

## URANIUM SERIES METHOD APPLIED TO FOSSIL BONE

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In connection with the application of the uranium series method for the dating of fossil bones, separation techniques were developed for the isolation of uranium and thorium from a bone matrix. These separations were achieved on columns of the strongly basic anion exchange resin Dowex 1, using media of hydrochloric acid (for the isolation of uranium) and nitric acid (for the isolation of thorium). For uranium and thorium, yields of about 100% and about 70%, respectively, were obtained. The measurement of the  $\alpha$ -activities was carried out following the electrolytic preparation of thin-layers of uranium and thorium on stainless steel discs. The  $\alpha$ -spectrometry was done by means of Si-surface barrier detectors. Results are presented for a sample of snake-bones found in an Austrian cave.

### Introduction

The U-series method for dating of fossils is based on the measurement of the activity ratios  $^{230}\text{Th}/^{234}\text{U}$  and  $^{231}\text{Pa}/^{235}\text{U}$ , respectively. It seems to be applicable for sample ages between about  $10^4$  and  $3 \cdot 10^5$  years. This is of special interest, since the lower limit is well within the range of the  $^{14}\text{C}$ -method, while the upper limit is about a factor of 10 higher than for conventional  $^{14}\text{C}$ -dating. It is thus possible to compare results of the U-series method with the well established  $^{14}\text{C}$ -method in case of younger samples, while samples older than about  $4 \cdot 10^4$  years are usually beyond the range of the  $^{14}\text{C}$ -method but can be dated by the U-series method. Recently accelerators have been used as ultra-sensitive mass spectrometers<sup>1</sup> in successful attempts to increase the sensitivity for  $^{14}\text{C}$ -measurements. It now seems to be possible in principle to date samples of ages up to about  $10^5$  years by  $^{14}\text{C}$ . This increases the region of overlap of the two methods. One has to keep in mind, however, that the  $^{14}\text{C}$ -method has also serious systematic uncertainties, when extended to an unknown region of ages (e.g. possible change of cosmic ray flux). Intercomparison is thus of importance for the further development of both  $^{14}\text{C}$ - and U-series method.

The U-series method has been successfully applied to pleistocene shells and corals.<sup>2</sup> A major improvement was the application to travertine, which made it possible to date fossil bones enclosed in travertine found in several caves.<sup>3</sup>

In case of the present investigation dealing with fossils from special Austrian caves, the lack of travertine forced us to apply the U-series method to bone material directly. The present paper deals with chemical separation techniques for the isolation of uranium and thorium from a bone matrix. Special attention was given to high yields. ( $^{231}\text{Pa}$  was determined by measurement of the  $\alpha$ -activity of its grand-daughter  $^{227}\text{Th}$ ).

Sample preparation and absolute  $\alpha$ -counting are described. One typical result of an actual age determination is presented and discussed in connection with an open system model for U-exchange of the bone samples with the surrounding soil.

### Experimental

#### *Isolation of uranium and thorium from bone samples*

After trial and error, it turned out that the chemical procedure developed earlier for the analysis of manganese nodules<sup>4</sup> could be adapted for the present case of separating uranium and especially thorium from a bone matrix.

*Dissolution of the samples.* The bone samples were purified with doubly distilled water using an ultrasonic bath and then dried in air. The dry samples were finely ground in a stone mill and the powder obtained was subjected to the following dissolution procedure:

The bone sample (2 g) is transferred to a Teflon "bomb" (a cylindrical, thick-walled Teflon container of about 50 ml capacity, equipped with a Teflon screw cap) and 2 ml of water, 20 ml of 40% hydrofluoric acid and 10 ml of 72% perchloric acid are added. The screw cap is tightly closed and the bomb is heated for 12 h at 110 °C in an oven. Subsequently the bomb is allowed to cool to room temperature and then its content is transferred to a platinum dish using 20 ml of 40% hydrofluoric acid as a rinse. The solution is evaporated on a steam bath until hydrofluoric acid is removed, and then evaporation to dryness is performed on a sand bath. To the residue 10 ml of concentrated perchloric acid are added and the mixture is taken to dryness on a sand bath. The same operation is repeated once more using another 10 ml of perchloric acid and finally the dry residue is taken up in 10 ml of 2M hydrobromic acid. Subsequently the HBr solution of the sample is transferred to a 250 ml beaker using 20 ml of 2M hydrobromic acid as a rinse. The resulting solution is evaporated to dryness on a steam bath, the residue is taken up in 10 ml of concentrated hydrobromic acid and the

solution is evaporated to dryness on a steam bath. This evaporation step is repeated using another 10 ml of the same acid. Subsequently, the residue is dissolved in 10 ml of 6M hydrochloric acid, and, while evaporating on the steam bath, altogether 5 ml of 30% hydrogen peroxide are added at about equal time intervals from the start to the end of the evaporation; for each addition, 1 ml of hydrogen peroxide is used. After evaporation to dryness the residue is taken up in 6M hydrochloric acid and the solution is evaporated to dryness again. Finally the residue is dissolved in 100 ml of 6M hydrochloric acid and from this solution (sample solution) uranium and thorium are isolated using the ion-exchange separation procedures described in the next section.

This dissolution method for bone samples using Teflon "bombs" is much more efficient for dissolving thorium compounds than dissolution procedures involving the use of e.g. sodium carbonate or hydrochloric acid.<sup>4</sup> Furthermore, silica is removed completely by using the bomb procedure, so that practically no losses of thorium and uranium may occur because of incomplete dissolution.

The repeated evaporations with concentrated perchloric acid serve to transform all insoluble fluorides into soluble perchlorates.

The sample has to be transferred from the platinum dish to a beaker because the bromine which is evolved on subsequent treatment of the residue with hydrobromic acid, will damage (dissolve) the platinum. Treatment with hydrobromic acid is necessary to convert the perchlorates of the elements to chlorides and bromides. (Hydrochloric acid proved to be a much less efficient reducing agent for perchlorate.) This reduction is necessary; if perchlorates were present in the sample solution (6M hydrochloric acid), they would interfere seriously with the adsorption of uranium as the anionic chloride complex on Dowex 1 from 6M hydrochloric acid. However, not only perchlorate but also bromide decreases the adsorption of uranium. Therefore, the residue of bromides is treated with 6M hydrochloric acid in the presence of hydrogen peroxide; the bromine formed is evaporated and the chlorides of the elements present are formed.

To prevent the oxidation from becoming too vigorous, the hydrogen peroxide is added portionwise during slow evaporation of the solution on the steam bath. The second evaporation with 6M hydrochloric acid in the absence of hydrogen peroxide is required to destroy any peroxide present after the oxidation procedure.

*Ion-exchange separations.* Since different uranium and thorium isotopes have similar  $\alpha$ -energies, a chemical separation of these two elements from one another is necessary because of the limited energy resolution of the  $\alpha$ -spectrometer.

To separate uranium from thorium and the main constituents of the bone matrix i.e. calcium and phosphate 50 ml of the sample solution is passed through an ion-exchange column containing 4 g of the strongly basic anion exchange resin

Dowex 1X8 (100–200 mesh; chloride form, pretreated with 100 ml of 1M hydrochloric acid and 50 ml of 6M hydrochloric acid in that order) at a flow rate which corresponds to the back pressure of the resin bed. Subsequently the resin bed is washed with 50 ml of 6M hydrochloric acid and this effluent is combined with the effluent obtained when the sample solution was passed. (These combined effluents contain the thorium.)

Afterwards uranium and co-adsorbed elements such as trivalent iron are eluted with 100 ml of 1M hydrochloric acid and the eluate is evaporated to dryness on the steam bath. The residue is dissolved in 50 ml of a mixture (THF–MG–HCl-mixture) consisting of 50% (v/v) tetrahydrofuran (THF), 40% (v/v) methyl glycol (MG; ethylene glycol monomethyl ether) and 10% (v/v) 6M hydrochloric acid and the solution is passed through an ion exchange column containing 4 g of the anion exchange resin Dowex 1 (pretreated with 100 ml of 1M hydrochloric acid and then with 100 ml of the THF – MG – HCl-mixture) at a flow rate which corresponds to the back pressure of the resin bed. Afterwards the resin bed is washed with THF – MG – HCl-mixture (100 ml) and 6M hydrochloric acid (100 ml) and uranium is eluted with 100 ml of 1M hydrochloric acid. This eluate contains all the isotopes of uranium which can be subjected to  $\alpha$ -spectrometric measurement if the amount of accompanying impurities is very low. If an impure eluate is obtained, the column operation using this mixed organic solvent system has to be repeated. For this purpose the impure eluate is evaporated to dryness on a steam bath, the residue is taken up in 25 ml of the THF – MG – HCl-mixture, and the resulting solution is passed through a 2 g column of Dowex 1 (pretreated as stated above for the 4 g column). Then the column is washed with 50 ml of the THF – MG – HCl-mixture and 50 ml of 6M hydrochloric acid. Subsequently uranium is eluted with 50 ml of 1M hydrochloric acid.

From the 6M hydrochloric acid, uranium (as an anionic chloride complex) is strongly retained by the anion exchange resin ( $K_{d_{\text{uranium}}} = 283$ ) while thorium is not adsorbed but passes into the effluent quantitatively, together with all the other elements which do not form anionic chloride complexes under these conditions (e.g. calcium). Also retained from 6M hydrochloric acid are trivalent iron and some trace constituents of bone matrices. Cobalt and copper, if present, are adsorbed to some extent but the bulk of these two elements passes into the effluent when, after the sorption of uranium, the resin is washed with 50 ml of 6M hydrochloric acid. On elution of uranium with 1M hydrochloric acid iron is co-eluted but can be separated from uranium by the second anion exchange operation using the THF – MG – HCl-system. From this mixed solvent system, uranium is strongly retained by the resin while, because of the CIESE effect<sup>5</sup> iron(III) passes into the effluent.

To isolate thorium from the combined 6M hydrochloric acid effluents (see above), the following procedure is used. The combined effluents are evaporated to dryness on a steam bath, the residue is taken up in 20 ml of concentrated nitric acid and the resulting solution is evaporated to dryness (steam bath). This evaporation step is repeated using another 20 ml of nitric acid. The final residue is dissolved in 50 ml of a nitric acid – oxalic acid mixture (to 7M nitric acid, solid oxalic acid is added to make the resulting solution 0.25M in oxalic acid) and the solution (sorption solution) is passed through an ion-exchange column containing 4 g of Dowex 1X8 (100–200 mesh; nitrate form, pretreated with 100 ml of 7M nitric acid) at a flow rate corresponding to the back pressure of the resin bed. Subsequently, the resin bed is washed with 150 ml of nitric acid-oxalic acid mixture and then with 50 ml of 7M nitric acid. Finally the thorium isotopes are eluted with 100 ml of 6M hydrochloric acid (thorium eluate). If an impure thorium eluate is obtained the separation of thorium is repeated on a separate column of the anion-exchanger. For this purpose the eluate is evaporated to dryness on a steam bath and chlorides are removed as described above by using two successive evaporations with concentrated nitric acid. Then the residue is dissolved in a few milliliters of the nitric acid – oxalic acid mixture and the solution is passed through a column containing 2 g of the same resin. Subsequently, the resin bed is washed with 100 ml of nitric acid – oxalic acid mixture and 50 ml of 7M nitric acid. Finally thorium is eluted with 50 ml of 6M hydrochloric acid and the eluate is evaporated using an infrared lamp. During the evaporation, a few milliliters of concentrated formic acid are added in order to completely destroy nitrate ions. The repeated evaporations with concentrated nitric acid convert the chlorides contained in the evaporation residue of the combined 6M hydrochloric acid effluents to nitrates (mainly calcium nitrate), so that they cannot interfere with the adsorption of thorium on Dowex 1 from the 7M nitric acid – 0.25M oxalic acid medium ( $K_d$  thorium = 189). Although adsorption of thorium as a negatively charged nitrate complex on Dowex 1 would also take place in the absence of oxalic acid, this compound was found to be a very desirable ingredient because it prevents the formation of hydrated metal oxides during the sorption process, which might block the ion-exchange column. The washing step with 7M nitric acid, which is employed after washing the resin with 150 ml of nitric acid – oxalic acid mixture, serves to remove oxalic acid as completely as possible. Consequently the organic matter which passes into the 6M hydrochloric acid-thorium eluate is kept to a minimum.

*Determination of chemical yields.* The percent losses of uranium and thorium which occur during the ion-exchange separations were determined by adding known activities of uranium and thorium to the sample solutions before carrying

out the column operations. For this purpose a spike solution was used which contained  $^{232}\text{U}$  in radioactive equilibrium with  $^{228}\text{Th}$ .<sup>6</sup> These isotopes are not present in the solutions of the bone samples and their  $\alpha$ -energies do not interfere with the  $\alpha$ -lines which have to be measured. In each case the magnitude of the activity of the spike was selected to be similar to the activity expected in the sample. Thus, to the sample solution a known amount of spike (in 6M hydrochloric acid) was added and the separations were carried out as described above.

Based on the assumption that the spike shows the same chemical behaviour as the isotopes of uranium and thorium contained in the bone samples, the activities of  $^{232}\text{U}$  and  $^{228}\text{Th}$  found in the final eluates of uranium and thorium respectively can be regarded to be reliable measures for the chemical yields.

For uranium and thorium, yields of about 100% and about 70%, respectively, were obtained. The lower chemical yield for thorium is caused by the high phosphate content of the bone matrix, which reduces the amount of thorium adsorbable on the anion-exchange resin. Nevertheless, these yields allowed the  $\alpha$ -spectrometric determinations of the activities of thorium and uranium to be performed with sufficient accuracy.

*Electrolytic preparation of thin-layers of uranium and thorium for  $\alpha$ -spectrometry.* Because of the phenomenon of self-absorption of  $\alpha$ -particles by the samples, an essential prerequisite for the exact measurement of  $\alpha$ -activities is the preparation of homogeneous thin-layers of uranium and thorium for  $\alpha$ -spectrometry.

*Uranium.* The quantitative, electrolytic deposition of trace quantities of uranium on a stainless steel disc from a 1,2-dichloroethane (DCE) – polyethylene glycol (PEG) solution was found to be a very suitable method to prepare homogeneous thin layers of uranium.<sup>7</sup> Since, however, the quality of the layers was found to be strongly reduced even in the presence of only trace amounts of iron, it was necessary to purify the uranium employing the two successive anion-exchange separations described above, using the THF–MG–HCl-system. Following the second separation, the 50 ml 1M hydrochloric acid eluate containing the uranium is evaporated to dryness on a steam-bath and the residue is dissolved in 0.5 ml of 1M hydrochloric acid and 4.5 ml of 1M ammonium thiocyanate. Subsequently, uranium is extracted twice with 2 ml each of 0.1% (w/v) PEG–DCE solution. The organic extracts are combined and transferred to a Teflon cell, the bottom of which is a stainless steel disk simultaneously acting as the cathode and as the carrier for the samples. The anode of this electrolytic “arrangement” is a spiral-formed, rotating platinum wire and the electrolysis is performed for 40 min using a constant current of 4.6 mA. After the electro-deposition of uranium, the steel disc is ignited whereby a thin layer of uranium oxide is formed.

*Thorium.* As in the case of uranium, the electrolytic preparation of a suitable thin layer of thorium requires that thorium is contained in a highly pure solution. Therefore, in most cases the two successive anion-exchange separation procedures described above using the nitric acid oxalic acid system have to be employed to obtain thorium solutions of high purity. To prepare the electrolyte, the residue obtained after the evaporation of the thorium eluate using an infrared lamp is first dissolved in 5 ml of 0.5M hydrochloric acid. Subsequently, the solution is diluted with 4 ml of 2M sodium chloride, 1 ml of 0.5M oxalic acid and 0.8 ml of 2M ammonium formate. The resulting solution (electrolyte solution) is transferred to a Teflon cell and the electrolysis is performed for 3 h using a constant current of 0.5 A.<sup>8</sup>

Before the electrodeposition of uranium or thorium, the stainless steel discs are carefully cleaned by heating them for 15 min (in a platinum dish) with a 2% (v/v) hydrofluoric acid-15% (v/v) nitric acid mixture (steam bath).<sup>9</sup> Afterwards the discs are rinsed with distilled water. As a result of this treatment a slightly roughened surface is formed on the disk, which favours the electrolytic deposition of uranium and thorium.

#### *Determination of the $^{231}\text{Pa}$ -activity*

The activity of  $^{231}\text{Pa}$  was determined indirectly by its daughter  $^{227}\text{Th}$ .  $^{231}\text{Pa}$  decays via  $^{227}\text{Ac}$  ( $T = 21.2$  y) to  $^{227}\text{Th}$  ( $T = 18.6$  d) which is also an  $\alpha$ -emitter. This means that after a period of 100 years  $^{231}\text{Pa}$  is in radioactive equilibrium with  $^{227}\text{Th}$ .<sup>10</sup> This time is short enough for an eventual mobility of  $^{227}\text{Ac}$  to have no effect on the  $^{227}\text{Th}$  activity. Therefore it was not necessary to develop another chemical separation technique for the determination of  $^{231}\text{Pa}$ . Because the daughter products of the spike  $^{228}\text{Th}$  would interfere with the  $\alpha$ -spectrometric determination of  $^{227}\text{Th}$ , it was not possible to use the "spiked" Th-fraction for this purpose. After the determination of the  $^{230}\text{Th}$  activity, a second chemical separation of Th from the bone matrix was performed without the spike. For the determination of  $^{227}\text{Th}$  the already known activity of  $^{230}\text{Th}$  was used as an "internal spike".

#### *Alpha-spectrometry*

The  $\alpha$ -spectrometry was done by means of Si-surface barrier detectors mounted in a vacuum chamber and connected to a low noise amplifier system and a multi-channel analyzer. The  $\alpha$ -spectra of the spiked uranium and thorium fraction were determined using a detector with 401 mm<sup>2</sup> active area. Contamination of the detector by daughter products of the spike, especially of  $^{228}\text{Th}$ , cannot be avoided

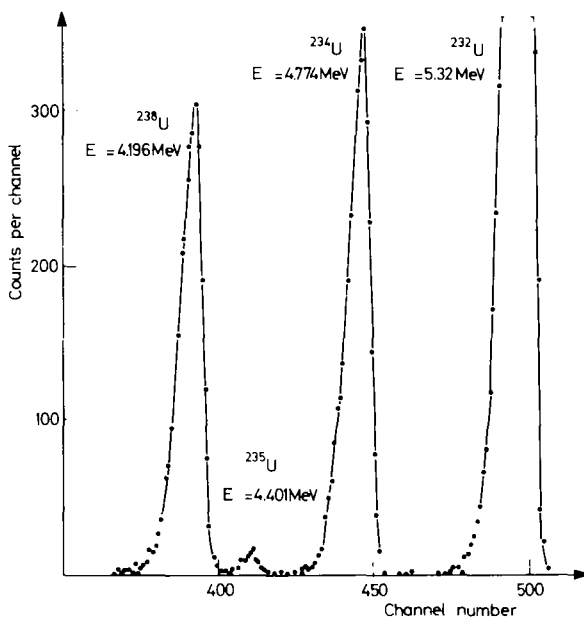


Fig. 1. Typical  $\alpha$ -spectrum of uranium separated from a bone sample ( $^{232}\text{U}$  was added as a spike)

completely.<sup>11</sup> Because the daughters of  $^{228}\text{Th}$ , interfere seriously with the small  $^{227}\text{Th}$  activity, this activity was determined separately using a second detector with an active area of  $600\text{ mm}^2$ . The overall  $\alpha$ -resolution was about  $100\text{ keV}$ . With this resolution, it was possible to separate and determine the  $\alpha$ -activities of interest. For a counting statistics of  $\sim 5\%$  ( $1\sigma$ -error), counting times of  $10\text{--}20$  hours were necessary, depending on the U-content of the sample, the quality of the separation and the deposition procedure. Fig. 1 shows a typical U-spectrum of a bone sample.

### Results and discussion

As an example for the application of the method described, results are presented for a typical sample. The sample "StM" consists of snake vertebra found in a cave near St. Margarethen (Burgenland) in the eastern part of Austria. The sample is especially interesting since these snakes (*Coluber gemonensis*, *Coluber vividiflavus*) prefer warm climate and an absolute age determination can be used to discriminate between a few distinct possibilities (Mindel/Riß- or Riß/Würm-Interglacial).<sup>12</sup> Table 1 shows the rather high U-content found in the sample together with the



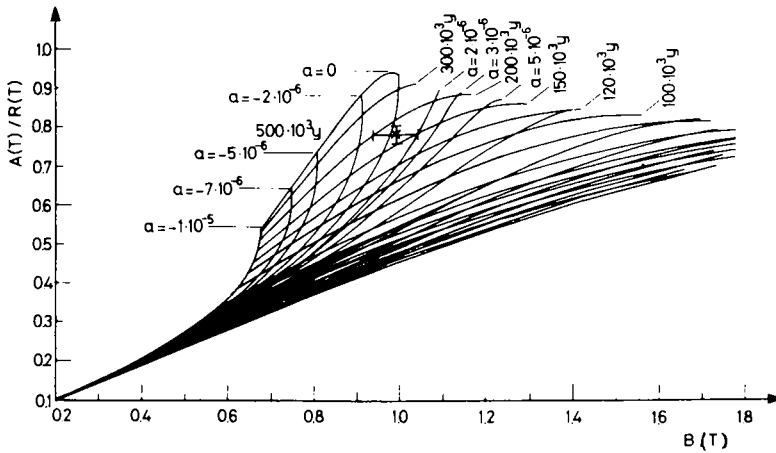


Fig. 2. Activity ratios as a function of sample age according to an open system model (see text). The measured values for the sample "StM" [ $^{234}\text{U}/^{238}\text{U} \equiv R(T) = 0.88$ ] are indicated. If the sample remained a closed system, the activity ratios should lie on the upward bent curve marked  $\alpha = 0$ . The special sample dated shows an activity ratio almost consistent with this assumption [ $A(T)/R(T) \equiv ^{230}\text{Th}/^{234}\text{U}$ ;  $B(T) \equiv ^{227}\text{Th}/^{235}\text{U}$ ]

Table 1  
Results of the measurements

| U ppm | $^{234}\text{U}/^{238}\text{U}$ | $^{230}\text{Th}/^{234}\text{U}$ | $^{227}\text{Th}/^{235}\text{U}$ | Age, year                        |
|-------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 158   | $0.885 \pm 2\%$                 | $0.78 \pm 3.3\%$                 | $0.99 \pm 5\%$                   | $175\,000^{+62\,000}_{-39\,000}$ |

measured activity ratios. These activity ratios are almost compatible with the assumption that the sample had remained a closed system. This can be seen from Fig. 2, where the measured activity ratios are displayed within the framework of an open system model<sup>1,3</sup> containing the closed system as a special case. Since the deviations from concordance between  $^{230}\text{Th}$ - and  $^{231}\text{Pa}$ -age respectively, are rather small, systematic errors due to the assumptions of the model are also expected to be small.

These assumptions are essentially<sup>1,3</sup>

(1) All products (including radiogenic  $^{234}\text{U}$ ) formed by radioactive decay remain in the sample,

(2) Uranium originally migrated into the sample can (besides radioactive decay) change with time according to a kind of "chemical decay constant" ( $\alpha$ ):

$$U(t) = U(0) e^{-\alpha t}$$

As can be seen from Fig. 2, the measured activity ratios in the case of the sample StM are compatible with a chemical decay constant  $\lambda \cong 0$ , which means an almost closed system. The error of the age given in Table 1 is determined by calculating the maximum and minimum age (using the measured activity ratios and their errors as given in Table 1) according to the open system model<sup>13</sup> displayed in Fig. 2.

From the two possibilities of age assessment mentioned above, the placement of the sample to the Mindel/Riß warm period seems to be adequate.

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