Chinese Chemical Letters 33 (2022) 3474–3477

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Isolation of ^{212}Pb from natural thorium for targeted alpha-therapy

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A R T I C L E I N F O

Article history:
Received 29 November 2021
Revised 5 February 2022
Accepted 7 February 2022
Available online 10 February 2022

Keywords:
^{212}Pb
Targeted alpha-therapy
Radiolabeling
Radionuclide generator
Solid-phase extraction

A B S T R A C T

Targeted alpha-therapy (TAT) is increasingly attractive due to its extraordinary antitumor efficacy. However, the supply of α-emitters for TAT is insufficient and under control by a limited number of countries. ^{212}Pb is a promising α-emitter with an optimal half-life (10.6 h) and favored decay chain. Of interest, ^{212}Pb can be extracted directly from natural thorium, which may be abundant in the mining waste of rare-earth, uranium, etc. Indeed, radioactive thorium waste has been a longstanding environmental challenge that needs immediate action. Developing an on-demand and facile process to isolate ^{212}Pb from natural thorium would be ideal to meet the above challenges, yet is difficult. In theory, the ratio of ^{212}Pb to ^{nat}Th is below 10^{-13} in commercially available thorium salts. As a pilot study, 2.2 MBq of ^{212}Pb was successfully extracted from a 5 L solution of thorium nitrate by using a Pb-selective resin. The radiochemical purity of ^{212}Pb is over 99.9% according to gamma-ray analysis. The purified ^{212}Pb was applied to radiolabel a couple of peptides used in clinics (*i.e.* PSMA, TATE and FAPI-04), and the radiochemical yields are >85%. Of note, ^{212}Pb can be repeatedly separated from the thorium solution every 2 days. In summary, a practical and scalable method was developed to isolate ^{212}Pb for potentially clinical use, which may be of great importance as it does not require either cyclotron or nuclear reactor.

TAT (Targeted alpha-therapy) treats cancer by using α-particles [1–3], of which the linear energy transfer (LET) is notably higher than that of β-particles [4], resulting in lethal damage in cancer cells. α-Particles often induce DNA double-strand break, which can hardly repair, and are less dependent on the oxygen level in tumors [5]. The short range of α-particles (50–100 µm) confines the killing region to the lesion and reduces the negative effects on normal tissues [6]. α-Emitters exhibit better therapeutic efficacy than that of β-emitters in clinical studies, features with much less therapeutic dose and less radiation resistance [7,8].

For instance, ^{225}Ac-labeled radiopharmaceuticals have demonstrated exceptional performance in clinical studies [9–11]. However, ^{225}Ac is conventionally generated from ^{229}Th or ^{233}U [12,13], and its annual worldwide manufacturing capacity is less than 3 Ci [14], posing a barrier to the growth of TAT [15]. ^{212}Pb ($t_{1/2} = 10.6$ h) decays to the stable ^{208}Pb, which is also an α-emitting nuclide and can be radiolabeled with DOTA or other TAT chelators [4,17].

Extracting ^{212}Pb from natural thorium will resolve the current shortage of α-emitters for TAT and provide a means of supplying α-emitters without relying on high-energy accelerators and reactors. Despite the fact that ^{212}Pb has been developed for TAT for many years, the available source of ^{212}Pb is rather insufficient. ^{212}Pb may be separated from the decay of ^{228}Th, ^{232}Th or ^{232}U [18,19]. Compare to ^{232}Th, ^{228}Th and ^{232}U are preferred due to the high content of ^{212}Pb [16,19]. However, both ^{228}Th and ^{232}U are not commercially available and can hardly be obtained by importation [20]. In contrast, ^{232}Th is abundant, especially in China [21]. The low ^{212}Pb content and the consequent technical challenges have hampered the investigation of directly extracting ^{212}Pb from natural thorium. Though it may be the only realistic strategy to prepare ^{212}Pb in China [20], the reports about isolating ^{212}Pb from natural thorium are limited [22,23].

In this work, we attempted to separate ^{212}Pb directly from natural thorium compounds for labeling radiopharmaceuticals. By solid-phase extraction with a Pb-selective resin (Pb-Spec^{TM}) [24], the ^{212}Pb was isolated directly from the thorium nitrate solution. 2.2 MBq of ^{212}Pb was obtained from 2.5 kg of thorium nitrate hydrate every 48 h, representing 54% of the theoretical maximal activity and 83% of the ^{212}Pb separation efficiency. To validate the quality of the prepared ^{212}Pb, it was applied to label several peptide conjugates of clinical importance, DOTATATE [25], PSMA-617 [26] and FAPI-04 [27,28], with good radiochemical yields (over 88%).

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https://doi.org/10.1016/j.cclet.2022.02.012
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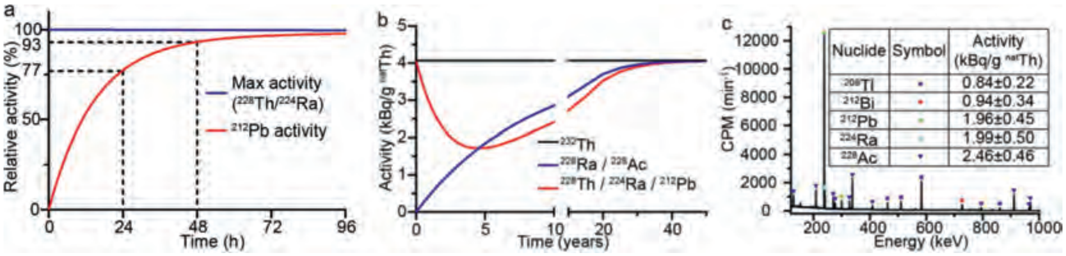

Fig. 1. (a) Recovering of ^{212}Pb from ^{228}Th and ^{224}Ra, (b) $^{232/228}$Th decay equilibrium curve, and (c) γ-spectroscopy of commercially available thorium compounds.

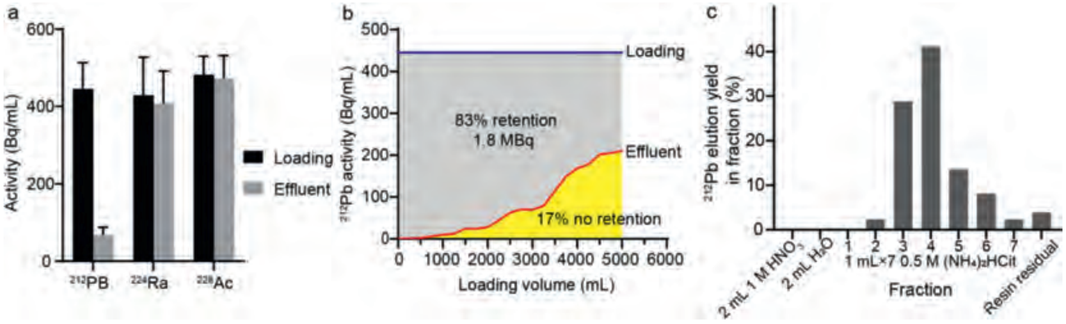

Fig. 2. Pb adsorption and elution by Pb-Spec^{TM} extraction column. (a) ^{212}Pb, ^{224}Ra and ^{228}Ac activities in the loading and effluent of the column. (b) ^{212}Pb activity curves in the extraction column loading and effluent. (c) Extraction column ^{212}Pb elution yield in each fraction.

^{232}Th is abundant and often considered as radioactive waste from mining. The ineffective utilization and treatment of thorium-containing slag not only wastes resources, but also causes major environmental issues when radionuclides from ^{232}Th enter the atmosphere as dust or enter the land and rivers *via* groundwater [29]. The decay of ^{232}Th produces ^{212}Pb, which is an α-emitter and can be exploited for TAT. The activity of ^{212}Pb in natural thorium is 4.07 kBq/g ^{232}Th at decay equilibrium [30], and it may be extracted directly from thorium compounds for radiopharmaceuticals. In the decay chain, ^{212}Pb was accumulated *via* ^{224}Ra (α, 3.7 d) ^{220}Rn (α, 55.6 s) ^{216}Po (α, 0.14 s) ^{212}Pb. According to the aforementioned, shown in Fig. 1a, ^{212}Pb in $^{232/228}$Th can be recovered 77% within 24 h and up to 93% within 48 h following the separation of ^{212}Pb, which satisfies the nuclide generator's requirement.

The decay equilibrium curve of $^{232/228}$Th is shown in Fig. 1b. Right after the isolation of thorium compounds, the activity of ^{212}Pb gradually declines due to the rapid decay of ^{228}Th, eventually reaching the lowest value of 1.7 kBq/g ^{nat}Th in the fourth year. Following that, as ^{232}Th decays to ^{228}Th, the ^{212}Pb activity grows gradually until it reaches decay equilibrium.

As shown in Fig. 1c, the γ-spectrum of commercial thorium nitrate. As an inexpensive and easily accessible thorium compounds, thorium nitrate contains radionuclides from the thorium decay chain, ensuring its purity as a raw material for ^{212}Pb generators. The acquired thorium compound had not reached decay equilibrium, as the ^{212}Pb activity was only 1.96 kBq/g ^{nat}Th, or 48% of equilibrium activity, resulting in additional thorium compound.

Thorium nitrate hydrate (2.5 kg) was dissolved in 5 L HNO$_3$ solution (1 mol/L) with a thorium concentration of around 1 mol/L. According to previous results, this solution had an activity concentration of around 386 kBq/L and total activity of 1.93 MBq.

Pb-Spec^{TM} is a extraction chromatographic resin by sorbing 4,4′,(5′)-di-(t-butyldicyclohexano)−18-crown-6 (DtBu-CH18C6) in isodecanol solution on an inert substrate for the separation and preconcentration of radiolead [24,31]. Lead has a good retention on Pb-Spec^{TM} under a wide range of nitric acid concentration (0.1–8 mol/L). Under 1 mol/L HNO$_3$, the D_w of the PB-Spec^{TM} was around 1.5×10^3 ($k' \sim 8 \times 10^2$) [24], indicating that the extraction column with 6 mL resin (2.3 g resin, Φ12.5 mm × 50 mm, 4 mL free column volume) was adequate as the extraction resin for a more efficient ^{212}Pb extraction.

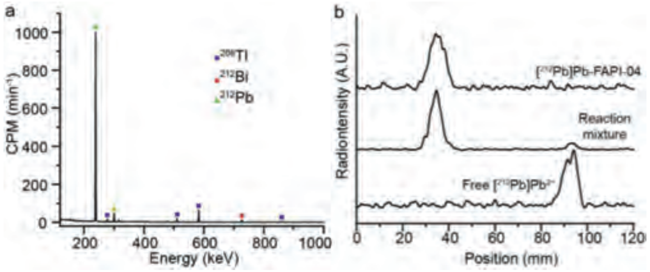

Fig. 3. (a) γ-spectrum of ^{212}Pb product. (b) Radio-iTLC of free [^{212}Pb]Pb^{2+}, reaction mixture, and purified ^{212}Pb-labeled compound during the ^{212}Pb labeling of FAPI-04.

To trap ^{212}Pb with high efficiency, 5 L of a 1 mol/L Th(NO$_3$)$_4$/1 mol/L HNO$_3$ solution was loaded onto the extraction column at a flow rate of 0.5 L/h. The column was then washed with 5 mL 1 mol/L HNO$_3$ and 5 mL H$_2$O to remove residual Th^{4+} and to reduce the acidity. Finally, Pb^{2+} was eluted with 7 mL 0.5 mol/L (NH$_4$)$_2$HCit [31], which is suitable for radiopharmaceutical labeling directly.

As shown in Fig. 2a, the column trapped Pb but not Th, Ra or Ac. The radioactivity of ^{212}Pb fell from 445 Bq/mL to 68 Bq/mL in the elution, with an 85% retention ratio, suggesting that the column can efficiently separate and enrich ^{212}Pb. The generator could rapidly regenerate for the next ^{212}Pb separation. The change of ^{212}Pb activity in the column effluent is described in Fig. 2b. At the beginning of loading, the column retained 100% of the ^{212}Pb, but as the loading volume increased, small amount of ^{212}Pb leakage was observed. The average extraction efficiency of ^{212}Pb was 83%, yielding 1.8 MBq ^{212}Pb. As demonstrated in Fig. 2c, 7 mL 0.5 mol/L (NH$_4$)$_2$HCit could efficiently elute 95% ^{212}Pb from the column. We analyzed the samples that had reached decay equilibrium in the next day, and found that the radioactivity of ^{212}Pb was 2.2 MBq at the end of purification.

The γ-spectrum of obtained ^{212}Pb was shown in Fig. 3a. All visible peaks were ascribed to ^{212}Pb and its daughter, confirming the

Table 1
Conditions and results of ^{212}Pb labeling.[a]

Compound	Precursors	^{212}Pb	Condition	RCY (%)[b]	Isolation yield (%)[c]
FAPI-04	50 µg (55 nmol)	370 kBq $^{212}Pb^{2+}$	pH 5.0~5.5	90.7 ± 3.2	86.0 ± 5.3
PSMA-617	50 µg (45 nmol)	1 mL 0.5 mol/L $(NH_4)_2$HCit	95 °C, 10 min	90.3 ± 5.0	84.3 ± 8.1
DOTATATE	50 µg (33 nmol)			88.3 ± 5.1	83.3 ± 5.8

[a] Precursors were combined with ^{212}Pb solution and then incubated for 10 min at 95 °C. Purification of the product is accomplished using C18 column extraction ($n = 5$).
[b] Radiochemical yield was determined *via* radio-iTLC of the reaction mixture. TLC-SG strips were loaded with samples and developed using 0.1 mol/L Na_3Cit as mobile phase. Strips were left for 4 h for the decay equilibrium, then evaluated with a Miniscan radio TLC scanner.
[c] The isolation yield was calculated based on the decay-corrected product, which was left for 4 h to reach decay equilibrium after C18 column extraction.

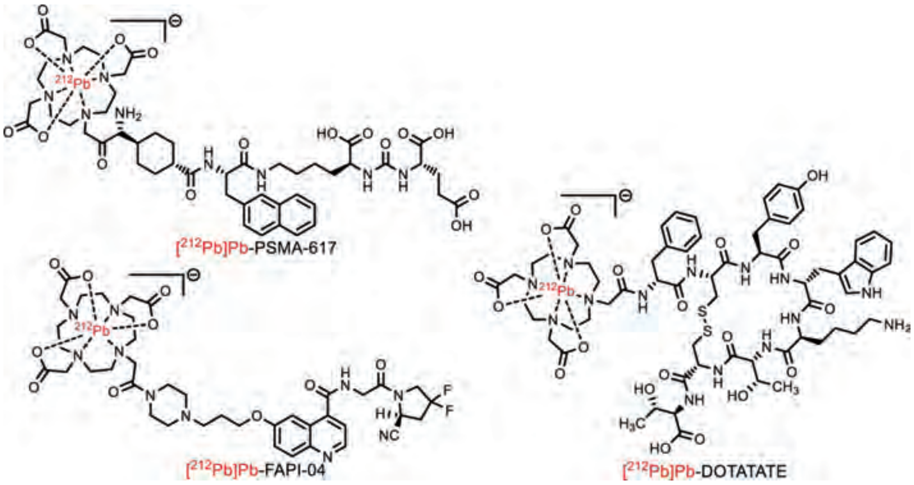

Fig. 4. Chemical structure of [^{212}Pb]Pb-FAPI-04, [^{212}Pb]Pb- DOTATATE and [^{212}Pb]Pb- PSMA-617.

greater purity of ^{212}Pb product obtained by this approach. To further evaluate the ^{212}Pb's purity, it was utilized to label a couple of peptides. Although Cit^{3-} can be coordinated with Pb^{2+}, it was demonstrated that 0.5 mol/L $(NH_4)_2$HCit did not affect the labeling of Pb^{2+}. Additionally, 0.5 mol/L $(NH_4)_2$HCit with a pH of 5.5 may be an ideal buffer for ^{212}Pb labeling with DOTA. The ^{212}Pb labeling reaction was carried out for 10 min at 95 °C, and the reaction's progress and yield were monitored using radio-iTLC (Na_3Cit as mobile phase, Fig. 3b). Table 1 summarizes the labeling conditions and results for [^{212}Pb]Pb-FAPI-04, [^{212}Pb]Pb-PSMA-617, and [^{212}Pb]Pb-DOTATATE (chemical structure shown in Fig. 4). All radiochemical yields (RCY) were greater than 88%, and isolated yields were greater than 83%, indicating that the ^{212}Pb produced using this method was reliable for radiopharmaceutical investigations.

In the above experiment, we successfully obtained 2.2 MBq ^{212}Pb from a 5 L thorium nitrate solution. However, to obtain more ^{212}Pb, additional thorium nitrate solution is needed, which may be difficult in the lab. Another solution to obtain ^{212}Pb based on ^{nat}Th is to extract $^{228/224}$Ra from the matrix and enrich them to isolate ^{212}Pb in a limited volume of $^{228/224}$Ra, which would be easier to generate a higher activity of ^{212}Pb. Therefore, the separation and enrichment of $^{228/224}$Ra from natural thorium compounds are ongoing to construct next-generation of ^{212}Pb generator. In addition, in the process of rare earth and uranium mining, 'radium removal residue' is a kind of radium-containing waste residue in the form of $Ba(Ra)SO_4$, and the amount of $^{228/224}$Ra is considerable. If the waste could be used as a source of $^{228/224}$Ra to prepare $^{228/224}$Ra-^{212}Pb generators, it would be a "one-stone two-bird" strategy that make full use of the hazardous radioactive waste to produce high-value medical radionuclides.

To summarize, this study aims to extract and purify α-emitter ^{212}Pb from natural thorium compounds for use in TAT radiopharmaceuticals. 2.2 MBq of ^{212}Pb was extracted from 5 L of thorium nitrate solution using Pb-Spec^{TM} with an extraction efficiency of 83% and a high purity. The ^{212}Pb in 0.5 mol/L $(NH_4)_2$HCit solution produced could be used directly for the radiolabeling of DOTATATE, PSMA-617 and FAPI-04, which all achieved greater than 88% RCY and 83% isolation yield. The aforementioned results demonstrate that modest amounts of ^{212}Pb can be isolated directly from natural thorium compounds for developing ^{212}Pb-labeled radiopharmaceuticals for targeted alpha-therapy.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Acknowledgments

This work was funded by Beijing Municipal Natural Science Foundation (No. Z200018), the Special Foundation of Beijing Municipal Education Commission (No. 3500–12020123), the National Natural Science Foundation of China (Nos. U1867209 and 21778003) and the Ministry of Science and Technology of the People's Republic of China (No. 2017YFA0506300) and Li Ge-Zhao Ning Life Science Youth Research Foundation (No. LGZNQN202004).

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