USTUR 100: TISSUE ASHING, SAMPLE DISSOLUTION, SAMPLE ALIQUOT SELECTION, AND TRACER ADDITION FOR ANION EXCHANGE ISOLATION OF RADIONUCLIDES

Purpose	Preparation of tissue actinide determination	**Method Number**	USTUR 100
Original Date	10/10/95	**Author**	Radiochemistry Staff
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No longer in use – see:

USTUR 105, Preparation of Tissue for Actinide Determination: Dry Ashing of Tissues

USTUR 110, Preparation of Tissue for Actinide Determination: Wet Ashing of Tissues

USTUR 115, Dissolution of Sample Ash

USTUR 120, Hydrofluoric Acid Digestion of Soft Tissues

USTUR 125, Sample Fusion using Potassium Fluoride

SAFETY NOTE: Before beginning this procedure, read all of the Material Safety Data Sheets for the chemicals listed in Section 3 of this procedure.

1. Principle of Method

1.1. The selected tissue is oven-dried, muffled at 500° C, alternately wet- and dry-ashed, and dissolved in acid (usually 6-8 M HCl).

1.2. An aliquot of the solution is taken, and tracer isotopes are added.

2. Apparatus

2.1. Beakers: borosilicate glass, various sizes.

2.2. Beakers: Teflon, with covers.

2.3. Balance: with at least two-decimal-place accuracy.

2.4. Watch glasses: sizes to fit beakers in use.

2.5. Drying oven, vented.

2.6. Muffle furnace: adjustable to 500° C.

2.7. Fume hood.

2.8. Hot plates: adjustable to 140° C.

2.9. Hot plates: magnetic stirring, adjustable to 140° C.

2.10. Stirring bars: Teflon-coated.

2.11. Bottles: I-chem; 8, 16, and 32 oz, with teflon-lined caps.

2.12. Platinum dish or crucible.

2.13. Blast burner.

3. Reagents

3.1. Nitric acid (concentrated, 69-71%, reagent-grade).

3.2. Hydrochloric acid (concentrated, 36.5-38%, reagent-grade).

3.3. Hydrochloric acid (8 M). Add 667 mL of concentrated HCl to 300 mL deionized (DI) H_2O. Dilute to 1000 mL with DI H_2O.

3.4. Hydrofluoric acid (48.0-51.0%).

3.5. Hydrogen peroxide (30%).

3.6. Plutonium-242 tracer solution.

3.7. Americium-243 tracer solution.

3.8. Uranium-232 tracer solution.

3.9. Thorium-229 tracer solution.

3.10. Quality control solutions. Make solutions of the appropriate isotope(s), bringing the total solution weight to 1000g. The concentration of each isotope should be approximately 0.5 dpm per 50g. (The value is accurately known.) Use 6-8 M HCl to make up the solution. Use Pu-238 and Am-241 for the Pu/Am quality control solution, natural U for the uranium solution, and thorium nitrate for the thorium solution.

3.11. Boric acid (reagent-grade).

3.12. Sodium bisulfate, reagent-grade.

3.13. Sulfuric acid (concentrated, 96-98%, reagent-grade).

3.14. Potassium Fluoride, anhydrous (reagent-grade).

4. Sample Drying

4.1. Record the sample number (using a permanent marker) on a clean, tared borosilicate glass beaker. Place the tissue in the beaker. Record the sample net weight on a sample control sheet (See Figure 1). Analytical balance must be capable of 1% precision.

4.2. Cover the beaker with a ribbed watch glass.

4.3. Dry sample for 2-5 days. Drying time is dependent on size and moisture content of the sample. Small tissue samples will dry within 2 days and may then be removed from the oven.

5. Sample Muffle-Ashing

NOTE: The very large muffle furnace exhibits temperature gradients. Care must be taken to use the correct temperature for samples on the bottom or in the middle of the furnace.

5.1. Transfer dry samples to a muffle furnace and heat samples according to the following schedule. Increase the temperature in increments of 50°C every 4 hours until the final temperature for the day and then hold.

NOTE: Liver samples and higher fat content samples need to have temperatures rise at a slower rate, based on the amount of fumes coming off samples. Between 200 and 300°C, the temperature should be increased by 25°C increments, holding at a given temperature until evolution of smoke stops. At 350°C, the temperature may have to be held longer than 4 hours, if samples evolve a lot of smoke.

Day 1 150°C start, move to 250°C
250°C hold

Day 2 250°C start, move to 300°C
300°C hold

Day 3 300°C start, move to 450°C
450°C hold

Days 4-6 450°C start, move to 500ºC; maintain temperature at 500°C for 72 hours, shut off furnace, and allow samples to cool.

5.2. Cool samples to room temperature. Record weight of bone ash for bone samples. For small samples you must use a balance capable of 1/100 of sample weight (e.g. for a 1 g sample the balance must be capable of 0.01 precision).

6. Acid-Ashing

6.1. To the muffled sample add, by rinsing the sides of beaker, sufficient amounts of concentrated HNO_3 to completely cover the ash. Place beaker on a hot plate set at 120°C-140°C.

6.2. Once the acid has warmed, add 1 to 2 mL of 30% H_2O_2 dropwise, allowing foaming to subside between additions. Bring solution back up to temperature. Then add 1-2 mL 30% H_2O_2 at 30 minute intervals. Keep solution volume at a minimum, only adding concentrated nitric to prevent sample drying out. Ashing is complete when sample loses its brown color. A minimum of 4 hours will be necessary for this step.

NOTE: These volumes correspond to a wet sample weight of 100g. Scale volumes up, if the sample weight is larger than this.

6.3. Once the sample loses its brown color, remove the watch glass and take the sample to dryness.

6.4. If sample ash remains black and viscous after repeating Steps 6.1 and 6.2 several times, proceed as follows.

6.4.1. Cover the beaker with a watch glass and reflux on a hot plate set at 140°C until the sample loses its viscosity.

6.4.2. Remove the watch glass and reduce the hot plate temperature to 100°C. Allow the sample to dry thoroughly. Use care to avoid splattering the sample. When the sample appears dry, increase the hot plate temperature to 140°C. Heat for several hours to ensure dryness.

6.4.3. Cover the sample beaker with a watch glass and place the sample in the muffle furnace. Set the furnace at 200°C, and increase the temperature by 100°C every 2 h until 450°C is reached. Hold 450°C until the ash whitens (usually requires several hours).

6.4.4. Remove the sample from the muffle furnace and repeat Step 6.1 and 6.2 until the ash material is white, cream, or purple in color.

NOTE: All lung and lymph node tissues require HF treatment. Proceed to Step 7.6.

7. Sample Ash Dissolution

7.1. To the beaker containing the ashed tissue sample, add 5-10 mL of concentrated HNO_3 to loosen ash from the beaker bottom. Place the beaker on a hot plate set to 140°C. Once sample is warm, add 5-10 mL of concentration HCl. Heat until the major part of ash has gone into solution.

NOTE: Experience has shown that the addition of small amounts of HNO_3 at this step will aid in the dissolution of the ash in the 8 M HCl added in Step 7.2.

7.2. Add 50-900 mL of 6-8 M HCl to the sample beaker. Place a watch glass on the beaker and reflux until the ash is dissolved.

NOTE 1: The amount of acid required to dissolve the sample ash will vary between samples and will, to some extent, depend on the mass of the sample ash and tissue type. The amount of HCl used should be limited to the quantity required for complete ash dissolution. In general, bone samples will require more acid than soft tissues, and large samples will require more acid than small samples. The final sample weight, measured in grams of HCl, will be adjusted in Step 8.2 to between 100 and 1000 g.

NOTE 2: If any obviously foreign solid material is present at this stage (e.g. surgical staples) it should be removed, placed in a vial and labeled. This material may be analyzed later. Record on sample sheet.

7.3. To check ash for undissolved particles, shine the light from a flashlight through the beaker bottom (in a darkened room). If the sample solution is clear, proceed to Step 8.

7.4. If light is reflected from particles of ash, additional treatment will be necessary. Place the sample on a stirring hot plate, add a Teflon-coated stirring bar, and stir-heat for one hour. If the sample remains undissolved, add 6-8 M HCl and stir-heat for several hours. If the sample remains undissolved, proceed to Step 7.6, except for bone samples.

7.5. Bone samples with undissolved ash.

NOTE: Bone samples are not treated with HF because the acid may precipitate calcium fluoride.

7.5.1. If the bone sample has not dissolved, evaporate the solution to dryness on a hot plate set at 140°C, then add enough concentrated HNO_3 to cover the salts. Cover the sample with a watch glass and reflux for 1 hour.

7.5.2. Remove the watch glass and dry thoroughly. Repeat Step 6.4.3 and Steps 7.1 through 7.4. If the sample is still not dissolved, filter through Whatman #42 paper, collecting the solution in a tared bottle appropriately labeled.

7.5.3. Dry the filter paper and then ash the paper in a muffle furnace. Start the temperature at 150°C and raise the temperature in 50°C increments to 250°, holding at a given temperature until fumes subside.

7.5.4. Raise the temperature in 100°C increments to 500°C and hold at 500°C until ash is mainly white.

7.5.5. Wet ash the muffled sample with concentrated HNO_3 and 30% H_2O_2 to remove any remaining dark color from the ash.

7.5.6. Follow section 7.6 to dissolve ash. A few drops of perchloric acid can be added to the HF/HNO_3 digestion to ensure complete dissolution.

Warning: Perchloric acid must be used with extreme caution!

7.5.7. Follow Steps 7.1 to 7.3, limiting the amount of 6-8 M HCl used so the solution can be added to the tared bottle (from Step 7.5.2) containing the filtered portion of the sample.

7.6. Hydrofluoric acid digestion of soft tissue

7.6.1. Transfer the sample into the proper size Teflon beaker using 6-8 M HCl. If necessary, use a rubber policeman to dislodge ash from the sides of the beaker.

7.6.2. Evaporate the sample to dryness at 120°C.

7.6.3. Add concentrated HNO_3 to completely cover the ash with approximately 2-3 cm height of acid.

7.6.4. Estimate the volume of HNO_3 added and add one-fifth that volume of HF.

CAUTION: Use extreme care with HF. Double gloves are required. Wash gloves after use.

7.6.5. Cover the Teflon beaker with a Teflon cover and reflux for 2 hours on a hot plate with a surface temperature of 250º C.

7.6.6. Remove beaker from the hot plate and cool until acid is not fuming. Add the same volume of HF calculated in step 7.6.4 and continue refluxing for another 2 hours.

7.6.7. If sample still contains undissolved particles repeat HF addition and reflux for another 2 hours.

NOTE: Larger samples may require extra HF additions and longer reflux periods.

7.6.8. Remove the cover and evaporate to dryness.

7.6.9. Wash down the beaker with concentrated HNO_3, having at least 1 cm depth of acid and heat to dryness. Repeat twice. With the first addition of concentrated HNO_3 add ~50 mg boric acid. If $HCl0_4$ was added earlier, ensure that it has completely been fumed off. (There should be no heavy white fumes if $HCl0_4$ is gone). If necessary, repeat this step additional times to remove $HCl0_4$.

7.6.10. Repeat steps 7.1 through 7.3.

7.6.11. If there still remains undissolved material in the solution, filter the sample through Whatman #42 paper.

7.6.12. Ash the filter paper following Steps 7.5.3 to 7.5.5.

7.6.13. The remaining undissolved material will be treated on an individual sample basis. Discuss treatment options with your supervisor before proceeding with section 7.7 or 7.8.

7.7. Sample Fusion using Sodium Bisulfate

This fusion should not be used on any residues from larger bone samples. The dissolved fusion cake can cause $CaSO_4$ to precipitate over time when added to the bulk of dissolved bone sample.

7.7.1. Transfer the undissolved sample material from step 7.6.12, using conc. HNO_3 ONLY, to a platinum dish.

7.7.2. Dry the sample down in the platinum dish on a hotplate. Cool.

7.7.3. Estimate the weight of undissolved material and add 10-20 times in weight of sodium bisulfate.

7.7.4. Heat the platinum dish over a blast burner, gradually making a melt of the sodium bisulfate.

NOTE: Use platinum tipped tongs to handle dish.

7.7.5. Continue heating the melt, gently swirling to aid dissolution, until the melt is clear and undissolved particles not seen.

7.7.6. If the material is not totally dissolved and H_2SO_4 is no longer fuming from the melt, cool the melt, add a few drops of conc. H_2SO_4, and then repeat steps 7.7.4 and 7.7.5 until material is completely dissolved.

7.7.7. When dissolution is complete, slowly swirl melt up the sides of the dish before allowing it to solidify. (The larger surface area aids in acid dissolution.)

7.7.8. Dissolve the cake (solidified melt) by adding enough 2 M HNO_3 to the platinum dish to cover the cake. Heat on hotplate until the cake loosens and dissolves.

NOTE: Never add HCl or HCl/HNO_3 to PT dish!

7.7.9. Transfer the resulting solution to an appropriate size beaker. Using 2 M HNO_3 to rinse the platinum dish.

7.7.10. Estimate the volume of solution and add enough conc. HCl to make the solution approximately 6 M HCl.

7.7.11. Make sure all solids are dissolved and add to original bottle containing the majority of the dissolved sample.

7.8 Sample Fusion using Potassium Fluoride (KF). Adapted from the INEL Radiological and Environmental Sciences Laboratory Analytical Chemistry Branch Procedures Manual, 1982.

7.8.1 Filter sample containing undissolved material into a vacuum filtration apparatus. Gelman Supor membrane filters are typically used.

7.8.2 Ash the membrane filter containing undissolved material in a 50-mL platinum dish with a Meker burner. Be careful to heat the membrane sample slowly, to prevent the membrane from igniting. If the sample does ignite, immediately extinguish by covering the dish with a watch glass.

7.8.3 Allow the platinum dish to cool. Rinse the sides of the platinum dish, containing the ashed membrane residue, with approximately 5-10 mL of concentrated nitric acid (16 M HNO_3) to wet-ash; heat to dryness on a hot plate.

7.8.4 Add approximately 5 mL of concentrated hydrofluoric acid (48% HF) to the dish and heat to dryness on a hot plate to removed siliceous material. **CAUTION:** Do not expose any unprotected skin to the vapors while drying.

7.8.5 Add approximately 1 g anhydrous KF (more or less depending on the amount of residue and the size of the platinum dish), 1-5 drops each of 16 M HNO_3 and 48% HF.

7.8.6 Fuse the residue with the KF over a Meker burner, swirling the liquid fusion across the bottom and lower sides of the dish to ensure complete sample fusion. The residue should be adequately fused when the liquid fusion is clear.

7.8.7 Once the fusion in cool, rinse the sides of the platinum dish by adding approximately 5-10 mL of 16 M HNO_3, add approximately 50 mg of boric acid to the fused solution, whirl, and heat to dryness. Repeat this step once. Do not add additional boric acid with repeat.

7.8.8 Add enough concentrate (16 M) HNO_3 to dissolve the fusion cake and heat to 120°C until dissolved. Transfer the dissolved cake solution to a beaker and rinse the dish twice with 16 M HNO_3. Place fusion solution on a stirring hot plate and examine the sample with a flashlight in a darkened

room to ensure complete dissolution of the fusion cake. **NOTE:** The volume of the fusion solution should not be more than 25 mL if 16 M HNO_3 is used for dissolution.

7.8.9 The fused solution may then be added to the original dissolved sample solution, the filtrate collected in Step 7.8.1.

8. Sample Storage

8.1. Cool the sample to room temperature and transfer to a tared bottle of appropriate size. Record the tare weight on the bottle. Rinse the beaker with 6-8 M HCl and pour rinse into the bottle. Repeat twice.

8.2. Using 6-8 M HCl, bring the solution level up to the nearest 25 g of weight. Record the solution weight on the control sheet and on the bottle. Mix solution thoroughly.

9. Sample Aliquot Selection for Radiochemical Analysis

9.1. Determine the sample aliquot volume required to detect the analyte of interest from the estimated gross concentration of alpha activity and ash weight of the tissue.

9.2. Tare the beaker selected to contain the aliquot volume and transfer an appropriate aliquot. Record the sample weight.

9.3. For each batch of 8 to 10 samples being run, weigh an aliquot of the appropriate quality control solution into separate beaker.

9.4. Add tracers. Make an appropriate blank, adding 75 mL of 6-8 M HCl into separate beaker.

NOTE: If very large sample aliquots are taken for uranium analysis, larger amounts of HCl should be added to blank.

9.5. Add appropriate tracers to aliquot.

CAUTION: Some tracers contain isotopes which may interfere with analysis depending on the methods used.

9.6. Place the sample beaker on a hot plate with a surface temperature of 100°C. Take the sample to dryness. Do not bake. Allow the sample to cool.

9.7. Proceed to appropriate procedure for isolation from prepared tissue solutions.

10. Source Material

10.1. H. A. Boyd, B. C. Eutsler, and J.F. McInroy, "Determination of Americium and Plutonium in Autopsy Tissue: Methods and Problems," in Actinides in Man and Animals, Proceedings of the Snowbird Actinide Workshop, Oct 15-17, 1979, M. E. Wrenn, scientific editor (R. D. Press, Salt Lake City, Utah, 1981), pp. 43-52.

10.2. LANL Procedures manual. RESL Procedure. Claude Sill's Method.