at 300 rpm and 80 °C for 17 to 18 hours next to the sample vial. Just before unpacking of the irradiated sample vial, 200 μL of a pH4.2 HCl solution was added to the control vial. 5 minutes after this addition, 5 μL was taken from the vial and put on an iTLC strip. Then after unpacking the irradiated sample, approximately 30 minutes after the pH4.2 HCl addition, another 5 μL is taken from the control vial and put on an iTLC strip. These strips were also measured as described in subsection 3.3.

In order to determine the activity of the measured vials, the Wallac was calibrated for the ^{166}Ho measurement of a vial containing 12 mL of MQ. This was done by irradiating a HoDOTA⁻ sample, after which the activity of the irradiated product was measured in the germanium detector. Then the irradiated sample was dissolved in 12 mL MQ in a scintillation vial and measured in the Wallac. From the results, a value for the activity per count measured by the Wallac on a 12 mL MQ sample was calculated.

The factor for converting Wallac measurements of vials containing 12 mL MQ to a ^{166}Ho activity in the vial was calculated using equation 3.4.

$$\tau = \frac{A_{cal}}{C_{cal} * 2^{\frac{t_{cal}}{t_{1/2}}}} \quad (3.4)$$

Here τ is the conversion factor in Bq cpm⁻¹, A_{cal} is the activity in the calibration measurement using the germanium detector in Bq and C_{cal} is the value found from the calibration measurement using the Wallac gamma counter in cpm. The standard error in the conversion factor was calculated using equation 3.5.⁶²

$$\varepsilon_{\tau} = \sqrt{\left(\frac{\varepsilon_{A_{cal}}}{A_{cal}}\right)^2 + \left(\frac{\varepsilon_{C_{cal}}}{C_{cal}}\right)^2} \quad (3.5)$$

Here $\varepsilon_{A_{cal}}$ is the estimated standard error in A_{cal} in Bq and $\varepsilon_{C_{cal}}$ is the estimated standard error in C_{cal} in cpm. The value for the activity of a vial as it would have been directly after irradiation was calculated by equation 3.6.

$$A = \tau * C * 2^{\frac{t}{t_{1/2}}} = \tau * \Gamma \quad (3.6)$$

Here A is the activity directly after irradiation in Bq, C is the value measured by the Wallac in cpm, t is the time between the Wallac measurement and the irradiation in s and $t_{1/2}$ is the half-life of ^{166}Ho in s. The specific activity was subsequently calculated by equation 3.7.

$$S = \frac{A}{V * c_{Ho}} = \frac{\tau * \Gamma}{V * c_{Ho}} \quad (3.7)$$

Here S is the specific activity of the sample in a vial as it would have been directly after irradiation in Bq mg⁻¹, V is the volume of the sample in L and c_{Ho} is the concentration of Ho in the sample as measured by the ICP-OES in mg L⁻¹.

The experiment on the Szilard-Chalmers method was done in quintuple. The standard deviation of the means of the found Ho concentrations were again calculated using equation 3.2. The standard deviations in the means of the found activities were calculated using equation 3.8.⁶²

$$s_A = A \sqrt{\left(\frac{\varepsilon_{\tau}}{\tau}\right)^2 + \bar{\Gamma}^{-2} * \frac{1}{n-1} \sum_{i=1}^n (\Gamma_i - \bar{\Gamma})^2} \quad (3.8)$$

Here s_A is the standard deviation of the activity in Bq. The standard deviation of the means of the found specific activities were calculated by equation 3.9.⁶²

$$s_S = S \sqrt{\left(\frac{\varepsilon_\tau}{\tau}\right)^2 + \left(\frac{\Gamma}{c_{Ho}}\right)^{-2} * \frac{1}{n-1} \sum_{i=1}^n \left(\frac{\Gamma_i}{c_{Ho,i}} - \overline{\left(\frac{\Gamma}{c_{Ho}}\right)}\right)^2} \quad (3.9)$$

Here s_S is the standard deviation of the specific activity in Bq mg⁻¹.

4. Results & discussion

4.1 Determining the ^{166}Ho -DOTA labelling efficiency at different incubation times

In order to be able to control and optimize the Ho-DOTA labelling, as necessary for achieving a high specific activity with a continuous flow Szilard-Chalmers system, the labelling efficiency of ^{166}Ho to DOTA was measured for different incubation times and reaction temperatures. The results are shown in Figure 4.1a, b and c for the 25, 40 and 80 °C experiments respectively. The figures show that at every temperature the Ho-DOTA labelling will occur, with higher labelling efficiencies at higher temperatures. The highest achieved ^{166}Ho labelling percentage is $96 \pm 3\%$ after 16 min at 80 °C. The error bars in the figures indicate the standard deviation of the means of the iterated measurements. The phosphor imager measurement on one of the 4 iterations of the 40 °C measurements indicated an unexpected measurement (see appendix 1), this measurement was therefore not included in the results shown in Figure 4.1b.

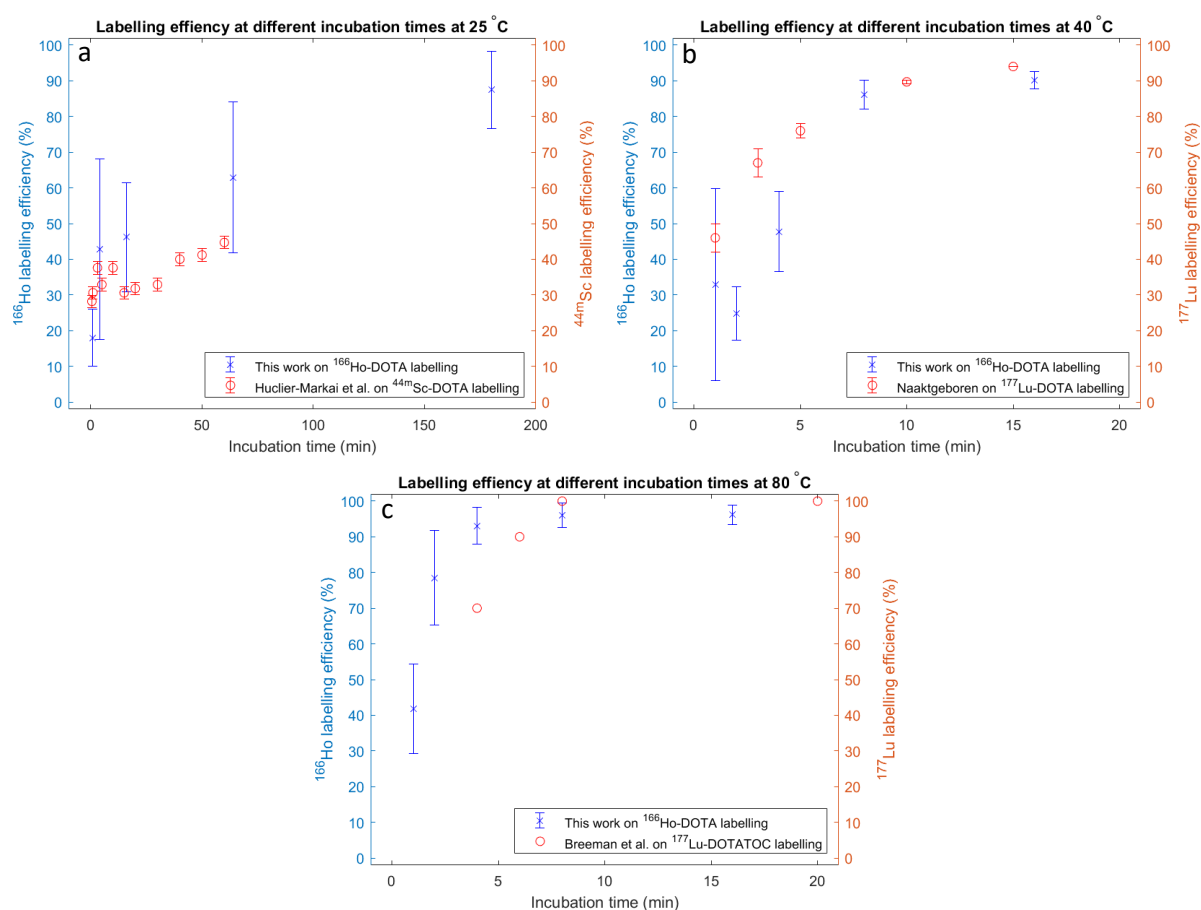


Figure 4.1. Error bar plots of labelling efficiencies against incubation time at 25, 40 and 80 °C (figure a, b and c respectively). Both the values found in this work on ^{166}Ho -DOTA and the values found by Huclier-Markai et al. on $^{44\text{m}}\text{Sc}$ -DOTA, Naaktgeboren on ^{177}Lu -DOTA and Breeman et al. on ^{177}Lu -DOTATOC are shown in figure a, b and c respectively. Notice the different time scale in figure a. Breeman et al. did not provide measurement uncertainties.^{29, 63, 64}

The results on the ^{166}Ho -DOTA labelling show quite large measurement uncertainties, especially for short incubation times. Inconsistent measurement results at short reaction times could be caused by inhomogeneous content distributions in the sample. Especially shortly after addition of the last substance to the sample, this will not be evenly distributed through the sample yet, while labelling is

already occurring. This inhomogeneity can cause some parts of the sample to show quicker labelling than others and this inhomogeneous reactions can also cause a spread in measured labelling efficiencies. Over time the sample will become more homogenous due to diffusion and shaking by the thermo shaker and therefore give more consistent labelling efficiency results. This homogenisation is expected to be quicker in warmer samples, which could be the cause of the smaller errors in the warmer experiments.^{65, 44} Another possible cause for the large errors is a continued labelling reaction after application of the sample on the iTLC strip. This was shown to happen, when DOTA⁴⁻ and Ho³⁺ showed labelling even when they were mixed on the strip itself (see appendix 2). When applying ¹⁶⁶Ho³⁺ and DOTA⁴⁻ on the same strip separately, the iTLC process could still indicate up to 30 % labelling. Uncertainty in the labelling efficiency caused by this effect is expected to decrease as the labelling efficiency in an applied sample increases. However, the effect should be taken into account in interpreting the ¹⁶⁶Ho labelling efficiency results shown in figure 4.1.

Figure 4.1 shows that for a higher reaction temperature of 80 °C, the ¹⁶⁶Ho labelling efficiency is already over 90 % after a 4-minute incubation time, while it takes over 3 hours to reach the same ¹⁶⁶Ho labelling efficiency at 25 °C. The low labelling efficiency at low temperatures was expected to be the case for Ln-DOTA labelling.¹⁴ The large difference in the labelling efficiency achieved at different temperatures is beneficial for the design of a continuous flow Szilard-Chalmers system as it allows controlling when isotopes label to the target compound by controlling the temperature. An advantage that could be achieved by the control of isotope labelling to the target compound is the possibility for a reduction in relabelling, which can increase the production yield.²²

When the results for the ¹⁶⁶Ho-DOTA labelling efficiency at different incubation times observed in this work are compared to literature, the results observed in this work are comparable with the results found in literature for labelling of various M³⁺-DOTA⁴⁻ complexes:

Figure 4.1a shows the 25 °C labelling observed in this work and the labelling of Sc³⁺ and DOTA⁴⁻ observed by Huclier-Markai et al..⁶³ Although the ionic size of scandium is somewhat smaller, its dominant trivalent state still makes a comparison of Sc-DOTA to Ho-DOTA labelling interesting.⁶⁶ Comparison of the labelling at 25 °C as observed in this work to that observed by Huclier-Markai et al., shows that both have low labelling efficiencies compared to higher temperatures.⁶³ The results from Huclier-Markai et al. are similar to what is observed in this work. This is unexpected, since the data from Huclier-Markai et al. was obtained from a 1:500 Sc³⁺:DOTA⁴⁻ ratio and the formation constant of ScDOTA⁻ is higher than that of HoDOTA⁻.^{33, 35, 63, 67} However, slower reaction kinetics for the Sc-DOTA than for the Ho-DOTA labelling could still explain the similar results observed with different ratios and formation constants.¹⁴

Figure 4.1b shows the observed labelling efficiencies at 40 °C and compares it to the results for Lu³⁺-DOTA⁴⁻ labelling efficiencies at different incubation times observed by Naaktgeboren.²⁹ The results are very similar for longer reaction times, but differ somewhat for shorter reaction times. The comparison of the results observed in this work and that observed by Naaktgeboren therefore indicate that the ¹⁷⁷Lu labelling at 40 °C at short incubation times is higher than that for ¹⁶⁶Ho.

Figure 4.1c shows that the high labelling efficiencies at short incubation times at 80 °C observed in this work are confirmed by the labelling efficiencies of Lu³⁺ to the DOTA group of DOTATOC observed by Breeman et al..⁶⁴ Even though Breeman et al. used a molar Lu:DOTATOC ratio of 1:1.2, which is higher than the 1:1 Ho:DOTA ratio used in this work, the results observed in this work show slightly higher labelling efficiencies. Still, both show near 100% radionuclide labelling within 10 minutes.⁶⁴

4.2 Determining the amount of isotope-exchange and labelling by $^{166}\text{Ho}^{3+}$ in a solution with near 100% of the DOTA labelled to Ho

In order to estimate the influence of isotope-exchange and relabelling on the specific activity achieved in the Szilard-Chalmers method, the amount of isotope-exchange and labelling by $^{166}\text{Ho}^{3+}$ was measured in a solution containing mostly HoDOTA^- and little DOTA^{4-} at different temperatures, $\text{Ho}:\text{DOTA}$ ratios and reaction times. The observed percentages of free $^{166}\text{Ho}^{3+}$ that undergo isotope-exchange or labelling in such a solution are shown in figure 4.2. The error bars in figure 4.2 indicate the standard deviation of the means of the iterated measurements. The results show that a low amount of isotope-exchange and relabelling of lower than 8 % occurs, even for 16 minutes reaction time.

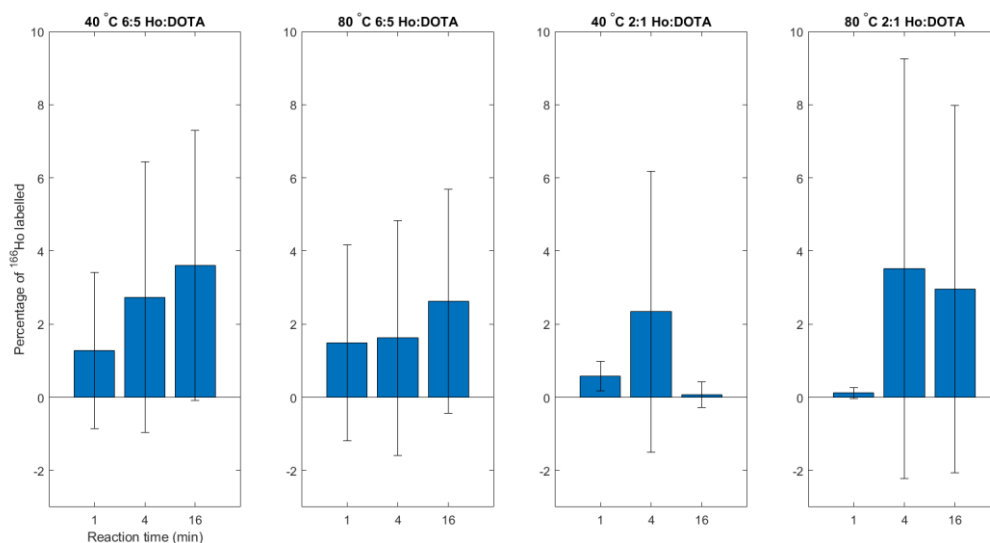


Figure 4.2. Plots of the percentage of ^{166}Ho bound to DOTA due to relabelling and isotope-exchange after different reaction times. Plots are given for the 40 and 80 °C measurements with the 6:5 and 2:1 $\text{Ho}:\text{DOTA}$ molar ratios.

Although the relative standard deviation is large, the absolute standard deviation is in most measurements less than 4% of the total amount of ^{166}Ho present in the sample. A 4% error in iTLC measurements is expected, regarding other iTLC measurements done in this work. As it was found that a labelling reaction can continue after sample application on an iTLC strip (appendix 2), there is some uncertainty in the actual reaction time. This could have contributed to some spread in the measured labelling efficiencies.

The percentages of DOTA labelled to Ho is the DOTA labelling efficiency. The DOTA labelling efficiencies in the controls checking whether near 100% of the DOTA was labelled with Ho before the start of the isotope-exchange and labelling reactions, i.e. at the moment of addition of ^{166}Ho to the sample, are shown in figure 4.3 for both measured temperatures and $\text{Ho}:\text{DOTA}$ ratios.

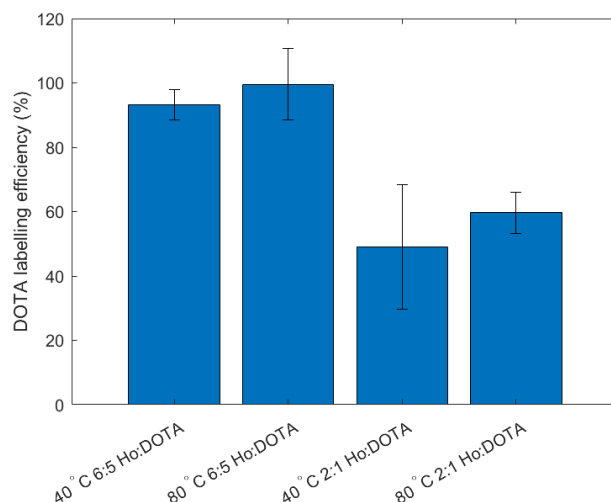


Figure 4.3. Plot on the ^{166}Ho labelling efficiency in the controls checking whether near 100% of the DOTA was labelled with Ho before the start of the isotope-exchange and labelling reactions. The results are shown for the 40 and 80 °C measurements with the 6:5 and 2:1 Ho:DOTA molar ratios.

The controls show that for the 6:5 Ho:DOTA controls the DOTA labelling efficiency was near 100 %, as expected for 20 minute 60 °C Ho-DOTA labelling with an abundance of Ho (See figure 4.1). These two controls indicate that little free DOTA^{4-} was left in the samples when the $^{166}\text{Ho}^{3+}$ was added, as is necessary to measure isotope-exchange as well as possible.

The controls show that near 100 % DOTA labelling was not achieved for the 2:1 samples. The abundancy of Ho^{3+} over DOTA was expected to give a high DOTA labelling efficiency. As this is not the case, the results for the relabelling and isotope-exchange of the 2:1 Ho:DOTA samples are deemed unreliable. As the only difference between the 6:5 and the 2:1 experiments were the used Ho^{3+} concentrations, the unexpected results of the controls of the 2:1 ratio could indicate that the Ho^{3+} in the solution can behave differently at high Ho^{3+} concentrations than at low concentrations.

Zhernosekov, Filosofov and Rösch found the varying results that 10.3-24.5 % of ^{166}Ho was found in the $^{166}\text{HoDOTA}^-$ complex after Szilard-Chalmers separation on a $^{165}\text{HoDOTA}^-$ target compound independent of irradiation time.²¹ The percentage of relabelling or isotope-exchange in the experiment by Zhernosekov, Filosofov and Rösch is therefore less than this 10.3-24.5 %. This is confirmed by the percentage of ^{166}Ho undergoing labelling or isotope exchange of less than 8% as observed in this work.²¹

4.3 Design of a cation exchange chromatography method for separation of Ho^{3+} from DOTA^{4-} and HoDOTA^-

In order to design a separation method for separating Ho^{3+} from HoDOTA^- and DOTA^{4-} the elution of Ho^{3+} and HoDOTA^- from a cation exchange resin using various eluents was investigated. The results for the elution profiles of the free Ho^{3+} and HoDOTA^- from a DOWEX 50W cation exchange resin are shown in figure 4.4 for various eluents. It shows that Ho^{3+} and HoDOTA^- can be separated via ion exchange by elution with pH4.2 HCl, since this eluent elutes only HoDOTA^- , followed by elution with 6 M HCl, which elutes the Ho^{3+} . The figure shows the normalized number of counts in the vials with the collected eluate. The normalized number of counts from the vial with the collected resin is also shown.

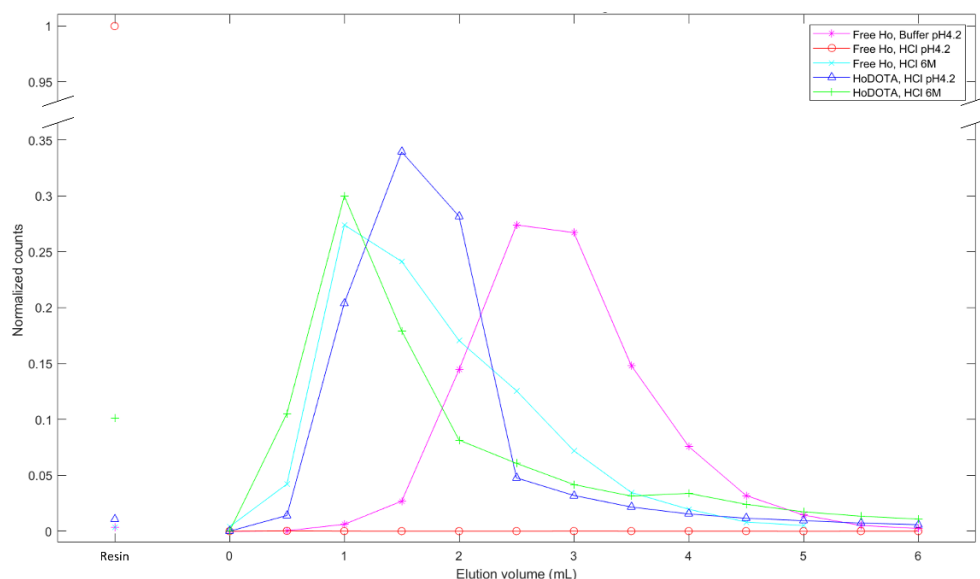


Figure 4.4. Elution diagram of the various investigated eluents on free Ho^{3+} and HoDOTA^- samples. The x-axis shows the total amount of added eluent volume. The y-axis shows the amount of normalized counts found in the eluted volume collected since the preceding data point. The amount of normalized counts measured from the resin is also shown.

Figure 4.4 shows that both free Ho^{3+} and HoDOTA^- are eluted by the 6 M HCl eluent. The figure also shows that the pH4.2 HCl eluent only elutes HoDOTA^- , while leaving the free Ho^{3+} in the resin. This is expected from the results found by Strelow, Rethemeyer and Bothma on the elution of the chemically similar trivalent group three elements and lanthanoids from a DOWEX 50W resin.⁶⁸ Based on the results for the pH4.2 and the 6 M HCl elution, a separation method for separation of free Ho^{3+} and HoDOTA^- was designed, where the resin selectively retains the free Ho^{3+} ions, while allowing the HoDOTA^- target compound to be eluted by a pH4.2 HCl solution, after which the Ho^{3+} is eluted by a 6 M HCl solution. Next to the HCl eluent, an acetic acid 1 M pH4.2 buffer was also tested as potential eluent. However, since the buffer solution was shown to elute the Ho^{3+} ions from the resin and it was expected to elute the HoDOTA^- as well, it was shown not to be useful for the separation of the two components. A cause for the elution of free Ho^{3+} with the acetic acid 1 M pH4.2 buffer eluent, could be that the Na^+ in the buffer occupies all binding sites in the resin. This is likely, since the amount of Na^+ in the buffer exceeds the binding capacity of the resin, which is less than 1 meq.⁶⁹ Ho^{3+} does bind stronger to the resin, but the high concentration of Na^+ could still force the Ho^{3+} out of the resin.⁶⁸ Another possible explanation for the elution of free Ho^{3+} with the acetic acid 1 M pH4.2 buffer eluent, is that the acetate in the buffer can bind the Ho^{3+} , due to which the Ho^{3+} weakens or loses its bond to the resin and is therefore eluted.⁷⁰

A comparison of the separation efficacy of the ion exchange chromatography and the iTLC, showed 33 and 40 % of the ^{166}Ho to be complexed to DOTA respectively for the same sample. The two results are quite similar given the uncertainty of the iTLC separation method, which can be more than 10% in samples with low labelling efficiency. Therefore the proposed ion exchange separation method seems to work.

4.4 Determining the specific activity achieved by the Szilard-Chalmers method in irradiation of a dried $^{165}\text{HoDOTA}^-$ sample

In order to determine what effect the Szilard-Chalmers method can have on the specific activity of produced ^{166}Ho , the specific activity of ^{166}Ho was measured after irradiation of a dried $^{165}\text{HoDOTA}^-$ sample and successive separation of the dissolved sample by ion exchange chromatography. This has resulted in a maximum achieved ^{166}Ho specific activity of $2.8 \pm 0.2 \text{ MBq mg}^{-1}$, corresponding to an increase of a factor 1.62 ± 0.03 compared to the specific activity achieved by an irradiation without use of the Szilard-Chalmers method.

The first two of in total five iterations of this experiment, showed that less than 40 % of the expected activity was collected from the irradiated sample. This was likely due to insufficient shaking and therefore bad homogenization after irradiation. It is unsure if there is a difference in the homogenization of the Ho^{3+} and the HoDOTA^- , therefore the ion exchange separation results of the first two iterations were unreliable and not included in any of the presented results.

The control measuring the percentage of ^{166}Ho labelled to DOTA after the first 30 minutes of Ho-DOTA labelling, showed $91.3 \pm 1.9 \%$ of the ^{166}Ho to be labelled to DOTA. After drying and dissolving again, the percentage of ^{166}Ho labelled to DOTA in the control solution was $70 \pm 4 \%$ and $73.8 \pm 1.0 \%$, before and after unpacking of the irradiated sample respectively. These results indicate that there is some breaking of HoDOTA^- complexes occurring during the drying process. However, most Ho is still complexed, which is important for the production of high specific activity $^{166}\text{Ho}^{3+}$ in the sample, as this requires low amounts of free $^{165}\text{Ho}^{3+}$. The reduction of the amount of ^{166}Ho labelled to DOTA during drying, might be caused by DOTA changing its binding characteristics due to a change in pH or due to DOTA becoming saturated in the solution during drying.

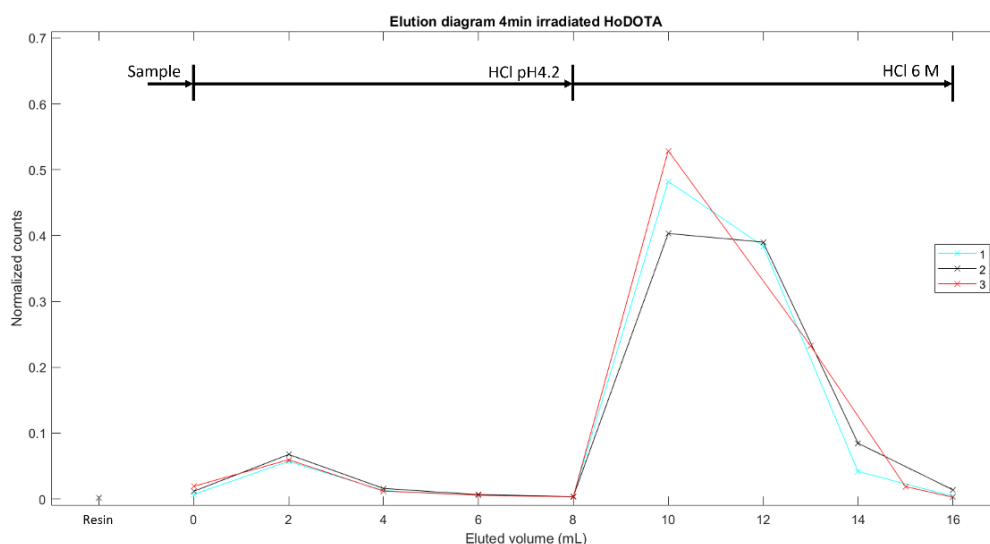


Figure 4.5. Elution diagram of the three iterations of the HoDOTA^- irradiation. The first 8 mL of eluent is pH4.2 HCl and the last 8 mL of eluent is 6 M HCl. The x-axis shows the total amount of added eluent volume. The y-axis shows the amount of normalized counts found in the eluted volume collected since the preceding data point. The amount of normalized counts measured from the resin is also shown.

Figure 4.5 shows that the ion exchange separation results are quite consistent. Little activity is in the eluate of the last pH4.2 HCl addition, indicating that most $^{166}\text{HoDOTA}^-$ is eluted before the use of the 6 M HCl eluent. Moreover, little activity is left in the resin after the last 6 M HCl addition, indicating most of the $^{166}\text{Ho}^{3+}$ is eluted at the end of the ion exchange separation. The different elution

volumes, of 3 mL and 1 mL respectively, in the 7th and 9th collected vial of the 3rd iteration were taken into account in every of the following calculations.

The percentage of ¹⁶⁶Ho that is labelled resulting from the ion exchange separation is 9.4±1.0 %. The iTLC measurement of the sample showed a labelling percentage of 36±7 %. The large discrepancy in the results of the two separation methods is likely caused by continued labelling of the sample on the strip after application, as is shown to happen (see appendix 2). Continued labelling after addition of the sample to the ion exchange column is expected to have a smaller effect, since there is less time between the sample application and the actual separation of the components in the sample. The ion exchange separation is therefore considered more reliable than the iTLC separation.

The ICP-OES measurements on the Ho concentration in the vials collected during sample loading, during the first 2 mL of HoDOTA⁻ elution by pH4.2 HCl and during the first 2 mL of Ho³⁺ elution by 6 M HCl, are shown in table 4.1.

The activity per count found from comparison of the measurement on the germanium detector and the Wallac gamma counter was 0.345±0.004 Bq cpm⁻¹. When using this value to convert the Wallac gamma counter measurement to the activity of the vials, the activity values in table 4.1 are found. This is useful since from these activities, the Ho concentrations and equation 3.7, the specific activity of the vials is found. These specific activities are also shown in table 4.1.

Table 4.1. Table showing the results of the experiment determining the specific activity achieved by the Szilard-Chalmers method on the Ho concentration, the activity and the specific activity in the sample loading, first HoDOTA⁻ elution and first Ho³⁺ elution vial.

Vial	Ho concentration (mg L ⁻¹)	Activity (kBq)	Specific activity (MBq mg ⁻¹)
Sample loading	0.39±0.17	2.2±1.1	0.46±0.03
First 2 mL HoDOTA ⁻ elution	1.64±0.12	10.3±0.8	0.53±0.05
First 2 mL Ho ³⁺ elution	2.4±0.4	80±12	2.8±0.2

The percentage of Ho labelled to DOTA in the sample that was separated, was calculated by equation 4.1.

$$L_{Ho} = \frac{N_l}{N_l + N_f} * 100 = \frac{[Ho]_s + \frac{[Ho]_l}{C_2} * \sum_{i=2}^5 C_i}{[Ho]_s + \frac{[Ho]_l}{C_2} * \sum_{i=2}^5 C_i + \frac{[Ho]_f}{C_6} * \sum_{i=6}^9 C_i} * 100 \quad (4.1)$$

Here L_{Ho} is the percentage of Ho labelled to DOTA and N_l and N_f are the amount of Ho labelled to DOTA and unlabelled respectively. $[Ho]_s$, $[Ho]_l$ and $[Ho]_f$ are the Ho concentrations for the sample loading, first HoDOTA⁻ and first Ho³⁺ elution respectively. C_i is the amount of counts measured on the Wallac from the i^{th} collected vial, where $i=1$ is collected during the sample loading, $i=2-5$ are collected during HoDOTA⁻ elution and $i=6-9$ are collected during Ho³⁺ elution. The found percentage of Ho labelled to DOTA in the separated sample is 37±2 %. The specific activity of the entire irradiated sample together was calculated using equation 4.2.

$$S_{ent} = \frac{\tau * \sum_{i=1}^9 C_i}{\left([Ho]_s + \frac{[Ho]_l}{C_2} * \sum_{i=2}^5 C_i + \frac{[Ho]_f}{C_6} * \sum_{i=6}^9 C_i \right) * V} \quad (4.2)$$

Here S_{ent} is the specific activity of the entire separated sample in Bq mg⁻¹, τ is again the conversion factor in Bq cpm⁻¹ and V is the volume eluted per sample in L. The Ho concentrations in equation 4.2 are in mg L⁻¹ and the measured amounts of counts are in cpm. The found specific activity of the entire separated sample was 1.72±0.11 MBq mg⁻¹. This means that the Szilard-Chalmers separation has increased the highest achieved specific activity by a factor, the enrichment factor, of 1.62±0.03 (see appendix 3 for the error calculations of this specific activity and enrichment factor).

Naaktgeboren has shown that spontaneous decomposition of LuDOTA⁻ complexes on the time span used to execute the irradiation and separation is negligible.²⁹ The same can be assumed to be the case for HoDOTA⁻, as Ho and Lu are both trivalent lanthanoids with similar ionic radius.⁴³ Combining this with the fact that only a small fraction of Ho atoms capture a neutron and undergo a Szilard-Chalmers reaction, it is found that the remaining processes of radiolysis and relabelling cause the Ho labelling with DOTA to decrease from the 70±4 % Ho labelling before irradiation to the 37±2 % after irradiation. Since relabelling is expected to only increase the Ho labelling to DOTA, this means that radiolysis during irradiation is expected to cause at least 47±7 % of the labelled Ho to be released from its bond to DOTA. Naaktgeboren found that approximately 10 % of labelled Lu in LuDOTA⁻ is released in a gamma dose similar to the gamma dose in the irradiation done in this work. Since HoDOTA⁻ and LuDOTA⁻ have similar chemical properties, HoDOTA⁻ is expected to have a similar radiolysis due to gamma radiation as found by Naaktgeboren. Since the total radiolysis of HoDOTA⁻ in this work is expected to have been 47±7 %, radiolysis due to neutron irradiation seems to have played an important part too.

By Zhernosekov, Filosofov and Rösch a specific activity enrichment factor of 90.0 was found using Szilard-Chalmers separation on HoDOTA with a 0.5 hour irradiation with a similar neutron flux as used in this work.^{21, 60, 71} Since it was established in section 2.3 that a shorter irradiation time will give a higher enrichment factor, it is expected that a 4-minute irradiation would result in an even higher enrichment factor.²¹ The specific activity after a 4-minute irradiation achieved in this work was a lot lower. Some of the discrepancy between the results found by Zhernosekov, Filosofov and Rösch and the results found in this work can be explained by Zhernosekov, Filosofov and Rösch using a 100% complexed irradiation target, where this work uses a 70±4 % complexed irradiation target.²¹ Zhernosekov, Filosofov and Rösch were able to achieve the absence of free Ho³⁺ in the irradiation target by filtering them out using ion exchange chromatography. Higher initial complexation will result in less free ¹⁶⁵Ho in the irradiation product and therefore a higher specific activity.

As established before, the results observed in this work indicated that radiolysis caused at least 47±7 % of Ho labelled to DOTA to have been released after irradiation for 4 minutes in a 5±1 *10¹² cm⁻² s⁻¹ neutron flux in the BP3 in the HOR.^{60, 71} From equation 2.16, it then follows that the radiolysis constant, which can be seen as a radiolysis cross-section, in this irradiation is at least 5±1 *10⁸ barn. This is a factor 7 *10² higher than the decomposition cross-section of 750000 barn found in by Zhernosekov, Filosofov and Rösch.²¹ As more decomposition will also result in a larger amount of free ¹⁶⁵Ho after irradiation, more radiolysis will decrease the specific activity. Decomposition of the HoDOTA⁻ complex during irradiation can therefore be another important cause of the difference in enrichment factor between the result found Zhernosekov, Filosofov and Rösch and that found in this work. A possible explanation for the lower complex decomposition observed by Zhernosekov, Filosofov and Rösch could be irradiation with more gamma shielding or with neutrons with lower

energies than in this work. Neutrons with lower energies are expected to ionise less particles,⁴¹ which on their turn are expected to cause less radiolysis of the HoDOTA⁻ complexes.

Using equation 2.12, the experiments done in this work can be combined to find the chance for a successful Szilard-Chalmers reaction of a HoDOTA⁻ complex, in which (n, γ) neutron capture by a complexed ¹⁶⁵Ho causes the newly created ¹⁶⁶Ho to escape the target compound. In this experiment an initial labelling before sample irradiation of 70 $\pm$ 4 % was observed. Moreover, the chance for radiolysis of a HoDOTA⁻ complex was expected to be at least 47 $\pm$ 7 %. The observed specific activity of free ¹⁶⁶Ho was 2.8 $\pm$ 0.2 MBq mg⁻¹, which corresponds to a ¹⁶⁶Ho/¹⁶⁵Ho ratio of 1.06 $\pm$ 0.08 * 10⁻⁷. A 4-minute irradiation in a neutron flux of 5 $\pm$ 1 * 10¹² cm⁻² s⁻¹ of ¹⁶⁵Ho, which has a ¹⁶⁵Ho(n, γ)¹⁶⁶Ho neutron capture cross-section of 58 barn, gives a chance for this neutron capture by ¹⁶⁵Ho of 7.0 $\pm$ 1.4 * 10⁻⁶ %. The amount of isotope-exchange in this experiment is uncertain, since the sample was only separated two hours after irradiation. From the experiment on the isotope-exchange it follows that 4 % of the ¹⁶⁶Ho is labelled to DOTA after 16 minutes of being dissolved. However, this 4 % was found at 40 °C while the isotope exchange in this experiment on determining the specific activity achieved by the Szilard-Chalmers method was at 25 °C. Less than 10 % of the ¹⁶⁶Ho was labelled after this experiment.²⁹ Therefore 4 $\pm$ 4 % of the ¹⁶⁶Ho undergoing isotope exchange is expected to be a good estimation. The chance for ¹⁶⁵Ho in a HoDOTA⁻ complex to perform a successful Szilard-Chalmers reaction in a 4-minute-irradiation in a 5 $\pm$ 1 * 10¹² cm⁻² s⁻¹ neutron flux calculated using equation 2.12 is then at least 7 $\pm$ 3 * 10⁻⁶ % (see appendix 4 for the error calculation). Although the relative error on this value is large, it does show that the chance of ¹⁶⁵Ho(n, γ)¹⁶⁶Ho neutron capture followed by a successful Szilard-Chalmers reaction is in the same order of magnitude as the chance of only the neutron capture. This is a beneficial result for the potential of Szilard-Chalmers reactions for creating high specific activity ¹⁶⁶Ho³⁺ using ¹⁶⁵HoDOTA⁻ complexes.

Use of a continuous flow Szilard-Chalmers system, instead of a batch irradiation, was found to increase the achieved specific activity by a factor 6 for holmium 8-hydroxyquinolate complexes by Safavi-Tehrani.²² This does show that a continuous flow Szilard-Chalmers system adds value.

5. Conclusion

5.1 Conclusion

The investigations on the processes at play showed promising results for the viability of high specific activity ^{166}Ho production by a continuous flow system in which a dissolved $^{165}\text{HoDOTA}^-$ target undergoes Szilard-Chalmers reactions and ion exchange separation.

In the experiments on the ^{166}Ho -DOTA labelling efficiency at different incubation times it was observed that higher temperatures showed higher labelling efficiencies. The highest achieved ^{166}Ho labelling efficiency of $96\pm 3\%$ was therefore achieved at $80\text{ }^\circ\text{C}$ after 16 minutes of labelling. Inhomogeneity and uncertainty in the reaction time may have resulted in high errors on the ^{166}Ho -DOTA labelling efficiency measurements, especially at low temperatures. However, the observed labelling efficiencies at 25 , 40 and $80\text{ }^\circ\text{C}$ showed good correspondence to literature on $^{44\text{m}}\text{Sc}$ -DOTA, ^{177}Lu -DOTA and ^{177}Lu -DOTATOC labelling respectively.^{31, 63, 64}

The experiments on the relabelling and isotope-exchange of free $^{166}\text{Ho}^{3+}$ and $^{165}\text{HoDOTA}^-$ have shown that only a small amount of $^{166}\text{Ho}^{3+}$ undergoes relabelling and isotope-exchange reactions. Both after 1, 4 and 16 minutes, less than 8 % of the initially free $^{166}\text{Ho}^{3+}$ was found in complexed form in a 6:5 molar ratio Ho:DOTA solution. Uncertainty in the reaction time may again have caused some uncertainty in the measurement results. However, literature confirms that the amount of ^{166}Ho ending up in a $^{166}\text{HoDOTA}^-$ complex after relabelling and isotope-exchange is less than 10.3-24.5 %.²¹

The experiment on the design of a cation exchange chromatography method for the separation of Ho^{3+} from DOTA^{4-} and HoDOTA^- has resulted in a successful separation method. A separation using a pH4.2 HCl eluent first, in order to elute only DOTA^{4-} and HoDOTA^- from the resin, and a 6 M HCl eluent second, in order to elute Ho^{3+} from the resin, was proven successful in separating Ho^{3+} from DOTA^{4-} and HoDOTA^- .

The experiment on the specific activity of ^{166}Ho achieved by use of the Szilard-Chalmers method have resulted in an achieved specific activity of $2.8\pm 0.2\text{ MBq mg}^{-1}$ in a 4 minute irradiation. The enrichment factor following from this specific activity was found to be 1.62 ± 0.03 . Literature shows achieved enrichment factors of even 90.0 in a 0.5 hour irradiation.²¹ The discrepancy in the results found in this work and that found in literature, can likely be attributed to a lower initial labelling efficiency of Ho^{3+} to DOTA^{4-} and much higher chance of target radiolysis in this work. The experiment on the specific activity achieved by the Szilard-Chalmers method in irradiation of a dried $^{165}\text{HoDOTA}^-$ sample has also shown that the chance for a successful Szilard-Chalmers reaction, in which the complexed target isotope undergoes a (n, γ) neutron capture, due to which the product isotope escapes the complex, is in the same order of magnitude as the chance for neutron capture only. This confirms the potential of the Szilard-Chalmers method on $^{165}\text{HoDOTA}^-$ in producing high activity ^{166}Ho .

5.2 Future research recommendations

In order to make a continuous flow Szilard-Chalmers system more worthwhile, the enrichment factor achieved by implementation of the system should be increased. The specific activity that could be achieved by the system could be increased by minimizing effects that create free target isotopes or

bound product isotopes. Future research should be focussed on minimizing these effects in order for higher enrichment factors to be achieved.

In contrast to the experiment on the Szilard-Chalmers method, drying of the target isotope-compound complex is not part of a continuous flow Szilard-Chalmers system. That means the reduction of the Ho labelling efficiency caused by the drying will not happen in such a system. Moreover, longer labelling time of multiple days or even weeks might improve the achieved Ho labelling efficiency and might form more stable complexes.³⁴ An initial labelling efficiency of 96 %, similar to what was shown to be possible in subsection 4.1, instead of 70 %, similar to what is left after drying the sample, would increase the achieved specific activity already by 28 % according to equation 2.12, where the chance for successful Szilard-Chalmers reactions is taken equal to the chance of neutron capture and the chance of radiolysis is taken to be 47 %. Therefore it would be interesting to investigate what specific activity is reached in the Szilard-Chalmers experiment when a dissolved, i.e. not dried, sample is irradiated and when the initial Ho-DOTA labelling is prolonged to multiple days. Zeisler and Weber have shown that there can be a difference between use of a dry and dissolved irradiation targets in the enrichment factor achieved by the Szilard-Chalmers method on various Ho-complexes.²⁰

According to figure 2.8,³¹ as established before, the expected part of radiolysis of HoDOTA⁻ complexes due to gamma radiation is approximately 10 % in a 4-minute irradiation in the BP3 irradiation facility of the HOR. This gamma dose on the sample can be reduced by gamma shielding. However, it is important that shielding material is used that shields as much gamma radiation while shielding as little neutron radiation as possible. After all, the neutrons are necessary for forming ¹⁶⁶Ho in the first place. Reduction of the gamma dose by 50 % can increase the achieved specific activity by 6 % according to equation 2.12, where the chance for successful Szilard-Chalmers reactions is taken equal to the chance of neutron capture, the chance for radiolysis by neutron irradiation is taken to be 37 % and the initial Ho labelling is taken to be 70 %. Therefore, it would be interesting to investigate ways to shield a sample from gamma irradiation without giving in too much on the neutron flux.

Radiolysis by neutron irradiation is shown to have a large effect on the achieved specific activity. After subtracting the radiolysis expected to be caused by gamma radiation from the calculated total radiolysis, neutron radiation is expected to cause radiolysis in approximately 37 % of the HoDOTA⁻ complexes in 4 minutes of irradiation. Reducing radiolysis by neutron irradiation is difficult, since shielding would also reduce the amount of created ¹⁶⁶Ho. Use of less energetic neutrons could be a solution for reducing radiolysis by neutrons, since less energetic neutrons are expected to ionise less particles, which is expected to cause less radiolysis.⁴¹ Moreover, less energetic neutrons are expected to only increase the amount of created ¹⁶⁶Ho.⁷² Reducing the radiolysis by neutron irradiation by 50 % would increase the achieved specific activity by 26 % according to equation 2.12, where the chance for successful Szilard-Chalmers reactions is taken equal to the chance for neutron capture, the chance for radiolysis by gamma irradiation is taken to be 10 % and the initial Ho labelling is taken to be 70 %. Therefore it would be interesting for future research to investigate ways to slow down or filter out high energy neutrons without reducing the neutron flux too much.

The isotope exchange has been shown to have a small effect of less than 8 % loss on the achieved specific activity. A 50 % improvement on this part will improve the specific activity achieved by less than 4 % and will therefore be less effective than improvements on the before mentioned processes.

In order to make a continuous flow Szilard-Chalmers system more worthwhile, research on the system should first focus more on improving the subprocesses, as done in this work. Only when that

has resulted in higher enrichment factors, research is ready to focus on the practicalities of implementing a large scale continuous flow Szilard-Chalmers system.

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Appendix

1. Unexpected results in a measurement on the ^{166}Ho -DOTA labelling at different incubation times

Figure A.1 shows the radioactivity distribution of the first and second iteration of the measurement on the ^{166}Ho labelling to DOTA at different incubation times at 40 °C. The results for the second iteration (the right five strips) show that the separation of $^{166}\text{Ho}^{3+}$ and $^{166}\text{HoDOTA}^-$ has not resulted in a strip containing radioactivity only on either ends, but it has resulted in strips with large amounts of activity half-way on the strips. This is an unexpected separation result and therefore the second iteration of the measurement on the ^{166}Ho labelling to DOTA at different incubation times at 40 °C is considered unreliable.

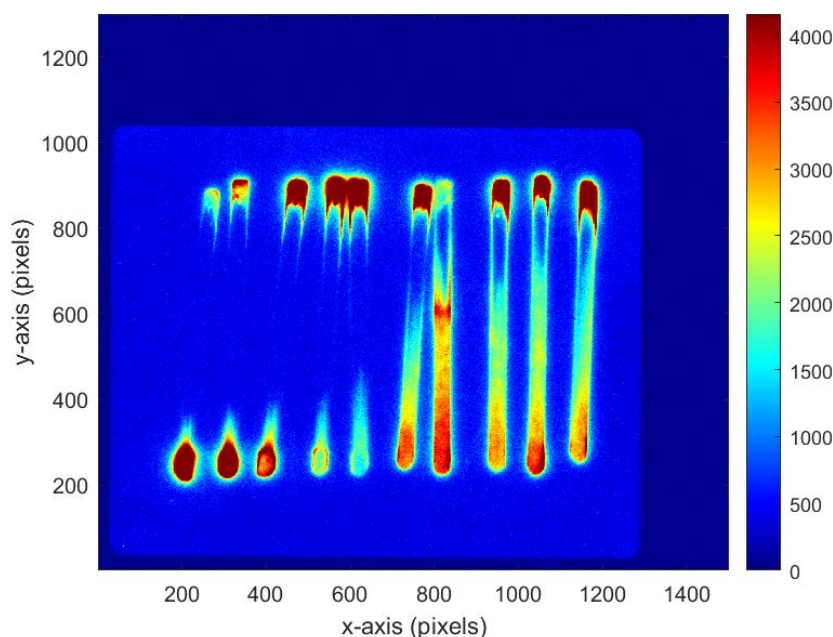


Figure A.1. Radioactivity distribution map of the iTLC strips resulting from the first (left five strips) and second (right five strips) iteration of the measurement on the ^{166}Ho -DOTA labelling at 40 °C. The colours indicate the count intensity on a location up to 30% of the highest measured count intensity. Every location with a count intensity above this 30% is coloured dark red.

2. Continued ^{166}Ho -DOTA labelling after application on an iTLC strip

Figure A.2 shows the separation found on three iTLC strips that have been treated as described in subsection 3.3, except for the sample application. On the left strip 2 μL DOTA 10.0 mM in pH4.2 HCl was added to the strip directly after which 3 μL HoCl_3 6.9 mM containing $^{166}\text{HoCl}_3$ in pH4.2 HCl was added on the same location on the strip. On the middle strip the same two addition were done in opposite order, i.e. first HoCl_3 and then DOTA. On the right strip 2 μL pH4.2 HCl was added after which 3 μL HoCl_3 6.9 mM containing $^{166}\text{HoCl}_3$ in pH4.2 HCl was added. Figure A.2 shows that on both the left and middle strip a significant amount of activity is found on either sides of the strip, while the right strip, which functions as a control on which no labelling is possible due to the absence of DOTA, only shows activity on the side of the strips where unlabelled $^{166}\text{Ho}^{3+}$ ends up. Figure A.2 therefore shows that ^{166}Ho labelling still occurs after application on an iTLC strip.

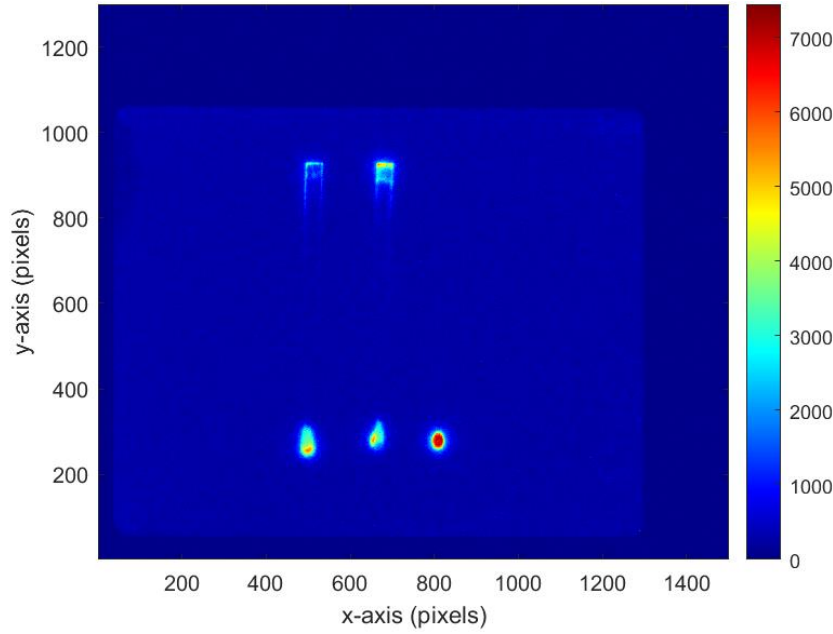


Figure A.2. Radioactivity distribution map showing the continued labelling of ^{166}Ho to DOTA after application on an iTLC strip. The colours indicate the count intensity on a location.

3. Standard deviation calculation of the specific activity of the entire separated irradiated sample and of the enrichment factor

The standard deviation in the specific activity of the entire separated irradiation sample as calculated in equation 4.2 is shown in equation A.1.⁶²

$$s_{S_{ent}} = \overline{S_{ent}} * \sqrt{\left(\frac{\varepsilon_{\tau}}{\tau}\right)^2 + (\overline{S_{ent}})^{-2} * \frac{1}{2} \sum_{i=1}^3 (S_{ent,i} - \overline{S_{ent}})^2} \quad (\text{A.1})$$

Here $s_{S_{ent}}$ is the standard deviation in the specific activity, ε_{τ} is the error in the conversion factor, τ is the conversion factor, $S_{ent,i}$ is the specific activity calculated from the i^{th} iteration and $\overline{S_{ent}}$ is the mean of the triplo results for the specific activity. The standard deviation in the enrichment factor is shown in equation A.2.⁶²

$$s_{\eta} = \bar{\eta} * \sqrt{\left(\frac{\varepsilon_{\tau}}{\tau}\right)^2 + (\bar{\eta})^{-2} * \frac{1}{2} \sum_{i=1}^3 (\eta_i - \bar{\eta})^2} \quad (\text{A.2})$$

Here s_{η} is the standard deviation of the enrichment factor, η_i is the enrichment factor calculated from the i^{th} iteration and $\bar{\eta}$ is the mean of the triplo results for the enrichment factor.

4. Standard deviation calculation of the chance for a successful Szilard-Chalmers reaction calculated using equation 2.12

The error in the chance for a target isotope incorporated in the target compound to undergo a successful Szilard-Chalmers reaction, in which the complexed target isotope undergoes a (n, γ) neutron capture, due to which the product isotope escapes the complex, is given in equation A.3.

$$\alpha_{\mu_{Sz}} = \mu_{Sz} * \sqrt{\frac{\alpha_f^2 + \mu_{cap}^2 * \mu_{non-iso}^2 * \left(\left(\frac{\alpha_{\mu_{cap}}}{\mu_{cap}} \right)^2 + \left(\frac{\alpha_{\mu_{non-iso}}}{\mu_{non-iso}} \right)^2 \right)}{(f - \mu_{cap} * \mu_{non-iso})^2} + \frac{\alpha_B^2 + B^2 * \mu_{rad}^2 * \left(\left(\frac{\alpha_B}{B} \right)^2 + \left(\frac{\alpha_{\mu_{rad}}}{\mu_{rad}} \right)^2 \right)}{(B - \mu_{rad} * B)^2} + \frac{\alpha_B^2 + B^2 * \mu_{rad}^2 * \left(\left(\frac{\alpha_B}{B} \right)^2 + \left(\frac{\alpha_{\mu_{rad}}}{\mu_{rad}} \right)^2 \right)}{(1 - B + \mu_{rad} * B)^2} + \left(\frac{\alpha_{\mu_{non-iso}}}{\mu_{non-iso}} \right)^2} \quad (A.3)$$

Here μ_{Sz} is the chance for a target isotope incorporated in the target compound to undergo a successful Szilard-Chalmers reaction. f is the fraction of free $^{166}\text{Ho}/^{165}\text{Ho}$, μ_{cap} is the chance for a target isotope to undergo an (n, γ) reaction and $\mu_{non-iso}$ is the chance for a product nuclide to not undergo isotope-exchange. B is the initial target isotope-complex labelling efficiency and μ_{rad} is the fraction of radiolysed target compounds. α_x denotes the standard deviation in x .