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# Solid phase extraction-based separation of the nuclear isomers $^{177m}\text{Lu}$ and $^{177}\text{Lu}$

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## ABSTRACT

A solid phase extraction based  $^{177m}\text{Lu}$ - $^{177}\text{Lu}$  separation method has been investigated for its feasibility to be used in the radionuclide generator. The use of 2,2',2''-(10-(2,6-dioxotetrahydro-2H-pyran-3-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid, (DOTAGA-anhydride) allowed grafting of DOTA (1,4,7,10-tetraazacyclododecane N,N',N'',N'''-tetraacetic acid) complex on the surface of commercially available amino propyl silica. The grafting of DOTA has been confirmed by several characterization techniques. The thermogravimetric analysis reveals that the 0.33 mmol DOTA groups have been grafted per gram of silica. However, during the Lu ion complexation, a 10 times lower Lu adsorption capacity of 0.03 mmol g<sup>-1</sup> could be achieved under the studied reaction conditions. The results indicate that the grafting of DOTA on solid affects the Lu coordination and also influences the kinetics of Lu-DOTA complexation. The weak coordination resulted in high  $^{177m}\text{Lu}$  leakage, while the unreacted DOTA groups interfere with the  $^{177}\text{Lu}$  release. This is evident from the 0.3%  $^{177m}\text{Lu}$  leakage combined with a  $^{177}\text{Lu}$  extraction efficiency of 25%. Overall, the results show a  $^{177m}\text{Lu}$ - $^{177}\text{Lu}$  separation with a maximum  $^{177}\text{Lu}/^{177m}\text{Lu}$  activity ratio of 25. But this is still far away from clinically acceptable activity ratio of 10,000 for which future work is recommended.

## 1. Introduction

Lutetium-177 ( $^{177}\text{Lu}$ ) is a radionuclide with tremendous potential in the field of nuclear medicine (Banerjee et al., 2015). [ $^{177}\text{Lu}$ ]Lu-DOTA-TATE has been approved for neuroendocrine tumor treatment and clinical studies involving the application of other  $^{177}\text{Lu}$  based radiopharmaceuticals in the treatment of prostate cancer, bone pain palliation among others are in progress (Banerjee et al., 2015). Recently, a  $^{177m}\text{Lu}/^{177}\text{Lu}$  radionuclide generator for  $^{177}\text{Lu}$  production has been proposed (De Vries and Wolterbeek, 2012; Bhardwaj et al., 2017) and is anticipated to bring significant advances in the development of  $^{177}\text{Lu}$  based radiopharmaceuticals (Bhardwaj et al., 2019). It offers unique advantage of onsite, on-demand  $^{177}\text{Lu}$  production without the need of a nearby radionuclide production facility. The development of the  $^{177m}\text{Lu}/^{177}\text{Lu}$  radionuclide generator involves the challenging separation of the physically and chemically identical nuclear isomers,  $^{177m}\text{Lu}$  and  $^{177}\text{Lu}$ . The  $^{177m}\text{Lu}$ - $^{177}\text{Lu}$  separation has been based on the internal conversion decay of  $^{177m}\text{Lu}$  and the proof of concept has been already established (Knapp and Dash, 2016; Bhardwaj et al., 2020). Further, the reported liquid-liquid extraction (LLE) based  $^{177m}\text{Lu}$ - $^{177}\text{Lu}$  separation technique has shown promising potential in producing clinically acceptable  $^{177}\text{Lu}$  quality (Bhardwaj et al., 2019). However, the

commercial applicability of LLE based radionuclide generators is limited by several shortcomings such as, ease of utilization, automation, reproducibility, undesired use of organic solvents and others (Bhardwaj et al., 2019).

Solid phase extraction (SPE) has been considered as one of the most convenient method that can allow circumventing the above-mentioned limitations (Dash and Chakravarty, 2014). Its operational simplicity, amenability to automation, and ability to obtain daughter radionuclide using low amount of eluting solvents makes it a very attractive separation technique. The SPE has been explored in the past for the development of  $^{99m}\text{Mo}/^{99}\text{Tc}$ ,  $^{68}\text{Ge}/^{68}\text{Ga}$  and  $^{188}\text{W}/^{188}\text{Re}$  radionuclide generators (Roesch, 2012; Muddukrishna et al., 1990; Braun et al., 1987; Sakr et al., 2017). Typically, in a SPE based radionuclide generator, the parent radionuclide is attached to a solid support and the produced daughter radionuclide is eluted in a liquid phase using an eluting agent (Dash and Chakravarty, 2014). SPE has never been applied for the separation of physically & chemically identical parent-daughter radionuclide pair. The SPE based  $^{177m}\text{Lu}$ - $^{177}\text{Lu}$  separation requires a solid support that should i) be chemically stable ii) allow  $^{177m}\text{Lu}$  complexation and iii) permit the elution of free  $^{177}\text{Lu}$  ions while retaining the complexed  $^{177m}\text{Lu}$  ions.

Amino propyl silica (APS) is one of the extensively studied and often

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used starting material for the preparation of different solid supports (Caravajal et al., 1988; Rostamzadeh et al., 2014; Yang et al., 1997; Sudhölter et al., 1985; Palmi et al., 2013). The presence of amine groups provides a facile way to couple it with a wide variety of functional groups such as acids, esters and others (Montalbetti and Falque, 2005). There are several reports involving the grafting of different molecules (Tamami et al., 2013; Kocyigit et al., 2012; Gangoda et al., 2016; Jal et al., 2004) and macrocyclic compounds (Sander and Wise, 1984; Hirose et al., 2003; Wu et al., 2016; Zhai et al., 2016) on APS surface. However, the application of majority of these solids lies in metal ion recovery (Kocyigit et al., 2012; Gangoda et al., 2016; Hirose et al., 2003; Wu et al., 2016; Zhai et al., 2016; Erdem et al., 2011) or use as silica supported metal catalysts (Tamami et al., 2013) and has never been used for any radionuclide generator development.

In this work the chelator DOTA, which is well known to complex Lu ions, has been grafted on the commercially available amino propyl silica support. The synthesized solid has been characterized and tested for its Lu adsorption behavior. Lastly,  $^{177\text{m}}\text{Lu}$  cations have been adsorbed on the solid surface and tested under different elution conditions that can allow the removal of  $^{177}\text{Lu}$  ions while keeping the leakage of complexed  $^{177\text{m}}\text{Lu}$  ions minimal.

## 2. Experimental

### 2.1. Synthesis of DOTA grafted silica (DGSi)

The grafting of DOTA on APS has been performed using the commercially available precursor, DOTAGA-anhydride, as shown in Fig. 1. Aminopropyl silica (WAT023513) was supplied by Waters as Sep-Pak Aminopropyl ( $\text{NH}_2$ ) Plus Light Cartridge. 2,2',2''-(10-(2,6-dioxotetrahydro-2H-pyran-3-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid (DOTAGA-anhydride) was purchased from Chematech. N, N'-Diisopropylethylamine (DIEPA) and Dimethylformamide (DMF) were purchased from Sigma Aldrich. All the chemicals were used as purchased without any further purification.

DOTAGA-anhydride (80 mg, 0.18 mmol, 3 eqv) was weighed and transferred in a glass tube containing 3–4 mL DMF. DIEPA (50  $\mu\text{L}$ , 10 times excess) was added to it in 3 equal portions at a time interval of 10 min with continuous stirring. Finally, Amino Propyl Silica (APS) (60 mg, 0.06 mmol, 1 eqv) was added and the reaction mixture was left for stirring at 80 °C for 2 h. After 2 h, the reaction mixture was brought to room temperature and the suspension was centrifuged. The separated

solid was washed with 0.1 M HCl deionized water and dried. The reaction conditions were based on the reported protocol involving the reaction between the propyl amine group and DOTAGA-anhydride in liquid phase (Bernhard et al., 2012).

### 2.2. Characterization

Scanning electron microscopy (SEM) experiments were conducted in a JEOL JSM-IT100 microscope operated at an accelerating voltage of 20 kV. The experiments were performed to characterize the surface morphology of the silica particles before and after DOTA immobilization. Solid-state  $^{13}\text{C}$ - Nuclear Magnetic Resonance Spectroscopy were performed at 17.6 TonaBruker Advance spectrometer equipped with a 4 mm triple channel MAS probe (Bruker, Karlsruhe, Germany). Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was carried out in a Nicolet 8700 equipped with a high temperature and pressure cell using a liquid nitrogen cooled MCT/A detector. Spectra were recorded from 4000 to 1000  $\text{cm}^{-1}$  wavenumbers with 128 scans and a resolution of 4  $\text{cm}^{-1}$ . The DRIFT spectra reported in the present work were obtained at 100 °C in order to remove any interference from the adsorbed water content. Thermo-gravimetric analysis (TGA) was performed on a Mettler Toledo TGA/SDTA1 with a sample robot (TSO 801RO) and gas control (TSO 800GC1). The temperature was linearly increased from 30 to 900 °C at a heating rate of 5 °C.min $^{-1}$  under an air flow (100  $\text{cm}^3 \text{min}^{-1}$ ). Grafting percentage of different DOTA moieties on the silica surface was calculated by subtracting the weight loss of the untreated aminopropyl silica particles from the loss after the modification with DOTA. The number of DOTA groups immobilized per g silica (mmol per g) was calculated using the equation below:

$$n_0 = \frac{\text{weight loss of the modified silica particles (g)}}{\text{total silica particle mass (g)} * M_w \text{ of the bonded organic molecules (g mol}^{-1}\text{)}} \quad (1)$$

Lastly, the presence of free amino groups was checked using the Kaiser test kit supplied by Sigma Aldrich, 60,017.

### 2.3. Lutetium-177 and lutetium-177 m sources

The lutetium-177 ( $^{177}\text{Lu}$ ) used in the study was produced by irradiating 1–2 mg of  $\text{LuCl}_3$  at the Hoger Onderwijs Reactor Delft (HOR) with a thermal neutron flux of  $4.72 * 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$  (epithermal neutron flux of less than  $7.08 * 10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$ ) and an irradiation time of 10 h, followed by 3 days of cooling period. The  $^{177\text{m}}\text{Lu}$  source was provided by IDB- Holland as a 1 mM hydrochloric acid solution with 5 MBq  $^{177\text{m}}\text{Lu}$  per g of solution that is in equilibrium with the corresponding  $^{177}\text{Lu}$  activity. For the  $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$  radionuclide generator experiments, about 0.2–0.3 MBq  $^{177\text{m}}\text{Lu}$  was used per experiment.

### 2.4. Study of Lu adsorption on the DOTA grafted silica

About 5 mg of the solids were used for batch adsorption studies. They were taken in an eppendorf and cold Lu ions spiked with  $^{177}\text{Lu}$  (about 10 KBq) were added to it in 4–5 times excess molar ratio. For amino silica, the adsorption was studied at three different pH values, 4.3, 5.6 and 7.3. The APS showed negligible retention of Lu ions at pH 4.3, therefore the adsorption for DGSi was studied only at pH 4.3. The pH during the adsorption was maintained using 0.5 M buffer sodium acetate-acetic acid buffer. The buffer and the Lu ions were added to the solid in an eppendorf. It was left stirring at 80 °C for about 2 h, followed by an incubation period of about 1 h at room temperature. The solid suspension was then transferred to 1 mL empty chromatographic column, 40 mm \* 5.6 mm (supplied by Bio Rad) using a pipette. These columns were connected with a luer lock syringe for a simple single step elution. The columns were eluted manually. First, the excess amount of liquid was flushed out of the column by applying pressure using the empty syringe.

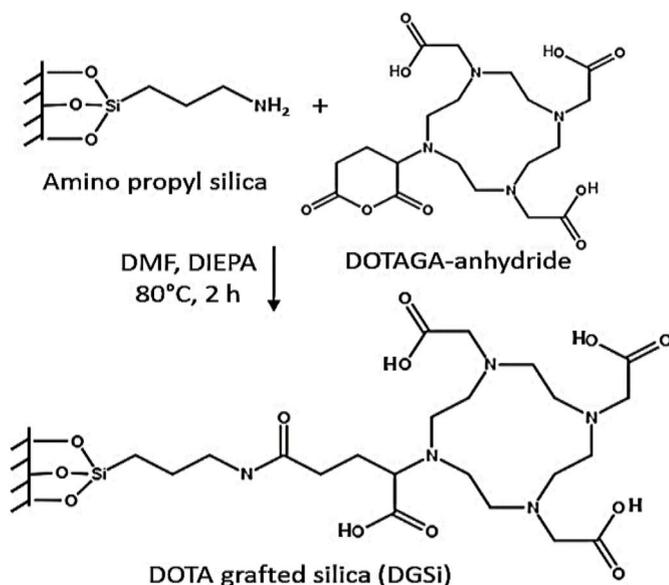


Fig. 1. Schematics of grafting of DOTAGA-anhydride on amino propyl silica.

It was followed by a wash with 10 mM sodium acetate-acetic acid buffer at pH 4.6 and 10 mM DTPA pH 5, (about 2 mL each). The initial activity and the decant liquids were collected and measured using a well type gamma counter (Wallac 2480 Automatic Gamma counter from Perkin Elmer Technologies) to measure the amount of active Lu ions retained by silica. The total Lu adsorption capacity  $q$  in mol  $g^{-1}$  was calculated using Equation (1),

$$q \text{ (mol.g}^{-1}\text{)} = \frac{\text{Counts}_{\text{initial}} - \text{Counts}_{\text{final}}}{\text{Counts}_{\text{initial}}} \times \frac{C_o * V}{1000 * m}$$

where,  $\text{Counts}_{\text{initial}}$  are the initial  $^{177}\text{Lu}$  counts before the adsorption and  $\text{Counts}_{\text{final}}$  represents the total  $^{177}\text{Lu}$  counts in the eluate liquid after the loading and the washing steps.  $C_o$ , represents the total concentration of cold Lu ions used in the experiments,  $V$  is the volume of lutetium solution, and  $m$  is the mass of silica used.

Instrumental Neutron Activation Analysis was also used to determine the adsorption capacity of the synthesized DOTA grafted silica. The cold lutetium ions were adsorbed on the surface of APS and DGSi, using the same protocol as mentioned previously in section 2.4. After the cleaning with DTPA, about 2 mg of the material was bombarded at the Hoger Onderwijs Reactor Delft (HOR) with a thermal neutron flux of  $4.72 * 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$  (epithermal neutron flux of less than  $7.08 * 10^{11} \text{ cm}^{-2} \text{ s}^{-1}$ ) and an irradiation time of 10 h, followed by 3 days of cooling period. The  $^{177}\text{Lu}$  activity of the silica particles was measured on a well-type germanium detector to perform a quantitative evaluation on the amount of Lu ions per g solid.

## 2.5. Gamma ray spectroscopy

The activity measurements were performed using a well-type HPGe gamma-ray detector. The energy and efficiency calibration of the detector was performed using a certified Eu-152 source, and the efficiency calibration for each lutetium peak was fine-tuned using a known  $^{177}\text{Lu}$ ,  $^{177\text{m}}\text{Lu}$  source provided by IDB- Holland to take true-coincidence summing effects into account.

## 2.6. Study of Lu elution behavior on the DOTA grafted silica

In this work, three different eluents namely 10 mM sodium acetate-acetic acid buffer solution (pH 4.3), 10 mM DTPA (pH 5), and 1% DEHPA in DHE were applied as eluting agent. Typically,  $^{177}\text{Lu}$  ions were complexed with 5–6 mg of DGSi using the protocol mentioned in this section. After the complexation and washing steps, the columns with a known initial  $^{177}\text{Lu}$  activity were obtained. A luer lock syringe was attached to one end of the column, and the eluents (10 mM pH-4.3 buffer solution, 10 mM DTPA (pH 4.6), 1% DEHPA in DHE) were passed through the column dropwise by manually inserting pressure on the syringe. For each eluent, the elution fraction volumes of 0.2 mL, 1 mL and 2 mL were collected and measured using gamma ray spectrometry to determine the percentage  $^{177}\text{Lu}$  activity leaked in each fraction.

## 2.7. $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$ separation

The  $^{177\text{m}}\text{Lu}$  ions were adsorbed on the surface of DGSi using the adsorption and washing protocol as detailed previously in sec 2.4. About 5 mg of the DGSi was taken in an Eppendorf. It was left in contact with about 0.30 mL of 1 mM Lu solution containing about 0.3 MBq  $^{177\text{m}}\text{Lu}$  and the pH during the absorption was maintained using 0.5 M buffer sodium acetate-acetic acid buffer. The reaction mixture was left stirring at 80 °C for about 2 h, followed by an incubation period of about 1 h at room temperature. It was then washed with pH- 4.3 NaAc buffer and 10 mM pH-5 DTPA solution. The washes were collected and measured using gamma ray spectroscopy to determine the amount of  $^{177\text{m}}\text{Lu}$  ions loaded on the silica. The  $^{177\text{m}}\text{Lu}$  containing solids were then transferred to 1 mL empty chromatographic column, 40 mm \* 5.6 mm (length \* diameter)

(supplied by Bio Rad) using a pipette. These columns were transferred in a 10 mL centrifuge tube followed by placing them in whirl-pak sampling bag (supplied by Sigma Aldrich, product number. Z527009) and they were moved inside a liquid nitrogen tank, to allow the  $^{177}\text{Lu}$  production during the accumulation period. After a  $^{177}\text{Lu}$  accumulation period of 7 days, the columns were eluted using pH- 4.3 sodium acetate-acetic acid buffer solution as eluent and elution fraction volumes of 0.2 mL were collected. Gamma ray spectroscopy was used to determine the  $^{177}\text{Lu}$ ,  $^{177\text{m}}\text{Lu}$  activity collected in each eluted fraction.

## 3. Results and discussion

The synthesized DOTA grafted silica have been characterized and tested for i) stability ii) Lu absorption capacity iii) Lu elution behavior and iv) the  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation performance, and the results are discussed below:

### 3.1. Synthesis and characterization

The surface morphology of the starting amino propyl silica and the synthesized DOTA grafted silica particles were examined by SEM analysis (Fig. 2).

Fig. 2(a) shows that the original amino propyl silica contained uniformly distributed particles of about 10–20  $\mu\text{m}$ . At the end of modification, a majority of the particles retained their size and remained unaffected (Fig. 2(b)). To further analyze the particles, several characterization studies were performed, as shown in Fig. 3. Fig. 3(a), (b) shows the IR,  $^{13}\text{C}$  NMR spectra of the amino propyl silica before and after the grafting of DOTA, respectively while Fig. 3(c) shows the TGA analysis, which allowed the quantification of the amount of DOTA molecules grafted on the surface of silica.

Fig. 3(a) shows the  $^{13}\text{C}$  NMR spectra of APS (in black) and DGSi (in blue). The  $^{13}\text{C}$  NMR of APS consists of three peaks at 10(C1), 27(C2) and 43(C3) ppm. They are assigned to the carbon chain of amino propyl group as  $\text{SiCH}_2(1)\text{CH}_2(2)\text{CH}_2(3)\text{NH}_2$ , accordingly (Caravajal et al., 1988). The  $^{13}\text{C}$  NMR spectra of DGSi showed additional broad peaks around 50 ppm and 170 ppm, which can be ascribed to the aliphatic  $\text{CH}_2$  groups and the carbonyl carbons of the DOTA, respectively. Additionally, an upfield shift from 27 ppm to 21 ppm was observed for the peak corresponding to C2 carbon of amino silica, along with a shoulder peak at 27 ppm. The upfield shift from 27 ppm to 21 ppm can be attributed to binding of amino groups with the DOTA groups, while the shoulder peak indicates a small part of unreacted amino groups (Caravajal et al., 1988).

Fig. 3(b) shows the IR spectrum of APS (in black), DGSi (in blue), and DGSi after Lu complexation (in red). The IR spectrum of APS exhibits a sharp peak around  $3675 \text{ cm}^{-1}$ , which is characteristic for the silanol groups present on the surface of silica (Capel-Sanchez et al., 2004; Dugas and Chevalier, 2003; Newby et al., 2011). The three peaks at  $3376$ ,  $3310 \text{ cm}^{-1}$  and  $1595 \text{ cm}^{-1}$  can be assigned to characteristic N–H stretching vibrations and to the  $\text{NH}_2$  deformation mode of free amino groups (Palmai et al., 2013; Socrates, 1994). The bands around  $3000\text{--}2800 \text{ cm}^{-1}$  belong to the C–H stretching vibrations (Capel-Sanchez et al., 2004; Kulkarni et al., 2008). The peak at  $1868 \text{ cm}^{-1}$  is characteristic of the Si–O vibration of the silica structure (Blitz et al., 1988) and the broad peak around  $1349 \text{ cm}^{-1}$  can be attributed to Si–O–Si asymmetric stretching vibration (Vansant et al., 1995). After the reaction with DOTAGA-anhydride, the peaks corresponding to NH stretching vibrations and to the  $\text{NH}_2$  deformation mode of free amino groups disappear with the appearance of new bands around  $1720 \text{ cm}^{-1}$ ,  $1652 \text{ cm}^{-1}$  (see DGSi spectra, in blue). The peaks at  $1720 \text{ cm}^{-1}$ ,  $1652 \text{ cm}^{-1}$  correspond to the free –COOH groups and –CO–NH– group which confirms the successful coupling of DOTA molecules to the amino propyl groups. Further, after the loading of Lu ions on the surface of DGSi, the peak at  $1720 \text{ cm}^{-1}$  disappears indicating the successful coordination of carboxylic acids with Lu ions (see Fig. 3(b), DGSi-Lu spectra, in red). Further, the DGSi also gave a positive Kaiser test, which confirms the

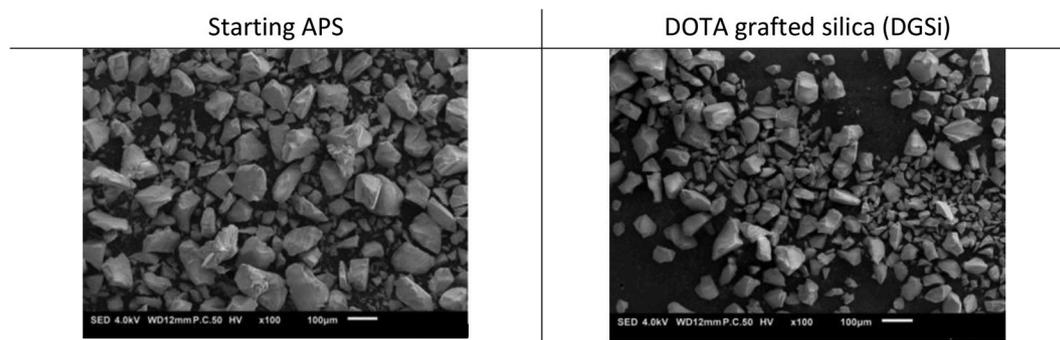


Fig. 2. SEM images of a) Amino propyl silica (APS) b) DOTAGA-anhydride grafted silica (DGSi).

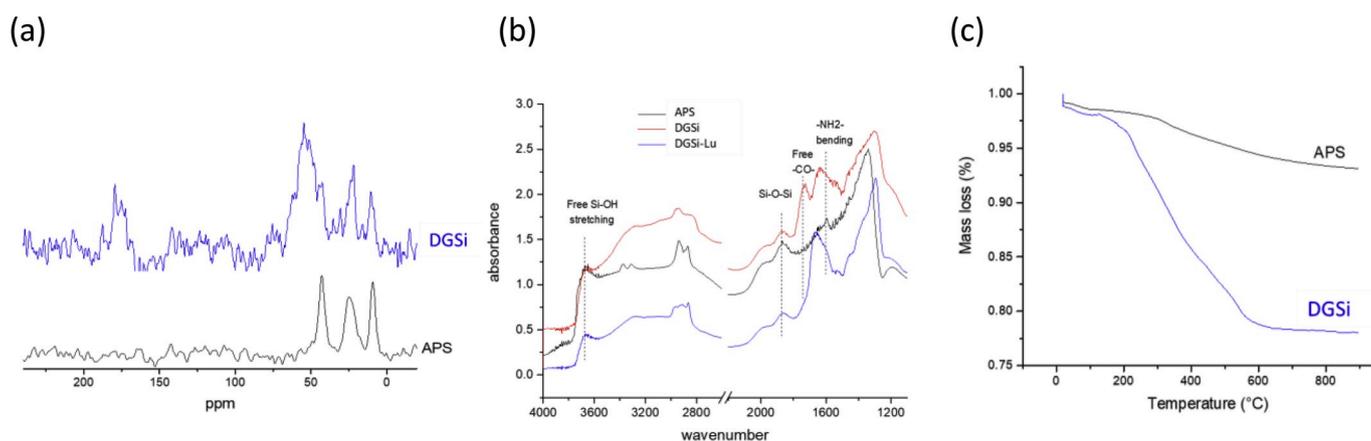


Fig. 3. Characterization studies (a) <sup>13</sup>C NMR spectra of aminopropyl silica (APS) (in black) and DOTAGA- Grafted Silica (DGSi) (in blue) (b) FT-IR spectra of aminopropyl silica (APS) (in black), DOTAGA- Grafted Silica (DGSi) (in blue) and DGSi after the coordination with Lu ions (in red) (c) TGA of aminopropyl silica (APS) (in black), DOTAGA- Grafted Silica (DGSi) (in blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

presence of unreacted primary amino groups.

Fig. 3(c) shows the TGA spectra of APS (in black) and DGSi (in blue). It can be seen that both solids exhibit a mass loss in the temperature range 200–900 °C that corresponds to the organic groups. For APS, a mass loss of 5.4% has been observed which matches the manufacturers specifications of 1 mmol amino propyl groups per gram silica. For DGSi, a much higher organic mass loss of 20.6% was observed. The increased organic mass loss is attributed to the DOTA groups grafted on the surface and corresponds to 0.33 mmol DOTA groups per g of silica (in accordance with Equation (1)).

To summarize, the characterization studies shown in Fig. 3 indicate the successful coupling of DOTA groups on the surface of amino propyl silica and establish a novel strategy to immobilize DOTA groups on the surface of commercially available silica. Additionally, under the studied reaction conditions, some free amino groups remain present on the surface of DGSi as indicated by the IR, <sup>13</sup>C NMR, and a positive Kaiser test.

### 3.2. Lutetium adsorption

The adsorption of lutetium on DOTA grafted silica can happen either via the chemical complexation of Lu ions with the DOTA ligands or by undesired physical adsorption on the surface. The pH showing minimal interference with the chemical complexation of Lu ions has been determined and the results are shown in Table 1.

Table 1 shows that for APS an increase in Lu adsorption capacity was observed with the increase in the pH. The lowest lutetium ion adsorption of 0.02 nmol g<sup>-1</sup> was obtained at pH 4.3 increasing to about 4 nmol g<sup>-1</sup> at pH 8.3. This is expected as the APS surface has been reported to have

Table 1

The lutetium adsorption capacity of amino propyl silica (APS) and DOTAGA grafted silica (DGSi).

pH	Lu uptake	
	APS	DGSi
4.3	0.02 ± 0.002 nmol g <sup>-1</sup>	0.03 ± 0.005 mmol g <sup>-1</sup>
5.6	0.3 ± 0.003 nmol g <sup>-1</sup>	Not tested
8.5	4.4 ± 0.022 nmol g <sup>-1</sup>	Not tested

an iso-electric point around pH 6–7<sup>16</sup>. An increase in the pH leads to an increased negative charge on the APS surface and thus a higher affinity for positively charged Lutetium ions. Thus, pH-4.3 has been used for the Lu complexation, as it is also considered as an ideal pH for the Lu-DOTA complexation.

For DGSi, a Lu adsorption capacity of 0.03 mmol g<sup>-1</sup> has been observed (see Table 1) in comparison to 0.02 nmol g<sup>-1</sup> observed for amino propyl silica. The increased Lu uptake confirms the successful immobilization of DOTA groups on the surface of APS. However, the observed Lu uptake was 10 times less than the amount of DOTA grafted on the surface (0.33 mmol. g<sup>-1</sup> calculated from Fig. 3(c)). This was unexpected, as lutetium has been known to form a stable cage like coordination with DOTA in a 1:1 stoichiometry under the studied reaction conditions (Desreux, 1980). This suggests that under the studied reaction conditions, not all the DOTA groups were accessible to lutetium ions. It can also be due to the changed coordination behavior or slowed kinetics of complex formation after the grafting of DOTA on a solid surface. The change in the coordination behavior of metal ions after the

immobilization of DOTA groups on solid surface was previously observed for Pd-DOTA complex (Wu et al., 2016). It was suggested that Pd(II) gets coordinated with neighboring carboxylic groups instead of the coordination with N atoms in the cage structure (Wu et al., 2016). Presently, we did not investigate the exact nature of Lu ion complexation with the surface and such study should be performed in future to have a better understanding of the coordination mechanism.

### 3.3. Lutetium elution behavior

The  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation requires an eluting agent that minimizes the leakage of the complexed  $^{177\text{m}}\text{Lu}$  ions while allowing the release of the freed  $^{177}\text{Lu}$  ions.  $^{177}\text{Lu}$  cations were complexed with the DGSi and then different eluents were passed through the column to study the Lu leakage. The percent  $^{177}\text{Lu}$  leakage was studied as a function of elution fraction volume, as shown in Fig. 4 below. Fig. 4(a), (b), 4(c) represent the elution fractions of 2 mL, 1 mL and 0.2 mL respectively, collected using three different eluents namely, pH-4.3 sodium acetate-acetic acid buffer, aqueous pH-5 solution with 10 mM DTPA, and 0.5% DEHPA in DHE.

Fig. 4(a) shows that for the studied eluents, the Lu leakage varies in the order of 0.4%–1.5%. The percent Lu leaked remain almost constant when three successive fractions of 2 mL were collected. The lowest Lu leakage of 0.4% was observed on using 0.5% DEHPA in DHE as an eluent. The weaker interactions between the hydrophobic organic solvent and hydrophilic silica surface can possible explain the detected low Lu leakage. However, Lu leakage of the order of 0.002% has been observed previously using Lu-DOTA complex in liquid phase. The much higher Lu leakage observed in the current study points to the fact the Lu is not coordinated in the expected stable cage like coordination with the DOTA complex (Bhardwaj et al., 2019).

In order to reduce the lutetium leakage, smaller fractions of 1 mL and 0.2 mL were collected, and the results are shown in Fig. 4(b) and (c). As expected, the decrease in the elution fraction volume leads to a proportional decrease in the percent Lu ions leakage. Further, for elution fraction volumes of 0.2 mL, the first fraction contained the Lu leakage ranging from 0.1% to 0.3%, and decreases to less than 0.05% in the subsequent fractions (see Fig. 4(c)). The 0.1% Lu leakage is still far from the previously observed Lu leakage of 0.002%, but for the studied SPE extraction it provides with a possibility to decrease the  $^{177\text{m}}\text{Lu}$  leakage during the  $^{177}\text{Lu}$ - $^{177}\text{Lu}$  separation.

Lastly, it should be mentioned that the use of ether as an eluent solvent damages the column materials, and they could not be reused. Therefore, a dihexyl ether-based eluent was not used in  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation experiments and only the pH-4.3 NaAc buffer was used with the collection of 0.2 mL elution fraction volumes.

### 3.4. $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$ separation

The  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation experiments were performed using DOTAGA grafted silica (DGSi). The  $^{177\text{m}}\text{Lu}$  ions were complexed with DGSi, and the solids were left at 77 K to allow for  $^{177}\text{Lu}$  accumulation for a period of 7 days. At 77 K, the rate constants for the chemical reactions (i.e. association-dissociation kinetics) are extremely low making the  $^{177\text{m}}\text{Lu}$  contribution coming from the dissociation of the Lu-DGSi negligible. At the end of accumulation, the  $^{177}\text{Lu}$  ions were eluted 10 mM NaAc buffer (pH-4.3) and elution fraction volume of 0.2 mL were collected. The  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio obtained in each elution fraction is shown in Fig. 5(a) and Fig. 5(b) displays the corresponding  $^{177}\text{Lu}$  extraction efficiency and the percentage of starting  $^{177\text{m}}\text{Lu}$  activity leaked.

Fig. 5(a) shows that the  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio varies in the collected elution fraction. The first fraction exhibited very low  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio of 5, which increases to 25 for the fourth to eighth fraction and decreases further in the subsequent elution fractions. The observed trend can be explained on the basis of the results shown in Fig. 5(b). As can be seen, the highest  $^{177\text{m}}\text{Lu}$  leakage of 0.3% was observed in the first fraction and decreases to around 0.01–0.04% in the successive elution fractions. Similarly, the highest  $^{177}\text{Lu}$  extraction efficiency of 25% was observed in the first fraction and decreases to less than 5% in the successive fractions. On combining all the fractions, an overall  $^{177}\text{Lu}$  extraction efficiency of about 50% has been achieved. Further, the Lu ion removal does not exhibit a sharp peak but a tailing profile over the period of eluted volume. This suggests that either there are multiple binding sites or that Lu that is released can re-associate which also explains the observed low  $^{177}\text{Lu}$  extraction efficiencies. This observation again points out to the weak coordination of  $^{177\text{m}}\text{Lu}$  ions possibly due to their interaction with more than one DOTA group, what would result in bridged complexes that are less stable.

To summarize, the SPE based separation offers the possibility of building an easy to automatize, user friendly  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation technique. The presented separation method could lead to  $^{177}\text{Lu}$  enrichment of 100 times compared to the  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio of 0.25 when present in equilibrium with each other. However, currently it is inapplicable in designing a  $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$  radionuclide generator because the obtained ratios are far from the clinically preferred  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio of 10,000. The main challenge lies in designing the solid supports which forms a stable coordination with  $^{177\text{m}}\text{Lu}$  and causes minimal interference during  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation. The currently used DOTA grafted amino propyl silica did not allow the formation of stable cage coordinated Lu:DOTA complex under the studied reaction conditions. Further, the unreacted DOTA groups interfered in  $^{177\text{m}}\text{Lu}$  complexation and  $^{177}\text{Lu}$  release, ultimately leading to poor  $^{177\text{m}}\text{Lu}$ - $^{177}\text{Lu}$  separation. In future, this can be possibly minimized by the use of solid supports having low functional group density.

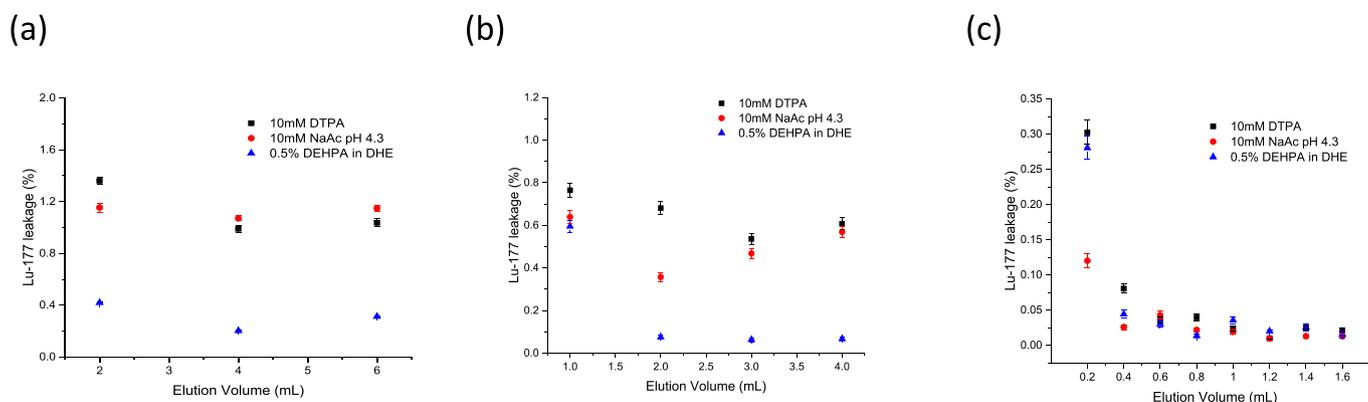
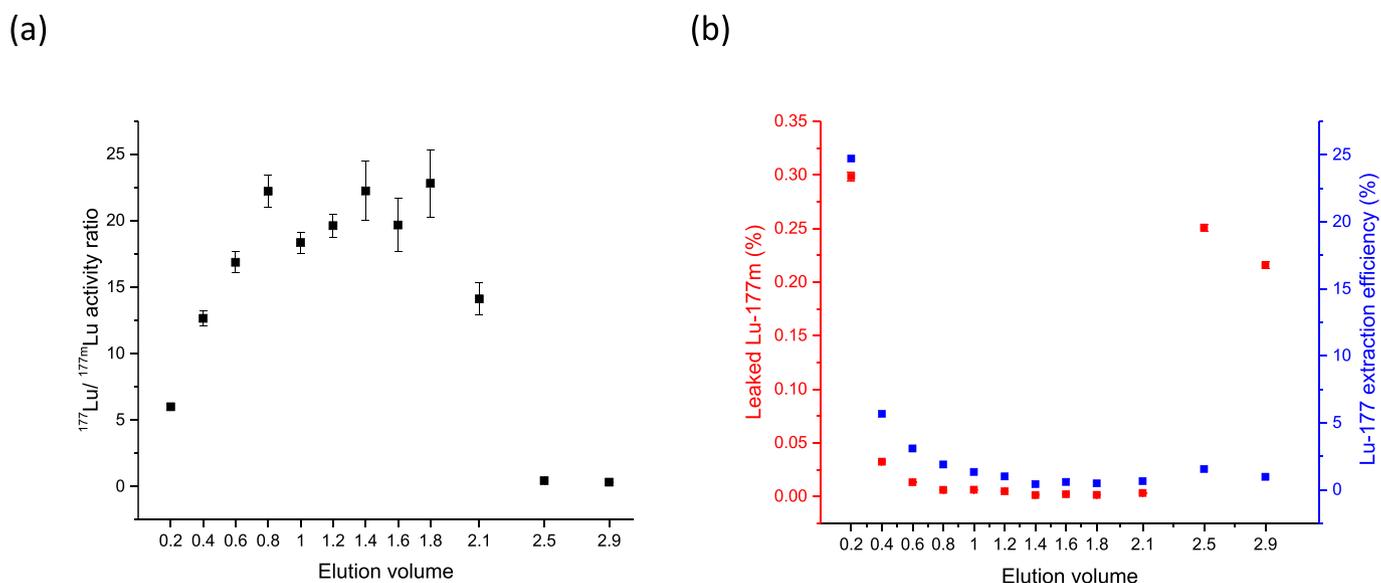


Fig. 4. The Lu elution profiles as a function of elution fraction 2 mL (a) 1 mL (b) 0.2 mL (c) obtained for the three different eluents namely, 10 mM sodium acetate-acetic acid buffer (pH- 4.3), 10 mM DTPA (pH-5) and 0.5% DEHPA in DHE.



**Fig. 5.** The  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio as a function of elution volume (fraction 0.2 mL) (a) and the corresponding  $^{177}\text{Lu}$  extraction efficiency, the leaked  $^{177\text{m}}\text{Lu}$  (b) obtained using pH-4.3 NaAc buffer as eluent. The presented data has been based on one experiment, and the error bars represents the error during the gamma ray spectroscopy measurements.

For instance, the use of a support with a functional group density of  $10 \mu\text{mol g}^{-1}$  (100 times lower than currently used APS) can significantly reduce the interference in  $^{177\text{m}}\text{Lu}$  complexation,  $^{177}\text{Lu}$  release and can potentially allow the loading of up to 2 GBq  $^{177\text{m}}\text{Lu}$  per mg solid.

#### 4. Conclusions

The presented work is the first step in designing a solid phase extraction based  $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$  separation. It establishes a strategy to immobilize DOTA groups on the surface of commercially available silica. The use of commercially available DOTAGA-anhydride allows easy and facile conjugation of DOTA moiety on silica surface. DOTA immobilized on silica was utilized as a solid support to facilitate the  $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$  separation. A high  $^{177\text{m}}\text{Lu}$  leakage during the  $^{177}\text{Lu}$  ( $^{177\text{m}}\text{Lu}$ ) separation impeded the achievement of activity ratios in the same order of magnitude as accomplished with liquid-liquid extractions. A maximum  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio of 25 could be achieved when the  $^{177\text{m}}\text{Lu}$  contribution was reduced to 0.01%. It is hypothesized that the grafting of DOTA on silica surface affected the Lu-DOTA complex formation and the stable cage coordination of Lu ions could not be achieved under the studied reaction conditions. Overall, the solid phase extraction presents an easy to automatize, user friendly and reproducible  $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$  technique. However, it needs further optimization and a careful evaluation of the complex formation and the kinetics of association and dissociation of Lu ions in order to reach high  $^{177}\text{Lu}/^{177\text{m}}\text{Lu}$  activity ratio.

#### Declaration of competing interest

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

#### CRediT authorship contribution statement

**Rupali Bhardwaj:** Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Hubert T. Wolterbeek:** Conceptualization, Funding acquisition. **Antonia G. Denkova:** Conceptualization, Project administration, Funding acquisition. **Pablo Serra-Crespo:** Methodology, Conceptualization, Writing - review &

editing, Supervision, Project administration, Funding acquisition.

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