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Radiochemical separation of ^{224}Ra from ^{232}U and ^{228}Th sources for ^{224}Ra/^{212}Pb/^{212}Bi generator

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ABSTRACT

The application of diagnostic and therapeutic radionuclides in nuclear medicine has grown significantly and has translated into the increased interest in radionuclide generators and their development. ^{224}Ra and its shorter-lived daughters, ^{212}Pb and ^{212}Bi, are very interesting radionuclides from Targeted Alpha Therapy point of view for treatment of small cancers or metastatic forms. The purpose of the present work was to develop a simple generator for rapid elution of carrier-free ^{224}Ra from ^{232}U or ^{228}Th sources by radiochemical separation based on extraction chromatography with the utilization of a home-made material. The bis(2-ethylhexyl) hydrogen phosphate (HDEHP) extractant was immobilized on polytetrafluroethylene (PTFE) grains and its ability to selectively adsorb ^{232}U and ^{228}Th, with simultaneous high elution recovery of ^{224}Ra, was checked over few years. The ^{224}Ra was quantitatively eluted with small volume (3–5 mL) of 0.1 M HNO_3 with low breakthrough (<0.005%) and was used for further milking of ^{212}Bi and ^{212}Pb from DOWEX 50WX12 by 0.75 M and 2.0 M HCl, respectively. The elaborated here methods allowed high recovery of ^{224}Ra, ^{212}Pb and ^{212}Bi radionuclides and their application in radiolabeling of various biomolecules.

1. Introduction

Targeted Alpha Therapy (TAT) is an attractive therapeutic modality that has been shown to be a potent weapon in the treatment of various cancers, especially in patients with developed multiple micrometastases (Poty et al., 2018a; Guerra Liberal et al., 2020). It utilizes radionuclides that emit during decay high-energy (4–9 MeV) α-particles, which due to their high Linear Energy Transfer (LET) *ca.* 80–100 keV/μm very easily interact with surrounding matter and create many ionization incidents along their path (Gott et al., 2016). For this reason α-particles are highly destructive *in vivo* over a very short range in tissues (<100 μm), depositing 1500-times more energy per unit path length than β^--particles (Guerra Liberal et al., 2020). The high effectiveness of α-radiation is mostly due to direct interaction with DNA and induction of double strand breaks, making cellular repair mechanisms ineffective which ultimately results in cancerous cell death (Poty et al., 2018b). Thus, α-particle emitting radionuclides are very promising in treatment of micrometastases, residual tumor cells after surgical resection of a primary lesion, hematologic and compartmental cancers.

There are several α-emitting radionuclides that might be used in nuclear medicine (Ferrier et al., 2019). One of them is ^{224}Ra that is a pure α-emitter with a half-life of 3.63 days, maximum energy of 5.69 MeV for emitted α-particle, and a relatively abundant γ-emission at 241.0 keV (4.1%). It decays via a series of six daughter nuclides emitting four α-particles and two β^--particles to finally become stable ^{208}Pb (Gott et al., 2016, NuDat 2.8). ^{224}Ra is derived from ^{228}Th ($T_{1/2}$ = 1.91 yrs) which can be obtained from a primordial ^{232}Th ($T_{1/2} = 1.40 \times 10^{10}$ yrs) or ^{232}U ($T_{1/2}$ = 68.9 yrs) (Fig. 1). Direct application of ^{224}Ra in nuclear medicine is not that much popular as in case of ^{223}Ra ($T_{1/2}$ = 11.43 d) which is currently the only α-emitting radionuclide, in the form of simple salt of [^{223}Ra]$RaCl_2$, approved in 2013 by FDA and EMA for treatment of metastatic castration-resistant prostate cancer. This is mostly due to the fact of its shorter half-life and also generation of the progeny radionuclides, ^{212}Pb ($T_{1/2}$ = 10.64 h) and ^{212}Bi ($T_{1/2}$ = 60.55 min), with significant half-lives which liberated from ^{224}Ra mother radionuclide may diffuse and accumulate in healthy tissues causing

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undesirable damage. Despite this fact, there are still ongoing preclinical trials on ^{224}Ra application in the treatment of cancers, either directly in a form of [^{224}Ra]$RaCl_2$ solution supplemented with EDTMP, to complex being in equilibrium ^{212}Pb and also re-direct it to bones, in case of treatment of osteolytic bones metastases from breast cancers (Juzeniene et al., 2018) or encapsulated in liposomes (Henriksen et al., 2004) and other micro- and nanocarriers as: calcium carbonate (Westrøm et al., 2018a, 2018b), polyoxopalladates (Gott et al., 2019), barium sulfate (Reissig et al., 2019, 2020) and nanozeolites (Piotrowska et al., 2013; Czerwińska et al., 2020). Recently, ^{224}Ra is also intensively tested in a unique intra-tumoral α-radiation-based tumor ablation treatment named *Diffusing Alpha emitters Radiation Therapy* (DaRT) which utilizes wire implants loaded with ^{224}Ra and surgically inserted inside the tumor (Arazi, 2020). In this procedure the ^{224}Ra remains at the tumor site and released daughters are dispersed uniformly within the tumor and during decay emit α-particles that eradicate cancer cells. DaRT approach was found to destroy solid tumors in preclinical studies but also in patients with cutaneous malignancies allowing direct application of ^{224}Ra in the treatment of non-resectable human cancers (Keisari et al., 2020). Unfortunately, lack of proper bifunctional chelators is the major drawback of radium radionuclides application in a receptor targeted therapy. The large Ra^{2+} cation, like other cations of alkali earth elements, forms weak complexes; therefore, labeling biomolecules with radium radionuclides is a very challenging mission. On the other hand, ^{224}Ra decay metallic products, ^{212}Bi and ^{212}Pb, are easily complexed with well-known acyclic and macrocyclic ligands such as DTPA, DOTA or TCMC, and those complexes attached to targeting vectors like PSMA (Banerjee et al., 2020) or trastuzumab (Meredith et al., 2018) may be potential new radiopharmaceuticals, especially with the longer-lived ^{212}Pb which may serve as an *in vivo* generator for ^{212}Bi (Ferrier et al., 2019; Guerra Liberal et al., 2020).

Since TAT has been shown to be very promising, many studies are performed on the radiolabeling of various biomolecules with α-emitters and thus observed is growing also interest in the preparation of generators which may serve for elution of α-radionuclides. In case of ^{224}Ra, it is mainly obtained from ^{228}Th either by utilization of ion-exchange resins or by extraction techniques. Atcher et al. (1987, 1988) described quantitative elution of ^{224}Ra with 8 M HNO_3 from ^{228}Th retained on the anion exchange resin BioRad AG1x8 (200–400 mesh), although no more details on the elution volume were added. Another procedure relies on the immobilization of ^{228}Th in 0.1 M HNO_3 on a DIPEX® actinide resin and regular elution of ^{224}Ra with 1–2 mL of 1 M HCl followed by the additional purification of crude eluate on the second column with the same procedure (Westrøm et al., 2018a). Alternative

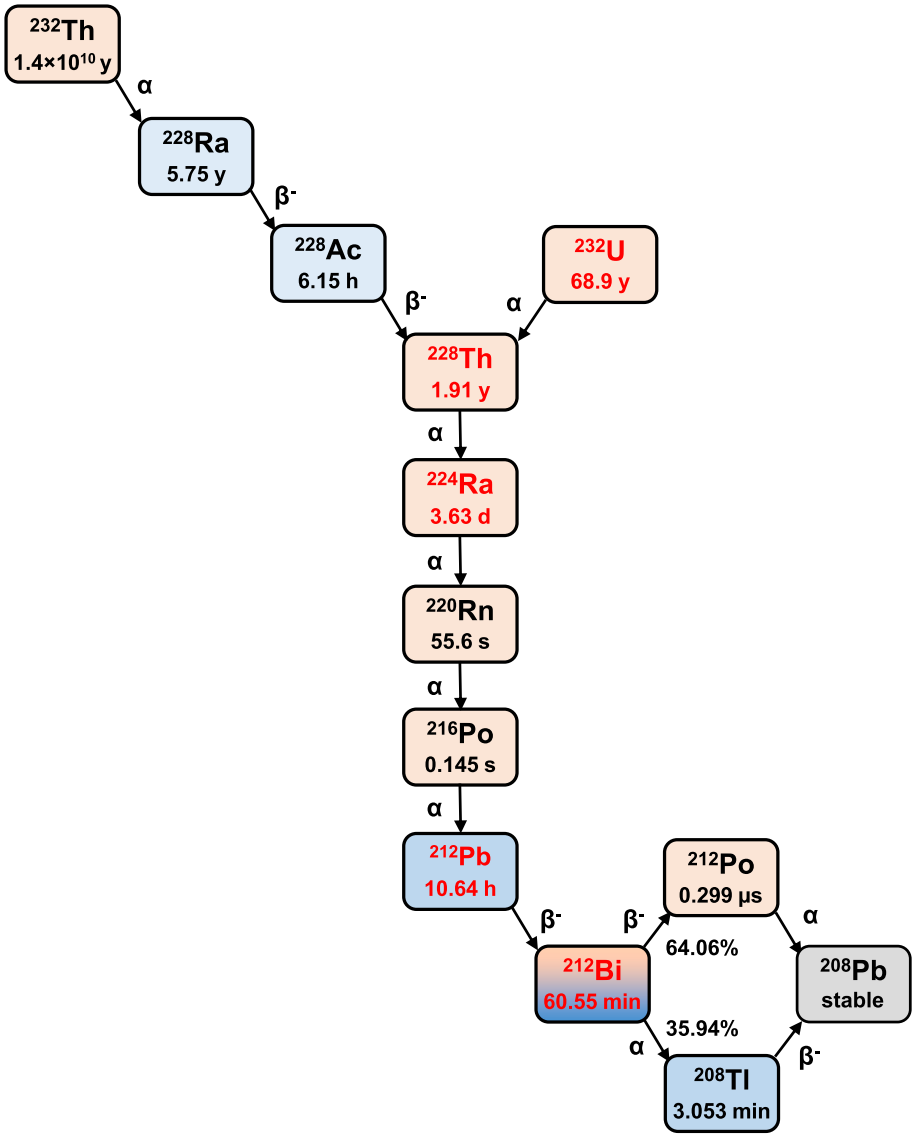

Fig. 1. Decay scheme of ^{232}Th and ^{232}U.

interesting approach was proposed by McAlister and Horwitz (2011) to combine cartridges filled first with UTEVA-resin, next one with Sr-resin and the last one with Prefilter-resin, and pass through this tandem 20 mL solution of ^{228}Th (being in equilibrium with its daughters) in 4 M HNO_3. ^{228}Th is selectively retained by the first column and ^{212}Pb on the Sr-resin, whereas ^{224}Ra passes through all three cartridges. The elution of ^{224}Ra is completed by additional rinsing of entire cartridges setting with 10 mL of 4 M HNO_3, giving a full recovery (>99%) in a quite large volume of highly acidic 4 M HNO_3.

Organic phosphonates are commonly used for extraction and separation of thorium, uranium and plutonium, especially from the nitric acid solutions (Siddall, 1959). One of them is bis(2-ethylhexyl) hydrogen phosphate (HDEHP) (Fig. 2) that is known as a very effective extractant for Th^{4+} ions from diluted solutions of mineral acids ($D > 10^4$), while Ra^{2+} is not extracted ($D \ll 1$) at these conditions (Marcus and Kertes, 1969). In the past, in our laboratory was developed a liquid generator in which ^{232}Th was extracted with 1 L of 2 M HDEHP solution in heptane and ^{224}Ra was quantitatively recovered with high separation factor by re-extraction with 50 mL of 0.1 M HNO_3 solution containing Ba^{2+} as a carrier (Narbutt and Bilewicz, 1998). This method gave good separation factor and recovery of ^{224}Ra; however, due to application of liquid-liquid extraction was not practical as large volume of organic phase was handled and additional three washes with heptane required longer time of procedure, high solvents consumption and generation larger volume of wastes. Therefore, the aim of this work was to develop a simple generator based on extraction chromatography for rapid elution of carrier-free ^{224}Ra from ^{232}U or ^{228}Th sources with a small volume of nitric acid; and further milking of ^{212}Pb and ^{212}Bi descendants from the separated ^{224}Ra eluates. We proposed to prepare an extraction chromatographic material by immobilization of HDEHP extractant on the PTFE grains to obtain a fine material for column preparation. Elution profile of ^{224}Ra was checked over several years to determine the percentage of mother radionuclides breakthrough. Thus obtained ^{224}Ra eluates were further used for preparation of generator to receive ^{212}Bi and ^{212}Pb radionuclides for labeling studies of various biomolecules.

2. Experimental

2.1. General reagents

Polytetrafluoroethylene (PTFE) in a form of a powder >40 μm particle size, bis(2-ethylhexyl) hydrogen phosphate (97%, HDEHP) and cation exchange resin of DOWEX 50WX12 (mesh 100–200, H^+) were from Sigma-Aldrich (USA). Acetone, nitric acid and hydrochloric acid as AnalaR NORMAPUR and ARISTAR purity grade were purchased from VWR (France). All other reagents used were of the highest grade commercially available. High purity deionized water (conductivity <0.07 μS) from the HLP5UV Hydrolab (Poland) system was used throughout. Uranium-232 was purchased in 1975 year from The Radiochemical Centre Ltd at Amersham (Buckinghamshire, England). Thorium-228 was obtained from European Commission Joint Research Centre (Karlsruhe, Germany). Both radionuclides were in secular equilibrium with all daughters, and were used as no-carrier-added in all performed studies.

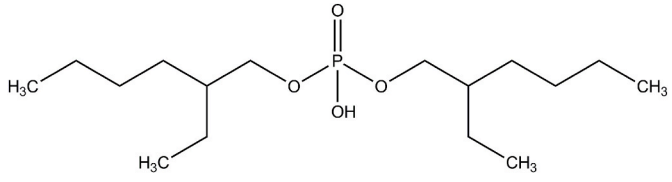

Fig. 2. Structure of a bis(2-ethylhexyl) hydrogen phosphate (HDEHP) extractant.

2.2. Radioactivity measurements

Radioactive samples were measured as a 1 mL aliquot in Eppendorf round bottom tube (2 mL of total possible volume) to keep constant geometry. Measurements were performed on a γ-spectrometer containing Coaxial High Purity Germanium (HPGe) detector (GX 1080) coupled with a multi-channel analyzer DSA-1000 and spectral files were analyzed by Genie 2000 software (Canberra, USA). The following γ-lines were chosen for determining the activity of each radionuclide, mostly ^{224}Ra, ^{212}Pb, ^{212}Bi and ^{208}Tl (Table 1) (NuDat 2.8).

2.3. Generators preparation

The column filling for extraction chromatography was prepared by washing commercially available PTFE powder several times with pure acetone followed by its evaporation at room temperature. Next, 30 g of PTFE was suspended in 50 mL of acetone containing 15 g of HDEHP in a round bottom flask, mixed for 60 min at room temperature and the excess of solvent evaporated on a Rotavapor R-210 (Büchi, Switzerland). Thus prepared impregnated material was stored under nitrogen in a desiccator.

Two Pyrex glass columns with ~10 mm inner diameter, ended with porous ceramic frit and closed with Teflon valve, were filled by gently tamping the dried HDEHP-PTFE material to form a bed 3 cm in length (*ca.* 5 g of material). Next, each column was washed with 2 L of 0.1 M HNO_3. On the first column (Generator 1) 2.0 MBq of ^{232}U in 0.5 mL of 0.1 M HNO_3 was loaded on the bed and mother radionuclide extracted on the top of the column at a flow rate of 10 drops/min followed by additional 10 mL 0.1 M HNO_3 rinse. A second generator (Generator 2) was prepared in a same way, but 3.5 MBq of ^{228}Th in 150 μL of 0.1 M HNO_3 was loaded (5 drops/min) on a column material. Both generators were left for two weeks to accumulate over 90% of the equilibrium amount of ^{224}Ra. Thus, once prepared generators were used for more than 5 years for elution of ^{224}Ra, most of the times elutions were performed twice a month.

^{224}Ra from both generators was eluted with 10 fractions (1 mL each) of 0.1 M HNO_3. Fractions were diluted and activities were measured by HPGe γ-spectroscopy to determine the elution profile of ^{224}Ra. Next, solutions were kept for 1–2 months to allow any present mother radionuclide (^{232}U or ^{228}Th) to have its daughters to be in secular equilibrium before being recounted again to check for potential breakthrough.

2.4. Total organic carbon (TOC) and inductively coupled plasma mass spectrometry (ICP-MS) analyses

The amount of carbon in ^{224}Ra eluates from both generators was analyzed by using the Analytik Jena AG multi N/C apparatus (Germany) with the TOC content determined with a carbon analyzer equipped with a Nondispersive Infrared Sensor (NDIR coupled with VITA method).

The trace metal analysis for eluates of ^{224}Ra and its descendants, ^{212}Pb and ^{212}Bi, obtained in the current studies were performed by ICP-MS technique using Elan DRC II (PerkinElmer, USA) device equipped with crossflow nebulizer with Scott double-pass spray chamber and Ni cones.

Table 1
Selected gamma-energies used for determination of radioactivity in samples (NuDat 2.8).

Nuclide	Half-life	Energy of emitted γ (keV)	Intensity (%)
^{224}Ra	3.63 days	240.99	4.10
^{212}Pb	10.64 h	238.63	43.6
^{212}Bi	60.55 min	727.33	6.67
^{208}Tl	3.05 min	583.19	85.0

2.5. Preparation of $^{224}Ra/^{212}Pb/^{212}Bi$ generator

^{224}Ra eluted in previous steps was used for the preparation of radionuclide generator to obtain its two daughters, ^{212}Pb and ^{212}Bi. For this purpose, a glass column (internal diameter 4 mm) was filled by gravity flow with 0.2 g of cation exchange resin DOWEX 50WX12 in 3 mL of 2 M HNO_3, rinsed twice with portions (3 mL each) of 2 M HNO_3 followed by final washing and preconditioning with 30 mL of 0.1 M HNO_3 in which resin was left. Next, ^{224}Ra fractions (in 0.1 M HNO_3) eluted from both generators were applied onto the column followed by a 3 mL rinse also with 0.1 M HNO_3. Fractions eluted during loading of ^{224}Ra onto resin were collected and measured to check potential leakage of ^{224}Ra. Thus prepared generator was left for 3 days to allow growth of ^{212}Pb and reach a secular equilibrium (>99%) with a parent ^{224}Ra. Generator was eluted starting with 0.75 M HCl followed by 2 M HCl and at the end with 0.75 M HCl, collected 0.5 mL fractions were counted for ^{212}Bi and ^{212}Pb activity. Those samples were also recounted a week later to check for any breakthrough of ^{224}Ra from a resin. In case of ^{212}Pb, other elution conditions like 1.5 M HCl or its mixture with 1.5 M NaCl at ratio 1:1 were also tested.

2.6. Proof-of-concept radiolabeling with ^{212}Bi and ^{212}Pb

An anti-HER2 nanobody (Nb) was a gift of Prof. Matthias D'Huyvetter (Vrije Universiteit Brussel, Belgium). This nanobody was conjugated with bifunctional chelating agents, such as *p*-SCN-Bn-CHX-A″-DTPA and *p*-SCN-Bn-TCMC (Macrocyclics, USA) according to procedures described earlier (Pruszyński et al., 2018). The ^{212}Bi and ^{212}Pb radionuclides eluted from a ^{224}Ra generator were evaporated to dryness and reconstituted in 100 μL of 0.8 M NH_4OAc (pH = 5.5) to which were added 100 μg (*ca.* 7 nmoles, 100–200 μL of 0.8 M NH_4OAc) of DTPA-Nb and TCMC-Nb conjugates, respectively. In case of ^{212}Bi, the mixture was left at room temperature for 20 min, whereas mixture with ^{212}Pb was incubated at 50 °C for 60 min. The percentage of formed ^{212}Bi-DTPA-Nb and ^{212}Pb-TCMC-Nb was determined by instant thin-layer chromatography (ITLC) on silica gel impregnated glass fiber sheets (Agilent Technologies) using 0.1 M citrate buffer pH 4.0 as a mobile phase. Radiolabeled conjugates were retained at the spot $R_f = 0.0$, while unbound radioactivity moved with the solvent $R_f = 1.0$.

3. Results and discussion

Earlier work from our laboratory described development of the liquid-liquid generator allowing quantitative recovery of ^{224}Ra, although in the presence of carrier added Ba^{2+}, from HDEHP heptane solution containing *ca.* 40 g of natural thorium (^{232}Th) (Narbutt and Bilewicz, 1998). The elaborated procedure was simple, although not that much practical due to handling large volume (1 L) of organic phase. The extraction chromatography combines the selectivity of liquid-liquid extraction with the rapidity of chromatographic methods (Horwitz et al., 2006). Therefore, in the present paper we decided to embed HDEHP extractant on the PTFE adsorbent beads and test prepared material for selective adsorption of ^{232}U and its descendant ^{228}Th. We used the organic polymeric material as a support instead of inorganic one to avoid matrix that contains any metallic components or impurities which may leak into the final eluate and influence on the radiolabeling of bioconjugates resulting in potential lower labeling yield. The PTFE was chosen as a support due to its excellent chemical resistance and inertness, especially in the presence of various acids and bases. The HDEHP is commonly used for effective solvent extraction of Th^{4+}, U^{4+} or UO_2^{2+} ions from diluted solutions of mineral acids with very high distribution coefficients ($D > 10^4$), while for cations of II group of elements, including Ra^{2+}, they are very low ($D \ll 1$) (Marcus and Kertes, 1969). Studies performed by Horwitz et al. (2006) confirmed that extraction mechanism in case of extractant agent loaded on the support is similar to conventional solvent extraction. Moreover, there is a direct correlation between both systems which allows prediction the retention ability of an chemical element in an extraction chromatography column based on its distribution coefficients determined by classical liquid-liquid extraction (Horwitz et al., 2006; Braun and Ghersini, 1975). Therefore, based on the abovementioned distribution coefficients, we assumed that prepared by us HDEHP-PTFE material should strongly catch ^{232}U or ^{228}Th applied to the extraction column while simultaneously allow elution of ^{224}Ra. However, thus prepared generators had to be checked for elution efficiency of carrier-free ^{224}Ra and its radiochemical purity, especially in a long-term (more than 5 years) stability and elution reproducibility.

The elution profiles of ^{224}Ra are presented in Fig. 3. The recovery yield of ^{224}Ra was almost quantitative for both generators with more than 95% obtained already in fractions up to 5 mL of 0.1 M HNO_3, and this volume was used in further operation of those generators. In case of Generator 2 (^{228}Th) the ^{224}Ra peak profile is more narrow (>90% in 3 mL) compare to wider peak of Generator 1 (^{232}U). Although the reason for such a behavior is unknown, the possible explanations could be either slightly too much packed bed of material in Generator 1 or larger volume of ^{232}U solution initially applied to the column, which could have resulted in its wider distribution in the filling material. All collected fraction samples were recounted two months later to check the characteristic gamma-lines of ^{224}Ra or its daughters (^{212}Pb, ^{212}Bi and ^{208}Tl) which would indicate a leakage of mother radionuclides. In both cases the measured radioactivity revealed that breakthrough was <0.003% (Fig. 3).

Both generators were repeatedly used, most of the times in bi-weekly cycles, for elution of ^{224}Ra or its descendants for studies performed in our laboratory. Periodically, also the elution profile of ^{224}Ra was checked (Fig. 4). The data are presented as a percentage of eluted ^{224}Ra activity in each fraction to total activity of ^{224}Ra expected to be eluted at that time from the generator. This form of data presentation allowed to compare if elution profile of ^{224}Ra is stable over time, especially in the case of Generator 2, where 1.91 years half-life of ^{228}Th resulted in gradual drop of ^{224}Ra activity eluted from this generator. This long-term study confirmed that elution profiles of ^{224}Ra, from both generators, were stable over last 5 years and most of the activity is still eluted within 5 mL of 0.1 M HNO_3. Moreover, recounting of samples from the current year (2020) revealed that breakthrough of mother radionuclides was still at very low level (<0.005%). This indicates a high stability of both generators that was also somehow confirmed by the determination of TOC in eluted 5 mL fractions. The amount of determined carbon in eluted samples was at the same level as in case of nitric acid used for their elution (<10 ppm). This confirms that HDEHP extractant is stably attached to the PTFE beads and does not undergo degradation and co-elution. The obtained results are very promising; however, due to the limited activities of both radionuclides at our repositories, we used only 2 MBq of ^{232}U and *ca.* 4 MBq of ^{228}Th. Therefore, from practical point of view, research on scale-up of both generators to activities allowing systematic pre-clinical or clinical application is necessary. Although at thus applied doses for a long period of time, the radiolysis effect may most likely cause degradation of both HDEHP extractant and PTFE matrix. We decided to use the organic polymeric support to avoid any metallic contamination of eluates though some inorganic (e.g. silica gel) might be more resistant to higher activities. Therefore, in our opinion it will be interesting to perform studies on embedding HDEHP or other organophosphorus extractants on various organic and inorganic matrices and check their properties on extraction of ^{232}U or ^{228}Th from mineral acids, elution profile and recovery of ^{224}Ra and its purity. Moreover, comparison with commercially available LN-resin (Eichrom®), where HDEHP extractant is immobilized on the poly (methyl methacrylate) support will be interesting. Unfortunately, currently we do not have access to higher activities of ^{232}U or ^{228}Th which might be used in the scale-up studies.

^{224}Ra eluates obtained from both generators were further loaded on a glass column filled with DOWEX 50WX12 resin for ^{212}Bi and ^{212}Pb separation. During loading of eluates either from one of the generator

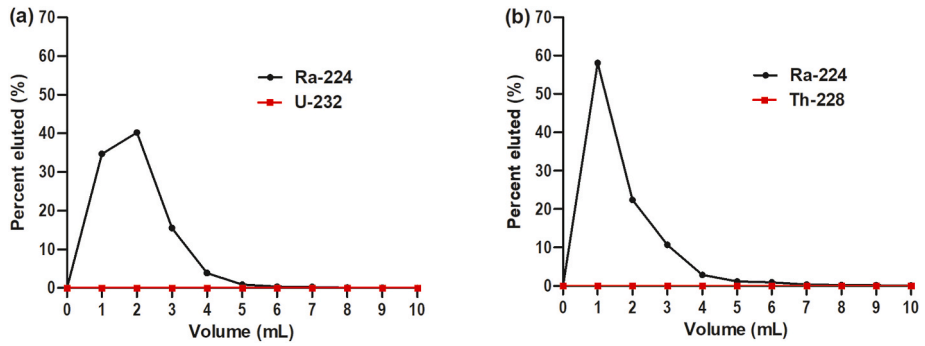

Fig. 3. The elution profiles of ^{224}Ra from (a) Generator 1 (^{232}U) and (b) Generator 2 (^{228}Th) with 0.1 M HNO_3.

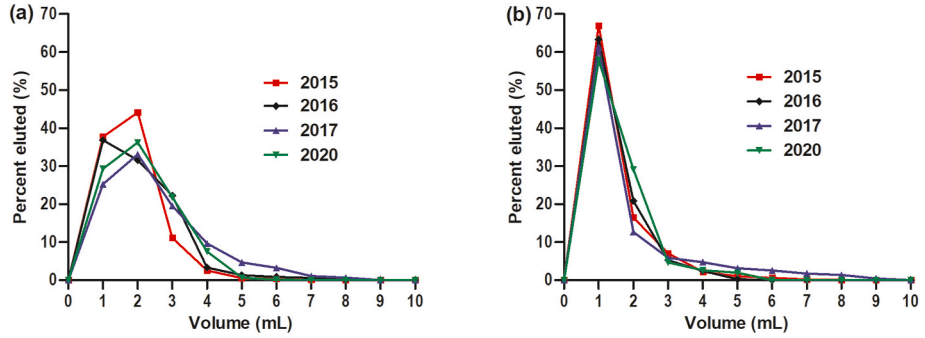

Fig. 4. The comparative elution profiles of ^{224}Ra over last 5 years from (a) Generator 1 (^{232}U) and (b) Generator 2 (^{228}Th) with 0.1 M HNO_3.

(total 5 mL of 0.1 M HNO_3) or both at once (total 10 mL) fractions were collected and checked for ^{224}Ra leakage. The performed HPGe measurements indicated very high retention of ^{224}Ra on the cationic resin. The elution profile of ^{212}Bi and ^{212}Pb was performed 3 days after loading when secular equilibrium (>99%) with ^{224}Ra was obtained (Fig. 5).

Both descendant radionuclides were recovered quantitatively from the generator, around 85% of ^{212}Bi was eluted already in first 1 mL (>95% by 1.5 mL) of 0.75 M HCl, whereas ~90% of ^{212}Pb was obtained in 1 mL (>95% in 1.5 mL) of 2.0 M HCl. Recounting of collected fractions after a week did not reveal any ^{224}Ra breakthrough. Moreover, this ^{224}Ra/^{212}Pb/^{212}Bi generator was often eluted every 24 h (~80% of ^{212}Pb in equilibrium with ^{224}Ra) without any observed breakthrough of ^{224}Ra. The ^{212}Pb radionuclide can be also eluted with 1.5 M HCl or its mixture (1:1 ratio) with 1.5 M NaCl; however, to achieve >95% elution larger volume of eluents are required 2 mL and 3 mL, respectively (Fig. 6). Therefore, for routine generator operation elution with 3 mL of 0.75 M HCl and 3 mL of 2.0 M HCl was chosen, although a fractionation (0.5 mL fractions collected) procedure was applied that allowed selection of fractions with the highest radioactivity. The scheme of the two column separation system for the elution of ^{224}Ra and its further processing to obtain either ^{212}Bi or ^{212}Pb is presented on Fig. 7.

To prove that obtained ^{212}Bi and ^{212}Pb are suitable for labeling of nanomolar amounts of bioconjugates, their eluates were evaporated to dryness and reconstituted in the labeling buffer containing *ca.* 7 nmoles of DTPA-Nb or TCMC-Nb and incubated at room temperature or 50 °C for 20 min and 60 min, respectively. Finally performed ITLC analysis

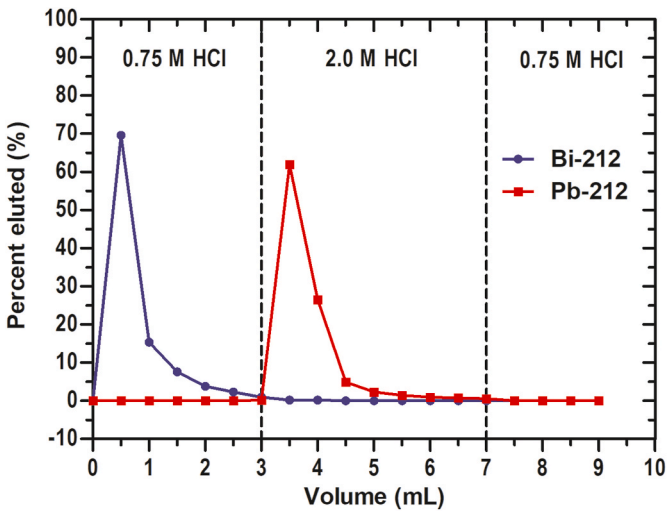

Fig. 5. The elution profiles of ^{212}Bi and ^{212}Pb with 0.75 M HCl and 2 M HCl, respectively, from ^{224}Ra immobilized on DOWEX 50WX12.

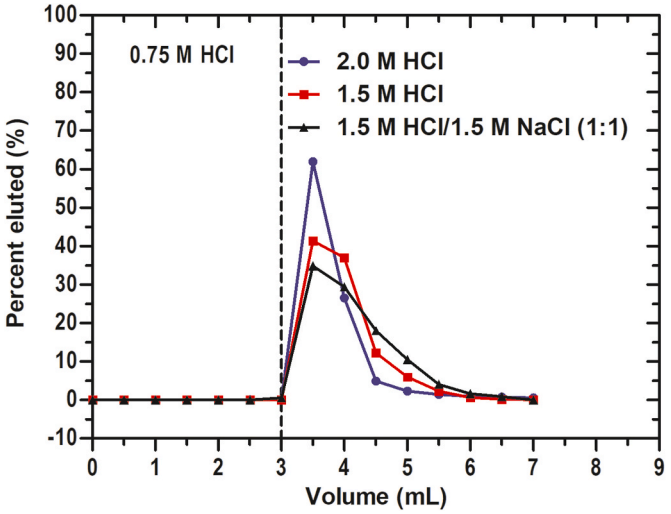

Fig. 6. The elution profiles of ^{212}Pb with various concentration of HCl and its mixture with NaCl from ^{224}Ra immobilized on DOWEX 50WX12.

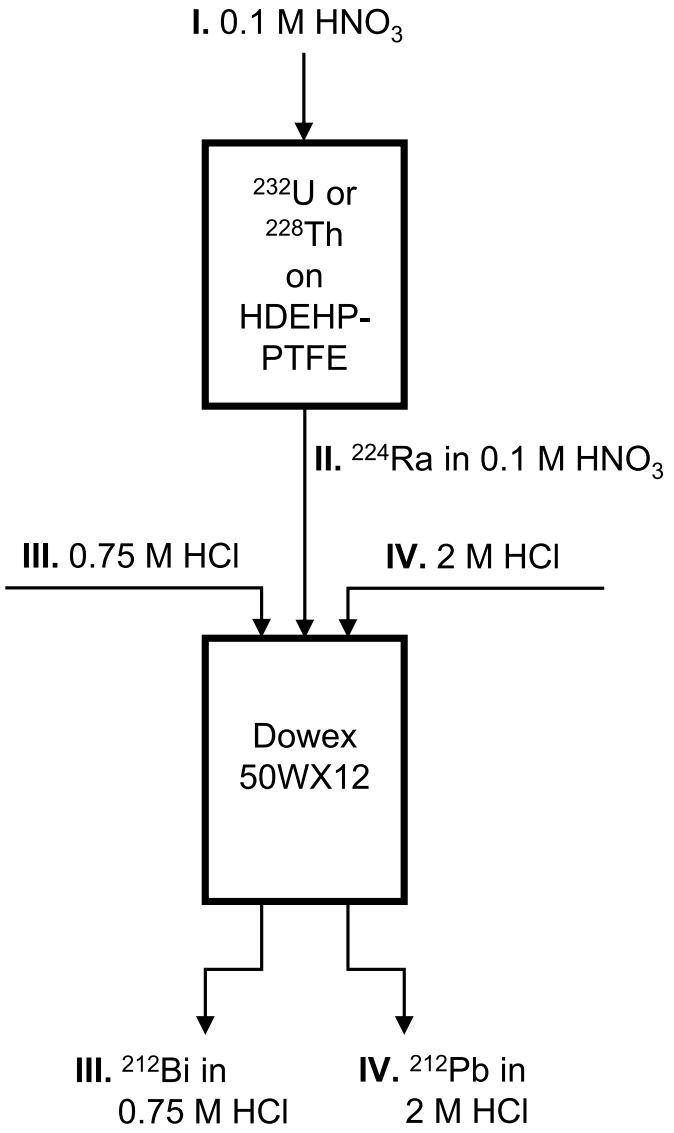

Fig. 7. The extraction chromatographic procedure for separation of ^{224}Ra, ^{212}Pb and ^{212}Bi from ^{232}U or ^{228}Th.

confirmed that both radiobioconjugates were obtained with high yield >95% for ^{212}Bi-DTPA-Nb and around 90% for ^{212}Pb-TCMC-Nb (Fig. 8).

This experiment directly proved that obtained by us eluates of ^{212}Bi and ^{212}Pb are of high purity and can be used for radiolabeling of nanomolar amounts of bioconjugates. The high purity of these eluates was also confirmed by their ICP-MS analysis. The amount of metallic elements which may interfere with chelator labeling and compete with radionuclides e.g. Fe, Zn, Cu etc. was below <10 ppb (ng/mL) for each metallic impurity.

4. Conclusions

The elaborated generators, based on the prepared HDEHP-PTFE material, exhibited high retention of ^{232}U and ^{228}Th, whereas desired ^{224}Ra can be quantitatively and easily eluted in less than 2 min with 0.1 M HNO_3 and high radionuclide purity. The developed separation procedure is simple and practical, and both generators operate reliably for more than 5 years currently without significant breakthrough of mother radionuclides. Moreover, application of extraction chromatography increased safety of working due to elimination of handling liquid extraction and use of toxic organic solvents and allows further automatization of separation process. Scaling up both generators should not be

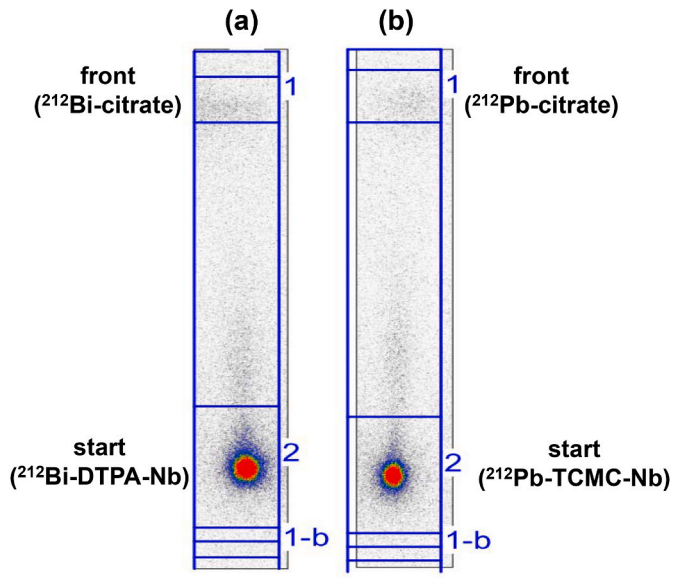

Fig. 8. The ITLC radiochromatograms of reaction mixtures after radiolabeling of (a) DTPA-Nb and (b) TCMC-Nb with ^{212}Bi and ^{212}Pb, respectively.

difficult from retention capacity point of view as the parent ^{232}U and ^{228}Th radionuclides are in non-carrier-added form, also the elution profile of ^{224}Ra should not be affected. However, increase of radioactivity may significantly influence on the radiation resistance of organophosphorus extractant and its support. Therefore, further studies on the immobilization of HDEHP or its derivatives on various organic and inorganic supports and their ability to extract ^{232}U or ^{228}Th from acids should be done in comparison to commercial LN-resin from Eichrom®. ^{212}Bi and ^{212}Pb descendants of ^{224}Ra can be easily eluted from a generator based on ^{224}Ra retained on DOWEX 50WX12 resin with high purity enabling radiolabeling of bioconjugates at nanomolar scale.

CRediT authorship contribution statement

Marek Pruszyński: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Project administration, Writing – review & editing. **Rafał Walczak:** Investigation, Formal analysis, Revising manuscript. **Magdalena Rodak:** Investigation, Formal analysis. **Frank Bruchertseifer:** Investigation, Resources, Revising manuscript. **Alfred Morgenstern:** Resources, Revising manuscript. **Aleksander Bilewicz:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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