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J Despotopulos, K Kmak, T Huynh

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Cation Exchange Separation of Radium and Actinium using Lactic Acid DTPA Buffer

Kelly N. Kmak[1], John D. Despotopulos[1], Tony L. Huynh[1]

[1]*Nuclear and Chemical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA, USA*

Abstract

The separation of actinium (^{228}Ac) from radium (^{223,228}Ra) on cation exchange resin with a diethylenetriaminpentaacetic acid-lactate buffer solution is demonstrated with series of columns. High yield, high radiopurity (~100%) separations of Ac from Ra are feasible with small columns in biologically compatible conditions and pHs. As Ac is eluted before Ra on these columns, further studies were performed to determine if this separation system could be applied to Ra/Ac isotope generators, but these were not successful. The separations presented in this work may be relevant for radiopharmaceutical purifications of ^{225}Ac which is typically obtained from its ^{225}Ra parent isotope.

Keywords

Radium; actinium; isotope generator; cation exchange; DTPA

Introduction

Actinium-225 is a candidate for Targeted Alpa Therapy (TAT) for cancer treatment [1] [2] [3] [4]. This isotope is typically derived from the decay of ^{229}Th (^{229}Th → ^{225}Ra → ^{225}Ac). As this method relies on a significant supply of ^{229}Th, other methods for producing ^{225}Ac are under investigation. Typically, these methods focus on separating ^{225}Ac from the ^{225}Ra

parent [3] as this is a route to isotopically pure ^{225}Ac without contamination of longer-lived Ac isotopes, such as ^{227}Ac.

Therefore, it is important to develop separations that not only separate Ra from Ac, but do so under conditions compatible with the end application of delivery into patients. While there are many established ways of separating Ra and Ac, most of these rely on high acid concentrations [1] [2] [3] [4], which is not optimal for radiopharmaceutical applications. Along with separations that do not require concentrated acids, there is research into ^{225}Ra/^{225}Ac isotope generators, which would not only require a biologically compatible eluant but also would require Ra to be retained by the resin while Ac elutes, which is the reverse elution order compared to many procedures in the literature [1] [2] [3] [4].

One promising avenue for such separations is the chelator diethylenetriaminpentaacetic acid (DTPA), Fig. 1. The use of DTPA in medicine is well established. It has approval from the Food and Drug Administration (FDA) to treat internal radioactive contamination [5] and DTPA derivations have been used for *in vivo* studies with ^{225}Ac for TAT applications [6] [7].

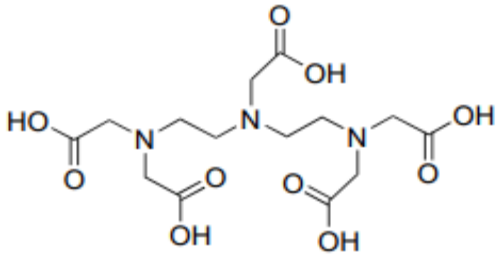


Fig. 1 Diethylenetriaminpentaacetic acid, the chelating ligand used in this work.

The use of DTPA to separate actinides and lanthanides based on pH is well established in both liquid-liquid extraction and column chromatography separations [8] [9]. These separation systems typically rely on a buffer of lactic acid, an organic phase (or resin) with a cation exchanger and DTPA to selectively chelate the desired actinide or lanthanide, pulling it into the aqueous phase. Such separation should be applicable for Ac due to its similarities to other trivalent actinides and lanthanides. Furthermore, a similar concept utilizing DTPA and cation exchange resin has been demonstrated for the separation of ^{223}Ra from its decay daughters, ^{211}Pb/^{211}Bi [10]. Critically, in this separation radium is not

eluted from cation exchange resin using DTPA solutions with a pH < 9 [10], which is much higher than the pH levels for extraction of the trivalent actinides and lanthanides (pH 3 – 4). This was utilized for liquid-liquid separation of Ra and Ac with buffered monochloroacetic acid and DTPA in Ref. [11], though the exact pH was not specified. Therefore, based on the literature data for trivalent actinides and radium, it should be feasible to separate radium from actinium with buffered DTPA solutions and cation exchange resin.

Studies were undertaken to assess whether Ac and Ra could be effectively separated with cation exchange resin using DTPA in a lactic acid/sodium lactate buffer solution. Column studies were performed to demonstrate conditions for a high yield, high purity separation of Ra and Ac. Using the parent-daughter pair ^{228}Ra/^{228}Ac, the separations were tested for applicability to isotope generator systems.

Experimental

Solutions were prepared from nitric acid (Ultrex II, J. T. Baker), L-(+)-lactic acid (≥98%, Sigma), sodium L-lactate (≥99.0%, Sigma) and diethylenetriaminepentaacetic acid (≥99% titration, Sigma). Millipore Milli-Q deionized water (18.2 MΩ cm) was used as needed for dilution. The solutions used in this work are shown in Table 1. To determine the pH of the solutions, a Mettler Toledo SevenExcellence Multiparameter system with a Mettler Toledo InLab Expert Pro-ISM Sensor was used; no pH adjustments were performed.

Table 1 DTPA-Lactate buffer solutions used for column elutions.

Solution #	[Latic Acid] (M)	[Sodium Lactate] (M)	pH	Total [Lactate] (M)	[DTPA] (M)
1	0.96	0.04	~3	1	--
2	0.02	0.98	4.5-4.7	1	0.05

Cation exchange resin (AG 50x12, 100-200 mesh) was cleaned and stored in dilute HCl. All columns were 2 mL snap-tip from Eichrom Technologies and packed under gravity flow with either 1.8 mL or 1 mL resin. Pre-packed 2 mL DGA resin (Eichrom Technologies, 50-100 µm) was used as received.

Initial experiments were conducted with ^{223}Ra and ^{228}Ac to simplify the counting. Later studies were performed with the parent-daughter pair ^{228}Ra/^{228}Ac. All isotopes were separated from long-lived decay chains (^{231}Pa for ^{223}Ra; ^{232}Th for ^{228}Ra/^{228}Ac) and prepared in 0.2 M HNO_3 solutions for column studies. Activity measurements were performed with an HPGe gamma-ray detector (ASPEC multi-channel analyzer, Ortec NIM electronics). Peak fitting was performed with Maestro software (Ortec). Samples were counted relative to the load solution (or a standard solution) in an identical geometry.

For all measurements, ^{228}Ac was identified based on its 911 keV gamma-ray emission, which has no interferences from either of the Ra isotopes (Table 2). The main gamma-ray emission for ^{223}Ra is at ~270 keV, which has an interference from a minor emission from ^{228}Ac. To resolve this, all experiments with these isotopes were counted twice, immediately after the experiments to quantify the ^{228}Ac, and then again 72 hours later, to allow for the decay of ^{228}Ac to background and quantification of ^{223}Ra. All counts were relative to separate ^{223}Ra and ^{228}Ac standards. Similarly, since ^{228}Ra has no detectable gamma-ray emissions, samples containing both ^{228}Ac and ^{228}Ra were also counted immediately after elution and then again 72 hours later. In this case, the second count allowed the quantification of ^{228}Ra via the in-growth of ^{228}Ac.

Table 2 Relevant nuclear decay data for the isotopes used in this work, including half-lives, decay mode, and significant gamma-ray energies and intensities [12].

Isotope	Decay Mode	Half-Life	Photopeak and Intensity
^{223}Ra	α	11.44 d	269.463 keV (13.3%)
^{228}Ac	β^-	6.15 h	270.245 (3.46%) 911.204 (25.8%)
^{228}Ra	β^-	5.75 y	--

Initial Column Studies

The first column studies were performed with both ^{223}Ra and ^{228}Ac. First, a load solution containing both isotopes was prepared in 2 mL 0.2 M HNO_3. This was loaded onto a 1.8 mL AG 50x12 column (Column 1) that had been preconditioned with 6 mL 0.2 M HNO_3. The load fraction was collected, followed by 4 mL of a lactate buffer (Solution #1), 12 mL of a DTPA-lactate solution (Solution #2), and finally 8 mL 4 M HNO_3. The flow rate of the columns was 0.3 mL/min. All fractions were 2 mL in volume. As mentioned previously, samples were counted twice: immediately after elution and again 72 hours later. The second column (Column 2) was nearly identical, but with a 1 mL load solution and 1 mL column volume. It was conditioned with 4 mL 0.2 M HNO_3 and eluted with 2 mL Solution #1, 6 mL Solution #2, 5 mL 4 M HNO_3. All fractions were 1 mL and were counted as described above.

Column Studies – DTPA Removal with DGA Resin

For the next column study, Column 2 was repeated identically (1 mL load; 1 mL bed volume) and the 3 highest activity ^{228}Ac fractions (containing ~85-90% of the activity) were combined, counted in the 3 mL geometry and then acidified with 5 mL 12 M HCl to a final concentration of 7.5 M HCl (total volume 7 mL). A 2 mL pre-packed DGA cartridge was conditioned with 8 mL 8 M HCl. The column was conditioned and eluted using an Eichrom 12-hole polycarbonate vacuum box; the eluent flow rate was ~1 mL/min (~5 inHg). The acidified ^{228}Ac solution was loaded; the load fraction was collected (7 mL) followed by 6 mL 8 M HCl and 18 mL 0.1 M HCl. All fractions were 3 mL and were counted relatively to the initial solution before acidification (3 mL geometry as described above). The load fraction was counted relative to the initial solution after acidification (7 mL) to ensure the geometry was consistent. Samples were counted relative to the load solution before acidification (3 mL) or after (7 mL) to ensure that every sample could be compared to the load solution in the same geometry. A second DGA column study was performed identically to the first except the 0.1 M HCl was replaced with Solution #1 diluted by half (0.48 M HLa, 0.02 M NaLa). Finally, a third column study was conducted

by repeating the cation exchange column identically to the first two, followed by acidification with 0.75 mL conc. HNO_3 (to a final concentration of 3.2 M HNO_3). The DGA column was conditioned with 8 mL 3 M HNO_3; the load fraction was collected followed by 6 mL 0.3 M HNO_3 and 12 mL ~0.5 M HLa (Solution #1 diluted by half).

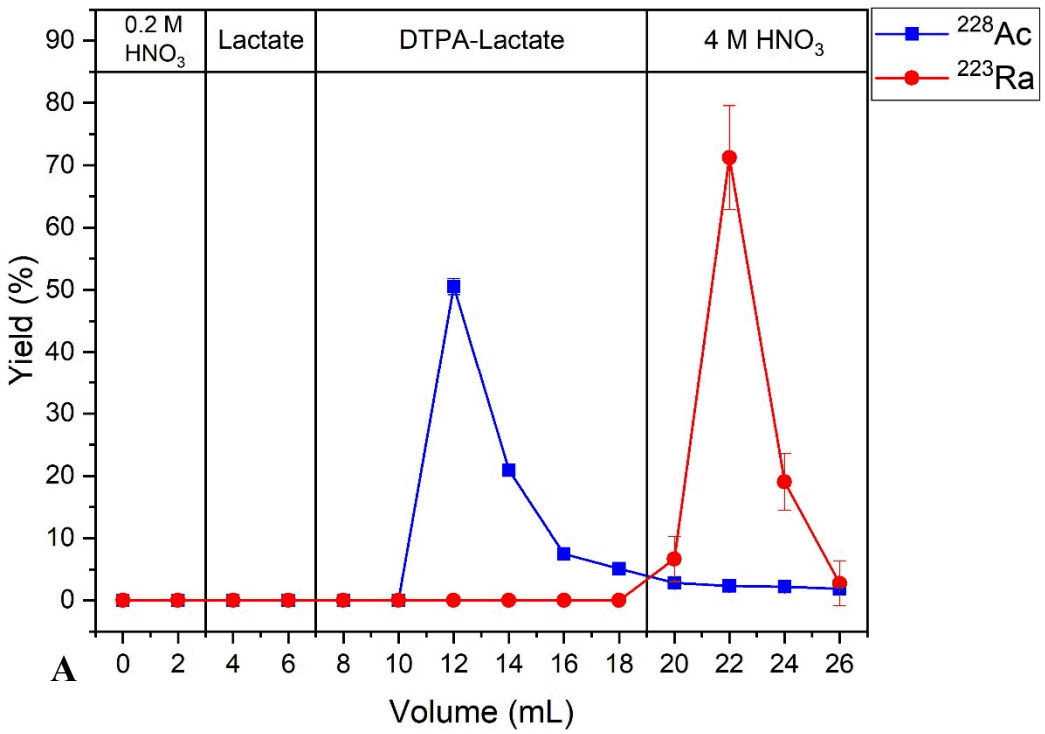
Column Studies – Isotope Generators

Based on the results from the initial column studies and Ref. [10], an isotope generator system was tested using this separation system. For these studies, two columns were prepared, one with 1.8 mL AG 50x12 resin, and the other with 1 mL AG 50x12 resin. The columns were conditioned with 6 mL and 4 mL of 0.2 M HNO_3, respectively. Load solutions of $^{228}Ra/^{228}Ac$, in equilibrium, were prepared in 200 µL 0.2 M HNO_3. Counting standards were prepared in 200 µL, 1 mL and 2 mL sizes. Each column was loaded and the empty tubes rinsed with 100 µL 0.2 M HNO_3. The load fraction and rinse were collected followed by two fractions of Solution #1, six fractions of Solution #2 and another two fractions of Solution #1. Fractions for the 1.8 mL column were 2 mL in volume; for the 1 mL column, fractions were 1 mL in volume. After loading, the columns were eluted 24 hours later and then every 48 hours for one week. These elutions consisted of one fraction of Solution #1 (what remained on the column from the previous elution), followed by six fractions of Solution #2 and two fractions of Solution #1. The experiment was stopped after 7 days due to ^{228}Ra breakthrough. For all isotope generator studies, the activity of ^{228}Ac was ~100 cps and the detection limit for ^{228}Ra was ~0.04 cps.

Results and Discussion

The elution of Column 1 and Column 2 are shown in Fig. 2. Both ^{228}Ac and ^{223}Ra are retained by the resin in dil. HNO_3 and the lactate buffer (pH ~3) with no DTPA. When DTPA is added to the eluant, Ac is selectively extracted, leaving Ra on the resin. Finally, Ra can be eluted with 4 M HNO_3. For Column 1 (Fig. 2a), the yield of ^{228}Ac in the DTPA-lactate fractions is 84 ± 2%, with the remainder eluting with the ^{223}Ra fractions; there is no detectable breakthrough of ^{228}Ra in the ^{228}Ac fractions (~100% radiochemical purity). For

Column 2 (Fig.2b), the yield of ^{228}Ac in the DTPA-lactate fractions is ~100% and as before there is no detectable breakthrough of ^{228}Ra in the ^{228}Ac fractions. The recovery of ^{223}Ra for both columns is ~100%. The elution is similar between the 1 mL and 2 mL columns, the elution band for ^{228}Ac is slightly sharper on the column with less resin, as expected, and this results in less tailing into ^{223}Ra fractions, as indicated by the yields for each column.



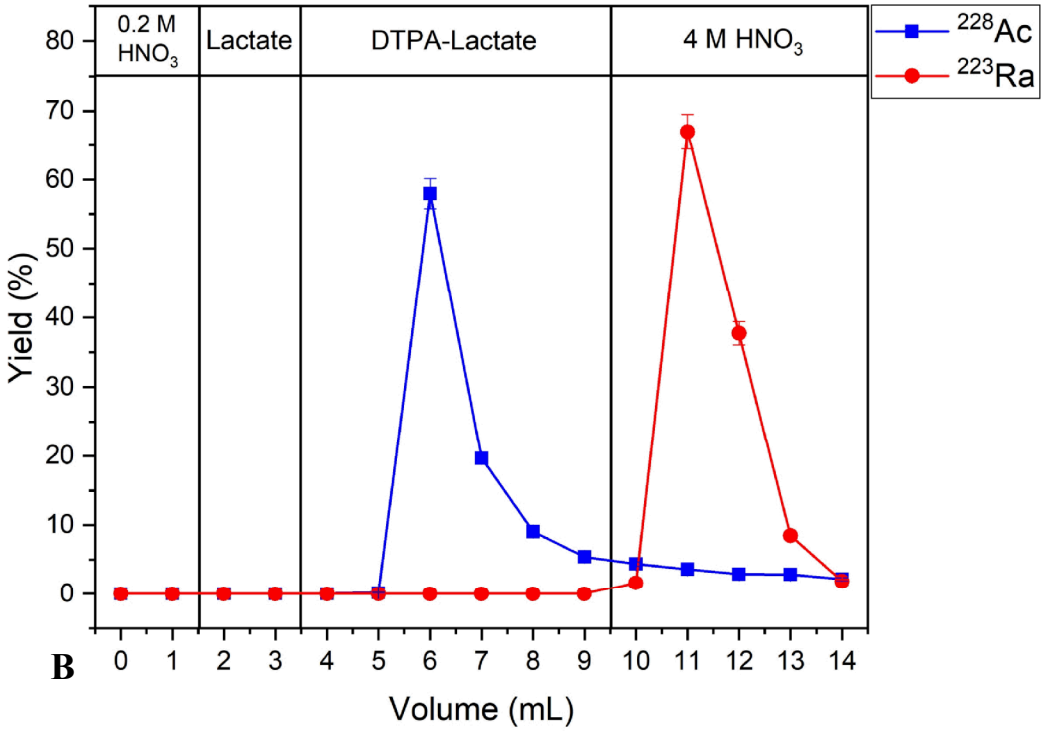


Fig. 2 Cation exchange separation of ^{228}Ac and ^{223}Ra. Error is from counting statistics, some error bars are smaller than the data points; lines are to guide the eye. (a) Column 1 – bed volume 1.8 mL; all fractions 2 mL. (b) Column 2 – bed volume 1 mL, all fractions 1 mL.

The chelation of trivalent actinides and radium with DTPA has been thoroughly studied in the literature [8] [9] [13] [14] [15] [16] [17]. The interaction between DTPA and these elements is highly pH dependent, which allows for specific separations, such as the separation of Am and Cm from the lanthanides separations [8] [9]. While the heavier trivalent actinides (Am, Cm, Cf) can be extracted with DTPA at pH values of 3 – 4 [16], Ac is more basic and requires higher pHs for effective chelation. For the synthesis of Ac-DPTA complex for medical studies, pH ~5 is utilized [6]. Radium is even more basic than Ac and requires higher pH values to extract with DTPA, typically pH >8 [10] [18].

In this work, it was desirable to use a low pH to maximize the retention of Ra, while still allowing for the elution of Ac, as such conditions would be most favorable for an isotope generator. A slightly lower pH (3-3.5) was tested for Ac elution but no ^{228}Ac was eluted under these conditions. Therefore, pH ~4.5 was used for column separations as this pH was sufficiently high to allow for Ac elution in a reasonable volume while still low relative to the ideal pH to elute Ra. Hydrolysis is not a concern for these elements, Ac does not hydrolyze until pH ~9 [19] and, while there is a lack of hydrolysis studies for Ra, it is more basic than actinium [21] and therefore would hydrolyze at higher pHs.

In the load solution and lactate buffer, both Ra and Ac (Ac^{3+} and Ra^{2+}, respectively) retain on cation exchange resin. The extracted species of Ac is likely $Ac(DPTA)^{2-}$ based on studies with other trivalent actinides and lanthanides [27]. DTPA is fully deprotonated in order to have a strong octadenate interaction with Ac. Finally, in 4 M HNO_3, Ra forms neutral nitrate species and therefore is eluted from the column along with any residual Ac, which is also a neutral species at this concentration.

As mentioned previously, many literature separations of Ra and Ac utilize high acid concentrations (>1 M HNO_3) and require Ra to be eluted first in chromatography separations [1] [2] [3] [4]. These methods are effective but not optimal for

radiopharmaceutical applications. High acid concentrations cannot be used directly for radiopharmaceuticals [20], requiring additional purification steps or dilution with large volumes. Furthermore, the elution order is critical for isotope generator applications, where the medically relevant ^{225}Ac would be produced from the decay of ^{225}Ra. For a resin-based isotope generator it is critical that the parent isotope can be retained on the resin while only the daughter isotope is eluted. This separation scheme allows for the elution of Ac in biologically friendly pH conditions, and Ac is eluted prior to Ra, which indicates potential for an isotope generator application.

If DTPA is undesired in the final Ac sample, it can be readily removed with DGA resin as shown in Fig. 3. From the cation exchange column, the Ac fractions with DTPA are acidified to a sufficiently low pH to prevent the chelation of Ac by DTPA. The solution is then passed through a DGA resin cartridge which retains Ac and allows DTPA, lactate and other salts, such as Na, to pass through the resin. Actinium can be stripped quantitatively (100% yield) in a variety of dilute acids including HLa and HCl, allowing for optimization of the procedure for the final application as desired. The removal of DTPA was assessed by residue mass: the ^{228}Ac fractions from the DGA columns were dried and compared to an identically run "blank" column (without a load solution). For all of the DGA columns, there was no excess residue as compared to the blank within the measurement limits (0.1 mg). The mass of DTPA in the ^{228}Ac load solution for these columns is 59 mg, therefore excess DTPA would be detectable, and the columns demonstrate a reasonably good removal of the chelator. While the separation is effective whether the solution is acidified with HCl or HNO_3 (Fig. 3), there are advantages and disadvantages to using either acid. For the HCl separation, a higher concentration of acid is required for the load solution and the wash (~8 M HCl vs. ~3 M HNO_3) which leads to a very low pH (< 0) for the subsequent ^{228}Ac fractions. However, trace nitrates are undesirable in many applications as they are more difficult to remove than chlorides, therefore separation in HCl media, followed by neutralization, could be preferable for some applications. For the separation in HNO_3, the pH of the eluted ^{228}Ac fractions is ~1, which would require less base to neutralize as compared to the HCl separation, which may also be advantageous under some circumstances.

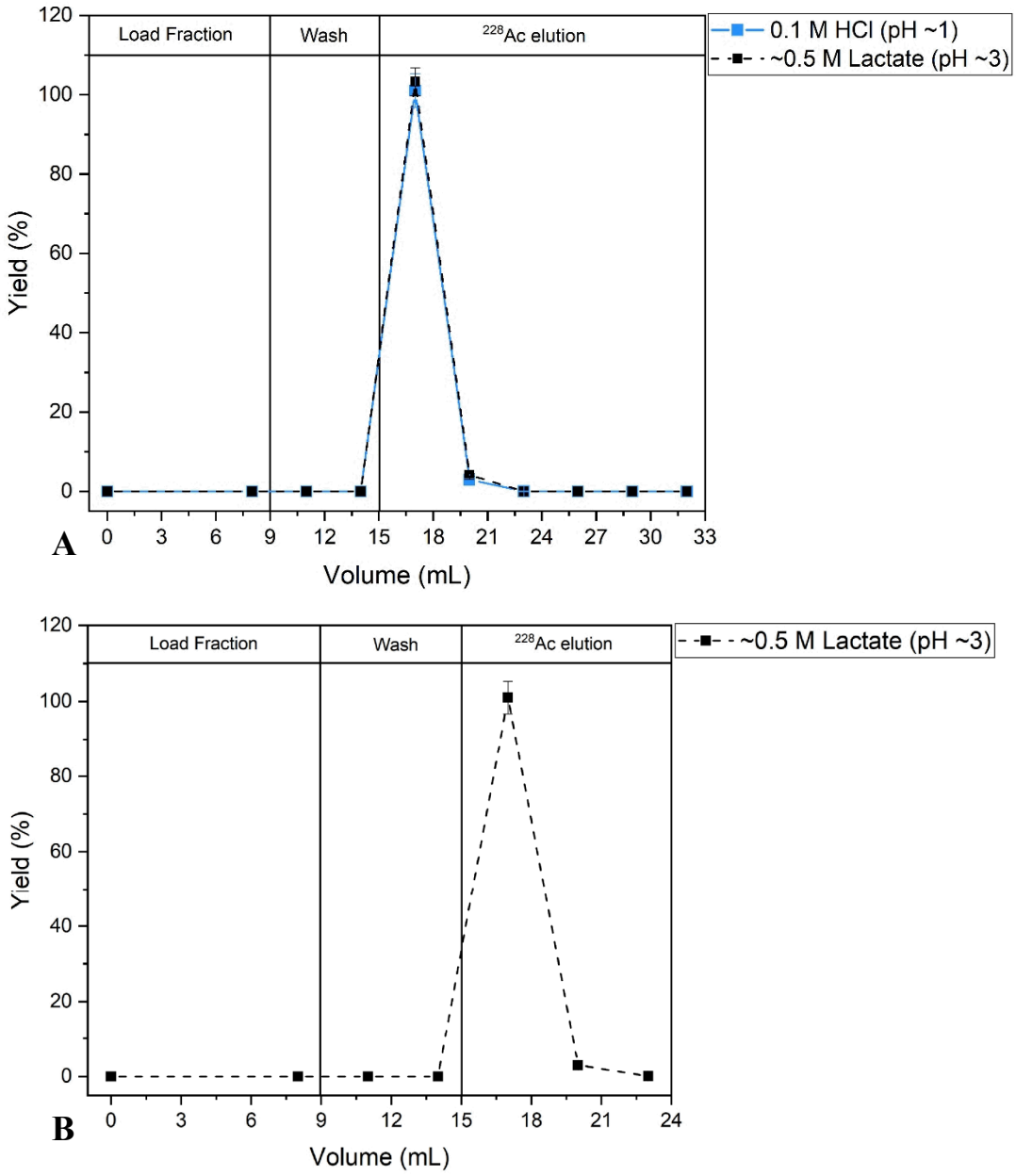


Fig. 3 The elution of ^{228}Ac from DGA from a (a) 7.5 M HCl load solution with 8 M HCl wash and (b) 3.2 M HNO_3 load solution with a 0.3 M HNO_3 wash. Error is from counting statistics, some error bars are smaller than the data points; lines are to guide the eye.

Based on the initial column studies, further experiments were conducted to assess whether this separation system would be applicable to an isotope generator to produce Ac from Ra. A generator relevant to radiopharmaceutical applications would be based on the ^{225}Ra/^{225}Ac parent-daughter pair, but these isotopes are difficult to produce and were not

available for this study. Therefore, the $^{228}Ra/^{228}Ac$ parent-daughter pair, which is readily obtained from ^{nat}Th, was used as a surrogate. While the 1 mL column performed better than the 1.8 mL column in the initial test, both sizes were used in the isotope generator studies. This is because resin degradation in isotope generators, particularly those with high levels of α activity, can reduce the extraction on the resin and larger bed volume can be useful to enhance retention.

The results from the isotope generator studies are shown in Fig. 4. Each column was loaded on Day 0, with 200 µL 0.2 M HNO_3 and one fraction of Solution #1 were collected as a load fraction and wash, respectively, before the elution was continued as shown in Figs. 4 and 5. The load fraction and wash are not plotted to ensure all elutions could be compared on the same axis (as there was no load fraction or wash required for later elutions) and neither had detectable activity. The column was stored in Solution #1, which is the first fraction collected (with no activity) in all elutions.

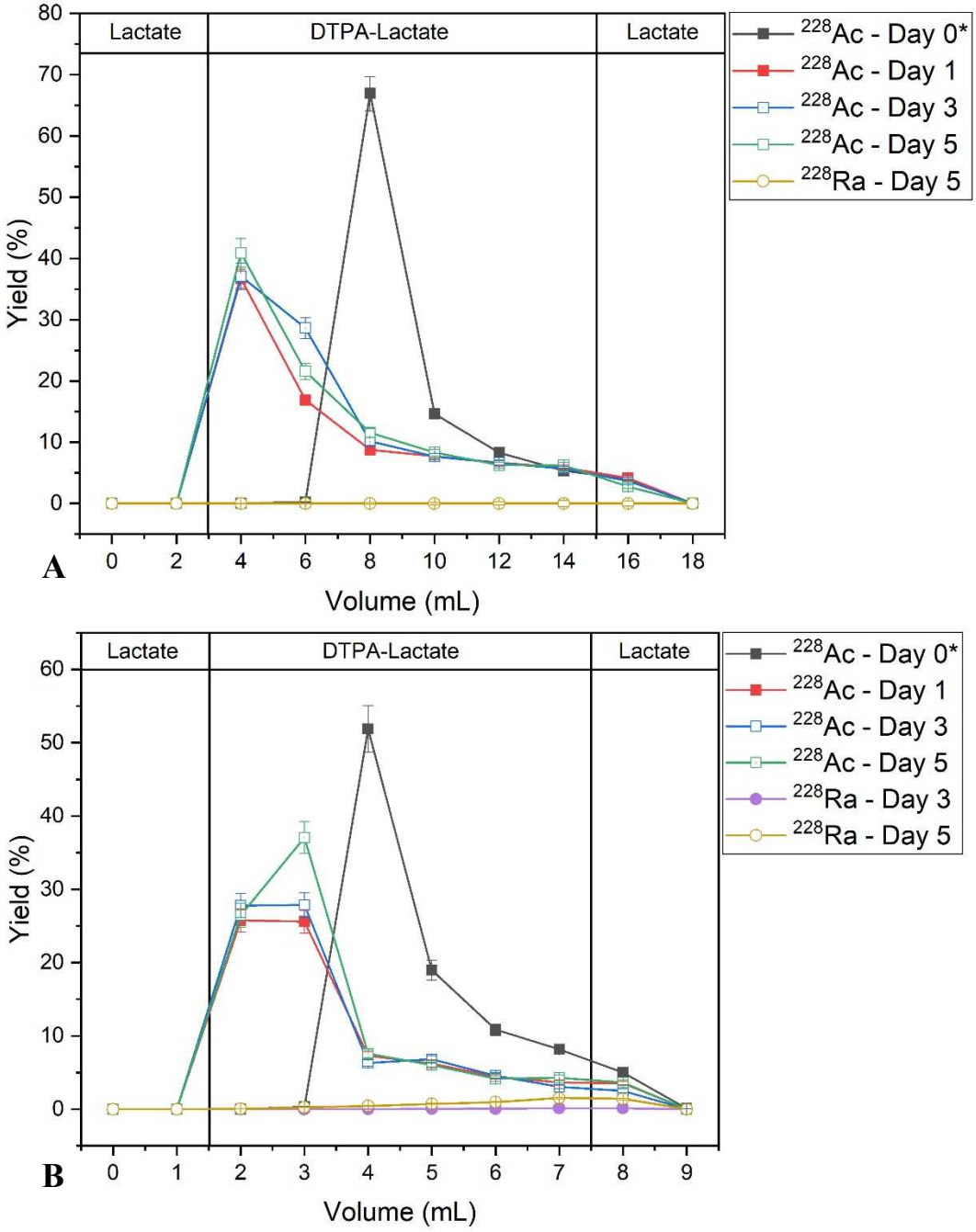


Fig. 4 ^{228}Ra/^{228}Ac isotope generator studies using cation exchange resin and DTPA-lactate solutions. Error is from counting statistics, some error bars are smaller than the data points; lines are to guide the eye. (a) Column 1 – bed volume 1.8 mL; all fractions 2 mL. (b) Column 2 – bed volume 1 mL, all fractions 1 mL.

These studies were not successful over a reasonable duration for Ra/Ac isotope generator applications. As the half-life of ^{225}Ra is 15 days [12], an isotope generator would need to last at least one half-life of the parent to have any practical use and an even longer lifetime (~4-5 half-lives) to be comparable to current nuclear medicine generators, e.g. ^{99}Mo/^{99m}Tc [21]. The 1 mL isotope generator tested had breakthrough of ^{228}Ra on only the second elution (Day 3), amounting to ~0.3% of the total activity and higher breakthrough on the third elution (~4%). The 2 mL isotope generator did not have break through until the third elution (Day 5) with the elution of ~1% of the total ^{228}Ra activity. The breakthrough is not due to resin degradation or capacity as these columns are relatively low activity (<1000 dps) and the radionuclides are carrier-free. Therefore, the breakthrough is due to slow ^{228}Ra elution in the DTPA-lactate solution.

Reference [10] demonstrated a ^{223}Ra/^{212}Pb generator based on elution of the daughter by DTPA at pH 5.5. The generator described in this work had a useful lifetime of 2 weeks based on the half-lives of the isotopes studied, rather than breakthrough. The difference in behavior as compared to the generators studied in this work is likely explained by the solution containing DPTA. The eluant used in Ref. [10] is 80% methanol, which has different hydrating properties and increases the extraction of Ra on cation exchange resin above what is possible at similar concentrations of HNO_3 in purely aqueous solutions [10] and therefore preclude Ra breakthrough for longer durations. Furthermore, the lactic acid used in this work is more complexing than HNO_3, which was used to adjust the pH in Ref. [10]. However, methanol itself is highly toxic, and methanolic-nitric solutions are also explosive and highly corrosive; such solutions require special handling and are problematic for radiopharmaceutical applications.

Conclusions

This work demonstrates a high yield, high radiopurity separation of Ac from Ra with recoveries of ~100% of both isotopes and no detectable Ra in the Ac fractions using DTPA-lactate solutions. While attempts to utilize this separation for isotope generators were unsuccessful due to early ^{228}Ra breakthrough, the successful column separation is viable

for many applications. In particular, the separation of Ra and Ac is important for the production of ^{225}Ac, a potential isotope for cancer treatment. Development of procedures for the separation of these elements without the need for strong acids is critical for separation methods appropriate for radiopharmaceuticals, which must be safe for use in humans. There are environmental applications for such columns as well, as the separation of ^{228}Ra and ^{228}Ac is important for the characterization of ^{228}Ra in water [18].

Future work is needed to optimize this separation system for isotope generator applications. Based on the results from this work as compared to Ref. [10], this will focus on the buffer solutions to develop an solution that is biologically-compatible, allows for elution of Ac in a small volume, and a can sustain more elutions before Ra breakthrough.

Acknowledgements

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Ethics Declarations

Conflict of Interest/Competing Interests

The authors declare that there is no conflict of interest or competing interest relevant to the content of this article.

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