

USDOE, RESL/ID  
Anal. Chem. Branch  
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P-2, 1977  
32p  
Fish, Vegetation  
Dry Ash, Ion Exchange  
Working Time 10 hr/6  
Yield: 85%  
Approx. D. L.  $1 \text{ E-}8 \mu\text{Ci/g}$ ,  
500 g, 20 min count

#### ABSTRACT

After dry ashing up to 500 g of sample, a cupferron precipitation is used to separate iron. The iron cupferrate can be used for the determination of Fe-55. Diverse ions are separated from phosphorus by ion exchange. The P-32 activity is measured by Cerenkov counting.

#### LIMITATIONS AND INTERFERENCES

Excessive amounts of calcium cannot be tolerated. For those cases where fish skeleton must be analyzed, only small portions can be taken. The 14.3 day half-life limits the analysis to young material.

#### REAGENTS

Magnesium Reagent Dissolve 55 g of  $\text{MgCl}_2 \cdot 6 \text{ H}_2\text{O}$  and 140 g of  $\text{NH}_4\text{Cl}$  in water. Add 350 mL of conc.  $\text{NH}_4\text{OH}$  and dilute to one liter. Filter if the solution is turbid and store in a plastic bottle.

#### INSTRUMENTATION

Liquid scintillation counting is done with a Packard Model 3385 liquid scintillation spectrometer.

PROCEDURE

1. Dry up to 500 g of fish muscle, 200 g of whole raw fish or up to 100 g of vegetation at 200°C in an aluminum pan lined with two layers of vellum tracing paper. Slowly increase the temperature to 550°C to insure that the fish oil does not boil. Predrying at 200°C for six or more hours generally removes most of the oil. Remove the sample at 400°C and grind up the ash to obtain a uniform gray ash after about 16 hours at 550°C.
2. Transfer the ash to a 600-mL beaker. Dissolve the residue in the pan with two 50-mL portions of 6M  $\text{HNO}_3$  and add to the beaker containing the ash.
3. Evaporate the sample to about 25 mL. A frothy brown residue will remain. In a perchloric fume hood, add 50 mL of concentrated  $\text{HNO}_3$  and 30 mL of concentrated  $\text{HClO}_4$  and evaporate the sample on a hotplate covered with asbestos. After the  $\text{HNO}_3$  has evaporated, dense white  $\text{HClO}_4$  fumes appear and the solution changes to a white slurry. Continue the evaporation until almost all the  $\text{HClO}_4$  has evaporated and the slurry changes to a shiny white residue.
4. Dissolve the residue in 100 mL of 6 N HCl and boil on the hot plate until about 50 mL remains. Filter the hot solution through a 5.5 cm diameter glass fiber filter and discard the residue.
5. Dilute the filtrate to about 150 mL and cool in an ice bath for 15 minutes. To the cool solution, add, with constant stirring, a solution of 5% aqueous cupferron. Use approximately 1 mL of cupferron solution for each 10 mL of acid solution. Add an excess of cupferron until a white precipitate forms.

6. Transfer this mixture to two 90-mL centrifuge tubes and centrifuge for 5 minutes. Filter the solution from both tubes through a 5.5 cm diameter glass fiber filter and rinse the filter with 50 mL of cool water which has been used to wash the beaker and centrifuge tubes. Save the filtrate for determination of  $^{32}\text{P}$  and the precipitate for the determination of  $^{55}\text{Fe}$ .
7. Add 10 mL of  $\text{HNO}_3$  and 10 mL of  $\text{HClO}_4$  to the filtrate and evaporate to fumes of  $\text{HClO}_4$ . Add additional  $\text{HNO}_3$  as necessary to complete oxidation of the organic material. If there is a large amount of phosphorus, salts may form before all the nitric acid has been removed. Heat cautiously to prevent bumping or spattering of the sample. Heat until most of the  $\text{HClO}_4$  has been driven off, but do not allow to dry.
8. Add sufficient 6 N  $\text{HCl}$  to dissolve all salts and heat to boiling. Boil for 10 minutes to hydrolyze any pyrophosphates.
9. Cool to room temperature. If salts form, add more 6 N  $\text{HCl}$  until they dissolve.
10. Pass the solution through an anion exchange column (Dowex 1 x 8, 20-50 mesh) in the  $\text{Cl}^-$  form. The column should be approximately 15-mm diameter by 150-mm long. Maintain a flow rate of about 5 mL per minute.
11. Evaporate the solution from the anion exchange column to fumes of perchloric acid and add sufficient 0.1 N  $\text{HCl}$  to dissolve all the salts.
12. Boil for 10 minutes to hydrolyze any pyrophosphates and cool to room temperature. If salts form on cooling, dissolve them by dropwise addition of 0.1 N  $\text{HCl}$ .

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13. Pass the solution through a cation exchange column (Dowex 50 x 8, 20-50 mesh,  $H^+$  form, with the same dimensions as the anion column) at a flow rate of 5 to 10 mL per minute. Wash the column with about 75 mL of 0.1 N HCl.
14. Evaporate the solution to about 100 mL. Cool and add 50 mL of magnesium reagent. Place in a water bath and when the precipitate has settled check for complete precipitation by adding additional reagent.
15. Cool in a water bath for 30 minutes to assure complete precipitation.
16. Using a vacuum, filter onto a 47-mm glass filter and wash 3 times with 0.1 N  $NH_4OH$ , then 95% ethanol and 0.1 N  $NH_4OH$ .
17. Vacuum dry for 20 to 30 minutes. Knock the precipitate from the filter paper into a 250 mL beaker - do not scrape the paper; any glass fibers scraped from the filter will cloud the counting solution.
18. Dissolve the  $MgNH_4PO_4$  in the smallest possible volume of 6 N  $HNO_3$ . Transfer the solution to a 22-mL counting vial for Cerenkov counting. If the solution is greater than 20 mL, note the total volume and then add 20 mL to the counting vial.
19. Measure the Cerenkov radiation using a liquid scintillation counting system.
20. Correct for the fraction of the sample used in step 18 and calculate  $\mu Ci/g$  of P-32 in the original sample.