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(54) **ALKYL-SUBSTITUTED HYDROXAMATE RESIN FOR USE IN A GENERATOR SYSTEM**

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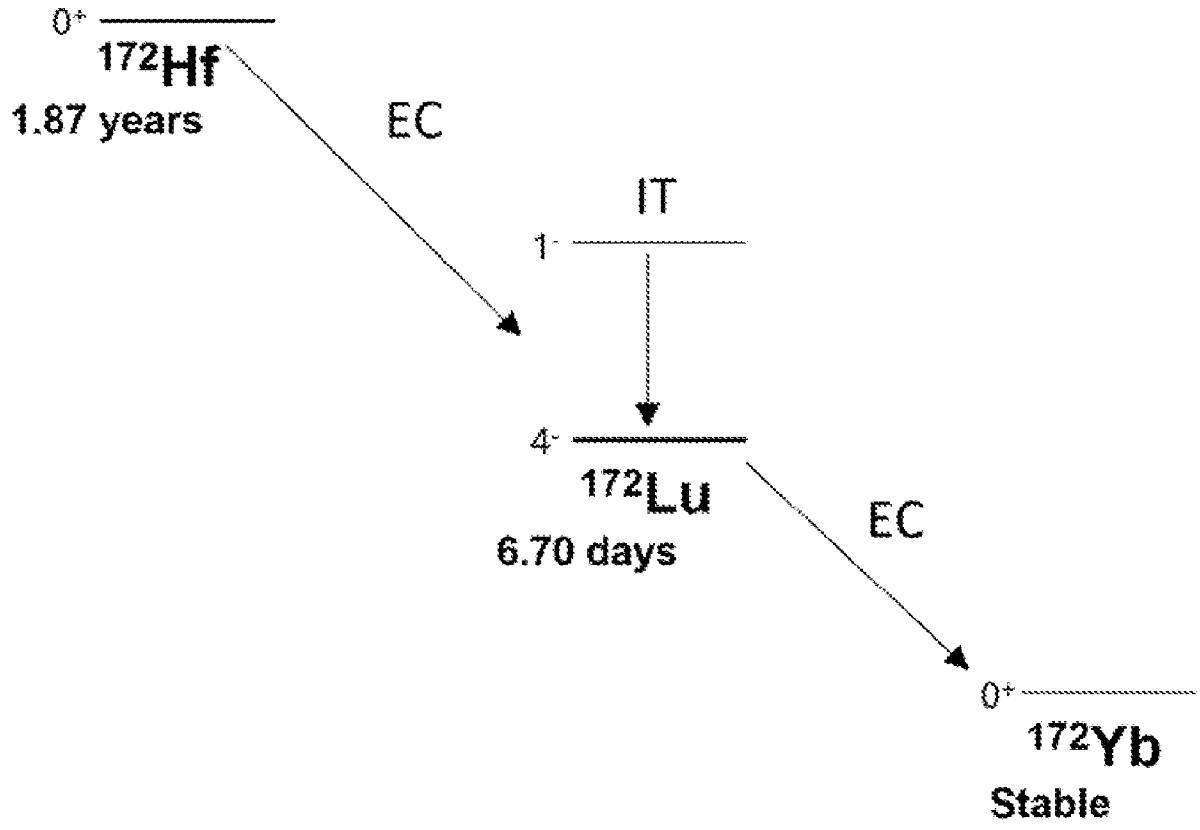
(52) **U.S. Cl.**
CPC ..... ***B01J 20/288*** (2013.01); ***B01J 20/265*** (2013.01); *B01J 2220/52* (2013.01)

(57) **ABSTRACT**

In one aspect, the disclosure relates to a hydroxamate-based resin for use in a $^{44}Ti/^{44}Sc$ generator system. In an aspect, the carboxylate groups of a commercially available resin can be synthetically modified to produce an alkyl-substituted hydroxamate resin. In one aspect, the carboxylate resin can be a commercial resin. The disclosure also relates to a $^{44}Ti/^{44}Sc$ generator system comprising an alkyl-substituted hydroxamate resin of Formula I and a method of producing $^{44}Sc$, the method comprising decay of $^{44}Ti$ in a $^{44}Ti/^{44}Sc$ generator system using an alkyl-substituted hydroxamate resin of Formula I. In an aspect, the alkyl can be methyl. The disclosure further relates to a $^{172}Hf/^{172}Lu$ generator system comprising the use of an alkyl-substituted hydroxamate resin of Formula I and a method of producing $^{172}Lu$ comprising decay of $^{172}Hf$ in a $^{172}Hf/^{172}Lu$ generator system using an alkyl-substituted hydroxamate resin. In an aspect, the alkyl can be methyl.



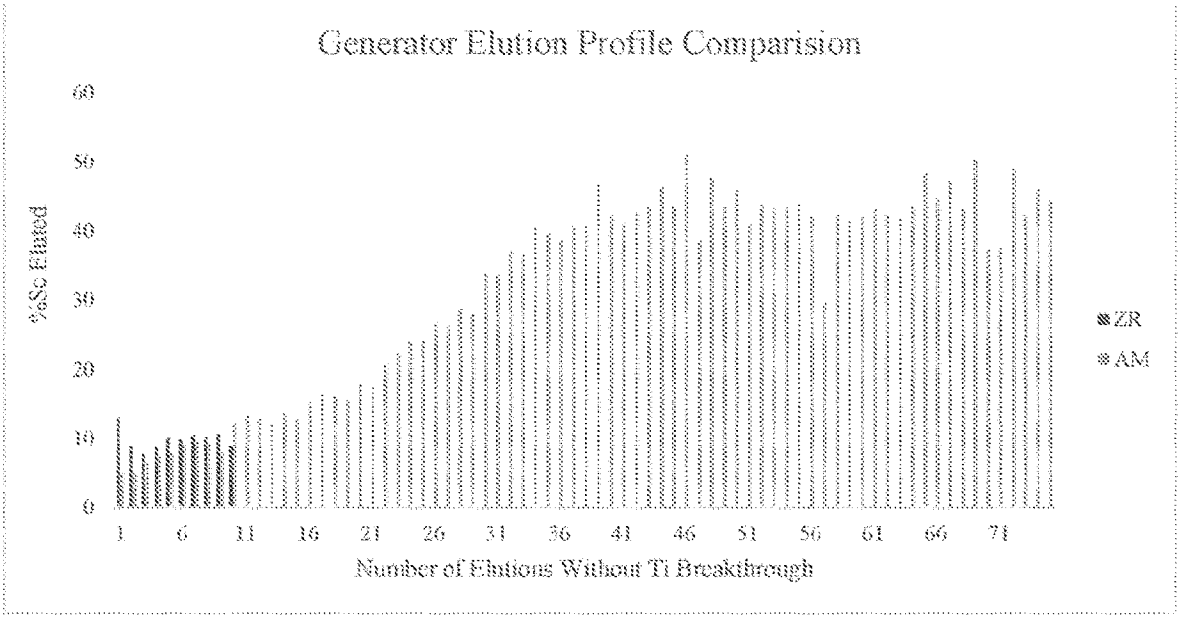

Generator Elution Profile Comparision
%Sc Eluted
60
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
Number of Elutions Without Ti Breakthrough
ZR
AM

FIG. 1

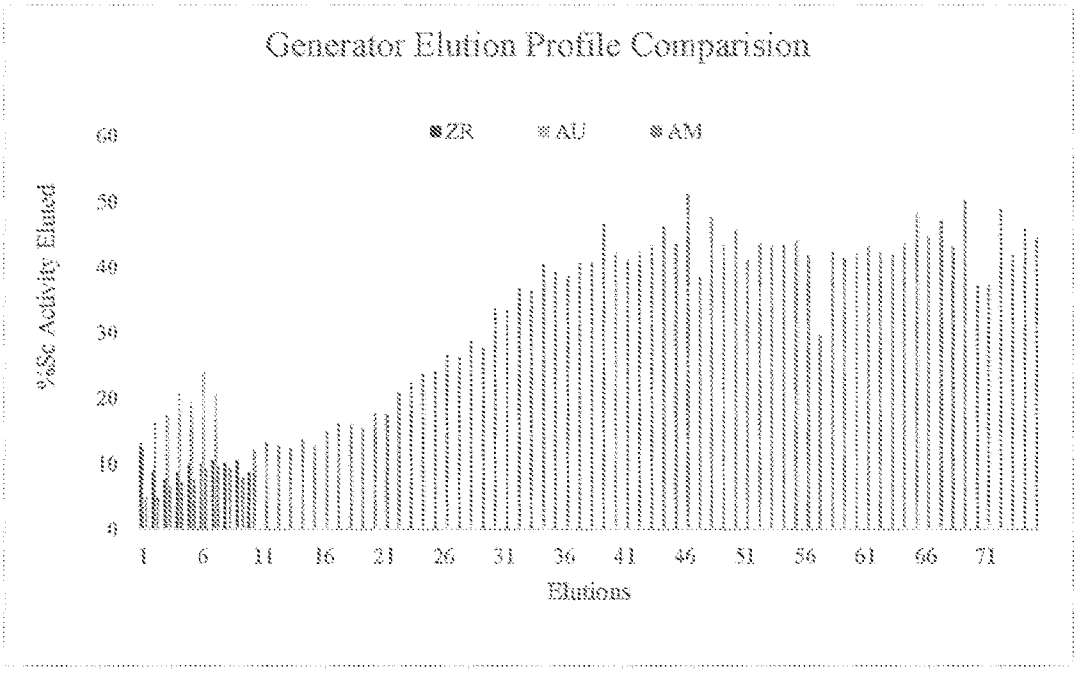

Generator Elution Profile Comparision
ZR
AU
AM
%Sc Activity Eluted
60
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
Elutions

FIG. 2

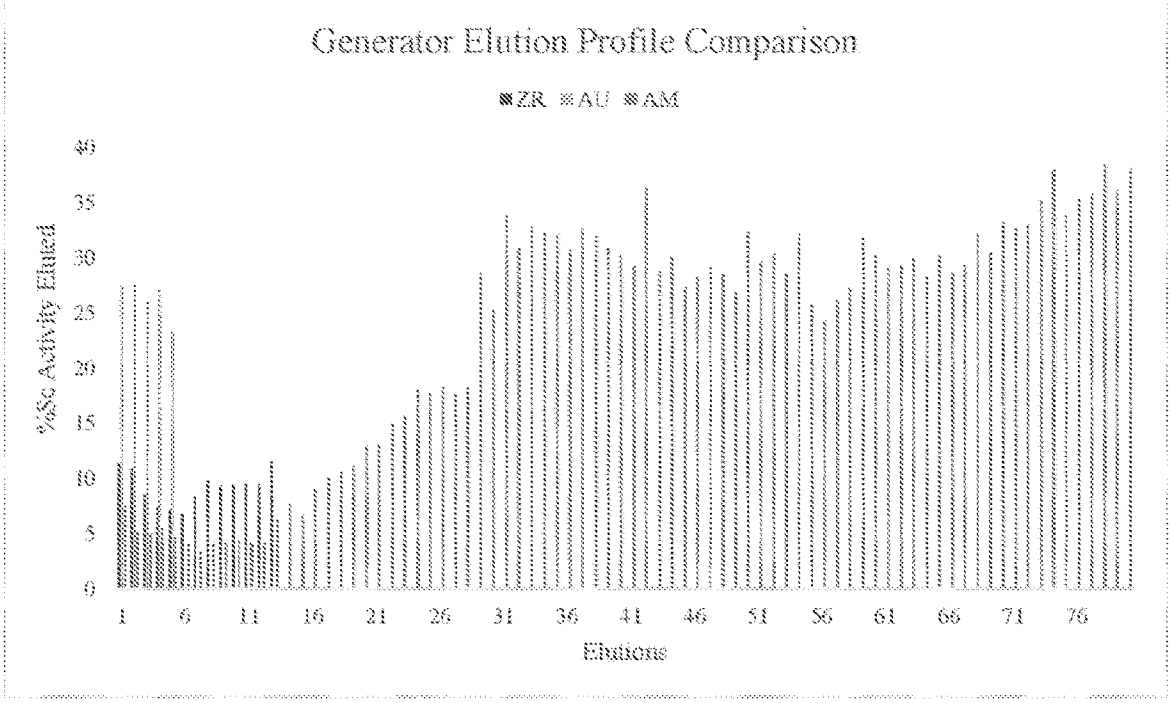

Generator Elution Profile Comparison
ZR
AU
AM
%Sc Activity Eluted
0
5
10
15
20
25
30
35
40
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
Elutions

FIG. 3

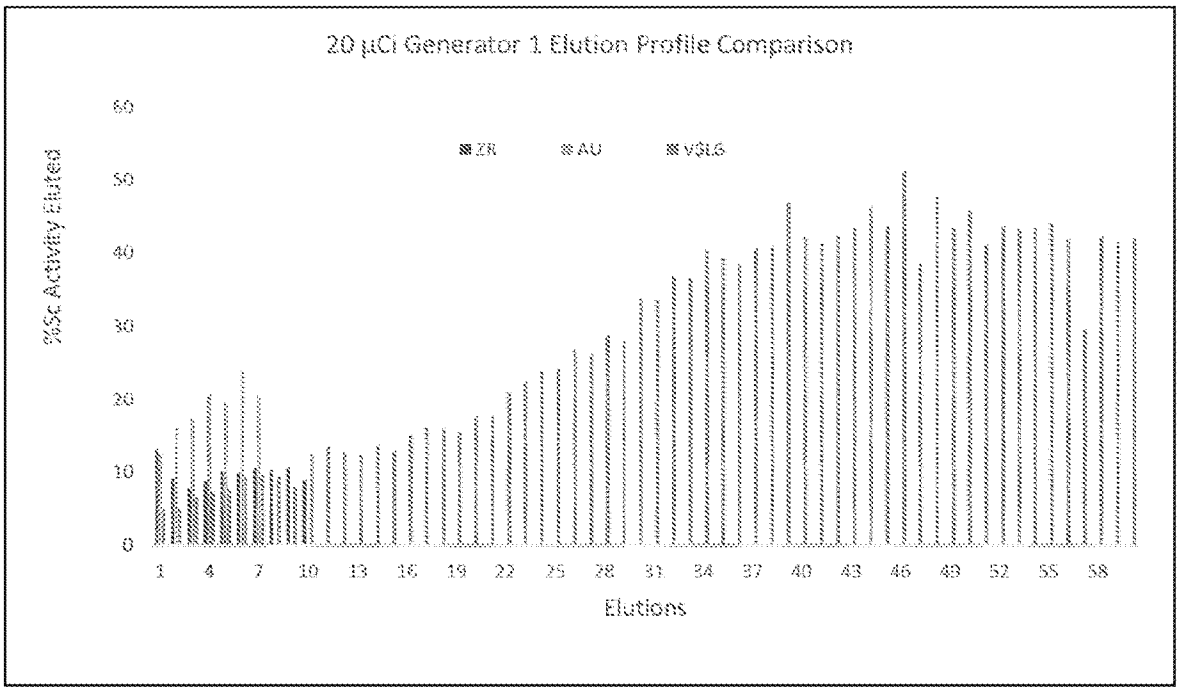

20 µCi Generator 1 Elution Profile Comparison
ZR
AU
VSLS
%Sc Activity Eluted
0
10
20
30
40
50
60
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
Elutions

FIG. 4

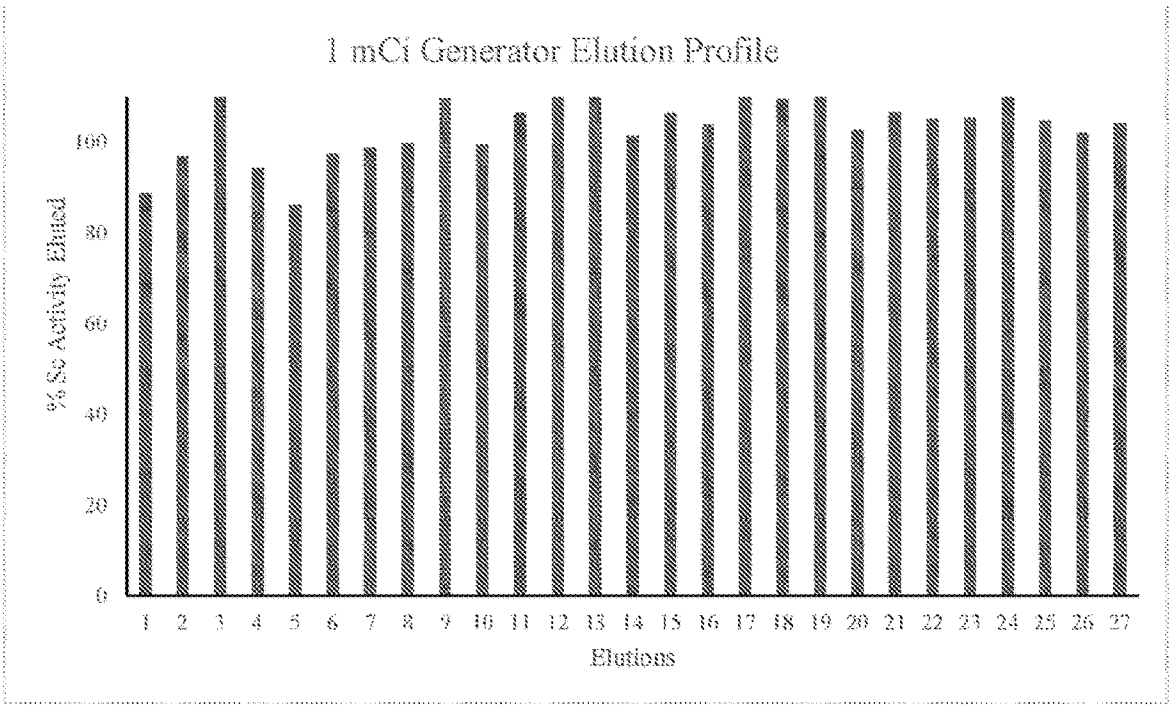

1 mCi Generator Elution Profile
% Sc Activity Eluted
0
20
40
60
80
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Elutions

FIG. 5

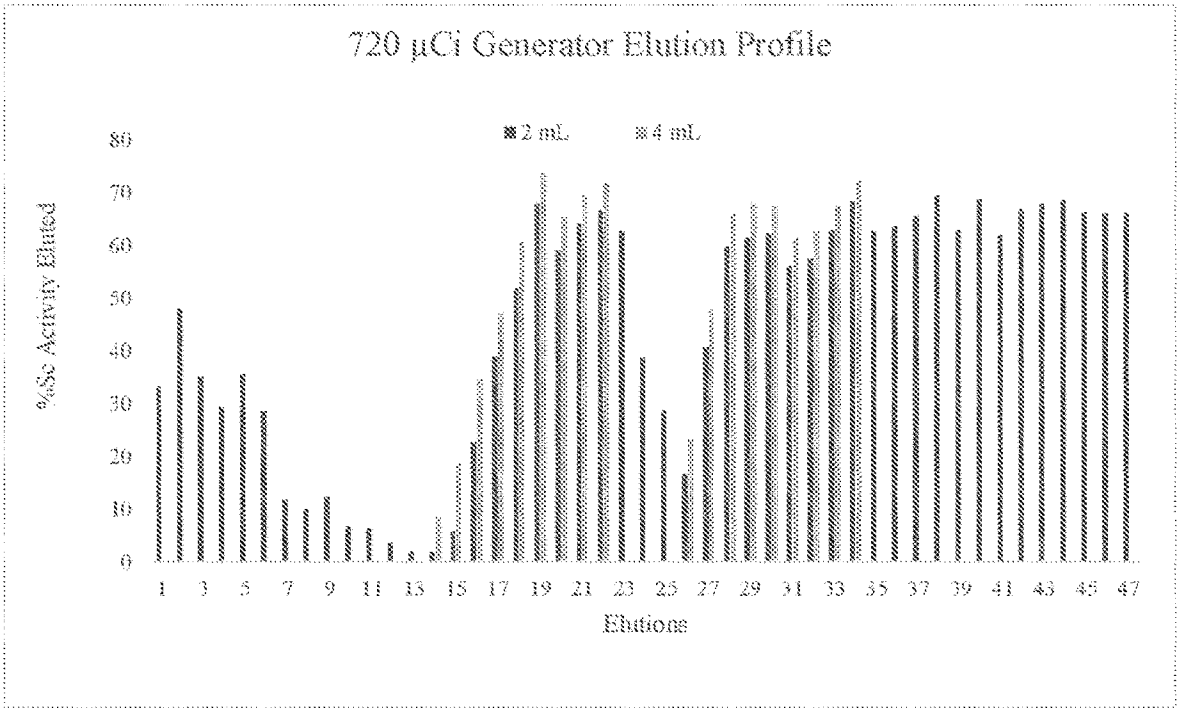

720 µCi Generator Elution Profile
2 mL
4 mL
%Sc Activity Eluted
0
10
20
30
40
50
60
70
80
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Elutions

FIG. 6

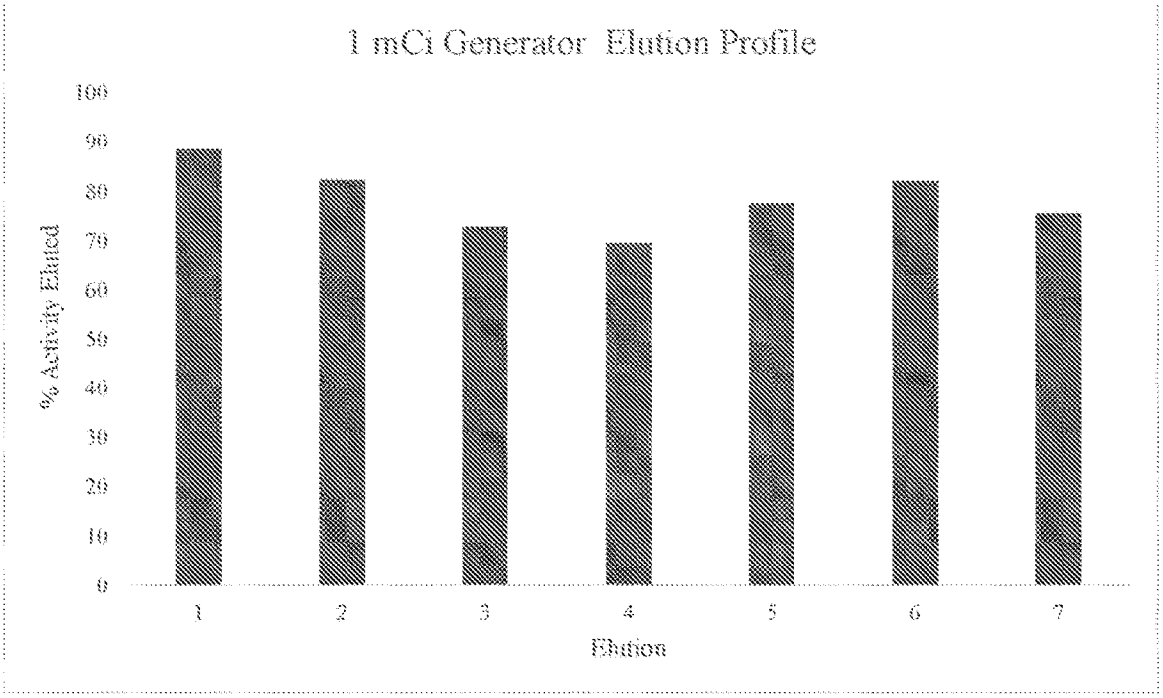

1 mCi Generator Elution Profile
% Activity Eluted
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
Elution

FIG. 7

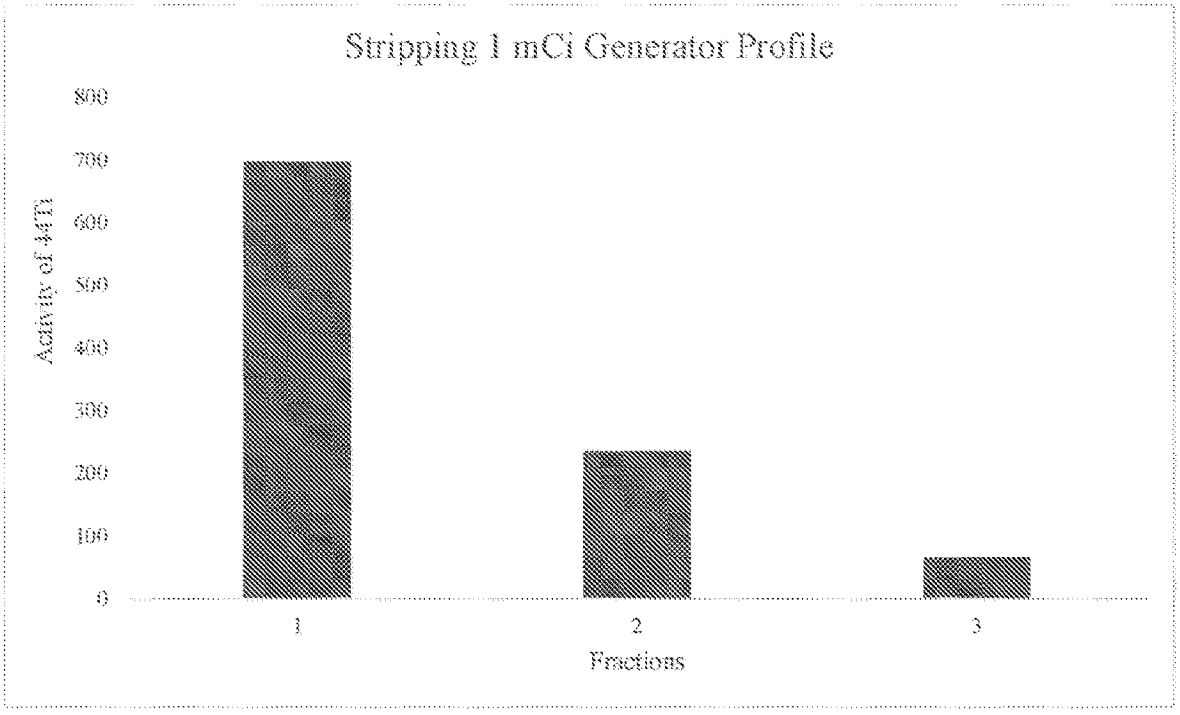

Stripping 1 mCi Generator Profile
Activity of 44Ti
800
700
600
500
400
300
200
100
0
1
2
3
Fractions

FIG. 8

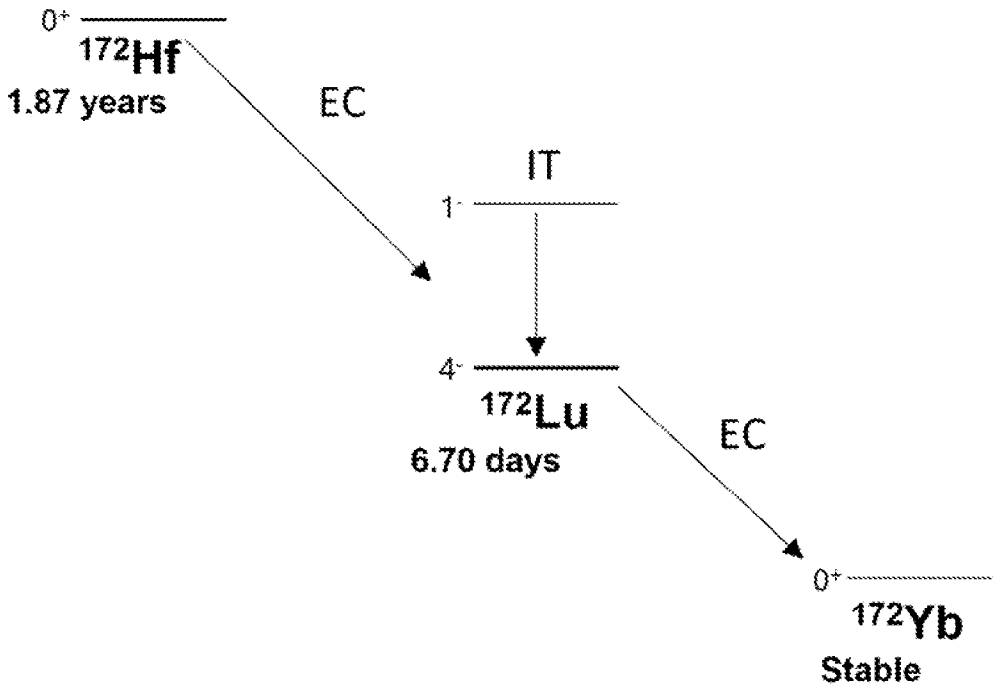

0+
172Hf
1.87 years
EC
IT
1-
4-
172Lu
6.70 days
EC
0+
172Yb
Stable

FIG. 9

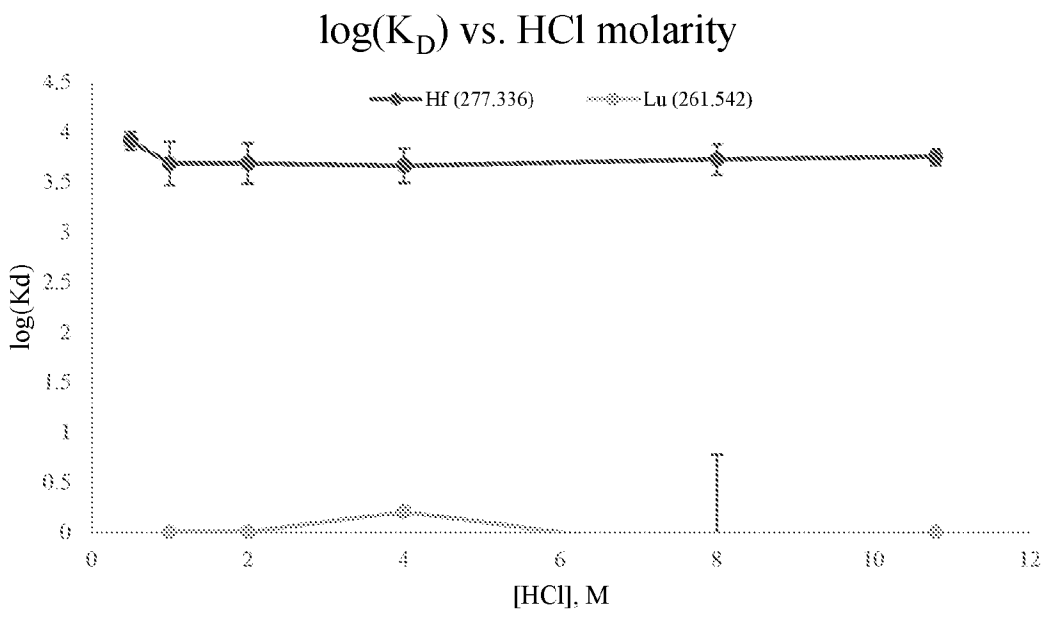

log(K_D) vs. HCl molarity
Hf (277.336)
Lu (261.542)
log(Kd)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
[HCl], M

FIG. 10

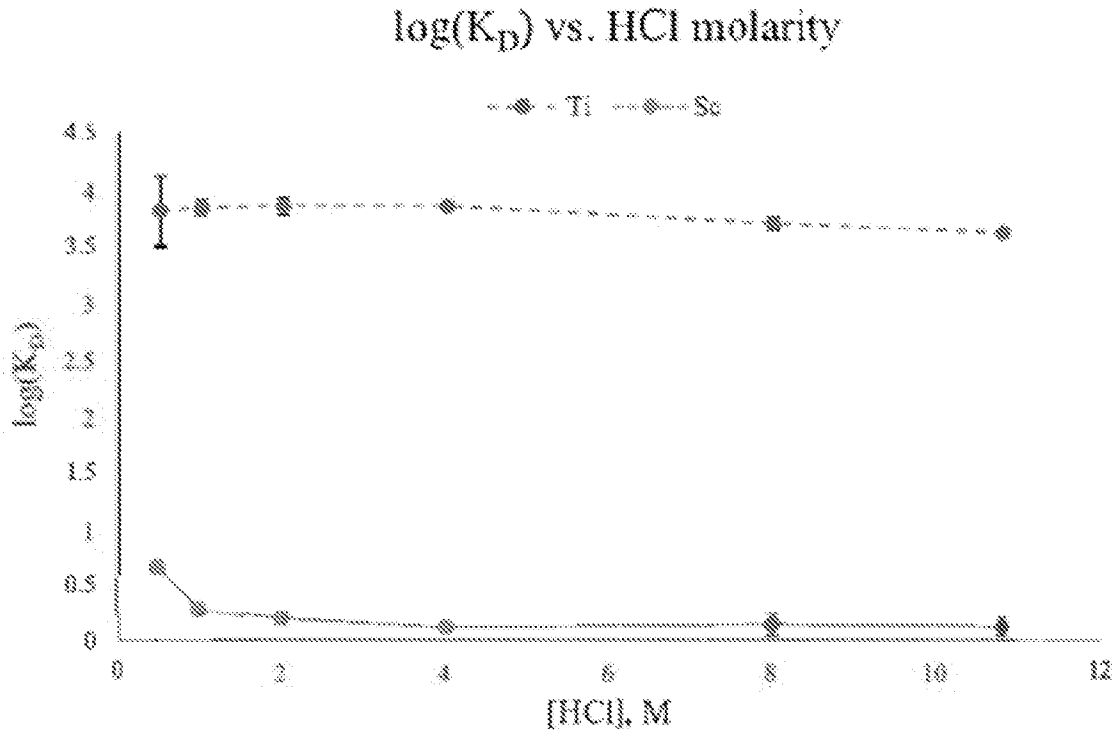

log(K_D) vs. HCl molarity
Ti
Sc
log(K_D)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
[HCl], M

FIG. 11

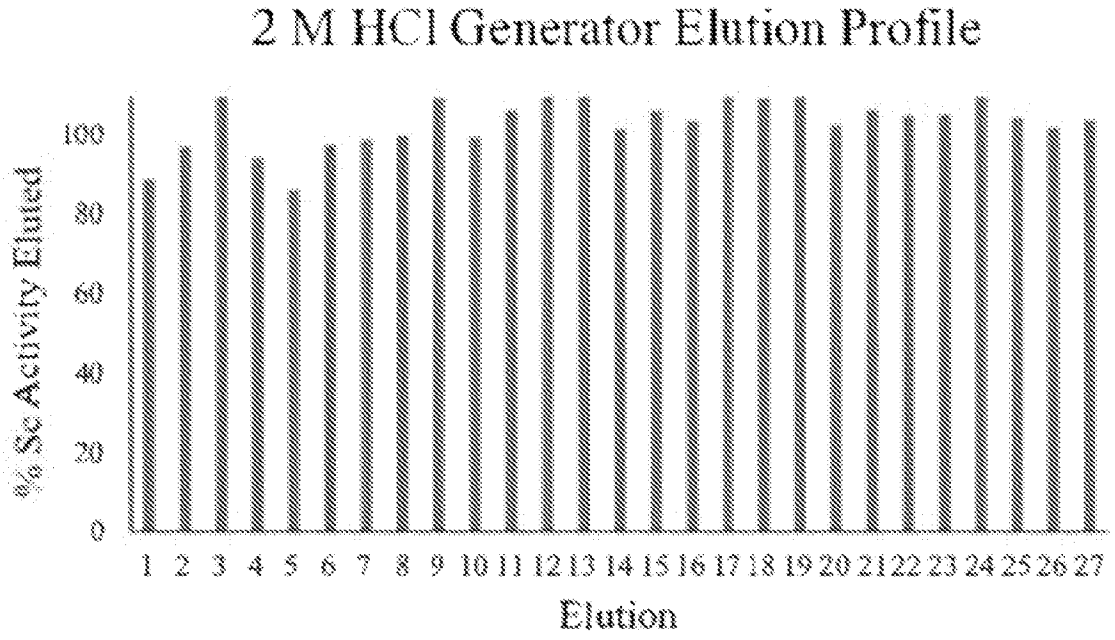

2 M HCl Generator Elution Profile
% Sc Activity Eluted
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Elution

FIG. 12

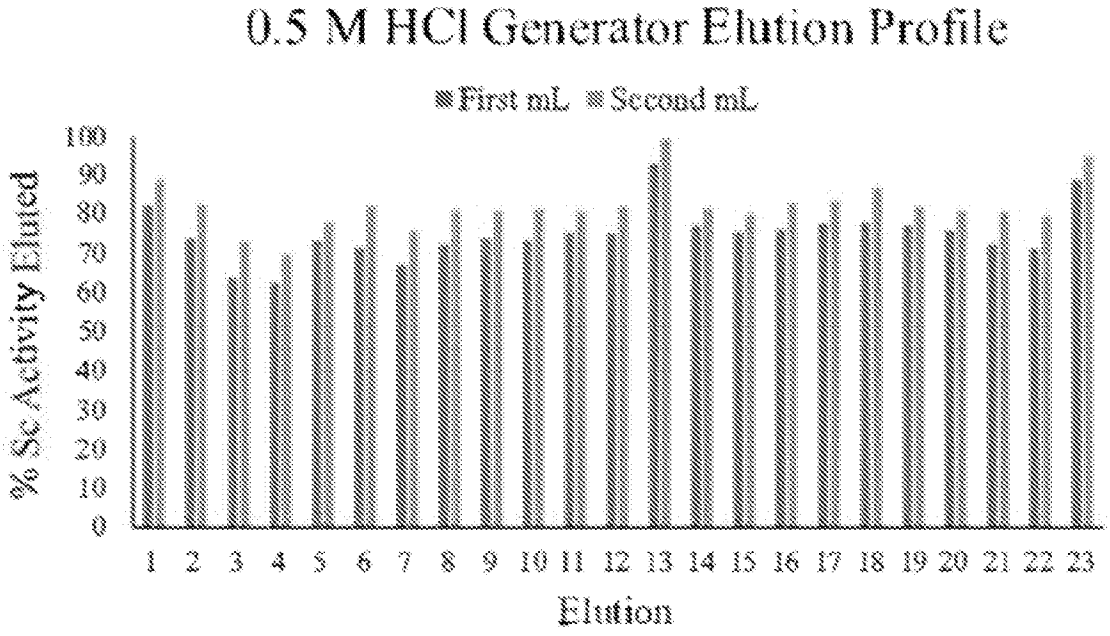

0.5 M HCl Generator Elution Profile
First mL
Second mL
% Sc Activity Eluted
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Elution

FIG. 13

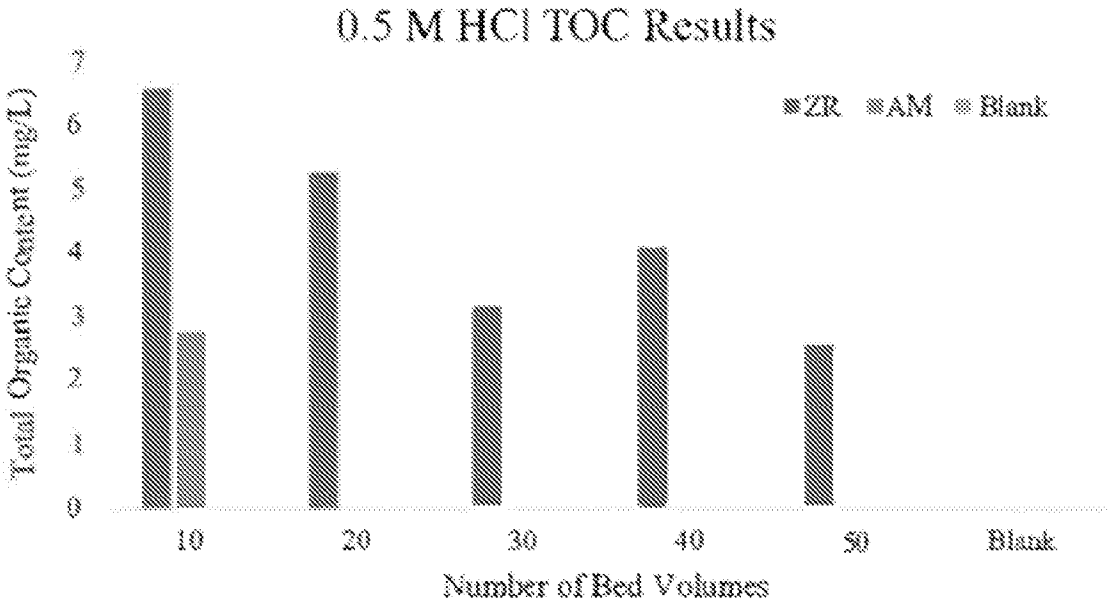

0.5 M HCl TOC Results
ZR
AM
Blank
Total Organic Content (mg/L)
7
6
5
4
3
2
1
0
10
20
30
40
50
Blank
Number of Bed Volumes

FIG. 14

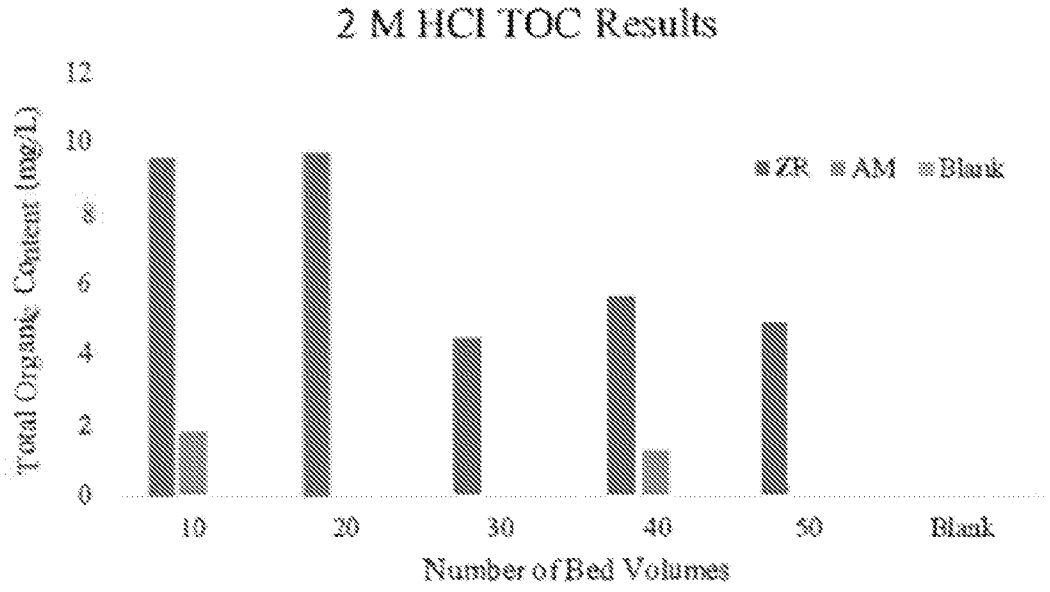

2 M HCl TOC Results
Total Organic Content (mg/L)
12
10
8
6
4
2
0
ZR
AM
Blank
10
20
30
40
50
Blank
Number of Bed Volumes

FIG. 15

# ALKYL-SUBSTITUTED HYDROXAMATE RESIN FOR USE IN A GENERATOR SYSTEM

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 63/344,194, filed on May 20, 2022, which is incorporated herein by reference in its entirety.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

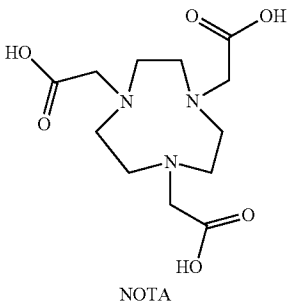
**[0002]** This invention was made with government support under contract number DE-SC0012704 awarded by the U.S. Department of Energy. The United States government has certain rights in the invention.

## BACKGROUND

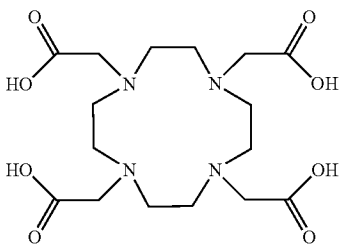
**[0003]** Scandium 44 ($^{44}$Sc) is of high interest as a potential positron emission tomography (PET) imaging radionuclide due to its promising characteristics such as high positron branching ratio (94.27%) and 3.97 hour half-life, making it a suitable candidate for radiolabeling of small to medium biomolecules since it matches their biological half-lives. $^{44}$Sc has even shown slightly superior image resolution in radiopharmaceutical imaging compared to the analogous $^{68}$Ga systems. Presently, $^{44}$Sc can be obtained in one of two ways; irradiation of $^{44}$Ca targets with low energy protons or through decay of the parent isotope $^{44}$Ti in a $^{44}$Ti/$^{44}$Sc generator system. While the solid targetry approach on calcium produces high yields of $^{44}$Sc, due to the short half-life of $^{44}$Sc the direct production requires access to a cyclotron at or near the imaging facility. More importantly during the irradiation several other Sc isotopes are produced which then requires extensive post-irradiation processing and purification before the $^{44}$Sc can be subsequently used for radiolabeling. Employing a generator system allows for an alternative approach to the production of $^{44}$Sc. In these systems a long-lived parent radionuclide ($^{44}$Ti) is adsorbed onto a solid phase matrix to facilitate chromatographic separations. Once the parent nuclide decays to the short-lived radionuclide of interest it is removed from the solid support through a facile process in a pure form. The $^{44}$Ti/$^{44}$Sc generator system would allow for daily elution of $^{44}$Sc without the need for on-site irradiation, and the long half-life of parent isotope $^{44}$Ti (60 years) would allow for a potentially long-lasting source of pure $^{44}$Sc.

**[0004]** Development of an acceptable $^{44}$Ti/$^{44}$Sc radionuclide generator needs to address several criteria such as efficient separation, low $^{44}$Ti breakthrough, long term stability and useful Sc eluates that will be suitable for subsequent radiolabeling (low volume, low pH, high purity). Several current generators employ the use of a commercial AG 1-X8 anion exchange resin eluting with dilute oxalic acid/hydrochloric acid mixtures, but there are numerous drawbacks to this method, such as the extremely large volumes needed to elute sufficient $^{44}$Sc activity, short generator lifetimes before $^{44}$Ti breakthrough, and the presence of competing oxalates in solution that will hinder subsequent radiolabeling.

**[0005]** More recently the use of commercially available ZR resin has been suggested as a possible resin for the $^{44}$Ti/$^{44}$Sc radionuclide generator system. The Triskem hydroxamate based ZR resin has been previously used to separate Zr from Y, and due to the chemical similarities between the Zr(IV)/Y(III) and Ti(IV)/Sc(III) pair, it also shows high selectivity for Ti and little selectivity for Sc over a range of acid concentrations. While the high retention of $^{44}$Ti on the ZR resin indeed seems promising, there is little to no information in the literature on the $^{44}$Sc elution activity or generator lifetime. One study conducted by Radchenko et al. suggests $^{44}$Ti breakthrough after 40 bed volume elutions of a conventional direct elution generator. Using ZR resin generators, the elution volumes needed compared to the AG 1-X8 generators is drastically decreased, but the generator lifetime before $^{44}$Ti breakthrough as well as $^{44}$Sc activity elution and purity of eluted $^{44}$Sc has been extremely inconsistent and unreliable. There is an organic impurity that is eluted along with the $^{44}$Sc which inhibits subsequent radiolabeling with DOTA, HOPO or NOTA under standard conditions. Thus, to date, there are no commercially available resins that provide a robust generator system that tightly retains $^{44}$Ti while supplying pure $^{44}$Sc in a convenient form for radiolabeling without the use of tedious post-elution processing and purification.

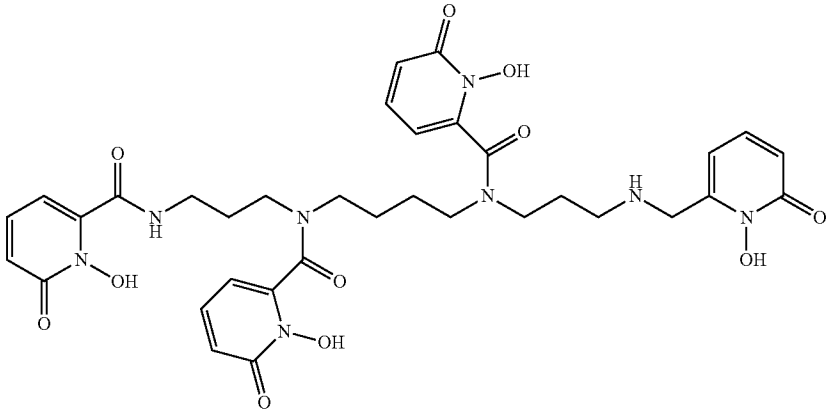


NOTA



DOTA

-continued



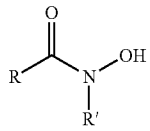
HOPO

**[0006]** Despite advances in radionuclide generator research, there is still a scarcity of generators that retain $^{44}$Ti while continuously supplying $^{44}$Sc that is free of organic impurities that can hinder subsequent radiolabeling. Such a system would eliminate the need for post-elution processing and purification and could operate at reduced elution volumes compared to known methods. These needs and other needs are satisfied by the present disclosure.

## SUMMARY

**[0007]** The present disclosure is directed to a hydroxamate-based resin for use in a $^{44}$Ti/$^{44}$Sc generator system. In an aspect, the carboxylate groups of a commercially available resin can be synthetically modified to produce an alkyl-substituted hydroxamate resin of Formula I (V$LG),

Formula I



**[0008]** wherein R can be a resin backbone that is a polymer-coated silica based media and wherein R' can be an alkyl group that can be branched or unbranched, is saturated, and has from about 1 to about 12 carbon atoms in its longest chain. In one aspect, the carboxylate resin can be an Accell Plus CM resin available from Waters™ (Milford, MA).

**[0009]** The disclosure also relates to a $^{44}$Ti/$^{44}$Sc generator system comprising an alkyl-substituted hydroxamate resin of Formula I and a method of producing $^{44}$Sc, the method comprising decay of $^{44}$Ti in a $^{44}$Ti/$^{44}$Sc generator system using an alkyl-substituted hydroxamate resin of Formula I. In an aspect, the alkyl can be methyl.

**[0010]** The disclosure further relates to a $^{172}$Hf/$^{172}$Lu generator system comprising the use of an alkyl-substituted hydroxamate resin of Formula I and a method of producing $^{172}$Lu comprising decay of $^{172}$Hf in a $^{172}$Hf/$^{172}$Lu generator system using an alkyl-substituted hydroxamate resin. In an aspect, the alkyl can be methyl.

**[0011]** Other systems, methods, features, and advantages of the present disclosure will be or become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present disclosure, and be protected by the accompanying claims. In addition, all optional and preferred features and modifications of the described embodiments are usable in all aspects of the disclosure taught herein. Furthermore, the individual features of the dependent claims, as well as all optional and preferred features and modifications of the described embodiments are combinable and interchangeable with one another.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0012]** Many aspects of the present disclosure can be better understood with reference to the following drawings. The components in the drawings are not necessarily to scale, emphasis instead being placed upon clearly illustrating the principles of the present disclosure. Moreover, in the drawings, like reference numerals designate corresponding parts throughout the several views.

**[0013]** FIG. **1** shows the amount of $^{44}$Sc activity eluted in 0.5 M HCl fractions of exemplary generators based on detection of $^{44}$Sc gamma peak at 1157 keV using HPGe γ spectroscopy. The ZR generator $^{44}$Sc activity elution is shown in the darker bars and the methyl hydroxamate resin (AM) is shown in the lighter bars.

**[0014]** FIG. **2** shows an elution profile of a generator experiment where loading occurs in 2 M HCl and elution occurs with 0.5 M HCl.

**[0015]** FIG. **3** shows an elution profile of a generator experiment where loading occurs in 0.1 M HCl and elution occurs with 0.5 M HCl.

**[0016]** FIG. **4** shows an elution profile of a generator experiment where loading occurs in 2 M HCl and elution occurs with 2 M HCl, where ZR and AU are as described previously and V$LG is equivalent to an AM resin.

**[0017]** FIG. **5** shows an elution profile of a 1 mCi AM generator experiment as described herein.

**[0018]** FIG. **6** shows an elution profile of a 720 μCi AM generator experiment as described herein.

**[0019]** FIG. **7** shows an elution profile of a 1 mCi AM generator experiment as described herein.

**[0020]** FIG. **8** shows a stripping profile of 1 mCi $^{44}$Ti generator using 2% $H_2O_2$ in 2 M HCl. Each fraction represents 8 bed volumes (8 mL).

**[0021]** FIG. **9** shows a decay scheme of $^{172}$Hf by EC to $^{172}$Lu ($t_{1/2}$=6.7 days) which then decays by electron capture to stable $^{172}$Yb.

**[0022]** FIG. **10** shows preliminary cold studies were conducted using the methyl hydroxamate resin to ascertain the distribution coefficient ($K_D$) for both Hf and Lu in varying concentrations of HCl to determine the extent of this generator system. As shown in the plot, Hf is retained strongly on the resin across all concentration of the acid, similarly to Ti. Lu, on the other hand, shows little to no retention across the HCl concentrations. Based on the similarities in the $K_D$ results of the Ti/Sc and Hf/Lu studies we believe that this resin would also serve as a suitable generator for Hf/Lu.

**[0023]** FIG. **11** shows Ti and Sc distribution coefficient dependencies on HCl concentration for AM resin.

**[0024]** FIG. **12** shows an elution profile for a 2 M HCl 37 MBq (1 mCi) AM generator.

**[0025]** FIG. **13** shows an elution profile for a 0.5 M HCl 37 MBq (1 mCi) AM generator shown as activity obtained in the first 1 mL fraction and the second 1 mL fraction.

**[0026]** FIG. **14** shows total organic content analysis of 0.5 M HCl eluent from ZR and AM resin columns.

**[0027]** FIG. **15** shows total organic content analysis of 2 M HCl eluent from ZR and AM resin columns.

**[0028]** Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

## DETAILED DESCRIPTION

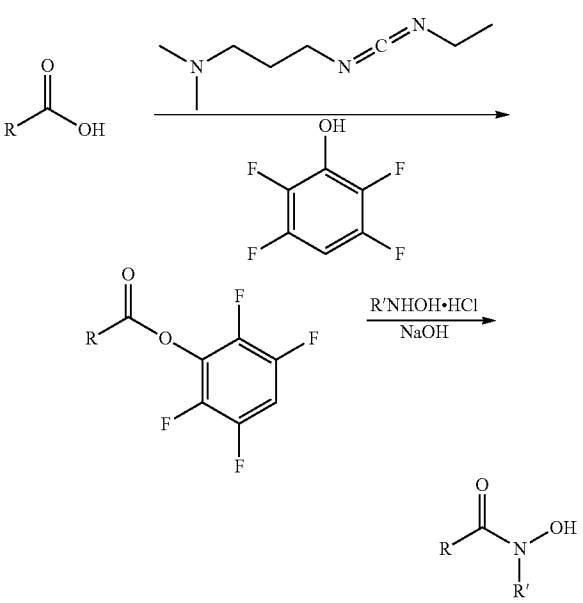
**[0029]** In one aspect, in the methods disclosed herein, the carboxylate groups of commercially available Waters™ Accell Plus CM resin and other commercially available resins can be synthetically altered to produce alkyl-substituted hydroxamate functional groups, such as, for example, methyl-substituted hydroxamate functional groups. In an alternative aspect, a commercially available hydroxamate resin can be used in the methods disclosed herein. In a further aspect, the silica-based media of the Accell resin allows for a chemically robust resin backbone, and, without wishing to be bound by theory, the alkyl substituent on the hydroxamate functional group lowers the $pK_a$ of the modified resin, compared to the protonated/unsubstituted form which can allow for stronger metal binding.

**[0030]** In an aspect, R' represents the alkyl substituent on the hydroxamate functional group. In a further aspect, alkyl groups can branched or unbranched, can be saturated, and can have from 1-12 carbon atoms in their longest chains, or can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 carbon atoms. Further in this aspect, non-limiting examples of suitable straight-chained, saturated alkyl groups include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl groups and dodecyl. In a preferred embodiment, the straight chain, saturated alkyl group can be a methyl group.

**[0031]** In another aspect, non-limiting examples of suitable branched, saturated alkyl groups include isopropyl, isobutyl, sec-butyl, t-butyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl (isopentyl), 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl (neopentyl), 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl groups, and 2-methyl-5-ethyldecyl. In a preferred embodiment, the branched, saturated alkyl groups include isopropyl and/or t-butyl.

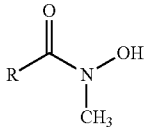
**[0032]** In one aspect, the disclosed alkyl-substituted hydroxamate resin can be synthesized in two steps using commercially available reagents under mild conditions (Scheme 1). Further in this aspect, R is a resin backbone that can be a polymer-coated silica based media. In a still further aspect, RCOOH can be preferably a Waters™ Accell Plus CM resin. In one aspect, R' is an alkyl group that can be branched or unbranched, can be saturated, and can have from 1-12 carbon atoms in its longest chain.

Scheme 1. Synthesis of alkyl-substituted hrdroxamate resin




**[0033]** In a preferred embodiment, R' is methyl. In one aspect, a methyl-substituted hydroxamate resin can have Formula II, shown below:

Formula II




**[0034]** In one aspect, a methyl-substituted hydroxamate resin is synthesized as follows. In an aspect, Accell Plus CM resin (1.0 g) is suspended in water (8.0 mL) in a Falcon tube and a solution of 2,3,5,6-tetrafluorophenol (TFP) in acetonitrile (1.0 mL, 1.2 M) and 1-ethyl-3-(3-dimethylaminopro-

pyl) carbodiimide hydrochloride (EDAC) (0.385 g) are added. In a further aspect, this is allowed to mix by inversion at room temperature for 1 hour after which an additional molar equivalent of TFP solution (1.0 mL, 1.2 M) and EDAC (0.385 g) are added to the Falcon tube. In one aspect, the solution is then mixed by inversion at room temperature for 2 hours. In a further aspect, the resin can then be isolated by vacuum filtration, washed with water and acetonitrile, and dried by continuous suction. In another aspect, the ester resin can then be converted to the N-methyl-substituted hydroxamate resin by reacting with N-methylhydroxylamine hydrochloride (0.837 g) in a methanolic 1 M NaOH solution at room temperature for 18 hours and mixing by inversion. In a still further aspect, the final resin can be isolated by vacuum filtration, washed with water and acetonitrile, and dried by continuous suction. In any of these aspects, the resins can be characterized using ATR-IR spectroscopy.

**[0035]** In any of these aspects, the resin can preferentially bind a parent isotope over a daughter isotope. In an aspect, the resin can have a distribution coefficient of greater than or equal to 5000 for the parent isotope and a distribution coefficient of less than or equal to 5 for the daughter isotope. In one aspect, the parent isotope can be $^{44}$Ti and the daughter isotope can be $^{44}$Sc. In an alternative aspect, the parent isotope can be $^{172}$Hf and the daughter isotope can be $^{172}$Lu.

**[0036]** Also disclosed are radionuclide generator systems having an elution bed, wherein the elution bed contains an alkyl-substituted hydroxamate resin as disclosed herein. In a further aspect, the radionuclide generator systems preferentially retain a parent isotope in contact with the alkyl-substituted hydroxamate resin while allowing a daughter isotope to be eluted. In some aspects, the elution bed can have a bed volume of from about 0.3 mL to about 2 mL, or of about 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or about 2 mL, or a combination of any of the foregoing values, or a range encompassing any of the foregoing values. In another aspect, the radionuclide generator system can be loaded with from about 20 μCi to about 10 mCi of the parent isotope, or about 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 850, 900, or 950 μCi, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mCi of the parent isotope, or a combination of any of the foregoing values, or a range encompassing any of the foregoing values. In an aspect, the parent isotope can decay while in contact with the alkyl-substituted hydroxamate resin, releasing the daughter isotope.

**[0037]** Also disclosed herein is a method for producing $^{44}$Sc, the method including contacting $^{44}$Ti with the disclosed alkyl-substituted hydroxamate resin, allowing at least a portion of the $^{44}$Ti to decay to $^{44}$Sc, and eluting the $^{44}$Sc. In an aspect, the $^{44}$Sc can be eluted with a dilute aqueous solution. In one aspect, the dilute solution can be an acid such as, for example, HCl, or can be saline, or any combination thereof. In another aspect, the HCl can have a concentration of from about 0.01 M to about 10.8 M, or from about 0.5 M to about 8 M, or of about 0.01, 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, or about 10.8 M, or a combination of any of the foregoing values, or a range encompassing any of the foregoing values. In one aspect, the $^{44}$Sc can be eluted with at least one bed volume of the acid, or can be eluted with two, three, four, or more bed volumes of the acid. In still another aspect, the elution step can be repeated at least once before the resin releases any $^{44}$Ti. In some aspects, depending on bed volume, $^{44}$Ti purity, and other factors, step (c) can be conducted at least 75 times before the resin releases any $^{44}$Ti.

**[0038]** In one aspect, the methyl-substituted hydroxamate resin can be tested in comparison to the commercially available ZR resin in side-by-side generator evaluations. In a further aspect, the robustness of the generator can be investigated by monitoring: the time it takes until $^{44}$Ti breakthrough, and the yield of $^{44}$Sc. Further in this aspect, generators were loaded with a 20 μCi $^{44}$Ti sample onto a 300 μL bed volume column in 2 M HCl and eluted daily with four bed volumes of 0.5 M HCl. In one aspect, each eluted fraction was monitored for $^{44}$Ti breakthrough and the total amount of eluted $^{44}$Sc activity was calculated using high purity germanium (HPGe) γ spectroscopy. In another aspect, once $^{44}$Ti breakthrough was observed the generator was discarded, the elution profile for the $^{44}$Sc comparison is shown below (FIG. **1**). In a still further aspect, during the 11th elution the ZR generator displayed $^{44}$Ti breakthrough, as observed through the detection of the 67.9 and 78.3 keV gamma peaks with an instrumentation error of <10%. The disclosed methyl hydroxamate resin (AM) generator was eluted for a total of 75 elutions before any $^{44}$Ti breakthrough was observed.

**[0039]** In an aspect, the lifetime of the generator using the methyl hydroxamate resin is almost seven times longer than the ZR resin generator, and overall showed increased $^{44}$Sc activity elution under the same conditions. In a further aspect, initial optimization studies also show drastically increased $^{44}$Sc activity elution by varying the concentration of the HCl eluent. In one aspect, on a 300 μL bed volume generator using the disclosed AM resin loaded with 100 μCi $^{44}$Ti, only 9% $^{44}$Sc was eluted in 4 bed volumes of 0.5 M HCl, but 86% $^{44}$Sc was eluted in the same volume using 2 M HCl with no observable $^{44}$Ti breakthrough.

**[0040]** Many modifications and other embodiments disclosed herein will come to mind to one skilled in the art to which the disclosed compositions and methods pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the disclosures are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. The skilled artisan will recognize many variants and adaptations of the aspects described herein. These variants and adaptations are intended to be included in the teachings of this disclosure and to be encompassed by the claims herein.

**[0041]** Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

**[0042]** As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure.

**[0043]** Any recited method can be carried out in the order of events recited or in any other order that is logically possible. That is, unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a

specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

**[0044]** All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

**[0045]** While aspects of the present disclosure can be described and claimed in a particular statutory class, such as the system statutory class, this is for convenience only and one of skill in the art will understand that each aspect of the present disclosure can be described and claimed in any statutory class.

**[0046]** It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosed compositions and methods belong. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly defined herein.

**[0047]** Prior to describing the various aspects of the present disclosure, the following definitions are provided and should be used unless otherwise indicated. Additional terms may be defined elsewhere in the present disclosure.

## Definitions

**[0048]** As used herein, "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps, or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps, or components, or groups thereof. Moreover, each of the terms "by", "comprising," "comprises", "comprised of," "including," "includes," "included," "involving," "involves," "involved," and "such as" are used in their open, non-limiting sense and may be used interchangeably. Further, the term "comprising" is intended to include examples and aspects encompassed by the terms "consisting essentially of" and "consisting of." Similarly, the term "consisting essentially of" is intended to include examples encompassed by the term "consisting of.

**[0049]** As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a radionuclide," "a resin," or "an eluent," includes, but is not limited to, mixtures or combinations of two or more such radionuclides, resins, or eluents, and the like.

**[0050]** It should be noted that ratios, concentrations, amounts, and other numerical data can be expressed herein in a range format. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms a further aspect. For example, if the value "about 10" is disclosed, then "10" is also disclosed.

**[0051]** When a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. For example, where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure, e.g. the phrase "x to y" includes the range from 'x' to 'y' as well as the range greater than 'x' and less than 'y'. The range can also be expressed as an upper limit, e.g. 'about x, y, z, or less' and should be interpreted to include the specific ranges of 'about x', 'about y', and 'about z' as well as the ranges of 'less than x', less than y', and 'less than z'. Likewise, the phrase 'about x, y, z, or greater' should be interpreted to include the specific ranges of 'about x', 'about y', and 'about z' as well as the ranges of 'greater than x', greater than y', and 'greater than z'. In addition, the phrase "about 'x' to 'y'", where 'x' and 'y' are numerical values, includes "about 'x' to about 'y'".

**[0052]** It is to be understood that such a range format is used for convenience and brevity, and thus, should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a numerical range of "about 0.1% to 5%" should be interpreted to include not only the explicitly recited values of about 0.1% to about 5%, but also include individual values (e.g., about 1%, about 2%, about 3%, and about 4%) and the sub-ranges (e.g., about 0.5% to about 1.1%; about 5% to about 2.4%; about 0.5% to about 3.2%, and about 0.5% to about 4.4%, and other possible sub-ranges) within the indicated range.

**[0053]** As used herein, the terms "about," "approximate," "at or about," and "substantially" mean that the amount or value in question can be the exact value or a value that provides equivalent results or effects as recited in the claims or taught herein. That is, it is understood that amounts, sizes, formulations, parameters, and other quantities and characteristics are not and need not be exact, but may be approximate and/or larger or smaller, as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art such that equivalent results or effects are obtained. In some circumstances, the value that provides equivalent results or effects cannot be reasonably determined. In such

cases, it is generally understood, as used herein, that "about" and "at or about" mean the nominal value indicated ±10% variation unless otherwise indicated or inferred. In general, an amount, size, formulation, parameter or other quantity or characteristic is "about," "approximate," or "at or about" whether or not expressly stated to be such. It is understood that where "about," "approximate," or "at or about" is used before a quantitative value, the parameter also includes the specific quantitative value itself, unless specifically stated otherwise.

**[0054]** As used herein, the term "effective amount" refers to an amount that is sufficient to achieve the desired modification of a physical property of the composition or material. For example, an "effective amount" of a resin refers to an amount that is sufficient to achieve the desired improvement in the property modulated by the formulation component, e.g. achieving the desired level of separation of $^{44}$Ti from $^{44}$Sc over the desired time period. The specific level in terms of wt % in a resin required as an effective amount will depend upon a variety of factors including the amount of resin, chemical identity of the resin including any substituents, initial $^{44}$Ti to $^{44}$Sc ratio, and eluent identity and concentration.

**[0055]** As used herein, the terms "optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

**[0056]** "Cold" as used herein refers to a reaction or molecule that is not radioactive. In an aspect, reactions and processes can be optimized using cold reagents and materials prior to performing the same reactions and processing with radioactive reagents and/or materials.

**[0057]** Unless otherwise specified, temperatures referred to herein are based on atmospheric pressure (i.e. one atmosphere).

**[0058]** Now having described the aspects of the present disclosure, in general, the following Examples describe some additional aspects of the present disclosure. While aspects of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit aspects of the present disclosure to this description. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of the present disclosure.

## EXAMPLES

**[0059]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the disclosure and are not intended to limit the scope of what the inventors regard as their disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in ° C. or is at ambient temperature, and pressure is at or near atmospheric.

### Example 1: Materials and Methods

#### Chemicals and Reagents

**[0060]** All chemicals were used without further purification. Hydroxylamine hydrochloride, N-methylhydroxylamine hydrochloride, N-phenylhydroxylamine, 2,3,5,6-tetrafluorophenol (TFP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), potassium hydroxide, $SOCl_2$, anhydrous diethylether, acetonitrile (ACN), and HPLC grade methanol (MeOH) were purchased from Sigma Aldrich. Optima grade hydrochloric acid (HCl) was purchased from Fisher Scientific (Pittsburgh, PA, USA) and diluted to suitable concentrations (6 M, 4 M, 2 M, 0.5 M, 0.1 M) with 18 MΩ water (25° C., Milli-Q, Millipore, Burlington, MA, USA). ZR resin was purchased from Triskem Intl. (Brunz, France), Amberlite IRC50 resin and Amberlite CG50 resin were purchased from Sigma Aldrich and Accell Plus CM resin was purchased from Waters (Milford, MA, USA). Deionized Milli-Q water (18 MΩ, Millipore) which had been purified by passing through a 10 cm column of Chelex 100 resin (Bio-Rad Laboratories, Hercules, CA, USA) was used in all reactions and solution preparations.

#### Measurement of Radioactivity

**[0061]** The absolute radioactivity of $^{44}$Sc and $^{44}$Ti was measured by γ-spectrometry using a high-purity germanium (HPGe) detector, samples were counted for 10 minutes. $^{44}$Ti was detected directly via the 67.87 keV (93.0%) and 78.32 keV (96.4%) γ-lines and $^{44}$Sc was detected via the 1157.02 keV (99.9%) γ-line.

#### Synthesis of Resins

**[0062]** Accell Resins: Ester Resin. Accell Plus CM resin (1.0 g) was suspended in Chelex water (8.0 mL) in a 15 mL Falcon tube. TFP solution (1.0 mL, 1.2 M in ACN, 1.20 mmol) and EDAC (0.385 g, 2.48 mmol) were added to the Falcon tube. The reaction was mixed by inversion at room temperature for 1 hour, after which an additional 1.0 mL of TFP solution (1.2 M in ACN, 1.20 mmol) and EDAC (0.385 g, 2.48 mmol) were added to the reaction to ensure complete conversion of the carboxylate groups to the ester resin. The reaction was mixed by inversion at room temperature for a further 2 hours after which the final resin was isolated by vacuum filtration and washed with 3×15 mL water and 3×15 mL ACN and dried by continuous suction. This resin can be stored dry under ambient conditions without any apparent degradation or hydrolysis. IR (ATR, selected bands, $\nu_{max}$): 3384, 1781, 1670, 1065, 956, 794, 452 $cm^{-1}$.

**[0063]** Accell Resins: UH Resin (AU). Hydroxylamine hydrochloride (0.695 g, 10.0 mmol) was dissolved in 1 M NaOH (1.0 mL) and MeOH (2.0 mL) to form the free base hydroxylamine. The ester functionalized resin (1.00 g) was added to this solution in a 15 mL Falcon tube and was mixed by inversion at room temperature for 18 hours. The hydroxamate resin was then isolated by vacuum filtration and washed with 3×15 mL water and 3×15 mL ACN. This resin can be stored dry under ambient conditions without any apparent degradation or hydrolysis. IR (ATR, selected bands, $\nu_{max}$): 3347, 1731, 1660, 1451, 1062, 795, 451 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0064]** Accell Resins: MH Resin (AM). The N-methylhydroxamate Accell resin can be synthesized by substituting N-methylhydroxylamine hydrochloride (0.837 g, 10.0 mmol) for the hydroxylamine hydrochloride in the above procedure. IR (ATR, selected bands, $v_{max}$): 3361, 1733, 1654, 1449, 1062, 795, 452 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0065]** Accell Resins: PH Resin (AP). N-phenylhydroxylamine (1.09 g, 10.0 mmol) was dissolved in MeOH (3.00 mL) and the ester functionalized resin (1.00 g) was added to this solution in a 15 mL Falcon tube. The reaction was mixed by inversion at room temperature for 18 hours and isolated and washed in accordance with the above procedure. IR (ATR, selected bands, $v_{max}$): 3355, 1724, 1662, 1451, 1061, 795, 452 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0066]** IRC50 Resins: Anhydride Resin. IRC50 resin (2.00 g) was suspended in $SOCl_2$ (6.00 mL) in a 25 mL round bottom flask fitted with a condenser, and the solution was refluxed for 4 hours. The resin was isolated by vacuum filtration and washed excessively with anhydrous diethyl ether to remove all unreacted $SOCl_2$ and dried by continuous suction. IR (ATR, selected bands, $v_{max}$): 1800, 1745, 1456, 1390, 1258, 1111, 1007, 960, 853, 738, 713 $cm^{-1}$.

**[0067]** IRC50 Resins: UH Resin. Hydroxylamine hydrochloride (0.500 g, 7.20 mmol) was dissolved in MeOH (5.0 mL) and added to a solution of KOH (0.404 g, 7.20 mmol) in MeOH (5.0 mL). The KCl salt precipitated from solution immediately, but the solution was stored in the fridge for an hour to ensure complete precipitation of the salt. The salt was removed by filtration and the MeOH solution added to the anhydride resin (1.00 g) in a 15 mL Falcon tube. The reaction was mixed by inversion at room temperature for 1 hour, after which 3 M KOH (2.4 mL in MeOH, 7.20 mmol) was added to the Falcon tube. The reaction was mixed by inversion at room temperature for 5 minutes after which the resin was isolated by vacuum filtration and washed with MeOH. The potassium salt resin is then suspended in 0.1 M HCl (5.0 mL) and mixed by inversion at room temperature for a few minutes to protonate. The final resin was isolated by vacuum filtration and washed with water and MeOH and dried by continuous suction. This resin undergoes slow hydrolysis under ambient conditions over several days. IR (ATR, selected bands, $v_{max}$): 3300, 1724, 1670, 1547, 1477, 1447, 1387, 1346, 1200, 1015, 969 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0068]** IRC50 Resins: MH Resin. The N-methylhydroxamate IRC50 resin can be synthesized by substituting N-methylhydroxylamine hydrochloride (0.601 g, 7.20 mmol) for the hydroxylamine hydrochloride in the above procedure. This resin undergoes slow hydrolysis under ambient conditions over several days. IR (ATR, selected bands, $v_{max}$): 3366, 1719, 1642, 1548, 1473, 1445, 1408, 1384, 1350, 1238, 1167, 1131, 1017, 966 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0069]** IRC50 Resins: PH Resin. N-phenylhydroxylamine (0.786 g, 7.20 mmol) was dissolved in MeOH (6.0 mL) and added to the anhydride resin (1.00 g) in a 15 mL Falcon tube. The reaction was mixed by inversion at room temperature for 1 hour after which 3 M KOH (2.4 mL in MeOH, 7.20 mmol) was added to the reaction and mixed by inversion for 5 minutes at room temperature. The phenylhydroxamate resin was then isolated and washed in accordance with the above procedure. This resin undergoes slow hydrolysis under ambient conditions over several days. IR (ATR, selected bands, $v_{max}$): 3361, 1692, 1652, 1540, 1473, 1446, 1403, 1343, 1256, 1181, 1019 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0070]** CG50 Resins: Anhydride Resin. CG50 resin (2.00 g) was suspended in $SOCl_2$ (6.00 mL) in a 25 mL round bottom flask fitted with a condenser, and the solution was refluxed for 4 hours. The IR of the resulting resin showed incomplete conversion, so the resin was refluxed for a further 4 hr in fresh $SOCl_2$ (6.0 mL). The resin was isolated by vacuum filtration and washed excessively with anhydrous diethyl ether to remove all unreacted $SOCl_2$ and dried by continuous suction. IR (ATR, selected bands, $v_{max}$): 1800, 1754, 1455, 1389, 1255, 1112, 1007, 960. 845, 737, 603 $cm^{-1}$.

**[0071]** CG50 Resins: UH Resin. The unsubstituted hydroxamate CG50 resin can be synthesized by substituting the CG50 anhydride resin (1.00 g) for the IRC50 anhydride resin in the above IRC50 UH resin procedure. IR (ATR, selected bands, $v_{max}$): 3294, 1722, 1674, 1547, 1475, 1445, 1386, 1342, 1250, 1191, 1007, 965 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0072]** CG50 Resins: MH Resin. The N-methylhydroxamate CG50 resin can be synthesized by substituting the CG50 anhydride resin (1.00 g) for the IRC50 anhydride resin in the above IRC50 MH resin procedure. IR (ATR, selected bands, $v_{max}$): 3382, 1720, 1649, 1548, 1472, 1444, 1406, 1386, 1344, 1245, 1166, 1130, 1019, 966 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0073]** CG50 Resins: PH Resin. The N-phenylhydroxamate CG50 resin can be synthesized by substituting the CG50 anhydride resin (1.00 g) for the IRC50 anhydride resin in the above IRC50 PH resin procedure. IR (ATR, selected bands, $v_{max}$): 3366, 1698, 1647, 1485, 1446, 1387, 1340, 1253, 1172, 1018, 965 $cm^{-1}$. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

IR Measurement

**[0074]** Attenuated total reflectance infrared (ATR-IR) spectroscopy was performed by using a Thermo Scientific Nicolet iS50 FTIR Spectrometer. The ATR-IR data were collected on dry solid samples and processed by using OMNIC version 9.3.32 software.

Preparation and Evaluation of $^{44}Ti/^{44}Sc$ Generators

**[0075]** Production of $^{44}Ti$ used in the radionuclide generators was conducted by irradiation of a scandium sputtering target (American Elements) at the Brookhaven Linac Isotope Producer (BLIP).

**[0076]** 10 mL tapered Bio-Rad® columns were rinsed with Chelex water and filled to the bed volume (BV) with the corresponding resin that had been weighed and slurried in Chelex water. A small piece of glass wool was added to the top of the resin for protection, and the column washed with Chelex water and 2 M HCl or 0.1 M HCl. A sample of $^{44}$Ti was evaporated to dryness and taken up in either 2 M HCl or 0.1 M HCl and loaded onto the columns by pipette. The solutions were allowed to elute by gravity. The generators were eluted 3 to 5 times per week with 4 BV of dilute HCl and collected and counted by fraction.

Total Organic Content (TOC) Studies

**[0077]** 10 mL tapered Bio-Rad® columns were rinsed with Chelex purified water and filled to a bed volume (BV) of 300 μL with the corresponding resin that had been weighed and slurried in Chelex purified water. Two columns were prepared with the ZR resin and two were prepared with the synthesized AM resin. One of each type of resin column was rinsed with 50 BV of 0.5 M HCl and the other two remaining columns with each type of resin was eluted with 50 BV of 2 M HCl. Samples were collected after 10, 20, 30, 40, and 50 BV of eluent and sent for total organic content (TOC) analysis at Long Island Analytical Laboratories Inc. (Holbrook, New York 11741). A blank of each acid used (0.5 and 2 M HCl) was also sent for analysis for comparison.

Radiolabeling Experiments

**[0078]** Dry Down Method. In order to obtain the $^{44}$Sc in a form suitable for radiolabeling, dry down processing was implemented where the generator eluted $^{44}$Sc was evaporated dry on a hot plate in an open beaker at 140° C. and re-dissolved in 0.25 M $NH_4OAc$ pH 4 buffer. Radiolabeling of generator eluted $^{44}$Sc with DOTA, HOPO and NOTA was performed by mixing the ligand stock solution (0.04 mg/mL, 0.25 M $NH_4OAc$ pH 4 buffer) with the post-processed $^{44}$Sc (i.e., dried and re-dissolved $^{44}$Sc) in a 1.5 mL Eppendorf tube and heating the solution at 90° C. for one hour in a thermomixer. The only parameter varied was the ligand concentration, which was pre-determined as a ligand molar excess over the $^{44}$Sc molar amount (calculated by activity). The activity of $^{44}$Sc used in radiolabeling varied from 25-50 μCi for the AM generator experiments, and 5-10 μCi for the ZR generator experiments due to varying generator and post-processing behavior.

**[0079]** Direct Radiolabeling Method. The $^{44}$Sc generator eluent (in 0.5 M HCl) was obtained and neutralized with dilute NaOH solution and then used directly (e.g., without dry down processing) in subsequent labeling reactions. Radiolabeling of generator eluted $^{44}$Sc with DOTA was performed by mixing the ligand stock solution (0.04 mg/mL, 0.25 M $NH_4OAc$ pH 4 buffer) with the neutralized $^{44}$Sc eluent in a 1.5 mL Eppendorf tube and heating the solution at 90° C. for one hour in a thermomixer. The only parameter varied was the ligand concentration, which was pre-determined as a ligand molar excess over the $^{44}$Sc molar amount (calculated by activity). The activity of $^{44}$Sc used in radiolabeling varied from 25-50 μCi.

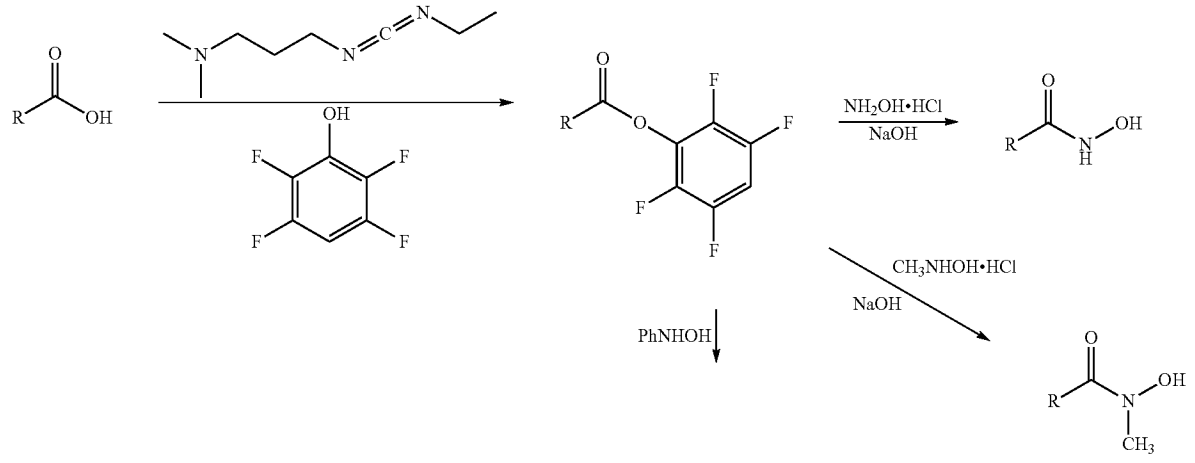
**[0080]** For both methods, the dry down method and the direct radiolabeling method the radiolabeling yield was determined using thin-layer chromatography (iTLC-SG strips, Agilent). The plates were developed in 0.04 M $NH_4OAc$/MeOH (50/50) pH 5 buffer and were counted for 1 minute on a BIOSCAN AR 2000. The $R_f$ values for the free $^{44}$Sc and labeled $^{44}$Sc (with all three ligands) were 0 and 0.9 respectively.

Example 2: Results and Discussion

Resin Synthesis and Characterization

**[0081]** The unsubstituted hydroxamate Accell resin (AU) was prepared following modified literature procedures by functionalizing the carboxylate groups of the Accell resin first to an ester, followed by conversion to the hydroxamate by reaction with hydroxylamine hydrochloride. The N-methylhydroxamate Accell resin (AM) can be synthesized using the same procedure substituting hydroxylamine hydrochloride for N-methylhydroxylamine hydrochloride. The N-phenylhydroxamate Accell resin (AP) can be synthesized from the ester functionalized resin using a similar procedure, but without the use of NaOH, as the N-phenylhydroxylamine is already in the free base form (Scheme 2). Addition of 1.0 mL of NaOH to the N-phenylhydroxylamine resin reaction results in hydrolysis of the ester resin back to the carboxylate form.

Scheme 2: Synthesis of Accell-based hydroxamate resins.



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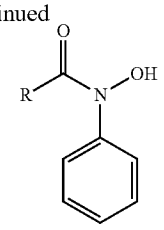
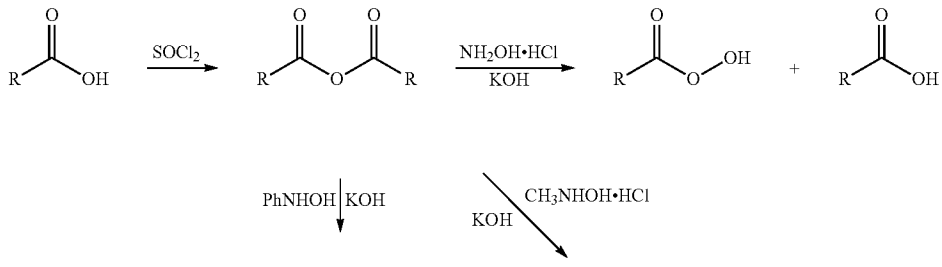


**[0082]** The characterization of the Accell resins by ATR-IR was difficult due to weak signal intensities caused by low ligand density and the overpowering silica peaks of the resin backbone. Because of this only the carbonyl region of the IR spectra was used in characterization. The starting Accell carboxylate resin shows asymmetric ($v_{as}$) and symmetric ($v_s$) C═O stretching frequencies at 1562 $cm^{-1}$ and 1408 $cm^{-1}$ respectively. In the intermediate ester resin this C═O stretching frequency shifts to higher energy at 1781 $cm^{-1}$. All three hydroxamate resins show a diagnostic C═O stretching frequency signal around 1730 $cm^{-1}$ as well as a second peak at around 1450 $cm^{-1}$, there is also evidence for the $v_{as}$ and $v_s$ carboxylate C═O peaks at approximately 1550 $cm^{-1}$ and 1400 $cm^{-1}$ respectively. There is no evidence of the carboxylate peaks in the ester resin, suggesting that there is some hydrolysis of the ester in the hydroxylamine reaction. The peaks seen at approximately 3400, 1650, 1065, 960, 800 and 450 $cm^{-1}$ in all of the Accell resin spectra are assigned as silica peaks, this was confirmed by direct comparison with the IR spectrum of crude silica gel. Formation of the hydroxamate functional group was also verified by the visual formation of a dark red complex upon addition of Fe(III) in dilute acid to the resin.

**[0083]** The procedure for the synthesis of the IRC50 and CG50 hydroxamate resins are the same but differ from the Accell resin synthesis. Attempts to use the same procedure as described above using IRC50 or CG50 resin did not result in formation of the ester functionalized resin in the first step, but rather formation of an anhydride. This was determined by characterization of the intermediate product using ATR-IR, which showed two C═O stretching frequencies at 1800 $cm^{-1}$ and 1750 $cm^{-1}$, this was true for both the IRC50 and CG50 resins. An alternate route to hydroxamate synthesis is conversion of carboxylates into acid chlorides which are then reacted with hydroxylamine, this has been suggested to work in the literature for similar resins. Reaction of IRC50 and CG50 with $SOCl_2$ under reflux resulted in the same anhydride product that was observed in the ester formation attempt (Scheme 3), this was evident by the identical IR spectrum of the product. Due to the high ligand density of the resins and the close contact of the neighboring carboxylate groups, all attempts at formation of the acid chlorides or esters (as in the Accell resin synthesis) always resulted in formation of the anhydride derivative without any apparent formation of the ester or acid chloride in any appreciable amounts.

**[0084]** There is literature evidence that it is possible to convert anhydrides into a 1:1 mix of hydroxamates and carboxylic acids by reaction with the free-based hydroxylamine and base. Based on this the hydroxamate IRC50 and CG50 resins were synthesized using modified literature procedures using the anhydride resin (Scheme 3). The hydroxylamine hydrochloride and N-methylhydroxylamine hydrochloride were free-based by reaction with one equivalent of KOH in MeOH, followed by removal of the KCl salt. This MeOH hydroxylamine solution was then added to the dry anhydride resin and allowed to react for 1 hour at room temperature. An additional molar equivalent of KOH in MeOH was then added briefly to catalyze the reaction to completion, but it was found that prolonged exposure of the hydroxamate resin to base resulted in hydrolysis back to the starting carboxylate resin. This product is presumably the potassium salt of the resin, so to protonate the hydroxamate groups the resin was washed in 0.1 M HCl. Exposure of this resin to ≥1 M HCl solutions resulted in hydrolysis of the hydroxamates back to carboxylates. Since the N-phenylhydroxylamine is already in the free base form this can be dissolved in MeOH and reacted directly with the anhydride resin, although addition of the catalytic KOH after the initial 1-hour reaction seemed to aid in the product formation.

Scheme 3: Synthesis of IRC50 and CG50 based hydroxamate resins



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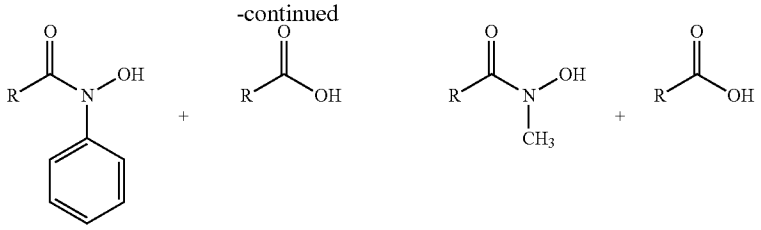
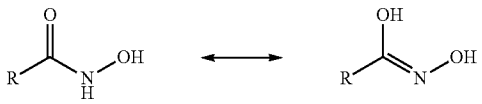


**[0085]** All six of these resins form dark red complexes in dilute acid solution with Fe(III) suggesting successful formation of the hydroxamate group. The IR spectra of resins show peaks due to both the carboxylate groups as well as the hydroxamate groups, with the C═O stretching frequency of the hydroxamate found around 1720 $cm^{-1}$ in all six resins. With the unsubstituted hydroxamate IRC50 and CG50 resins there was also sometimes an IR peak around 1680 $cm^{-1}$ (and no peak at 1720 $cm^{-1}$) that was not seen in the N-methyl or N-phenyl derivatives. This is likely due to the C═N stretching frequency that is present in the resonance form of the unsubstituted hydroxamate (Scheme 4).

Scheme 4: Resonance form of unsubstituted hydroxamate functional group



$K_D$ Studies of AM Resin

**[0086]** Results of the distribution coefficient studies of Sc and Ti on the AM resin in varying concentration of HCl are shown in FIG. **11**. As can be seen clearly in the plot, Ti is strongly retained on the resin across all HCl concentrations with $K_D$ values above 7000 across acid concentrations from 0.5 to 8 M, and a slight decrease to 5000 for the higher concentrations (8 and 10.8 M). Sc alternatively shows little to no retention across all HCl concentrations, all $K_D$ values for Sc are below 2, except for 0.5 M HCl which is slightly increased to a $K_D$ value around 4. These values are an improvement for both retention of Ti and weak affinity of Sc compared to the hydroxamate based ZR resin across a similar range of HCl concentrations. The slight increase of $K_D$ values for Sc at low acid concentrations (0.5 M HCl) suggests that further dilute solutions of HCl (<0.5 M) may not be suitable to obtain appreciable amount of Sc from the resin in small volumes, but the large separation factor between Ti and Sc across all HCl concentrations tested here is extremely promising that this resin will be a good candidate for a $^{44}Ti/^{44}Sc$ radionuclide generator.

Generator Performance

**[0087]** Study 1: Load in 2 M HCl, Elute in 0.5 M HCl. All generators had 300 μL BV and were loaded on the same day with 20 μCi of $^{44}Ti$ as well as three 300 UL BV "blank" generators loaded with 13 μCi of $^{44}Ti$ using the same procedure with unmodified IRC50, CG50 and Accell resins to ensure the carboxylate groups of the parent resin were not contributing to any metal binding. All nine synthesized resins were tested as well as commercially available ZR resin, the mass of resin used in each generator is given in Table 1. All generators were then subsequently eluted with four bed volumes of 0.5 M HCl daily (or semi-daily for the longer lasting generators) to monitor the amount of $^{44}Sc$ eluted as well as any $^{44}Ti$ breakthrough.

TABLE 1

Masses of Resins Used for 300 μL BV Generator in a 10 mL Tapered Column

| | Mass (mg) | | | |
|---|---|---|---|---|
| Resin | Blank | Unsubstituted | Methyl | Phenyl |
| Accell | 115 | 125 | 120 | 110 |
| IRC50 | 110 | 170 | 160 | 150 |
| CG50 | 80 | 100 | 105 | 110 |
| ZR | | 80 | | |

**[0088]** Unsurprisingly all unfunctionalized resins (in the carboxylate form) showed complete $^{44}Ti$ breakthrough during the load and failed to bind appreciable amounts of either metal (Table 2). For the remaining resins, during the 2 M HCl load experiment it was found that all functionalized IRC50 and CG50 resins failed to bind the Ti and displayed complete $^{44}Ti$ breakthrough either during the load or in the subsequent elution (Elute 1). This is likely due to hydrolysis of the hydroxamate groups in the 2 M HCl as described previously. Similar results were found by Holland et al with the unsubstituted Accell based hydroxamate resin, where the loading $^{89}Zr(IV)$ efficiency was found to be >99.9% at HCl concentrations of ≤2 M, but at higher concentrations the binding affinity of the resin decreases drastically. The AU and AM resins, however, displayed 100% $^{44}Ti$ loading efficiency, and the ZR resin showed a small amount of $^{44}Ti$ breakthrough during the load leading to 93.5% $^{44}Ti$ loading efficiency.

TABLE 2

2M HCl Load Generator Experiment $^{44}TI$ Breakthrough

| | % Ti Breakthrough | |
|---|---|---|
| Resin | Load | Elute 1 |
| Accell Blank | 100 | — |
| IRC50 Blank | 97 | — |
| CG50 Blank | 100 | — |
| ZR | 6.5 | — |
| Accell UH (AU) | 0 | 0 |
| Accell MH (AM) | 0 | 0 |
| Accell PH (AP) | 72 | 18 |
| IRC50 UH | 96 | 3 |
| IRC50 MH | 100 | — |
| IRC50 PH | 72 | 26 |
| CG50 UH | 93 | 4 |
| CG50 MH | 100 | — |
| CG50 PH | 100 | — |

**[0089]** The ZR, AU and AM generators were eluted daily with 4 bed volumes of 0.5 M HCl. The resulting $^{44}$Sc activity eluted as well as any potential $^{44}$Ti breakthrough was monitored through HPGe γ spectroscopy. The AU generator displayed the highest eluted $^{44}$Sc activity, almost double that of the ZR and AM generators, but also was the first to display $^{44}$Ti breakthrough. The AU generator was eluted seven times (total 28 BV) over seven days before 0.45% of the load activity $^{44}$Ti breakthrough was observed on the 8th day, due to the $^{44}$Ti breakthrough this generator was no longer eluted. The ZR generator was eluted 10 times (total 40 BV) over 10 days before 0.16% of the load activity $^{44}$Ti breakthrough was observed. The AM generator was eluted 75 times (total 300 BV) over several months before 0.3% of the load activity $^{44}$Ti breakthrough was observed. The elution profile of these three generators is displayed in FIG. **2**. Over the elution period of the AM generator there was a steady increase of $^{44}$Sc activity eluted from 10% to a maximum of 50%. After this slow increase the elution activity seemed to plateau and remain constant around 40-50%. The reason behind this increasing eluted activity trend is still unknown, although it is hypothesized to be due to protonation of open hydroxamate sites over time by the eluting acid allowing for more $^{44}$Sc to be eluted and no captured by open sites. If the reason for the increasing $^{44}$Sc activity was due to the movement of $^{44}$Ti down the column thereby movement of the $^{44}$Sc towards the end of the column then we would expect to observe $^{44}$Ti breakthrough shortly after the maximum $^{44}$Sc activity was reached, which was not observed. Further studies are needed to ascertain the cause of this observed elution trend.

**[0090]** Interestingly, the activity of eluted $^{44}$Sc was initially highest for the AU generator (around 20%), while the ZR and AM generators eluted lower activities in the same volume of 0.5 M HCl (both around 10%). However, over time the elution activity for $^{44}$Sc from the AM generator increased to a maximum of 50% and consistently eluted 45% for the remainder of its lifetime. The reason for this increased activity over time is still unclear, but it may be a result of the protonation of hydroxamate groups in the resin by the dilute HCl over time leading to fewer open coordination sites for the free $^{44}$Sc to bind.

**[0091]** A scaled-up generator experiment was similarly conducted using the AM resin to test the system in a more clinically relevant scale as it showed extremely promising results in the small scale 7 40 kBq (20 μCi) generator study. A 1 mL BV column (0.400 g AM resin) was loaded with 37 MBq (1 mCi) of $^{44}$Ti in 2 M HCl. The generator was eluted daily with 4 BV of 2 M HCl and the fractions counted to monitor the $^{44}$Sc elution activity as well as any potential $^{44}$Ti breakthrough. The elution profile of this generator is shown in FIG. **12**. Remarkably, this generator showed consistent quantitative elution of $^{44}$Sc on a daily basis using the same volume of eluent (normalized to the column BV) as the small-scale study. The drastic increase in eluted activity may be due to the increased activity: resin ratio, by loading a larger amount of $^{44}$Ti onto a smaller mass of resin there may be less coordination sites open to bind the free $^{44}$Sc making it more facile to elute. The lack of $^{44}$Ti breakthrough observed in the loading procedure suggests that the loading capacity of the resin was not saturated, so it may be possible to load even higher activities onto a 1 mL BV of AM resin. Further studies are needed to determine the saturation point of the resin and what affect this has on subsequent $^{44}$Sc elution behavior.

**[0092]** Attempts to further improve this generator towards direct radiolabeling conditions were conducted by eluting in more dilute HCl. A 0.5 mL BV column (0.200 g AM resin) was loaded in 2 M HCl with 37 MBq (1 mCi) of $^{44}$Ti and then eluted daily with 4 BV of 0.5 M HCl. The elution profile of this generator is shown in FIG. **13**. This generator consistently eluted 80-85% $^{44}$Sc in the 4 BV fractions (2 mL), with 90% of this activity being eluted in the first 1 mL allowing for isolation of 28-30 MBq (750-800 μCi) of $^{44}$Sc in 1 mL of 0.5 M HCl. Thus, producing a scandium product that should be highly favorable for use in direct radiolabeling experiments.

**[0093]** From the generator studies that have been conducted to date using the AM resin, there has not been any observation of resin degradation or structural damage from radiation from the $^{44}$Ti or $^{44}$Sc, even in the higher activity 1 mCi generators. There is a risk that with higher activity generators the hydroxamate-based resin may undergo radiolytic damage from the high energy gammas from $^{44}$Sc or the positron emission, but further studies are needed.

**[0094]** Study 2: Load in 0.1 M HCl, Elute in 0.5 M HCl. The blank generators were only tested in the 2 M HCl loading experiment as all three showed 100% $^{44}$Ti breakthrough during the load in the first fraction. The IRC50 resins were also not tested as they had undergone hydrolysis and degraded prior to this study. All generators had 300 μL BV and were loaded on the same day with 20 μCi of $^{44}$Ti and the same mass of resin was used as in study 1 (Table 1). All generators were then subsequently eluted with four bed volumes of 0.5 M HCl daily (or semi-daily for the longer lasting generators) to monitor the amount of $^{44}$Sc eluted as well as any $^{44}$Ti breakthrough.

**[0095]** Similar results were found in the 0.1 M HCl loading experiments, with the ZR, AU and AM resins giving the longest lifetimes without $^{44}$Ti breakthrough. The IRC50 resins were not tested in the 0.1 M HCl loading experiments as all three of the functionalized resins had degraded in air overtime (few weeks). The slow hydrolysis of the IRC50 resins was evident by the changing IR spectra as well as failed Fe(III) binding tests. The lower molarity HCl loading solution did slow down hydrolysis of the resins evident by the lower $^{44}$Ti breakthrough (Table 3), but all three CG50 resins as well as the Accell phenylhydroxamate resin (AP) showed consistent breakthrough over the load and two subsequent elutions, so they were abandoned and no longer eluted daily.

TABLE 3

0.1M HCl Load Generator Experiment $^{44}$Ti Breakthrough

| | % Ti Breakthrough | | |
|---|---|---|---|
| Resin | Load | Elute 1 | Elute 2 |
| ZR | 3 | 2 | 0 |
| Accell UH (AU) | 0 | 0 | 0 |
| Accell MH (AM) | 0 | 0 | 0 |
| Accell PH (AP) | 20 | 10 | 6 |
| CG50 UH | 42 | 7 | 4 |
| CG50 MH | 64 | 19 | 2 |
| CG50 PH | 69 | 15 | 3 |

**[0096]** Similar to study 1, the AU resin was the first to display $^{44}$Ti breakthrough after 5 elutions (total 20 BV) and the ZR resin after 13 elutions (total 52 BV) (FIG. **3**). The AM generator was eluted for a total of 80 times (320 BV) before 0.2% of the load activity $^{44}$Ti breakthrough was observed. Again, the AU generator initially eluted the highest activity of $^{44}$Sc at 25%, while the ZR and AM eluted lower amounts at 10% and 5% respectively. Similar to what was seen in the 2 M HCl load test, the amount of $^{44}$Sc eluted from the AM generator increased over time to about 35% and consistently eluted 30-35% for the remainder of its lifetime. It is worth noting that overall, the elution activities in this experiment have been lower for the AM generator compared to the 2 M HCl experiment, suggesting that loading in the higher molarity acid is preferred. This and the same pattern of increasing activity eluted over time may again be linked to the degree of protonation of the resin hydroxamate groups. Further studies are needed to ascertain the association between acid concentration/pH and resin performance.

**[0097]** Study 3: Load in 2 M HCl, Elute in 2 M HCl. Due to the strong performance of the AM resin compared to all other synthesized resins, only the AM resin was used in further studies in direct comparison to commercially available ZR resin. The low elution activity of $^{44}$Sc in the two previous studies prompted the use of higher molarity HCl to elute the generators in attempts to increase the amount of $^{44}$Sc being eluted in the same volume of eluent. Both the ZR and AM generators had 300 μL BV and were loaded on the same day with 20 μCi of $^{44}$Ti and used the same mass of resin that was used in the previous studies. Both generators were loaded in 2 M HCl and were subsequently eluted using 4 bed volumes of 2 M HCl daily to monitor the amount of $^{44}$Sc eluted as well as any $^{44}$Ti breakthrough. The elution profile of both generators, along with an AU (V$LG) generator, is shown in FIG. **4**.

**[0098]** It is immediately evident that the use of higher concentrated acid did indeed increase the amount of $^{44}$Sc being eluted with values of around 70% for the AM generator compared to 45% (study 1) and 30% (study 2). It is also evident that the lifetime of both the ZR and AM generators was decreased with the use of 2 M HCl, this was more drastic for the AM generator that displayed $^{44}$Ti breakthrough (0.45%) during the 20th elution (after 76 BV). The lifetime of the AM generator under these conditions was still more than twice that of the ZR generator, but it appears that the use of more concentrated HCl as eluent may be less desired as it possibly expedites the $^{44}$Ti breakthrough. Further studies are needed varying the concentration of HCl eluent to better ascertain the behavior of the AM resin under varying pHs.

**[0099]** Another AM generator was prepared around the same time both loading and eluting in 2 M HCl on a much bigger scale to assess the viability of this resin in a clinically relevant generator system. A 1 mL BV column (0.400 g AM resin) was loaded with 1 mCi of $^{44}$Ti, this generator study was conducted in a hotcell due the high activity. This generator was eluted daily with 4 BV of 2 M HCl and the fractions counted to monitor the $^{44}$Sc elution activity as well as any potential $^{44}$Ti breakthrough. The elution profile of this generator is shown below in FIG. **5**. Remarkably, this generator has shown consistent quantitative elution of $^{44}$Sc on a daily basis using the same volume of eluent as the other studies. Also interestingly, this generator was eluted 27 times (total 108 BV) without any sign of $^{44}$Ti breakthrough which differs from the results found in the smaller 300 μL BV 20 μCi generator eluting and loading in the same conditions. It is possible that the breakthrough observed in the study 3 generator was an anomaly, but more experiments are needed to prove this. The drastic increase in eluted activity may be due to the increased activity: resin ratio, by loading a larger amount of $^{44}$Ti onto a smaller mass of resin there may be less coordination sites open to bind the free $^{44}$Sc making it more facile to elute. The lack of $^{44}$Ti breakthrough observed in the loading procedure suggests that the loading capacity of the resin was not saturated, so it may be possible to load even higher activities onto a 1 mL BV of AM resin. Further loading studies are needed to determine the saturation point of the resin and what affect this has on subsequent $^{44}$Sc elution behavior.

**[0100]** Study 4: Large Scale Load in 2 M, HCl Elute in 0.5 M HCl. The long lifetime of the small-scale (20 μCi, low activity) generators eluted in 0.5 M HCl and the high activity elution of the 1 mCi generator eluted in 2 M HCl led to the study of larger scale (high activity) generators eluting in 0.5 M HCl to see if higher activities of $^{44}$Sc could be eluted in 0.5 M HCl. A 500 UL BV AM resin (0.200 g) generator was loaded with 720 μCi of $^{44}$Ti in 2 M HCl and this generator was eluted daily with 0.5 M HCl fractions. Initially this (720 μCi, high activity) generator was eluted with 4×0.5 mL fractions (total of 2 mL), but the eluted activity decreased drastically over the first 13 elutions as can be seen in FIG. **6**. In an attempt to increase the eluted activity, the volume of the fractions was increased to 1 mL each, so 4×1 mL fractions (4 mL total) were collected from elutions 14-22. There was an immediate increase in eluted activity seen in these elutions, and initially fractions 3 and 4 contained the majority of this activity, but over time this shifted to fractions 1 and 2 containing the majority of the activity as can be seen in FIG. **6**.

**[0101]** Since the eluted activity had increased and 90% of the activity was present in the first two fractions, the fraction volume was decreased back to 0.5 mL for elutions 23-25. The eluted activity immediately decreased as was observed before, so elutions 26-34 were again conducted with 1 mL fractions and the eluted activity again increased. As was observed previously, 90-95% of the eluted activity in these elutions were found in the first 2 fractions (first 2 mL) which suggests that a 2 mL volume should be sufficient to extract the majority of the eluted $^{44}$Sc, but when the generator is eluted in 4×0.5 mL fractions (2 mL) this activity decreases drastically. This trend suggests that the elution activity dependence may not be volume driven but rather kinetically driven, since 2×1 mL fractions elutes 70% more activity than 4×0.5 mL fractions. Both of these methods use the same volume of 0.5 M HCl to elute, but 1 mL fractions are collected more rapidly than 2×0.5 mL fractions. To test this the generator was eluted using 2×1 mL fractions (2 mL total) for elutions 35-47, and the eluted activity remained around 70% as was seen in elutions 26-34 which used 4 mL total volume. $^{44}$Ti breakthrough was observed in elution 48 so this generator was abandoned, but this study did prove that a higher activity: resin ratio does increase the amount of activity eluted from the generator (similar to what was observed in the 1 mCi generator) and that kinetics of the elution may impact the eluted activity more than the volume of eluent used. The small-scale generator study that was loaded and eluted in the same solutions only gave 40-45% $^{44}$Sc eluted in 4 BV of 0.5 M HCl while this generator eluted 70% in 4 BV of 0.5 M HCl.

**[0102]** Another AM resin generator of this type was prepared for ongoing studies, but a smaller 5 cm length×0.5 cm ID column was used to increase the length of the resin bed by using a column with a smaller inner diameter. This should increase the lifetime of the generator by increasing the path length that the $^{44}$Ti must travel before breakthrough is observed. For a 500 μL BV, the length of the resin bed in the 10 mL tapered BioRad columns is 1 cm while in the smaller 5 cm columns this length increases to 2 cm. This increased length should increase the lifetime of this generator before $^{44}$Ti breakthrough is observed, ideally by double. This strategy of increasing the length of the resin bed by use of a column with a small inner diameter has been used in the literature, where an HPLC type 150 mm×2.1 mm ID PEEK column is employed. Future studies using this column will be conducted with the disclosed generator system, which should allow for enhanced optimization and longer lifetimes as the resin bed will be 10× longer than previous generators that used the 10 mL BioRad columns which have a 1 cm ID.

**[0103]** Using the smaller 5 cm column, a 500 UL BV AM resin (0.200 g) generator was prepared loading 1 mCi of $^{44}$Ti in 2 M HCl. This generator was eluted daily with 2×1 mL fractions of 0.5 M HCl and the elution profile is shown in FIG. **7**. In this study the generator has been eluted 7 times, and the eluted $^{44}$Sc activity has been around 80% with no sign of $^{44}$Ti breakthrough. This generator may be eluted additional times until breakthrough is observed.

**[0104]** Since both this 1 mCi AM generator and the 720 μCi AM generator are able to elute high activities of $^{44}$Sc in small volumes of dilute (0.5 M) HCl, the eluent from these generators were used for direct radiolabeling studies. The results of these studies are summarized in the radiolabeling section. Direct radiolabeling was not possible with any of the other generators mentioned in this study since 2 M HCl is too acidic to be used in this sort of labeling reaction, and the smaller scale generators did not elute high enough activities in small volumes to be of use. This is why the dry down method was used prior to radiolabeling studies with $^{44}$Sc eluted from these generators. It is also worth mentioning that none of the ZR generators prepared in this study eluted sufficient activities of $^{44}$Sc in appropriate volumes of dilute HCl, so this resin is not conducive to produce $^{44}$Sc for use in direct radiolabeling reactions.

Eluent Variability Study

**[0105]** The use of 0.5 M and 2 M HCl as generator eluent with AM resin has already been verified, but the use of other eluents such as buffers was also of interest. If the generator can be eluted successfully using a mild buffer there would be no need for post-elution processing prior to radiolabeling of the $^{44}$Sc, which would be the most ideal for use in clinical studies. In order to be adequate for direct radiolabeling, the $^{44}$Sc needs to be eluted in small volumes of slightly acidic (pH 2-7) media without any competitive binding ligands such as oxalates. To test the elution behavior of the AM resin using different eluents, a 300 UL BV 100 μCi $^{44}$Ti generator was loaded in 2 M HCl and eluted using varying solvents. Between each change of eluent, the column was flushed with water to ensure no residual solvent was left. Each solvent was tested only a few times to ascertain the viability of its use to elute large quantities of $^{44}$Sc in small volumes without $^{44}$Ti breakthrough. The generator was eluted with 4 BV of each solvent and the results of the elution are shown in Table 4.

TABLE 4

Elution Behavior of 100 μCi AM Generator with Varying Eluents

| Eluent | % $^{44}$Sc Eluted | % $^{44}$Ti Breakthrough |
|---|---|---|
| 0.25M $NH_4OAc$ pH 4 | 2.1 | 0 |
| 0.25M $NH_4OAc$ pH 4 | 0.6 | 0 |
| 0.1M Na Citrate pH 4 | 31.8 | 0.8 |
| 4M HCl | 59.6 | 0 |
| 4M HCl | 36.8 | 0 |
| 4M HCl | 58.0 | 0 |
| 6M HCl | 67.2 | 0 |
| 6M HCl | 77.1 | 0 |
| 6M HCl | 80.7 | 0 |

**[0106]** The use of sodium citrate buffer as an eluent was only attempted once as there was evident $^{44}$Ti breakthrough observed, and the use of $NH_4OAc$ buffer was only conducted twice as the activity of $^{44}$Sc was negligible. From these results it is evident that the use of a radiolabeling buffer as an eluent is not viable, as the $NH_4OAc$ is not able to efficiently remove the $^{44}$Sc, and while the sodium citrate was successful in eluting modest activities of $^{44}$Sc, the citrate also appears to be a competitive binder for the $^{44}$Ti and leads to breakthrough. Similar to what was seen in the smaller generator studies, by increasing the molarity of HCl eluent the amount of $^{44}$Sc eluted is also increased. This is demonstrated by the gradual increase of eluted activity from 0.5, 2, 4 and 6 M HCl. This strongly suggests that pH is a strong contributing factor in the elution of $^{44}$Sc from the AM resin. While the percentage of $^{44}$Sc eluted in small volumes of 4 and 6 M HCl is high, it is far too acidic to be used in subsequent radiolabeling experiments without diluting or neutralizing (which is difficult at these high molarities), so it is a less ideal option.

Distribution Coefficient ($K_D$) Studies

**[0107]** $K_D$ values were obtained using the batch contact method. 20 mg of AM resin was prepared in 1.5 mL Eppendorf vials. Stock solutions for each metal were prepared by diluting High Purity ICP standards (100 ppm for Sc and 1000 ppm for Ti, both in 2% $HNO_3$) in HCl at various concentrations (0.5, 1, 2, 4, 8 and 10.8 M) to a final concentration of 10 ppm. Initial studies were conducted by first drying down the ICP standards and re-dissolving in the appropriate HCl concentration, but the results did not vary statistically from those obtained by the direct dilution method, so it was determined that the small amount of $HNO_3$ present in the initial metal solutions did not affect or contribute to the resulting distribution coefficients. 1 mL of these standard solutions was then added to the vials containing the resin which were then sealed and mixed by inversion at room temperature for 24 h to allow for equilibration. Following mixing, the tubes were centrifuged and a 500 μl aliquot obtained, diluted in 2% $HNO_3$ and submitted for ICP-OES analysis to determine the concentration of analyte remaining in the aqueous phase. This was compared to the concentration of the initial cold metal standard and the distribution coefficient calculated using the following equation, where C8 and CA are the elemental concentrations of the analyte in solution before and after equilibrium respectively (Equation (1)). Each experiment was done in triplicate at each concentration range.

$$K_D = \frac{(C_B - C_A)/w}{C_A/v} \quad \text{Equation (1)}$$

Total Organic Content

**[0108]** To determine and compare the amount of organic impurities that are potentially being eluted from the extraction chromatographic ZR resin and the covalently bound synthesized AM resin, small columns containing the resin were prepared and eluted with 0.5 M and 2 M HCl. The eluent was collected and sent for total organic content analysis. The results of the analysis arc shown below, the 0.5 M HCl in FIG. **14** and the 2 M HCl in FIG. **15**. In both cases the acid used did not show any organic content in the blank samples and therefore did not contribute to any organic content found in the column eluent samples. For both acid concentrations there is considerably more organic impurities being eluted from the ZR resin column, where there is little to no organic impurities being eluted from the AM resin columns. These results suggest that $^{44}$Sc eluted from an AM resin generator has the potential to be directly radiolabeled without any post-elution processing or purification as there are no organic impurities being eluted from the resin itself that would inhibit $^{44}$Sc radiolabeling.

Specific Activity of Generator Produced $^{44}$Sc

**[0109]** To determine the specific activity of the AM resin generator produced $^{44}$Sc, the eluted fractions from the 1 mCi 2 M HCl eluted generator were collected and analyzed by ICP-OES (Supplementary Materials) to determine the amount of cold Sc in the collected eluent. All four fractions from each elution 1, 5, 10, 15, 20, and 25 were combined, diluted to 8 mL in 2% $HNO_3$ and each analyzed by ICP-OES. The analyzed Sc peaks from elution 20 had an RSD of 43% so no reliable data was obtained, but all remaining elution samples had RSD values below 10% and the resulting Sc concentration and amount are given in Table 5.

TABLE 5

Resulting Scandium ICP-OES Data from 1 mCi 2M HCl Generator Elutions

| Elution | Combined Fractions Volume (mL) | ICP Sample Sc Concentration (ppm) | Combined Fractions Sc Concentration (ppm) | Amount of Sc in Combined Fractions (mg) | % RSD |
|---|---|---|---|---|---|
| 1 | 3.885 | 0.001 | 0.0021 | 0.008 | 1.03 |
| 5 | 3.195 | 0.001 | 0.0025 | 0.008 | 1.09 |
| 10 | 3.374 | 0 | 0[a] | 0[a] | 9.14 |
| 15 | 4.148 | 0 | 0[a] | 0[a] | 8.11 |
| 20 | 3.129 | 0 | — | — | 43.41 |
| 25 | 3.633 | 0.001 | 0.0022 | 0.008 | 2.45 |

[a]Below detection limit.

**[0110]** These results demonstrate that there is very little cold scandium in the eluted fractions from the AM resin generator, suggesting that the eluted $^{44}$Sc is carrier free, or at the very least that the amount of cold scandium in the elutions is below the detection limit of the ICP-OES.

Stripping of $^{44}$Ti from AM Resin

**[0111]** Stripping of the $^{44}$Ti from the AM resin was tested using several different solvents. Ideally the stripping solution should be able to isolate the full amount of $^{44}$Ti in relatively small volumes in a form that is not chemically detrimental to the $^{44}$Ti. Initially, concentrated HCl and $HNO_3$ were tested; four bed volumes of each concentrated acid were passed through study 3 generator (300 μL BV, 20 μCi) in attempts to remove the $^{44}$Ti (Table 6). Remarkably neither acid eluted appreciable amount of $^{44}$Ti. This suggests that once the Ti is bound, the AM resin tightly retains Ti- and is robust and fairly resistant to harsh low pH conditions.

TABLE 6

Stripping Attempts Using 4 BV of Concentrated Acids

| Acid | % $^{44}$Sc Eluted | % $^{44}$Ti Eluted |
|---|---|---|
| HCl | 95.5 | 0.5 |
| $HNO_3$ | 88.0 | 2.6 |

**[0112]** The use of dilute $H_2O_2$ in HCl has been used to efficiently strip $^{44}$Ti from the ZR resin so that was also tested. 2% $H_2O_2$ in 2 M HCl was used to strip the 1 mCi generator, and 99.8% $^{44}$Ti was recovered in 24 BV, although 70% was recovered in the first 8 BV (FIG. **8**). These stripping conditions use mild solvents that do not interfere with the chemical form of the $^{44}$Ti and do not contain any chelating agents that would need to be removed in post-processing experiments. The volume used may be optimized by use of varying amounts of % $H_2O_2$ and varying concentration of HCl, but further studies are needed for this optimization.

Radiolabeling Experiments

**[0113]** To assess the purity of the eluted $^{44}$Sc, radiolabeling experiments were conducted on $^{44}$Sc eluted from both the 1 mCi AM generator eluted in 2 M HCl, as well as a 100 μCi 300 μL BV ZR generator as direct comparison. All of these radiolabeling studies using $^{44}$Sc eluted from these generators were conducted using the dry down method mentioned previously. Both of these generators were eluted using 4 BV of 2 M HCl, and a portion of the eluent was evaporated to dryness at 140° C. on a hotplate, and then re-dissolved in 0.25 M $NH_4OAc$ pH 4 buffer in order to be radiolabeled. Both eluents were processed and radiolabeled in the exact same manner to ensure a direct comparison between the two different generator-produced $^{44}$Sc samples. The radiolabeling experiments were done using three different chelators (DOTA, NOTA and HOPO), but in each case the experiment conditions were the same. The appropriate amount of ligand was added to the $^{44}$Sc solution and heated at 90° C. for one hour in a thermomixer. The radiolabeling yield was then determined using radio TLC conditions as described above. The DOTA and HOPO

reactions were done using a 50,000 ligand molar excess, and the NOTA using a 100,000 ligand molar excess due to the lower thermodynamic stability of Sc-NOTA complexes. Each radiolabeling experiment was conducted in triplicate using different generator eluents (done on different days) and the TLCs were also each done in triplicate to ensure consistent results. The results of these radiolabeling experiments are summarized in Table 7.

TABLE 7

Radiolabeling Yields Determined by RadioTLC

| | AM | ZR |
|---|---|---|
| DOTA | 98.75% ± 0.68 | 38.19% ± 7.74 |
| NOTA | 97.42% ± 0.59 | 10.71% ± 12.19 |
| HOPO | 86.86% ± 7.43 | 38.77% ± 12.61 |

**[0114]** The error associated with the ZR generator experiments is slightly higher due to the lower activity used, and the separation of peaks in the HOPO and NOTA experiments were not as clean as with DOTA leading to slightly higher errors in those experiments. Overall, it is clear that the AM generator produced $^{44}$Sc labeled much better than the ZR $^{44}$Sc under the same conditions, suggesting that it is of higher purity. It is worth noting that the ZR $^{44}$Sc solution after processing is yellow in color, where the AM solution is completely colorless. This color is likely due to an organic-based impurity, possibly from the ZR resin, as the $^{44}$Sc or radiolabeled complexes should be completely colorless. The characterization of this impurity is currently unknown but seems to hinder the radiolabeling of the ZR generator eluted radioisotope.

**[0115]** The $^{44}$Sc eluent from the 720 μCi and 1 mCi generators that were loaded in 2 M HCl and eluted in 0.5 M HCl were used in direct radiolabeling experiments since the eluent was in a form conducive to this method of labeling. The $^{44}$Sc generator eluent in 0.5 M HCl was initially neutralized with equal amounts of 0.5 M NaOH and then added to the appropriate amount of ligand stock solution in 0.25 M $NH_4OAc$ pH 4 buffer. This solution had an overall pH of 4. This solution was then heated at 90° C. for 1 hour in a thermomixer and the radiolabeling yield was then determined using radio TLC conditions as described previously. An initial experiment was conducted with 50,000 molar equivalents of DOTA (similar to what was conducted in the dry down method) and the radioyield was 99.46%+0.05%. This excess was then decreased to 25,000 molar equivalents of DOTA and the resulting radioyield remained high at 99.28%+0.08%. This was then decreased further to 10,000 molar equivalents of DOTA, and the average radioyield from four separate reactions was 85.10%+10.57%. A comparison labeling study was done with 10,000 molar equivalents of DOTA using the dry down method which gave a radioyield of 9.36%+0.16%. The direct radiolabeling method not only allows for more quick and facile labeling studies as post-elution processing is not needed, but it also showed drastically higher yields at lower ligand amounts compared to the dry down method. Further studies are needed to optimize these labeling studies, but the use of AM resin generator produced $^{44}$Sc obtained in 0.5 M HCl shows extremely promising direct radiolabeling results which is currently not possible with $^{44}$Ti/$^{44}$Sc generators in the literature.

**[0116]** One of the biggest drawbacks to currently employed $^{44}$Sc generators is that the eluent needs post-elution purification in order to use the $^{44}$Sc in subsequent radiolabeling reactions. This is due to the presence of competing chelators in the eluent such as the oxalates present in oxalic acid. To overcome this, current methods employ the use of a second column to purify the $^{44}$Sc by removal of the oxalates and isolation of the $^{44}$Sc in a radiolabeling conducive buffer. To avoid post-elution processing of the generator eluent and allow for direct radio labeling of the $^{44}$Sc several criteria need to be met: the eluent must be free of competing chelators, the eluent must not be highly acidic, and the volume: activity ratio must be low in order to keep radiolabeling reaction total volumes reasonable. The generator presented in this work meets all of these criteria and should allow for use of the eluted $^{44}$Sc directly from the generator without the need of time-consuming post-elution processing and loss of activity.

**[0117]** To test this, preliminary radiolabeling studies were conducted on the generator eluent from the 37 MBq (1 mCi) generator eluted with 0.5 M HCl with DOTA under standard radiolabeling conditions. The $^{44}$Sc was obtained in 0.5 M HCl and then brought to a pH of 4 with the addition of 0.5 M NaOH. This solution was then added directly to an Eppendorf vial containing DOTA stock solution in 0.25 M $NH_4OAc$ at a pH of 4, with a resulting pH of 4 for the reaction solution. The solution was heated at 90° C. for 60 min and the radioyield determined by iTLC. This reaction was conducted using 925 kBq (25 μCi) of $^{44}$Sc and 1.59 nmol of DOTA resulting in a 99.24±0.21% yield, which is consistent with other generator produced $^{44}$Sc samples that have been processed using chromatography post-elution. More studies are needed to further optimize and study the radiolabeling efficiencies of this generator produced $^{44}$Sc, but these preliminary results show extremely promising evidence for the ability to directly radiolabel $^{44}$Sc from this generator system.

Example 3: Alternative Radionuclide Systems

**[0118]** In another embodiment, the present generator may be a $^{172}$Hf/$^{172}$Lu generator system comprising the use of an alkyl-substituted hydroxamate resin and a method of producing $^{172}$Lu comprising decay of $^{172}$Hf in a $^{172}$Hf/$^{172}$Lu generator system using an alkyl-substituted hydroxamate resin.

**[0119]** $^{172}$Hf has a half life of 1.87 years and decays by electron capture (EC) to $^{172}$Lu ($t_{1/2}$=6.7 days) which then decays by electron capture to stable $^{172}$Yb (FIG. **9**). This decay scheme matches what is needed for a radionuclide generator system, mainly the succinct decay of a long-lived parent radioisotope ($^{172}$Hf) to a short-lived daughter radioisotope ($^{172}$Lu) which can be used in nuclear medicine. $^{172}$Lu is of interest as an imaging agent in preclinical studies.

**[0120]** In the case that Hf is most stable in the (IV) oxidation state and Lu is only stable in the (III) oxidation state, this is similar to Ti(IV) and Sc(III). Although the ionic radii of Hf and Lu are larger than that of Ti and Sc, they possess similarities in bonding, so the hydroxamate groups of the resin will likely selectively bind Hf over Lu and allow for retention of $^{172}$Hf on the resin matrix and elution of $^{172}$Lu.

**[0121]** Preliminary cold studies were conducted using the methyl hydroxamate resin to ascertain the distribution coefficient ($K_D$) for both Hf and Lu in varying concentrations of

HCl to determine the extent of this generator system (FIG. **9**). As shown in the plot, Hf is retained strongly on the resin across all concentration of the acid, similarly to Ti. Lu, on the other hand, show little to no retention across the HCl concentrations. Based on the similarities in the $K_D$ results of the Ti/Sc and Hf/Lu studies we believe that this resin would also serve as a suitable generator for Hf/Lu.

**[0122]** It should be emphasized that the above-described embodiments of the present disclosure are merely possible examples of implementations set forth for a clear understanding of the principles of the disclosure. Many variations and modifications may be made to the above-described embodiment(s) without departing substantially from the spirit and principles of the disclosure. All such modifications and variations are intended to be included herein within the scope of this disclosure and protected by the following claims.

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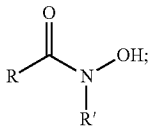
**1**. An alkyl-substituted hydroxamate resin of Formula I:

Formula I



wherein R comprises a resin backbone that is a polymer-coated silica based media and wherein R' comprises an alkyl group, and wherein the alkyl group is branched or unbranched, is saturated, and has from about 1 to about 12 carbon atoms in its longest chain.

**2**. The alkyl-substituted hydroxamate resin of claim **1**, wherein R' is methyl.

**3**. The alkyl-substituted hydroxamate resin of claim **1**, wherein the resin preferentially binds a parent isotope over a daughter isotope.

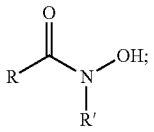
**4**. The alkyl-substituted hydroxamate resin of claim **3**, wherein the resin has a distribution coefficient of greater than or equal to 5000 for the parent isotope and a distribution coefficient of less than or equal to 5 for the daughter isotope.

**5**. The alkyl-substituted hydroxamate resin of claim **3**, wherein the parent isotope comprises $^{44}$Ti and the daughter isotope comprises $^{44}$Sc.

**6**. The alkyl-substituted hydroxamate resin of claim **3**, wherein the parent isotope comprises $^{172}$Hf and the daughter isotope comprises $^{172}$Lu.

**7**. A radionuclide generator system comprising an elution bed, wherein the elution bed comprises an alkyl-substituted hydroxamate resin of Formula I:

Formula I



wherein R comprises a resin backbone that is a polymer-coated silica based media and wherein R' comprises an alkyl group, and wherein the alkyl group is branched or unbranched, is saturated, and has from about 1 to about 12 carbon atoms in its longest chain.

**8**. The radionuclide generator system of claim **7**, wherein R' is methyl.

**9**. The radionuclide generator system of claim **7**, wherein the radionuclide generator system preferentially retains a parent isotope in contact with the alkyl-substituted hydroxamate resin while allowing a daughter isotope to be eluted.

**10**. The radionuclide generator system of claim **9**, wherein the resin has a distribution coefficient of greater than or equal to 5000 for the parent isotope and a distribution coefficient of less than or equal to 5 for the daughter isotope.

**11**. The radionuclide generator system of claim **9**, wherein the parent isotope comprises $^{44}$Ti and the daughter isotope comprises $^{44}$Sc.

**12**. The radionuclide generator system of claim **9**, wherein the parent isotope comprises $^{172}$Hf and the daughter isotope comprises $^{172}$Lu.

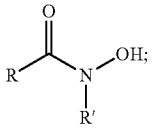
**13**. The radionuclide generator system of claim **7**, wherein the elution bed has a bed volume of from about 0.3 mL to about 2 mL.

**14**. The radionuclide generator system of claim **9**, wherein the system is loaded with from about 20 μCi to about 10 mCi of the parent isotope.

**15**. A method for producing $^{44}$Sc, the method comprising:

(a) contacting $^{44}$Ti with an alkyl-substituted hydroxamate resin of Formula I:

Formula I



wherein R comprises a resin backbone that is a polymer-coated silica based media and wherein R' comprises an alkyl group, and wherein the alkyl group is branched or unbranched, is saturated, and has from about 1 to about 12 carbon atoms in its longest chain;

(b) allowing at least a portion of the $^{44}$Ti to decay to $^{44}$Sc; and

(c) eluting the $^{44}$Sc.

**16**. The method of producing $^{44}$Sc according to claim **15**, wherein R' is methyl.

**17**. The method of claim **15**, wherein the $^{44}$Sc is eluted with a dilute aqueous solution.

**18**. The method of claim **15**, wherein the dilute aqueous solution comprises HCl, saline, or any combination thereof.

**19**. (canceled)

**20**. The method of claim **18**, wherein the $^{44}$Sc is eluted with at least one bed volume of the HCl acid.

**21**. The method of claim **15**, wherein step (c) can be repeated one or more times before the resin releases any $^{44}$Ti.

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