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TITLE:

Separation of Uranium and Thorium for ^{230}Th-U Dating of Submarine Hydrothermal Sulfides

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KEYWORDS:

Extraction, chromatography, uranium and thorium nuclide, ^{230}Th-U dating, submarine hydrothermal sulfides, dating

SUMMARY:

The protocol describes a method to purify and separate the U and Th nuclide in submarine hydrothermal sulfide sample with Fe co-precipitation and extraction chromatography for ^{230}Th-U disequilibrium dating.

ABSTRACT:

The age of a submarine hydrothermal sulfide is a significant index for estimating the size of hydrothermal ore deposits. Uranium and thorium isotopes in the samples can be separated for ^{230}Th-U dating. This article presents a method to purify and separate U and Th isotopes in submarine hydrothermal sulfide samples. Following this technique, the separated U and Th fractions can meet measuring requirements by multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS). The age of the hydrothermal sulfide sample can be calculated by measuring the present-day activity ratios of ^{230}Th/^{238}U and ^{234}U/^{238}U. A super clean room is necessary for this experiment. Cleaned regents and supplies are used to reduce the contamination during the sample processes. Balance, hotplate, and centrifuge are also used. The sulfide sample is powdered for analysis and less than 0.2 g sample is used. Briefly, the sample is

weighed, dissolved, added to ^{229}Th-^{233}U-^{236}U double spike solution, Fe co-precipitated, and separated on an anion-exchange resin extraction column. Approximately 50 ng U is consumed for ^{230}Th-U dating of sulfides sample by MC-ICPMS.

INTRODUCTION:

Submarine hydrothermal sulfides have been a steady source of metals like iron, copper, zinc and lead. They are also seen as economically viable resources of silver and gold. The location and size of the deposits are a record of the history of hydrothermal venting on the seafloor. Dating of a hydrothermal sulfide can provide important information regarding the formation and alteration mechanism of the sulfide ore deposit, seafloor hydrothermal activity history, and growth rate of large sulfide deposits[1-3]. ^{238}U-^{234}U-^{230}Th disequilibrium dating is an effective isotopic method of age estimation for hydrothermal sulfides[4-12], where the purification and separation of U and Th isotopes is necessary. This text describes a protocol for U and Th isotopes separation and ^{230}Th-U dating of sulfides sample by MC-ICPMS.

Geological materials which contain U and Th remain undisturbed for several million years, and a state of secular equilibrium between all the nuclides in the radioactive series is established. However, a combination of chemical solubility and nuclear recoil factors often create disequilibrium, in which the members of the decay series are separated from each other through processes such as deposition, transport and weathering. For example, when a sulfide deposit is formed, the state of ^{238}U, ^{234}U and ^{230}Th is of disequilibrium, and the long-lived ^{238}U can decay gradually towards short-lived ^{234}U and ^{230}Th subsequently. Assuming (i) the system remains closed with respect to U and Th isotopes, and (ii) initial amount of ^{230}Th and ^{232}Th incorporated into sulfide samples is zero, it is possible to determine the time of deposition by measuring the present-day activity ratios of ^{230}Th/^{238}U and ^{234}U/^{238}U. However, the initial amount of Th is not zero in the sample, and we assume the initial ^{230}Th/^{232}Th atomic ratio is $4.4 \pm 2.2 \times 10^{-6}$. The applicable dating range of this method is approximately ~10–6 x 10^5 years[13, 14]. However, the large difference between the abundance of uranium and thorium makes measurement challenging. Hence, it is very important to establish a chemical procedure for U-Th dating by MC-ICPMS.

In the past 30 years, most studies focused more measurements of carbonate materials[14-17] and less on sulfide deposits[11-12, 18-19]. Alpha-particle counting methods have traditionally been used for the study of ^{230}Th/^{238}U disequilibrium of submarine hydrothermal sulfides[1]. However, analytical uncertainty of 5–17% is a limiting factor that affects the precision of age determination of sulfides[1, 8-9]. These techniques generally suffer from the use of relatively large columns and reagent volumes and the need for multiple column passes for purification and separation U-Th from a sample. Recent developments in MC-ICPMS have greatly improved the precision of U-Th isotopic measurements (<5‰ for ages)[14] and have significantly reduced the sample size (<0.2 g) required for analysis. In these works, many chemical separation procedures have been developed, and have achieved excellent chemical yields with low chemical background[12-13].

Here we present a chemical-based protocol to obtain samples that are sufficiently clean for MC-ICPMS analysis. It is suitable for the dating of hydrothermal sulfide samples of age <6 x 10^5 years[14].

With this technique, the separated U and Th isotopic fractions can meet measuring requirements by MC-ICPMS. The age of the hydrothermal sulfide sample can be calculated from the extent of disequilibria between ^{230}Th and ^{234}U and between ^{234}U and ^{238}U by using the described activity decay equation.

PROTOCOL:

1. Preparing the sample, reagents, and containers

1.1. Clean the fume hood, hotplate and the balance room bench for the chemical experiment with sprayed alcohol or ultrapure water.

1.2. Prepare sub-boiled acids (2 M HCl, 8 M HCl, 7 M HNO_3, and 14 M HNO_3), clean beakers and any apparatus before sample processed.

NOTE: Sulfide samples presented in this study were collected from newly discovered hydrothermal zones in the South Atlantic. Approximately 60 mg of powdered sample was used in this process. Sample was collected into glass vials and put in the sample storage cabinet.

2. Weigh the samples

2.1. Prepare cleaned 30 mL perfluoroalkoxy (PFA) beakers. Label twice outside the beaker (to prevent erasure).

2.2. Weigh the blank beakers.

NOTE: The balance used is accurate to ±0.0001 g provided that all the vessels have had their static electricity completely removed.

2.3. Read the weight and record it.

2.4. Pour the sample into the beaker. Cover with a lid and weigh the samples.

NOTE: Sample weight depends on the ^{230}Th content. ^{230}Th level varies with the U concentration and age of the sample. In general, a total of 100 ng of total U is sufficient for the sample.

2.6 Add some (~1 mL) ultrapure water using a bottle, rinse the inner wall and shake the beaker carefully.

NOTE: Add enough ultrapure water cover all the samples.

3. Dissolve and spike the sample

3.1. Place the sample-containing beaker in the fume hood.

3.2. Open the beaker lid. Add 3 mL of HNO_3 (14 M) or aqua regia into the sample using a pipette.

3.3. Place the beaker on the hotplate, set the hotplate temperature to 170 °C and dissolve the sample completely.

NOTE: If there are still insoluble substances in the solution, add 12 M HCl, 22.6 M HF and 10.6 M $HClO_4$, and use a pressurized closed tank to ensure complete dissolution of samples.

3.4. Leave the solution to cool for at least 30 min. Add 0.1–0.3 g ^{229}Th-^{233}U-^{236}U spike solution of known activity into the solution.

NOTE: Generally, the optimal ratio of $^{235}U/^{233}U$ is ~10–20:1 in the mixed solution.

3.5. Place the solution onto the hotplate, set the temperature to 170 °C and leave it on the hotplate until it dries.

NOTE: Evaporation must be done slowly when the sample approaches dryness.

3.6. Dissolve the sample in 2 drops of HNO_3 (0.04 mL, 14 M), and dry it on the hotplate at 170 °C again.

4. Ferric hydroxide co-precipitation for U-Th

4.1. Prepare cleaned 15 mL centrifuge tubes, label and place them in the tube stand.

NOTE: Add approximately 10 mg of Fe(III) ($FeCl_3$ in 12 M HCl) into the centrifugal tube carefully if samples contain almost no Fe.

4.2. Add several drops (0.1 mL) of 2 M HCl into the beaker. Shake the beaker gently and dissolve the sample completely.

4.3. Transfer each sample into a centrifuge tube.

4.4. Add several drops of ammonia (~0.1 mL) until the acid is neutralized; when pH is 7–8, a reddish-brown precipitate appears. U and Th isotopes are precipitated by the $Fe(OH)_3$.

NOTE: The clear solution contains unwanted ions such as metal-elements, Mg^{2+}, NO_3^- and NH_4OH.

4.5. Cap the centrifuge tubes. Centrifuge at 2340 x *g* for 7 min. Discard the supernatant

4.6. Add some ultrapure water to wash the precipitate. Centrifuge as above and repeat this step twice more.

4.7. Dissolve the precipitate with 1.5 mL of 7 M HNO_3. Transfer it into the corresponding beaker.

4.8. Add 1 drop of $HClO_4$ (to remove organic matter), and dry it on the hotplate at 170 °C for about 30 min.

5. Preparation of anion exchange column

5.1. Prepare small polytetrafluoroethylene (PTFE) columns (~2.5 mL column size) as shown in **Figure 1**; insert the frit into each column slowly at the bottom on the bench.

5.2. Pipet cleaned anion-exchange resins into the columns. Put the columns on the holder.

5.3. Fill the whole column with ultrapure water. Add 1 drop of 14 M HNO_3.

NOTE: This step is performed in order mainly to remove the trace elements in the column.

5.4. Add 2 column volumes (CV) of 7 M HNO_3 to remove the trace elements. Then repeat this step.

[Place Figure 1 here]

6. Purification and separation of U and Th fractions

6.1. Dissolve the sample in 0.5 mL of 7 M HNO_3. Load it onto the column carefully.

6.2. Let it drip across the column into the waste beaker.

6.3. Add 2 CV and 1 CV of 7 M HNO_3 successively into the column. Iron and other metal-elements in the sample are removed while U and Th are retained by the resin in this step.

6.4. Add 2 CV and 1 CV of 8 M HCl into the column successively to elute thorium fraction. Collect the thorium fraction using a 7 mL capacity cleaned PFA beaker. Add 1 drop of $HClO_4$ into the beaker and dry the fraction on a hotplate at 170 °C for about 30 min.

6.5. Elute uranium fraction from the resin with 2 CV of 0.1 M HNO_3 twice. Collect the eluate in the cleaned PFA beaker. Add 1 drop of $HClO_4$ and dry it on the hotplate at 170 °C for about 30 min.

6.6. Prepare and label 2 mL capacity vials.

6.7. Dissolve each sample in 1 drop HNO_3 and dry it on the hotplate at 170 °C for less than 5 min until 0.5 drop is left. Transfer them along with 0.2 mL of 2% HNO_3 + 0.1% HF into the corresponding vials for instrument measurement.

[Place Figure 2 here]

7. MC-ICPMS measurement

7.1. Measure the U and Th fractions collected through the above chemical purification process using a high-resolution MC-ICPMS instrument.

NOTE: U and Th isotopic ratios can be obtained by using the instrument by applying secondary electron multiplier (SEM)[21] technique. The instrument parameters[13] are listed in **Table 1**. Thorium age was calculated using the following equation:

$$1-\left[\frac{^{230}\mathrm{Th}}{^{238}\mathrm{U}}\right]_{act}=e^{-\lambda_{230}T}-\left(\left[\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right]-1\right)\left(\frac{\lambda_{230}}{\lambda_{230}-\lambda_{234}}\right)\left(1-e^{(\lambda_{234}-\lambda_{230})\mathrm{T}}\right)$$

Initial ratio of ^{234}U to ^{238}U was measured as follows:

$$\left(\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right)_{initial}=\left(\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right)_{measured}\times e^{\lambda^{234}\mathrm{U}\times\mathrm{T}}$$

[Place Table 1 here]

REPRESENTATIVE RESULTS:

Using this procure, a submarine hydrothermal sulfide sample can be completely dissolved. Following this protocol, the Th fraction was eluted from the hydrothermal sulfide sample using 8 M HCl. Meanwhile, the U fraction of the hydrothermal sulfide sample was eluted with 0.1 M HNO_3. U and Th fractions were dissolved in the 2% HNO_3 (+0.1% HF) solution (see **Figure 2**) and stored in 2 mL capacity vials. The mixture was then analyzed by MC-ICPMS.

With the MC-ICPMS instrument, U and Th isotopes ratio and the age of submarine hydrothermal sulfide is determined precisely. The ages were calculated by an iterative method[13]. The test results are listed in **Table 2**. U content ranged from 178.0 to 5118.2 ng·g^{-1}, and Th content ranged from 603 to 7,212 pg·g^{-1}. Five samples had ages of 567 ± 52, 1,585 ± 27, 3,345 ± 132, 14,211 ± 727 and 21,936 ± 91 years B.P. (B.P. stands for “before year 2000 A.D.”). Sample consumption was about 60 mg except S32 wherein only 17 mg sample was consumed.

[Place Table 2 here]

FIGURE AND TABLE LEGENDS:

Figure 1: Ion-exchange column filling with anionic exchange resin.

Figure 2: Uranium and thorium fractions of the submarine hydrothermal sulfides.

Table 1: Instrument parameters for measuring U-Th isotopes by MC-ICPMS (using the instrument listed in the Table of Materials).

Table 2. ^{230}Th dating results for submarine hydrothermal sulfides. The error shown is 2s error.
[a]Sample mass for separation of uranium and thorium nuclide and U and Th analysis.
[b]All ratios are radioactivity ratios, which are calculated based on the decay constants λ_{238} = 1.55125 x 10^{-10} a^{-1} as described by Jaffey et al.[20], λ_{234} = 2.82206 (±0.00302) x 10^{-6} a^{-1} as described by Cheng et al.[15], and 9.1705 (±0.0138) x 10^{-6} a^{-1} as described by Cheng et al.[15].
[c]Calculated ^{230}Th age following the equation in section 7.
[d]Corrected ^{230}Th ages assuming the initial ^{230}Th/^{232}Th atomic ratio to be 4.4 ± 2.2 x 10^{-6}. These are the values for a material at secular equilibrium, with the bulk earth ^{232}Th/^{238}U value of 3.8. The errors are arbitrarily assumed to be 50%.
[e]B.P. stands for "Before year 2000 A.D.".
[f]Using the equation in section 7.

DISCUSSION:

Some critical steps must be followed to ensure success of this protocol. Ensure that all operations are carried out in clean chemistry room under fume hood with clean air circulation. Purify all regents in this process in advance and clean the apparatus before use. Dissolve the samples completely in the process of making the 7 M HNO_3 solution which is then loaded onto the 7 M HNO_3-conditioned resins. If there is any insoluble substance in the sample, it will be redissolved after drying. Additional important steps are suggested: (i) avoid the cross contamination from the adjacent samples during the sample processing; (ii) for each elution step allow the liquid to drain completely before the next step; and (iii) complete the process from the conditioning of the columns to collecting Th and U fractions within 2 h, otherwise the strong acid tends to break down the resin.

The major limitation of this technique is related to the ^{238}U and ^{232}Th concentration of the sample. It is best to choose samples with U > 50 ppb and Th < 10 ppb. The AG 1-X8 resin used can be replaced by UTEVA resin in the process.

With this method, five submarine hydrothermal sulfides samples from the South Atlantic were measured. Ages were 567 ± 52 to 21,936 ± 91 year B.P., indicating that this region has been experiencing hydrothermal activity events from 21,936 ± 91 years B.P.

U-Th purification and separation refers to isotopic methods of age estimation based on the measurement of uranium (^{238}U and ^{235}U), thorium (^{232}Th), and certain members of the intermediate daughter nuclides in the three naturally occurring radioactive decay series for hydrothermal sulfide sample. It is also useful to determine the U and Th concentration of deep-sea sediments[19]. The technique can be applied to the dating of carbonate and phosphate, and to environmental tracer studies, assisting in building the age framework for the formation of minerals.

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DISCLOSURES:

Authors have nothing to disclose.

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Figure 1

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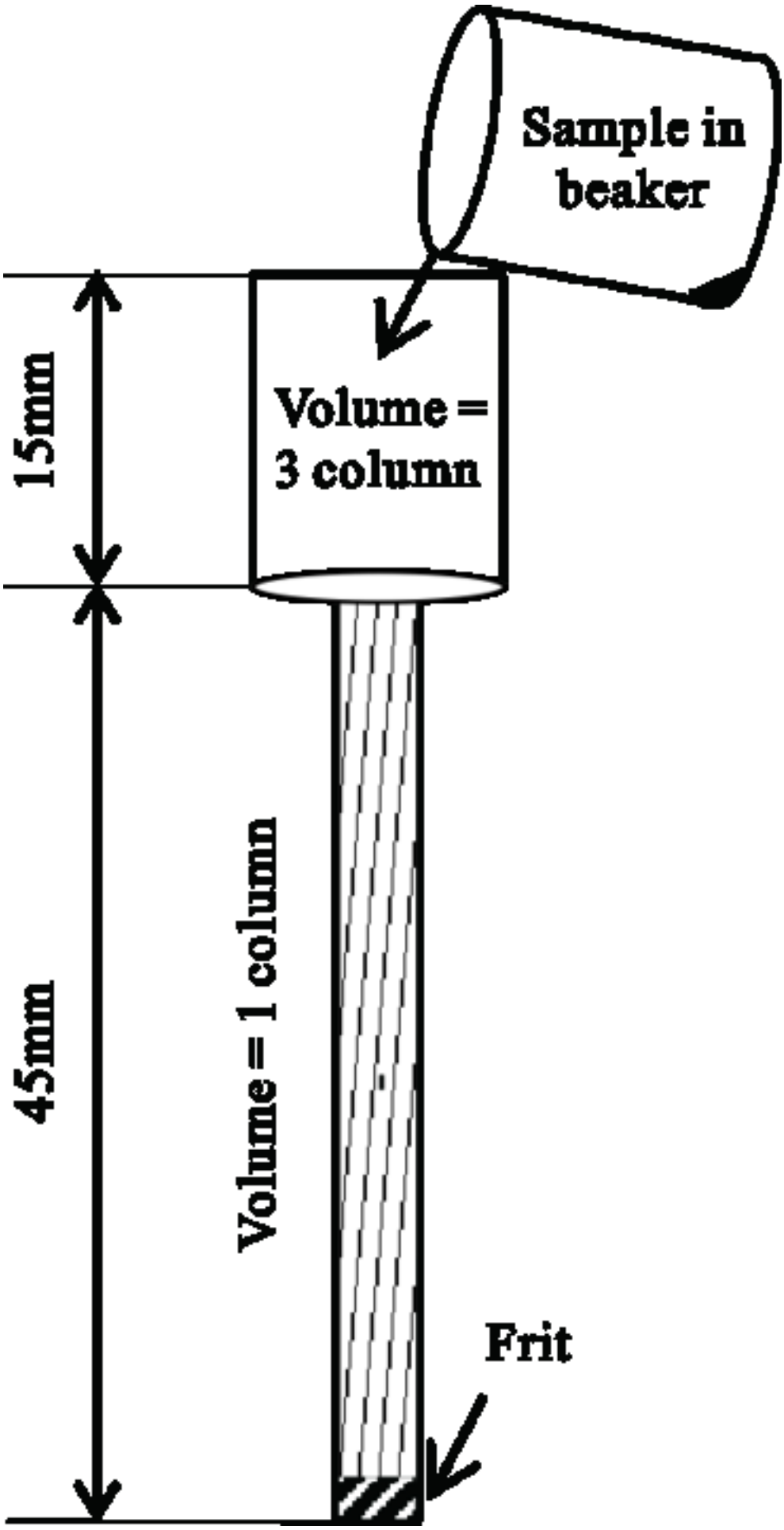

Figure 2

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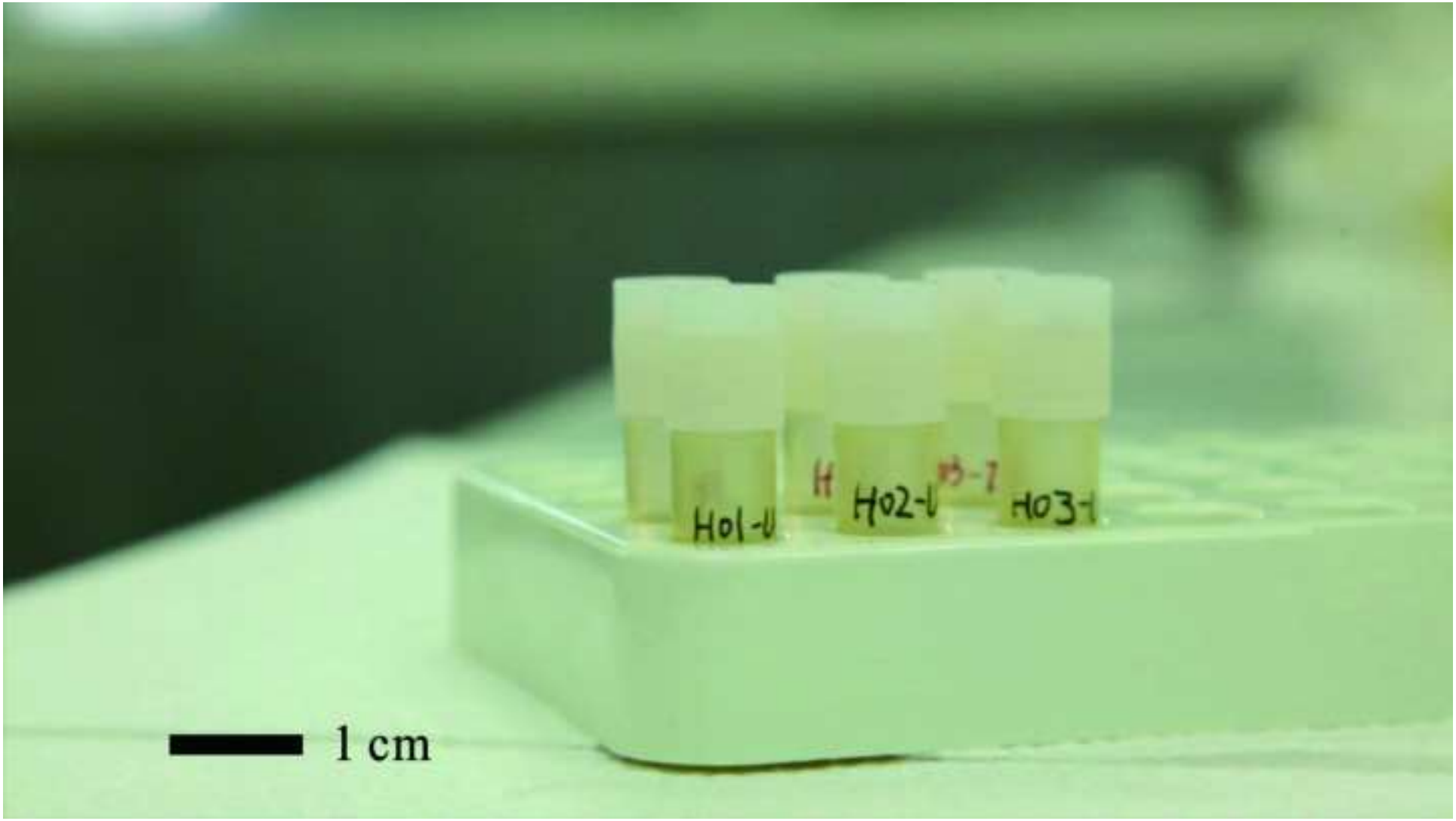

Table 1. Instrument parameters for measuring U-T

Instrument	Parameter
MC-ICPMS	RF power
	Cool gas
	Auxiliary gas
	Sample gas
	Low resolution
	Sample injection rate
CETAC Aridus II	Ar Sweep Gas
	Nitrogen Gas
	Spray Chamber Temperature
	Membrane Oven Temperature

h isotopes by Neptune Plus MC-ICPMS

Value
1325 W
16.00 L min^{-1}
1.78 L min^{-1}
1.00 L min^{-1}
300~400
50~60 μL min^{-1}
2~5 L min^{-1}
2~10 mL min^{-1}
110 °C
160 °C

Table 2. ^{230}Th dating results for submarine hydrothermal sulfides. The error is 2s error.

Sample No.	Sample Mass (mg)[a]	^{238}U (ng g^{-1})	^{232}Th (pg g^{-1})	^{230}Th/^{232}Th[b]	^{234}U/
S12	58	182.8 ±0.2	7212 ±144	11.7 ±0.3	1.156
S15	57	569.3 ±0.7	1200 ±24	310.3 ±6.3	1.166
S32	17	5118.2 ±10.4	5173 ±104	51.9 ±1.2	1.157
Y3	55	178.0 ±0.2	865 ±17	23.0 ±0.8	1.162
Y4	59	347.1 ±0.4	603 ±12	11.7 ±0.8	1.159

[a] Sample mass for separation of uranium and thorium nuclide and U and Th analysis.

[b] All ratios are radioactivity ratio, which calculated based on the decay constants λ_{238}=1.55125 ×10^{-10} a^{-}

[c] Calculated ^{230}Th age following the equation

$$1-\left[\frac{^{230}\mathrm{Th}}{^{238}\mathrm{U}}\right]_{act}=e^{-\lambda_{230}T}-\left(\left[\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right]_{act}-1\right)\left(\frac{\lambda_{230}}{\lambda_{230}-\lambda_{234}}\right)\left(1-e^{(\lambda_{234}-\lambda_{230})T}\right)$$

[d] Corrected ^{230}Th ages assume the initial ^{230}Th/^{232}Th atomic ratio of 4.4 ±2.2 x10^{-6}. Those are the value

[e] B.P. stands for “Before year 2000 A.D.”.

[f]

$$\left(\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right)_{initial}=\left(\frac{^{234}\mathrm{U}}{^{238}\mathrm{U}}\right)_{measured}\times e^{\lambda_{^{234}\mathrm{U}}\times T}$$

^{238}U[b]	$^{230}Th/^{238}U$[b]	^{230}Th Age(yr)[c] (uncorrected)	^{230}Th Age (yr BP)[d, e] (corrected)	$(^{234}U/^{238}U)_{initial}$[f]
±0.002	0.1511 ±0.0018	15221 ±193	14211 ±727	1.163 ±0.002
±0.002	0.2140 ±0.0007	22006 ±84	21936 ±91	1.177 ±0.002
±0.003	0.0172 ±0.0002	1628 ±20	1585 ±27	1.158 ±0.002
±0.002	0.0366 ±0.0010	3484 ±100	3345 ±132	1.164 ±0.002
±0.002	0.0067 ±0.0004	629 ±42	567 ±52	1.159 ±0.002

$^{-1}$ as described by Jaffey et al.(1971)[20], λ_{234}=2.82206 (±0.00302)×10^{-6} a^{-1} as described by Cheng et al.(2013)[15], an

s for a material at secular equilibrium, with the bulk earth $^{232}Th/^{238}U$ value of 3.8. The errors are arbitrarily assum

d 9.1705(±0.0138)×10^{-6} a^{-1} as described by Cheng et al.(2013)[15].

ıed to be 50%[15].

Name of Material/ Equipment	Company
AG 1-X8 anion-exchange resin	BIO-RAD
Ammonia solution	Kanto Chemical CO., INC.
Glass vials	BOTEX
Hydrochloric acid	Sinopharem chemical reagent Co. Ltd
Hydrofluoric acid	EMD Millipore CO.
Neptune Plus	Thermo Fisher Scientific CO.
Nitric acid	Sinopharem chemical reagent Co. Ltd
Perchloric acid	Kanto Chemical CO., INC.
Ultrapure water	Merck Millipore
Wipe paper	Kimberley-Clark
2 ml vial	Nelgene
229Th-233U-236U spike	None
7 ml PFA beaker	Savillex
10 ml centrifuge	Nelgene
30 ml PFA beaker	Savillex

Catalog Number	Comments/Description
140-1441	Separating rare elements
1336-21-6	Reagent
None	Sample collection
7647-01-0	Reagent
7664-39-5	Reagent
None	Apparatus
7697-37-2	Reagent
32059-1B	Reagent
None	Producted by Mill-Q Advantage systerm
0123-12	Wipe and clean
5000-0020	Sample collection
None	Reagent
200-007-20	Sample treatment
3110-1000	Sample treatment
200-007-20	Sample treatment

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Title of Article:	Separation of uranium and thorium for ^{230}Th-U dating of submarine hydrothermal sulfides
Author(s):	Lisheng Wang, Xuefeng Wang, Jun Ye, Zhibang Ma, Weifang Yang, Jule Xiao

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Title:	Separation of uranium and thorium for ^{230}Th-U dating of submarine hydrothermal sulfides
Signature:	Lisheng Wang
Date:	February 16, 2019

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Responses to the Editors' Comments

Dear Editors,

We are most grateful to editors for giving us the opportunity to modify the manuscript (JoVE59098, "Separation technology of uranium and thorium nuclide by extraction chromatography for ^{230}Th-U dating of submarine hydrothermal sulfides"). We have made all corrections following your comments in this revised version. Here presented is a list of changes and answers to each point. Please find our detail responses (in blue font) to the comments (in black font) below.

Detailed answers to the editors' comments are as follows.

Best regards,
Lisheng Wang

Editorial and production comments:

Editorial Changes

Changes to be made by the Author(s) regarding the written manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Thank you. We have made all corrections following reviewers' comments and suggestions in this new version.

2. Please sort the material table alphabetically.

Thanks for the comments. We have sort the material table alphabetically in the revised manuscript.

3. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

Thanks for the good suggestion. We have transferred every table in the form of .xlsx file in the revised manuscript. Please see Table 1 and Table 2.

4. Please shorten the title to be more concise.

Thank you for your comments. We have shortened the title to "Separation of uranium and thorium for ^{230}Th-U dating of submarine hydrothermal sulfides".

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and

“would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
Thanks. Following the suggestion, we have revised in the protocol section.

6. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.
Thanks for the good suggestion. We have added more details around the protocol steps in this revised version.

7. For all materials used, please specify the volume and concentration used. We need the specific amount used.
Thanks. We have added the specific volume and concentration in the text. Please see it in the new version.

8. What is the HNO_3 concentration?
Thank you for your comments. We have modified it in the manuscript.

9. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).
Thanks for the good suggestion. We have modified it in step 4.7.

10. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.
Thank you for your comments. We have modified it in the new manuscript.

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Thanks. We have rechecked all figures and ensured the correct citation. The data and figure without citation were new and have not been published. Please see all changes in the new version.

12. Please do not abbreviate journal titles.
Thank you for your comments. We have modified them.

Changes to be made by the Author(s) regarding the video:

1. Please increase the homogeneity between the written protocol text and the video.
2. Please reflect the revised title in the video as well.
3. Audio issues

- 0:11-0:47 - The audio and video are not synchronized. This needs to be corrected.
- 9:54 - The reading of "Eight" is clearly from a different recording session and sounds like a different person's voice. It isn't really necessary to read the chapter number out loud, so we would recommend cutting the "Eight" out.
- 11:05, 11:36 - There is an off-screen voice audible here. It should be cut out.

4. Frame size/proportions issues

- All of the video, with the exception of the title cards, has thin black borders on the left and right sides of the frame. The video needs to completely fill the frame.

5. Editing issues

- 1:47, 1:50, 3:47, 3:53, 3:58, 4:00, 4:04, 4:24, 4:42, 4:59, 5:02, 5:04, 5:06, 5:09, 5:11, 5:15, 5:19, 5:29, 5:30, 5:33, 5:38, 5:39, 5:40, 6:12, 6:18, 7:03, 7:05, 7:19, 7:23, 7:28, 7:43, 7:45, 8:22, 8:35, 8:37, 8:39, 8:42, 9:07, 9:09, 9:11 - The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.
- 4:58-5:06, 5:38-5:44 - This sequence of actions is paced too quickly. Each individual shot should be given more time on screen so that this doesn't feel rushed.
- 5:58-6:01, 8:23-8:33 - Narration should be added to explain what we are seeing in these shots, or these shots should be cut out.

Thank you for your comments. We have made all changes according to your comments. We have reflected the revised title and increased the homogeneity between the text and video. We have modified the audio, frame size/proportions and editing issues. Please see the modified text and video in the revised version.

Responses to the Reviewers' Comments

Dear Reviewer,

We are most grateful for valuable comments which have been very helpful in improving our manuscript (JoVE59098, "Separation technology of uranium and thorium nuclide by extraction chromatography for ^{230}Th-U dating of submarine hydrothermal sulfides"). We have made all corrections following your comments in this revised version. Here presented is a list of changes and answers to each point. Please find our detail responses (in blue font) to the comments (in black font) below.

Detailed answers are as follows.

Best regards,
Lisheng Wang

Reviewer #1:

Manuscript Summary:

Manuscript is prepared properly, clearly and in understandable way. Presented procedure is well organized and be useful for other researcher or analyst uses. Corresponding video material is also prepared in good manner. The paper and video are interesting and show a possibility to perform analysis of Th/U system using very small samples. I recommend this manuscript together with video to publishing, after some minor corrections.

Thank you very much. We have made all corrections following reviewer' comments and suggestions in this new version.

Major Concerns:

I recommend to precise a title, changing it for example into: Separation procedure of uranium and thorium by extraction chromatography and MC-ICPMS determination for 230Th-U dating of submarine hydrothermal sulfides. As concerns video material in introduction there is no good synchronization between a voice and picture.

Thanks for the good suggestion. I have modified the title according to your proposal. Please see it in the modified title.

Minor Concerns:

In the Protocol at point 3.4 I propose to substitute a word "spike" for "spike solution".

Thank you for your comments. I have changed it in the revised manuscript. Please see it in the revised manuscript and the video.

Point 4. Instead of "chloride" please introduce "hydroxide".

Thanks. I have modified it according to your suggestion. Please see it in Point 4.

Point 4.1. Instead of "$FeCl_3$" I propose to put "Fe(III)". I understand that this is Authors intention.

Thank you for your comments. I have modified it according to your comments. Please see it in the revised manuscript.

Responses to the Reviewers' Comments

Dear reviewer,

We are most grateful for valuable comments which have been very helpful in improving our manuscript (JoVE59098, "Separation technology of uranium and thorium nuclide by extraction chromatography for ^{230}Th-U dating of submarine hydrothermal sulfides"). We have made all corrections following your comments in this revised version. Here presented is a list of changes and answers to each point. Please find our detail responses (in blue font) to the comments (in black font) below.

Detailed answers are as follows.

Best regards,
Lisheng Wang

Reviewer #2:

Review of "Separation technology of uranium and thorium nuclide by extraction chromatography for 230Th-U dating of submarine hydrothermal sulfides" by Wang et al.

I watched the video and read the accompanying short article by Wang et al. I can confirm that the video delivers what it promises in the abstract and the technique presented could in general be reproduced following the video (at least as far as clean lab preparation, the measurement is not discussed). I recommend publishing this video after small changes, mostly in the written text file.

Thank you. We have made all corrections following reviewers' comments and suggestions in this new version.

I would cut some redundant words from the title: technology, nuclide.

Thank you for your comments. The title has been changed in the revised manuscript and video.

I had a few comments while watching the video, mostly parts of narration that I could not follow even after replaying:

1:50 Put the sample into the beaker, ….. (difficult to understand)

Thank you for your comments. We have changed it in the revised manuscript. It is modified to "Pour the sample into the beaker". Please see it in step 2.4.

2:38 You show open beakers on the hot plate. Does your sample dissolve so easily that you don't even close the beakers for dissolution? If that's not the case then the video is misleading.

Thank you for your suggestion. For some samples, it is enough to dissolve completely. But not all samples are completely soluble. If there are still insoluble substances in the solution, add 12M HCl, HF and $HClO_4$, and use pressurized closed tank to ensure complete dissolution of samples. I have added one note in step 3.3. Please see it in the video and the revised manuscript.

2:50 Does the spike mass matter? Someone else could be using a different concentration spike, in which case your instructions wouldn't be applicable.
Thank you for your comments. The spike mass is very important. Generally, the optimal ratio of $^{235}U/^{233}U$ is 10 ~20 in the mixed solution. We have added one note in step 3.4.

3:12 Your XXX must be slow - can't understand this word at all. Operation?
Thank you for your comments. It's an operating instruction. We have modified it in the manuscript. Please see it in step 3.5.

6:37 This step is in order to elute trace elements in the column XXX – unintelligible
Thank you for your comments. We have changed it to "Note: this step is in order to remove the trace elements in the column mainly". Please see it in the step 5.3.

What is the reason and significance of adding a drop of perchloric acid? This is not explained anywhere.
Thank you for your comments. The reason and significance of adding a drop of perchloric acid is to remove organic matter in the sample and brought from process of experiment. I have added the reason in step 4.11, please see it in the revised manuscript and the video.

Some comments on the article:
2nd paragraph of introduction, assumption (ii). Is the initial amount of Th really zero??? I doubt it.
Thank you for your comments. About assumption (ii), the initial amount of Th is not zero in the sample. Here we chose sample with very low content of ^{232}Th for dating. When there is a lot of ^{232}Th, an accurate age will be possible only after a strict initial ^{230}Th correction. When we correct the initial ^{230}Th, we assume the initial $^{230}Th/^{232}Th$ atomic ratio is $4.4 \pm 2.2 \times 10^{-6}$, which is the value for a material at secular equilibrium, calculated by the bulk earth $^{232}Th/^{238}U$ value of 3.8. The errors are arbitrarily assumed to be 50%. The correction method also appears in the annotation of Table 2. Please see it in the 2nd paragraph.

Discussion, end of 3rd paragraph. Until present or till 567 +/- 52. Please be precise.
Thank you for your comments. "Ages were 567 ± 52 to 21936 ± 91 yr B.P., indicating this field had been experienced hydrothermal activity event from 21936 ± 91 yr B.P.." Please see it in the revised version.

The article file has numerous language issues that I didn't highlight here (as suggested in reviewer guidelines). They can be easily corrected though.
Thank you for your comments. We have revised the language issues of the whole paper in the revised manuscript. Please see it in the revised manuscript and the video.

Responses to the Reviewers' Comments

Dear reviewer,

We are most grateful for valuable comments of our manuscript (JoVE59098, "Separation technology of uranium and thorium nuclide by extraction chromatography for ^{230}Th-U dating of submarine hydrothermal sulfides"). Here presented is a list of answers to the reviewer. Please find our responses (in blue font) to the comments (in black font) below.

Detailed answers are as follows.

Best regards,
Lisheng Wang

Reviewer #3:

This manuscript clearly describes a method to purify and separate the U and Th nuclide in submarine hydrothermal sulfide sample with Fe co-precipitation, extraction chromatography and MC-ICPMS for ^{230}Th-U dating.

Considering its novelty and practical value, I would suggest the publication of this work.

Thank you very much, we are so grateful for your good comments of our manuscript.