GEOLOGICAL SURVEY CIRCULAR 830

Palynomorph Preparation Procedures Currently Used in the Paleontology and Stratigraphy Laboratories, U.S. Geological Survey

Palynomorph Preparation Procedures Currently Used in the Paleontology and Stratigraphy Laboratories, U.S. Geological Survey

By L. Imogene Doher

GEOLOGICAL SURVEY CIRCULAR 830

1980

United States Department of the Interior
CECIL D. ANDRUS, *Secretary*

Geological Survey
H. William Menard, *Director*

Free on application to the Branch of Distribution, U.S. Geological Survey, 604 South Pickett Street, Alexandria, VA 22304

CONTENTS

ILLUSTRATIONS

TABLE

Table 1. Sequence of treatment of different sediment types

[This is a guide for technicians and is not necessarily limiting in scope; variation in the sequence of treatment may be necessary or desirable for some samples]

Sequence	Treatment	Page
	Silts, shales, carbonates, cherts, welded tuffs, and other siliceous rocks	
1	Cleaning the sample	2
2	Breaking down the sample	2
3	Removal of carbonates	3
4	Removal of silica and silicates	4
5	Oxidation: Organic residues—Schulze's Solution	8
6	Release of palynomorphs and humic compounds: Coals and organic residues—KOH procedure	9
7	Separation of organic residues by zinc bromide flotation	12
8	Recovery of dinoflagellates	14
9	Final cleaning of residues	15
10	Treatment of palynomorphs—Staining	20
	OR: Bleaching	21
11	Slide preparation	22
	Peats and modern soils	
1	Release of palynomorphs and humic compounds: Peats and modern soils—KOH procedure	10
2	Separation of organic residues by zinc bromide flotation	12
	OR: Final cleaning of residues	15
3	Treatment of palynomorphs—Staining	20
	OR: Bleaching	21
4	Slide preparation	22
	Lignites and coals	
1	Cleaning the sample	2
2	Breaking down the sample	2
3	Oxidation: Other coals—Schulze's Solution	7
4	Release of palynomorphs and humic compounds: Coals and organic residues—KOH procedure	9
5	Separation of organic residues by zinc bromide flotation	12
6	Final cleaning of residues	15
7	Treatment of palynomorphs—Staining	20
	OR: Bleaching	21
8	Slide preparation	22
	Paleozoic coals	
1	Cleaning the sample—Paleozoic coal samples	2
2	Breaking down the sample—Paleozoic coal samples	3
3	Oxidation—Paleozoic coals	6
4	Release of palynomorphs and humic compounds: Paleozoic coals—KOH procedure (see also: Weathered Paleozoic coals—KOH procedure)	8
5	Slide preparation—Mounting in canada balsam	24

INTRODUCTION

This manual represents a compilation of procedures currently in use at the U.S. Geological Survey pollen laboratory in Denver, Colorado. Most of the techniques described did not originate in this laboratory but have been gathered from the various sources referenced; techniques are subject to continuing revision and improvement. Subsequent editions of this manual are anticipated as the evolution of techniques progresses.

Although the procedures have been given in detail for the benefit of beginning technicians, it should be emphasized that in both choosing and carrying out procedures, a large amount of flexibility is desirable. (See table 1). The treatment of each sample must be tailored to its individual characteristics; only experience can suggest the proper variations.

To organize the instructions as much as possible, the text is formatted as follows: procedures are numbered and shown in the left column; additional details, precautions and comments are shown in the right column after corresponding numbers or letters in parentheses. General explanatory material, special notes, important cautions, and other miscellaneous information pertaining to a set of instructions appears in a headnote immediately before the start of the instructions.

PRELIMINARY TREATMENT

Cleaning the sample

MOST SAMPLES

The amount of sample processed for silts, shales, carbonates, cherts, welded tuffs, and other siliceous rocks: 30-40 grams; lignites and coals: 20 grams; Paleozoic coals: 50 grams; peats and modern soils: approximately 60 grams. Clean surface of material thoroughly by one or more of these methods.

1. Hold sample under running water and scrub with brush; blow with compressed air.

2. Scrape sample with scalpel or knife, discarding all scrapings.

3. Place crushed sample in 250-ml polyethylene beaker. Fill beaker with distilled water (tap water sometimes contaminates sample), stir, and allow to settle for approximately 10 minutes. Decant all water.

(1) After blowing with compressed air, sample may be placed on paper towel on hot plate (not over 100° F or 37.7° C) to dry.

PALEOZOIC COAL SAMPLES

Clean by only one of the following methods:

1. Outcrop samples of unweathered coal, mine samples, and diamond drill cores of coal may be washed with running water and a scrub brush or they may be placed in a beaker of water in an ultrasonic generator tank (40,000 Hz) for several minutes to remove surface contamination.

2. Place weathered samples in a beaker. Fill beaker with water. Stir and allow to settle for 3 minutes.

(1) If samples are cleaned in an ultrasonic generator tank the water should be decanted and discarded.

(2) Solid pieces settle rapidly and fines are poured off. Repeat washing until water clears. Sample should be placed in an aluminum foil tray in hood until surface is dry.

Breaking down the sample

Special equipment: ceramic mortars and pestles

MOST SAMPLES

1. Break sample into pea size fragments using mortar and pestle.

(1) Mortar and pestle should be cleaned after each use. Use an abrasive cleaner; scrub with a stiff-bristle brush and rinse under running water.

PALEOZOIC COAL SAMPLES

1. Break along the banding with a chisel-pointed hammer into sheets or plates. Break sheets, by hand, into pieces approximately one-quarter of an inch in diameter.

(1) The use of mortar and pestle should be avoided if good megaspore recovery is expected.

a. Broken outcrop or grab samples: run through a Jones sampler 1/ or riffle two times and take a suitably sized sample for maceration.

(a) Usually 40-50 grams.

b. Mine column and core samples: cut on a carborundum saw so that a complete ribbon or column sample of uniform size is obtained.

(b) Usually slightly less than one-fourth of an ordinary 2-inch core.

DISAGGREGATION

NOTE: Polycarbonate tubes are used in most of the following procedures. DO NOT use glacial acetic acid, acetone, undiluted nitric acid, methanol, ethanol, or xlyene in polycarbonate tubes. These reagents react with polycarbonate tubes and will destroy them.

Removal of carbonates—HCl procedure

1. To clean sample in 250-ml polyethylene or Pyrex beaker (Pyrex if heat will be used) add 10 pct aqueous hydrochloric acid (HCl) to slightly more than cover sample and stir.

(1) If no reaction (i.e. bubbling) occurs using 10 pct aqueous HCl, proceed to "Removal of silica and silicates, HF procedure" (p. 4).

2. If a reaction occurs, add 100 ml of 10 pct HCl to beaker. Stir frequently.

(2) If sediment threatens to foam out of beaker, add a few drops of water to slow reaction. A hot plate may be used to speed the reaction. Heat must be used when dissolving dolomites.

3. In order that disaggregated particles do not remain in HCl for too long, after 15 minutes decant into a 100-ml centrifuge tube, add distilled water to fill tube, centrifuge approximately 2 minutes at 2500 rpm, and decant. Add 10 ml distilled water to tube, and agitate; fill tube with distilled water; centrifuge and decant. Add 100 ml 10 pct HCl to beaker and leave for 15 minutes longer. Repeat decanting into tube, washing and adding fresh 10 pct HCl to beaker until reaction ceases.

4. After all reaction has ceased decant into the 100-ml tube (leaving large particles in beaker); centrifuge and decant.

(4) Repeat this step, centrifuging and decanting, until all material in beaker (except large particles) is transferred.

1/ The use of commercial trade names is for descriptive purposes only and does not constitute endorsement of these products by the U.S. Geological Survey.

5. Add about 100 ml distilled water to material remaining in beaker. Allow larger particles to settle and decant into tube; centrifuge and decant.

(5) Repeat this step 3 times to be sure material remaining in beaker is neutral. Check with pH paper to determine neutrality.

6. Add 10 ml distilled water to tube, mix, then fill tube with distilled water, centrifuge and decant.

(6) Repeat washing (usually 6-8 times) until liquid is neutral. Check with pH paper to determine neutrality.

Removal of silica and silicates—HF procedure

NOTE: Precautions always to be observed when using hydrofluoric acid: Always use in fume hood with fan ON. Check hood with vaneometer. Vaneometer reading should be no less than 75 ft/min. Wear rubber gloves, apron, and face shield. The centrifuge should be placed directly in front of fume hood, or in hood, since HF fumes are fatal if enough are inhaled.

MOST SAMPLES

1. Place sample from mortar in 250-ml polyethylene beaker. Add a small amount of 10 pct HCl (to cover sample) to check for calcium carbonate. If a reaction occurs go back to "Removal of carbonates—HCl procedure" (p. 3).

(1) About 100 ml of hydrofluoric acid (HF) is sufficient to disaggregate the sample. If all Ca salts are not removed with 10 pct HCl before adding HF to the sample, a precipitate will appear. This is usually CaF_2 and is extremely insoluble ($Ksp = 4 \times 10^{-11}$). Thorough treatment with 10 pct HCl and careful washing to eliminate dissolved Ca salts before adding HF is very important.

2. After "Removal of carbonates—HCl procedure", step 6, add a small amount of HF to the 100-ml tube; mix and decant into original 250-ml polyethylene beaker. Add more HF to rinse tube until all residue is transferred.

3. Place beaker on platform rotator.

(3) If no rotator is available, using a polyethylene stirring rod, stir the sample at 5-minute intervals.

4. If no reaction occurs with the addition of 10 pct HCl, allow sample to settle 5 minutes and decant the HCl. Add HF slowly (about 100 ml, depending upon size of sample) while stirring. If a violent reaction occurs and the sediment threatens to foam out of the beaker, squirt distilled water into the beaker (from a wash bottle) or place sample in cold water bath while adding HF. Place beaker on platform rotator. (This constant agitation will speed disaggregation of the sample.)

(4) Continue agitation until the sample is disaggregated. The length of time that sample is left in HF will depend upon the volume of the sample and the lithology. Most samples will be disaggregated within 1-2 hours. Since HF seems to have a corrosive action on pollen and spores it is best to stir constantly until the sample is disaggregated and wash immediately. (Some samples may have to remain in HF overnight or sometimes for days to disaggregate. These samples, however, do not usually contain very good pollen or spores.)

5. When the sample is disaggregated (fine sediment settled on bottom of beaker), add distilled water to fill beaker; allow to settle for one hour and carefully decant about half the liquid.

(5) Alternate Method: When the sample is disaggregated, add distilled water to fill beaker. Decant into 100-ml polycarbonate centrifuge tube; centrifuge and decant. Repeat decanting into tube, centrifuging and decanting, until all liquid is transferred from beaker to tube. NOTE: Large, heavy particles on bottom of beaker should not be decanted into tube. These remain in beaker for later treatment. After all floating material is transferred to tube, centrifuged and decanted, agitate sediment in tube with mixer, fill with distilled water, centrifuge, and decant. Repeat until the pH of the water is neutral.

6. Again add distilled water to fill beaker, let settle one hour, and decant about half of the liquid.

(6) This step may be repeated several times.

7. After the second decantation, transfer residue to a 100-ml polycarbonate centrifuge tube, leaving large, heavy particles in beaker. Fill tube with distilled water; centrifuge and decant.

(7) Repeat until all material (except large, heavy particles) has been transferred to tube.

8. Add 5 ml distilled water to sediment in tube, stir; fill tube with distilled water. Centrifuge and decant.

(8) Repeat washing until the pH of the water is neutral.

9. Optical Check. Using a dropper, place a drop of the washed sediment on a slide with a drop of distilled water and check with microscope to determine whether there are large organic aggregates that should be broken down.

10. If maceration is fairly complete, but large aggregates of organic material remain, see "Oxidation: Organic residues—Schulze's Solution" (p. 8).

11. If maceration is complete and palynomorphs seem clean, proceed to "Separation of organic residues by zinc bromide flotation" (p. 12).

PALEOZOIC COAL SAMPLES

If silica is observed in water mounts following the procedures under "Release of palynomorphs and humic compounds: Paleozoic coals—KOH procedure" step 4 (p. 8), perform the following procedures:

1. Transfer residue to 250-ml polyethylene beaker. Add HF to cover sample and stir.

(1) Observe all precautions noted on page 4! HF has a corrosive action on spores and pollen, so the treatment should be as short as possible. Two to three minutes should be sufficient.

2. After two to three minutes transfer sediment to 100-ml centrifuge tube. Add distilled water to fill tube; centrifuge and decant.

(2) Centrifuge should be in or directly in front of hood because inhalation of HF fumes can be fatal.

3. Add water to fill tube; stir, centrifuge and decant.

(3) Repeat washing until water is neutral.

OXIDATION

NOTE: Oxidation procedures should always be carried out in hood. DO NOT BREATHE the gases given off during oxidation as they are toxic. Wear rubber gloves, apron, and face shield. CAUTION: The presence of pyrite in a sample may cause an explosion when Schulze's Solution is added. Always have a beaker of cold water available to dilute the mixture if necessary.

Paleozoic coals—Schulze's Solution

1. Place cleaned sample in 1-quart mason jar.

2. Pour a very small amount of Schulze's Solution into jar (enough to wet sample).

(2) Preparation of Schulze's Solution: For low rank coals and organic residues mix one part reagent grade HNO_3 (70 pct) with one part of a saturated aqueous solution of $KClO_3$ (1:1). For high rank coals and very resistant organic residues mix two (2:1) or three (3:1) parts reagent grade HNO_3 with one part of a saturated aqueous solution of $KClO_3$. (The use of a higher concentration of HNO_3 on high rank coals can reduce time required to oxidize samples and may produce better results.)

3. If no extreme reaction occurs it is safe to pour in 200-250 ml Schulze's Solution.

(3) In coals of high volatile A rank and higher, the reaction to 3:1 Schulze's Solution may be slight. (A slight reaction is indicated when a light yellow gas is released on first contact with Schulze's Solution and for the first minute.) Little or no reaction to Schulze's Solution usually indicates a long period of oxidation will be required. Better spore recovery may be obtained through the use of a greater concentration of HNO_3. The use of fuming HNO_3 (90 pct), in place of reagent grade HNO_3 (70 pct), is recommended.

4. Stir sample at intervals with a glass rod.

5. Optical Check: After a suitable interval (depending upon the speed of the reaction) withdraw a small amount of liquid with a dropper and put it into a 15-ml Pyrex centrifuge tube to which about 10 ml of water has been added; centrifuge and decant. Place one drop of the washed sediment on a slide, add one drop of 5 pct KOH and cover with a cover glass. Check the degree of oxidation with microscope.

(5) If palynomorphs are released with the addition of 5 pct KOH to the drop of sediment and if they are clean and sufficiently light in color, then the oxidation process is completed.

6. When coal pieces are softened and when microscope check indicates that the oxidation process is complete add water to fill jar.

(6) If sample is not completely oxidized but coal pieces are slightly soft, the addition of hot water to the mason jar often will finish the oxidation phase of the maceration. Softening of coal is determined by resistance to pressure from the glass rod.

7. After a settling period of 3 hours siphon water from sample.

8. Add water to fill jar. Let settle 3 hours and repeat siphoning process.

(8) This step must be repeated until the liquid is essentially neutral. It may be necessary to repeat the process as few as 6 times or as many as 10 or 12 times depending on the amount of sample and the concentration of HNO_3 used.

9. Proceed to "Release of palynomorphs and humic compounds: Paleozoic Coals—KOH procedure" (p. 8).

Other coals—Schulze's Solution (from Schulze, 1855)

Treatment of samples with Schulze's Solution should always be done in a fume hood. Have distilled water at hand. DO NOT BREATHE the gases given off during oxidation as they are toxic.

1. Place cleaned sample in 250-ml polyethylene beaker. Cover sample with saturated aqueous solution of $KClO_3$ or $NaClO_3$ (about 30 ml), and add an equal amount of HNO_3 for moderate treatment (1:1).

(1) If sediment threatens to foam out of beaker a squirt or two of water will slow the reaction. For more rapid oxidation use up to 90 ml HNO_3 (3:1).

2. After 5 minutes pipette off (use dropper with bulb) a few drops of mixture and put into 15-ml Pyrex centrifuge tube containing distilled water; centrifuge and decant.

(2) If sample reacts vigorously (foaming yellow-brown gas given off), the reaction should be stopped after 10-15 minutes. If sample reacts little or not at all, it may be left overnight or as long as one week. If a sample must be left for more than a day, the spent Schulze's Solution should be decanted and a fresh solution added every day that the sample must be left.

3. Using dropper, place a drop of sediment from centrifuge tube onto a slide and add one drop of 5 pct KOH (aqueous). Place cover glass over and check for degree of oxidation with microscope.

(3) If fragments do not break down (no brown color given off) when 5 pct KOH is added, pipette off test fractions at 15 minute intervals and check with microscope.

4. When sediments are sufficiently oxidized (dark brown color given off when 5 pct KOH is added), oxidation may be stopped by adding distilled water to fill beaker.

5. Pour material from beaker into 100-ml polycarbonate centrifuge tube which has been half filled with distilled water. Centrifuge and decant.

(5) Repeat until all material is transferred from beaker to tube. It is necessary to dilute the Schulze's Solution by filling the centrifuge tube half full of water because HNO_3 will react with the polycarbonate tube.

6. Add 10 ml distilled water to tube, agitate; fill tube with distilled water; centrifuge and decant.

(6) Repeat until water is clear and neutral.

7. Depending upon the type and condition of the sample after oxidation with Schulze's Solution, it may require treatment with a strong base such as 5 pct KOH, or with a milder base such as an organic detergent or an 80 pct acetone-water mixture. (See "Release of palynomorphs and humic compounds: Coals and organic residues—KOH procedure" (p. 9) or "Release of palynomorphs and humic compounds: Coals and organic residues—Organic detergent procedure" (p. 10).)

(7) Tschudy (1958) suggests the use of an 80 pct acetone-water mixture as a substitute for a strong base following Schulze's Solution for samples which contain degraded palynomorphs. Some samples may be treated by $ZnBr_2$ flotation (see "Separation of organic residues by zinc bromide flotation" (p. 12)) directly after treatment with Schulze's Solution.

Organic residues—Schulze's Solution

After treatment with HF many samples will require oxidation with Schulze's Solution. NOTE: Follow same precautions as in "Other coals—Schulze's Solution" (p. 7).

1. After the sample has been decanted into a 100-ml tube and washed (leaving large heavy particles in beaker), add about 10 ml of saturated solution of $KClO_3$ or $NaClO_3$ (aqueous) to sediment in tube. Agitate on mixer and transfer back to polyethylene beaker. Rinse remaining sediment from tube with about 10 ml of the saturated solution of $KClO_3$ or $NaClO_3$ and pour into beaker.

2. Follow steps 2-7 under "Other coals—Schulze's Solution" (p. 7).

RELEASE OF PALYNOMORPHS AND HUMIC COMPOUNDS

Paleozoic coals—KOH procedure

CAUTION: Use KOH with great care because it will destroy pollen and spores if treatment is continued for too long a time; two to five minutes is usually sufficient.

1. After the last siphoning of water (see "Oxidation: Paleozoic coals" step 8 (p. 7)) add 10 pct KOH (aqueous) to the sample.

(1) Amount of 10 pct KOH added depends on size of sample. A normal reaction to the base treatment is the release of a brown color in the liquid.

2. Check for spores within 3 minutes after addition of the base. To make this check use the following method (water mount): Place a drop of the sediment on a slide with a drop of water. Place cover glass over this. Check with microscope to determine if palynomorphs are free and clear or if they will need more time in KOH.

(2) Usually 15 to 30 minutes treatment with a base is sufficient to free palynomorphs. Some samples may require no more than 3-5 minutes. Periods up to an hour are used, but if microfossils are not present by that time, the sample is either not oxidized enough, is oxidized too much, or it is unfossiliferous.

3. When spores are released and are clear (as seen with the microscope), add water to fill the jar.

4. After 3 hours, siphon off water. Add water to fill jar.

(4) This step is repeated until the liquid is neutral. It may be necessary to repeat the process 6 to 12 or more times, depending upon the size of the sample and the amount of humic matter released.

5. If silica is observed in water mounts (clear, angular, glass-like shards) see "HF Technique: Paleozoic coal samples" (p. 5).

6. If no silica is present following the KOH treatment, screen the sample by washing through a 65-mesh screen.

(6) The large spores, megaspores, cuticle, and resin rodlets are retained on the screen, giving a >65-mesh residue which is stored in 2-oz poly-seal screw-cap vials in 95 pct methanol until ready to be examined. (See "Mounting in canada balsam: Translucent megaspores" (p.25).)

7. Decant the <65-mesh residue into a 100-ml polycarbonate centrifuge tube; centrifuge and decant.

(7) Continue decanting and centrifuging until all material is transferred to tubes.

8. Add 10 ml water to tube, stir; fill tube with water; centrifuge and decant.

(8) Repeat washing until water is clear.

9. Transfer residue to 15-ml Pyrex centrifuge tube; fill with water; centrifuge and decant.

10. Proceed to "Mounting in canada balsam" step 1 (p. 24).

Weathered Paleozoic coals-KOH procedure

1. Place 1/4 of the riffled sample in a 250-ml Pyrex beaker and add 10 pct KOH (aqueous) to cover. If a brown color is released, wait 5 minutes. With a dropper, place one drop of the sediment on a slide and add one drop of water. Put a cover glass over this and check with microscope.

(1) If no reaction occurs with the addition of KOH, it is necessary to oxidize the sample. (See "Other coals—Schulze's Solution" (p. 7).) If abundant spores and pollen grains are present after the KOH treatment, it is unnecessary to oxidize the sample with Schulze's Solution.

2. Check with microscope every five minutes to ascertain condition of spores. When spores are free and clear add water to fill beaker.

(2) If spores and pollen are present but appear thin, try washing sample in 10 pct KOH and halting the reaction almost immediately. If spores and pollen still appear thin, try a dilute solution of organic detergent in place of the 10 pct KOH. (See "Release of palynomorphs and humic compounds: Coals and organic residues—Organic detergent procedure" (p. 10).) When palynomorphs are free, proceed to step 3.

3. Transfer material to 100-ml centrifuge tube, centrifuge and decant. Add water to residue; stir; centrifuge and decant.

(3) Repeat washing until water is clear and neutral.

4. Screen sample by washing through a 65-mesh screen.

(4) Store >65-mesh residue as in step 6 of "Release of palynomorphs and humic compounds: Paleozoic coals—KOH procedure" (p. 8).

5. Proceed to step 7 of "Release of palynomorphs and humic compounds: Paleozoic coals—KOH procedure" (p. 8).

Coals and organic residues—KOH procedure (from Kosanke, 1950)

This treatment usually follows Schulze's Solution for coals and black organic residues. CAUTION: Use KOH with great care because it will destroy pollen and spores if treatment is continued for too long a time; two to five minutes is usually sufficient.

1. After thorough washing as in step 7 of "Other coals—Schulze's Solution" (p. 7), add 5 pct KOH (aqueous) to 100-ml tube to cover sediment and stir.

2. Fill tube with 5 pct KOH; stir, centrifuge and decant.

(2) KOH may be heated before adding to the tube. This will speed the reaction. This step should take about three to five minutes.

3. Fill tube with distilled water; stir, centrifuge and decant.

(3) Warm water, if used in this step, will speed the process. Repeat washing until liquid is clear and neutral.

4. If there are large pieces of debris present in the sediment it may be screened through a 50-mesh stainless steel screen.

(4) The >50-mesh fraction should be checked microscopically for megaspores. The stainless steel screen should be flamed after use to prevent cross-contamination.

5. If megaspores are present the >50-mesh fraction should be stored in 2-oz poly-seal screw-cap vials in 95 pct methanol until ready to be examined. (See "Mounting in canada balsam: Translucent megaspores" (p.25).)

6. Proceed to "Separation of organic residues by zinc bromide flotation" (p.12).

Coals and organic residues—Organic detergent procedure

In samples with oxidized or corroded palynomorphs, use the following procedure after treatment with Schulze's Solution.

1. After thorough washing of residue and when liquid is neutral add about 20 ml warm organic detergent solution to sediment in 100-ml polycarbonate tube. Mix well.

(1) Prepare organic detergent solution by adding 3 grams of detergent (for example, Labtone) to 1000 ml distilled water (pH approximately 9); stir to mix thoroughly and warm on hot plate.

2. Fill tube with warm organic detergent solution; centrifuge and decant.

3. Fill tube with warm distilled water; stir, centrifuge and decant.

(3) Repeat washing until water is clear and neutral.

Coals and organic residues—Sodium metasilicate procedure

In some samples palynomorphs may have foreign material clinging to them after the foregoing preparations. The use of a sodium metasilicate ("metso") solution will, in many cases, release this material from the grains. It is advisable to place a drop of the residue on a slide, add one drop of metso, and check microscopically. It will be obvious under the microscope whether or not the metso is effective in cleaning the fossils. NOTE: Combined with short centrifuging this method will remove fine material from sediment.

1. Add 5 ml of metso solution to residue in 15-ml Pyrex centrifuge tube. Mix.

(1) Prepare metso solution by adding 2 grams of sodium metasilicate crystals to 500 ml of distilled water.

2. Fill tube with distilled water; stir, centrifuge, and decant.

(2) Repeat washing until decant is clear and neutral.

Peats and modern soils—KOH procedure

1. Place dry sample in 600-ml Pyrex beaker.

2. Add 5 pct KOH (aqueous) to slightly more than cover.

3. Place on hot plate and bring to boil. Simmer 1-2 minutes.

4. Remove from hot plate, fill beaker with distilled water and stir.

5. Transfer mixture from beaker to 100-ml polycarbonate centrifuge tube; centrifuge and decant.

(5) If there are large clumps of debris present, residues should be washed through a 50-mesh screen into the 100-ml tube. Discard the >50 mesh residue.

6. After all material is transferred to the centrifuge tube, add distilled water to fill tube; centrifuge and decant.

(6) Repeat washing until water is clear and neutral.

7. If residue is sandy, either (1) treat with HF (see "Removal of silica and silicates: HF procedure—Paleozoic coal samples" (p. 5)); or (2) proceed to "Separation of organic residues by zinc bromide flotation" (p.12).

8. Acetylate if necessary to remove cellulose content of palynomorphs. (See "Dissolution of celluloses by acetylation" below.)

DISSOLUTION OF CELLULOSES BY ACETYLATION

Precautions when mixing and using acetylation mixture: KEEP ACETYLATION MIXTURE AWAY FROM WATER. Wear rubber gloves, apron, and face shield. Mix reagent in hood. Examine Pyrex tubes (before using) for cracks or leaks, because leakage of the acetylation mixture into water will cause an explosion.

1. Add glacial acetic acid (CH_3COOH) to residues in 15-ml Pyrex centrifuge tube to fill; stir, centrifuge, and decant.

(1) Purpose of glacial acetic acid washes is to dehydrate residues.

2. Repeat glacial acetic acid wash.

3. Add acetylation reagent to tube (approximately 5 ml) and stir gently. Leave stirring rod in tube.

(3) Acetylation reagent: Nine parts acetic anhydride $(CH_3CO)_2O$ and one part sulfuric acid (H_2SO_4). Add acid to anhydride. Mix fresh just prior to use as reagent will take on water if left standing.

4. Place tube carefully in gently boiling water bath for 3-1/2 to 4 minutes. Stir occasionally.

(4) CAUTION: Do not allow water to boil high next to centrifuge tube lip; water produces a violent exothermic reaction with the reagent.

5. Remove from water bath and add glacial acetic acid to fill tube. Stir, centrifuge, and decant.

6. Repeat glacial acetic acid wash as in step 1.

7. Add distilled water to fill tube; stir, centrifuge, and decant.

(7) Repeat washing with distilled water until there is no odor of acetic acid (6-8 washings).

SEPARATION OF ORGANIC RESIDUES BY ZINC BROMIDE FLOTATION
(from Funkhouser and Evitt, 1959)

1. Remove as much water from sediment as possible by centrifuging and decanting.

2. To the sediment in 100-ml centrifuge tube add $ZnBr_2$ solution to cover sediment about 1/2 inch and stir thoroughly, using vortex mixer. It is necessary to mix sediment and $ZnBr_2$ solution completely, because some sediments clump when the $ZnBr_2$ solution is added and a clean and complete separation of the organic fractions cannot be achieved.

3. Fold clear plastic tubing (thin-walled Tygon—see Funkhouser and Evitt, 1959, p. 372) and place in 50-ml polycarbonate tube which has been lubricated with glycerin to facilitate removal.

4. Pour well-mixed sediment suspension into tubing, equalizing the amounts on each side. Rinse the 100-ml tube with more $ZnBr_2$ solution and pour into tubing so that all sediment is transferred; after transfer rinse tube well with water. If the total amount of sediment in the tube measures more than 20 mm deep (as measured on the tube) the sample should be divided in half and floated in two tubes.

5. Centrifuge 20 minutes at 2500 rpm. (International Equipment Co. centrifuge models 1 and 2 will operate at such high speeds.) If safety limits do not permit this speed use a lower speed for a longer time to achieve the same results.

(1) To acidify sediment before adding $ZnBr_2$ solution, add 10 ml of 10 pct HCl (aqueous) to sediment in 100-ml centrifuge tube; stir thoroughly and fill tube with distilled water; centrifuge and decant. It is necessary to acidify the sediment because the addition of $ZnBr_2$ solution to sediment in which some water remains may cause a white precipitate (zinc hydroxide) to appear.

(2) $ZnBr_2$ solution: Prepare by adding one part distilled water (which includes a small amount of 10 pct aqueous HCl) to two parts $ZnBr_2$ (using hydrometer), making a specific gravity of approximately 2.042. The solution must be acidified or a white precipitate (zinc hydroxide—$Zn(OH)_2$) will appear when water is added to $ZnBr_2$.

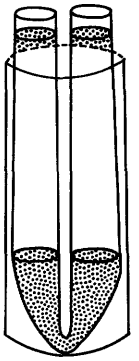


Figure 1.—Separation of sediment after centrifuging (step 5).

(5) It is necessary to place tubes in such a manner that both sides of the tubing are in line with the direction of rotation. The tubing will fold over at the top and spill part of the contents if placed at right angles to direction of rotation.

6. After centrifuging, remove tubing from the centrifuge tube; pinch tubing below the float with fingers or needle nose pliers and pour into the 100-ml tube which originally contained the sediment.

7. Still pinching the tubing, rinse top out with 10 pct HCl and pour this into the centrifuge tube, also.

(7) After transferring the float and rinsing the tubing with 10 pct HCl, the sediment and the remaining $ZnBr_2$ solution may be set aside for later checking to see if palynomorphs remained with the heavy mineral portion of the residue.

8. Add about 20 ml 10 pct HCl (to acidify wash water and avoid a precipitate of $Zn(OH)_2$) to float in 100-ml tube. Fill remainder of tube with distilled water and stir thoroughly. Centrifuge for 3 minutes at 2500 rpm and carefully decant. If interface forms in tube, decant only to meniscus; add more water, stir, centrifuge 3 minutes, and decant.

9. After two washes with distilled water the residue should be transferred to 15-ml Pyrex centrifuge tubes.

(9) If there are large pieces of debris present, screen the material by pouring into the 15-ml tubes through 50-mesh stainless steel screens formed to fit into small funnels. All stainless steel screens should be washed and flamed after use.

10. Wash, centrifuge, and decant 6 times.

11. If the organic residue now contains some mineral grains (derived from material adhering to the walls of the tubing in which separation was made) transfer sediment to a 15-ml polyethylene tube (with dropper). Rinse original tube with water and add to material in polyethylene tube. Fill polyethylene tube with water; stir, centrifuge, and decant.

12. Add 5 ml HF to material in polyethylene tube (in hood). Stir and leave for 5 minutes.

13. Fill tube with distilled water; centifuge, and decant.

(13) Repeat washing 6 times.

RECOVERY OF DINOFLAGELLATES

If a sample is being prepared for dinoflagellates the disaggregation and treatment remain the same as for pollen and spores through "Separation of organic residues by zinc bromide flotation" step 10 (p. 13). From that point, treatment continues as follows:

1. Using a filter assembly with a hand-operated vacuum pump (see fig. 2) place a 20-micron screen over the top of the assembly and secure with ring. Make sure that screen is not stretched tightly over opening. Screen should be depressed into a cone. Pour residue through screen, activate vacuum pump, and rinse with 10 pct methyl alcohol with wash bottle.

2. Remove screen from filter assembly and place in clean beaker. Holding screen by one corner and using a wash bottle, rinse material from screen into beaker.

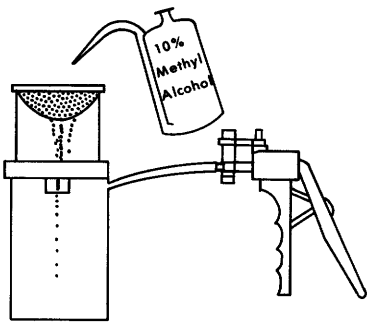
(2) Fine residue (<20 micron) may be discarded. However, if pollen and spores are also to be studied this residue should be poured into a 15-ml centrifuge tube, centrifuged, and decanted. If palynomorphs require stain, proceed to "Staining" (p. 20).

3. Transfer material from beaker to original 15-ml centrifuge tube, centrifuge, and decant.

4. Put 2-3 ml of distilled water in tube, mix on vortex mixer, fill tube with distilled water, centrifuge, and decant.

(4) Repeat washing 4 times.

5. If palynomorphs require stain, proceed to "Staining" (p. 20).



Vacuum Pump

Figure 2.—Filter assembly with vacuum pump.

FINAL CLEANING OF RESIDUES

Settling procedure A: Gravity settling in 2 tubes (removal of heavy debris)

When the preparation is completed and the sediment is ready for slide preparation, some of the heavy particles may be removed by taking advantage of their differential settling rates. By saving all residues, one may experiment with these techniques without losing portions of the sample.

1. Add 10 ml of distilled water to 15-ml tube containing sediment. Add a small amount of a wetting agent (such as Darvan #4) to prevent clumping; stir well, or place in ultrasonic generator tank for 30 seconds.

2. Allow to settle about 30 seconds.

3. Pour suspended material into a second tube, allowing only the heavy settled fragments to remain in the first tube.

4. Using a dropper, place some of these fragments on a slide and check with microscope.

(4) If palynomorphs are present in heavy settled residue, decrease settling time. If no palynomorphs are present in the heavy settled residue, it may be discarded.

5. Repeat steps 1-4, with appropriate settling interval, until residue is as free as possible of large fragments.

(5) With each change in the settling interval, microscope checks must be made after transfer.

Settling procedure B: Gravity settling with 2 tubes and dropper (removal of heavy debris)

1. Repeat step 1 of "Settling procedure A" (above).

2. Allow to settle 30 seconds.

(2) The settling period before either decanting or removal with a dropper should be either shortened or lengthened, depending upon the relative amounts of pollen and spores and larger fragments present. (Larger fragments settle more rapidly than pollen and spores.)

3. With dropper, draw material from the tube, leaving only heavy settled residue on the bottom.

4. Check some of residue with microscope.

(4) If palynomorphs are present in the heavy settled residue, decrease the settling time. If no palynomorphs are present in the residue, it may be discarded and the settling time increased.

5. Repeat steps 1-4, with appropriate settling interval, until residue is as free as possible of large fragments.

(5) With each change in the settling interval, microscope checks must be made.

Settling procedure C: Gravity settling with 4 tubes and dropper
(removal of fine debris)

This is basically the same technique as "Settling procedure B" (removal of heavy debris). This may be used to remove fine debris from samples. However, the method requires a total of four clean, 15-ml Pyrex centrifuge tubes for each sample. (See fig. 3).

1. Fill 15-ml tube containing residue with water; stir vigorously, place in holder, and allow to settle for 10 minutes.

2. With dropper, remove top half of the water, and place in second 15-ml centrifuge tube.

(1) Time intervals may be decreased or increased until almost all fine debris has been discarded.

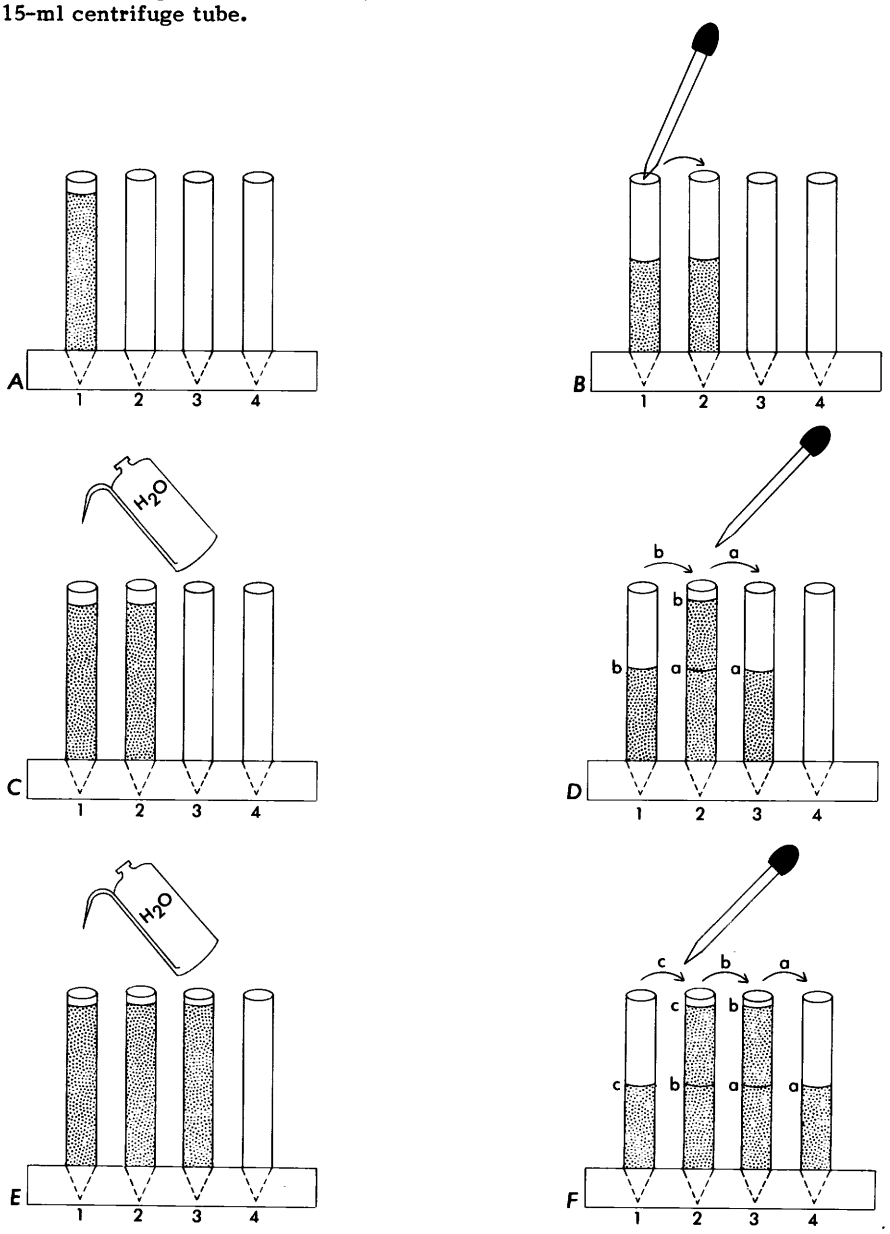


Figure 3.—Settling procedure C: Gravity settling with 4 tubes and dropper (removal of fine debris). A-F illustrate steps 1-6, respectively.

3. Fill first and second tubes with water; stir vigorously, and allow to settle for 10 minutes.

4. Remove top half of water from second tube and place in third tube. Remove top half of water from first tube and place in second tube.

5. Fill tubes with water; stir and allow to settle for 10 minutes.

6. With dropper, remove top half of water from third tube and place in fourth tube. Remove top half of water from second tube and place in third tube. Remove top half of water from first tube and place in second tube.

7. Fill first tube with water; stir, and allow to settle for 10 minutes (or longer if fine portion contained palynomorphs). Tubes 2 and 3 are already filled and should not be stirred.

8. Repeat steps 6 and 7.

(6) Now the pipetted mixture in the fourth tube should be centrifuged and checked microscopically. In most cases it will contain nothing but fine debris and may be discarded. If palynomorphs are present in the fine portion, increase settling time.

(8) Note: Repeat steps 6 and 7 until all fine debris has been discarded.

Settling procedure D: Gravity settling with alcohol washes (removal of fine debris)

Through the use of alcohol washes, much of the debris in a sample will be even more finely divided. Use the following procedure.

1. Add 2-3 ml 10 pct methanol to residue in 15-ml Pyrex centrifuge tube, stir; fill with 10 pct methanol; centrifuge and decant.

2. Add 2-3 ml 25 pct methanol to residue in tube, stir; fill tube with 25 pct methanol; centrifuge and decant.

3. Add 2-3 ml 50 pct methanol to residue in tube, stir; fill tube with 50 pct methanol; centrifuge and decant.

4. Add 2-3 ml 75 pct methanol to residue in tube, stir; fill tube with 75 pct methanol; centrifuge and decant.

5. Add 2-3 ml 95 pct methanol to residue in tube, stir; fill tube with 95 pct methanol; centrifuge and decant.

6. Add 2-3 ml 75 pct methanol to residue in tube, stir; fill tube with 75 pct methanol; centrifuge and decant.

7. Add 2-3 ml 50 pct methanol to residue in tube, stir; fill tube with 50 pct methanol; centrifuge and decant.

8. Add 2-3 ml 25 pct methanol to residue in tube, stir; fill tube with 25 pct methanol; centrifuge and decant.

9. Add 2-3 ml 10 pct methanol to residue in tube, stir; fill tube with 10 pct methanol; centrifuge and decant.

10. Add 2-3 ml water to residue in tube, stir; fill tube with water; centrifuge and decant.

11. Perform steps 1-8, "Settling procedure C" (p.16).

Short centrifuging (removal of fine debris)

Fine debris may be removed by centrifuging in 15-ml Pyrex tubes for short periods of time. Note: If a sample contains very small (10 micron) pollen or spores, short centrifuging may not be successful. Try "Settling procedure C" (p.16) instead.

1. Place tubes in centrifuge and turn it on; when centrifuge has reached 1500 rpm turn off.

(1) A small clinical centrifuge is best for this purpose.

2. Let centrifuge coast to a stop and remove tubes.

(2) DO NOT USE CENTRIFUGE BRAKE!

3. Decant into labeled beakers (one for each sample).

4. Add distilled water to tubes; stir, and repeat short centrifuging.

5. Decant into beakers a second time.

(5) Repeat washing, short centrifuging, and decanting until no float is seen in the tubes after centrifuging.

6. Check decanted portions microscopically.

(6) This can be simplified by transferring decanted material from beakers to clean 15-ml tubes, centrifuging about 2 minutes and decanting. Repeat until all material in beakers is transferred.

7. If no pollen or spores are present in the decanted portions this material may be discarded.

8. If there are a number of pollen and spores present in the decanted fractions, transfer material from second set of tubes to first set and repeat steps 1-6, increasing the centrifuging time.

(8) If there is floating material after longer centrifuging this material should be decanted and checked microscopically as in step 6.

Swirling procedure: Separation of particles according to size and specific gravity (from Funkhouser and Evitt, 1959)

The organic particles within a sample may be separated differentially according to size and specific gravity by swirling them in a watch glass using a technique similar to that used in gold panning.

1. To the sediment in a 15-ml centrifuge tube, add about 10 ml distilled water.

(1) To prevent clumping of particles: Before swirling, add about 0.25 ml (small squirt from squeeze bottle) of dispersing agent (such as a saturated solution of Darvan #4 or a non-ionic detergent) to the tube. Mix well. Place tube in ultrasonic generator tank. Agitate for 5 minutes.

2. Place a 3-inch watch glass on a smooth, level, hard, light-colored surface.

3. Place a small amount (5 ml) of the aqueous suspension in watch glass. Allow to settle for one minute.

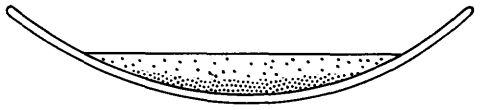


Figure 4.—Particles partly settled before swirling in watch glass.

4. Holding the edges of the watch glass between the thumb and fingers, resting the heel of the hand on surface and keeping the rim of the glass level, move the glass smoothly in circles. Circles should be about 4 mm.

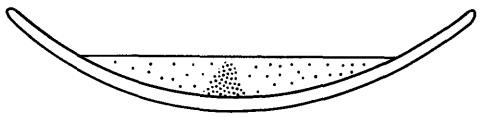


Figure 5.—Particles segregated after swirling in watch glass.

5. Tilt watch glass so the heavier particles in the center are strewn out for removal with a dropper.

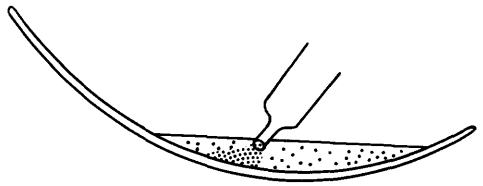


Figure 6.—Removal of heavier particles with a dropper. Watch glass tilted to strew particles out.

6. Place finer particles remaining in suspension in 15-ml tube; centrifuge, and check microscopically.

7. Repeat swirling with particles in the second watch glass. Pipette off all material held in suspension and place in 15-ml tube. Centrifuge and check microscopically.

(2) A light-colored background is helpful when removing particles from the watch glass after swirling.

(3) If a suspension is very thick and dark, with heavy, opaque particles, put a smaller amount in the watch glass to swirl at one time.

(4) Do not move glass rapidly or with a side to side motion as this will cause turbulence of the surface of the liquid.

(5) These particles may then be placed in another watch glass for even finer separation. The watch glass may be placed on the microscope stage and the sediment checked to determine success of the separation.

(6) Short centrifuging of these finer particles may help further concentrate the fossils. (See "Short centrifuging "(p. 18).) Note: If there is a great size difference among the pollen and spores present, the larger ones will be in the heavy fraction and the smaller ones in the fine fraction.

8. Check remainder of material in second watch glass microscopically.

(8) These particles should be heavy fragments and may be discarded. If there are a number of palynomorphs remaining in this fraction repeat steps 1-5 until a clean separation is achieved.

TREATMENT OF PALYNOMORPHS

Staining

After the various oxidation procedures outlined in this manual, many palynomorphs become translucent and require staining. Preparation of staining solutions: Triturate 0.1 gram Safranin O or Bismarck Brown Y in 100 ml ethanol (absolute ethyl alcohol) or 90 pct methanol (methyl alcohol). Alternate method: Add 0.1 gram of Safranin O or Bismarck Brown Y to 100 ml 90 pct ethanol or methanol in storage bottle (ground glass seal) and place bottle in ultrasonic generator tank. Agitate for 15 minutes.

1. Fill 15-ml Pyrex tube containing residue to be stained with 10 pct methanol. Stir, centrifuge, and decant.

(1) It is necessary to begin methanol washes with dilute aqueous solutions and ever succeeding more concentrated solutions of methanol to prevent clumping of sediments. If 95 pct methanol is added directly, most sediments will clump and are almost impossible to disperse.

2. Fill tube with 25 pct methanol; stir, centrifuge, and decant.

3. Fill tube with 50 pct methanol; stir, centrifuge, and decant.

4. Add 5 ml 75 pct methanol to tube. Stir. Add 5 ml Safranin O or Bismarck Brown Y staining solution to tube. Stir.

5. After 15-min interval, using a dropper, remove small amount of residue, dilute it with a drop of water, and check microscopically to determine degree of staining of palynomorphs.

(5) Some palynomorphs are more resistant to stain than others so microscope checks must be made.

6. When grains are sufficiently overstained, add 75 pct methanol to fill tube; centrifuge, and decant.

(6) The grains must be overstained since further washing with methanol or ethanol is necessary before mounting and these reagents will destain palynomorphs.

7. Add 2-3 ml 95 pct methanol to tube; stir and fill with 95 pct methanol. Centrifuge and decant.

8. Proceed to step 6, "Mounting in Vinylite AYAF" (p.22) or step 5, "Mounting in canada balsam" (p.24).

Occasionally palynomorphs may be opaque even after oxidation procedures. Structural features are obscured, so it is necessary to bleach such grains in order to make morphologic determinations and photomicrographs. In many cases, palynomorphs may be opaque but corroded. Because these grains will sometimes disintegrate with the use of sodium hypochlorite, a milder bleach such as hydrogen peroxide (30 pct) is recommended.

SODIUM HYPOCHLORITE PROCEDURE

CAUTION: Sodium hypochlorite (ordinary household aqueous bleach—5.25 pct by weight) corrodes pollen and spores over prolonged periods and may cause changes in grain size. Use great care in controlling the length of time that samples are bleached.

1. To residue in 15-ml Pyrex centrifuge tube add 5 ml distilled water; stir and add 5 ml sodium hypochlorite.

(1) Further dilution of the sodium hypochlorite can be made if the mixture seems to be damaging the grains. The check with the microscope (step 3) will determine if this is necessary.

2. Add 2 drops of 10 pct HCl (aqueous) and stir.

(2) For some samples this bleaching process may take no more than a few seconds—in others, 2-3 hours or overnight.

3. Place a drop of the suspension on a slide; place cover glass over drop and check with microscope.

(3) A cover glass is necessary because fumes may harm the objective. Several checks may be necessary before the bleaching is completed.

4. When grains are the desired color fill tube with distilled water; stir, centrifuge, and decant.

(4) Repeat washing 3 times.

5. Add 5 ml metso solution to tube, stir, and fill tube with distilled water. Centrifuge and decant.

(5) For preparation of metso solution see "Sodium metasilicate procedure" step 1 (p. 10).

6. Add distilled water to tube; stir, centrifuge, and decant.

(6) Repeat washing until water is clear.

HYDROGEN PEROXIDE PROCEDURE

1. Add 5 ml 30 pct hydrogen peroxide to sediment in 15-ml Pyrex centrifuge tube.

2. Dilute by adding 5 ml distilled water. Stir.

3. Place a drop of the suspension on a slide, cover with cover glass, and check with microscope.

(3) Use of a cover glass is necessary so that fumes do not harm the objective. Several checks may be necessary before the bleaching is completed.

4. When grains are the desired color fill tube with distilled water; stir, centrifuge, and decant.

(4) Repeat washing 6 times.

SLIDE PREPARATION

Sampling of Residues

Accuracy in sampling the total residue and in proportional representation of materials is essential to meaningful interpretation of the assemblage. If the residue in the vial is in a small amount of liquid a representative sample can be insured by agitating the sediment. This may be done by expelling air from the dropper bulb through the liquid several times before withdrawing the drop for mounting. Residues should be centrifuged before slide preparation until almost all liquid is removed.

Mounting in Vinylite AYAF

Some individuals prefer this mounting medium for palynomorphs because it is possible to mount palynomorphs in the same plane on the cover glass, and, thus, traversing slides is made simpler. It is not recommended that pollen and spores be stained during mounting through the use of AYAF to which stain has been added. This method results in a background of much the same color as the palynomorphs, making photomicrography very difficult. Preparation of Vinylite AYAF (refractive index = 1.46): Add 100 ml 95 pct methanol or ethanol to 20 grams AYAF (prepare in a bottle which can be tightly sealed). Add 4 ml dibutyl phthalate (plasticiser). Let stand until AYAF pellets are dissolved (this will take 2-3 days). Agitate bottle frequently to speed mixing. Note: Do not use canada balsam for mounting AYAF slides because AYAF will become cloudy after a period of time.

1. Add 2-3 ml 10 pct methanol to residue in 15-ml Pyrex centrifuge tube, stir; fill with 10 pct methanol; centrifuge and decant.

(1) If residue has been through staining process (see "Staining" p. 20 , steps 1-3 are omitted.

2. Add 2-3 ml 25 pct methanol to residue in tube, stir; fill tube with 25 pct methanol; centrifuge and decant.

3. Add 2-3 ml 50 pct methanol to residue in tube, stir; fill tube with 50 pct methanol; centrifuge and decant.

4. Add 2-3 ml 75 pct methanol to residue in tube, stir; fill tube with 75 pct methanol; centrifuge and decant.

5. Add 2-3 ml 95 pct methanol to residue in tube, stir; fill tube with 95 pct methanol; centrifuge and decant.

6. Add 2-3 ml absolute methanol to tube, stir; fill tube with absolute methanol; centrifuge and decant.

(6) Excess water remaining in the preparation will cause the mixture on the cover glass to become white and cloudy.

7. With dropper, transfer sediment to storage vial (see "Storage in Vinylite AYAF" p. 28 ; rinse centrifuge tube with absolute methanol and add this to the vial.

(7) An efficient method of removing alcohol from the sediment involves filling the storage vial with the sediment and absolute methanol and centrifuging the vial (with cap on). (A smaller, empty vial, placed in the bottom of the carrier, will prevent the top vial from slipping downward and wedging itself into the carrier.) Using a dropper with bulb, remove the methanol leaving only the sediment and a very small amount of alcohol.

8. Add approximately 2 ml AYAF solution to storage vial, mix well, screw on cap, and label. Let stand 4-5 hours or overnight before making slides.

9. Using a dropper, stir AYAF-sediment mixture by expelling air through the dropper. Place a drop of AYAF-sediment mixture on a clean cover glass.

(9) If residue is very concentrated the drop should be smaller than a drop from a sparse suspension.

10. Add 3-4 drops AYAF from the dropper bottle and mix with the dropper used to remove sediment and AYAF from the storage vial.

(10) Number of drops of AYAF solution depends on size of cover glass and concentration of residue: 22x30 mm cover glass — 3 drops of AYAF; 22x40 mm cover glass—4 drops of AYAF. If a suspension is quite sparse it is not necessary to place the drops of AYAF on the cover glass. Simply place 3-4 drops of the sparse suspension on the cover glass and proceed to step 11. (Droppers used in mounting with AYAF must be rinsed in absolute methanol before washing.)

11. Place a second clean cover glass directly over the first.

(11) The mixture will spread evenly to the edges of the cover glass.

12. Hold the sandwich by its long edges and pull the two cover glasses apart laterally.

13. Place cover glass prepared-side up on slide and put on slide warmer (40°C). Allow to dry at least 2 hours before mounting.

(13) Slides should be labeled temporarily with a wax pencil, felt pen, india ink, or with a tape label. (Steps 13-17 apply to both halves of the sandwich.)

14. Remove slide and cover glass from warmer, and put approximately 3-4 drops (amount depends on size of cover glass) of Histoclad mounting medium on the slide. Invert cover glass and, beginning with one end at the edge of the mounting medium, allow it to drop slowly into the medium.

(14) Do not allow cover glass to fall immediately but let down gradually so that medium spreads without leaving air pockets. In order to elevate the cover glass on one end and allow it to drop slowly, a teasing needle or toothpick may be held under the end of the cover glass. The consistency of the Histoclad should be such that it flows evenly under the lowered cover glass. This procedure should take approximately 10 seconds if the Histoclad is of the proper consistency. If the Histoclad is too thick there is a tendency for air bubbles to form. Use xylene or toluene to thin Histoclad.

15. Allow slide to cure at room temperature for 4-5 days.

(15) After 4-5 days the slide will be dry enough to be used with care but curing time may vary from 2-3 weeks.

16. When slide is fully cured, clean any excess mounting medium from edges of cover glass with a razor blade.

17. Slide is now ready for permanent labeling.

Mounting in canada balsam

<65-MESH RESIDUE

1. Add 2-3 ml 25 pct methanol to residue in 15-ml Pyrex centrifuge tube, stir. Fill tube with 25 pct methanol; centrifuge and decant.

2. Add 2-3 ml 50 pct methanol to tube; stir. Fill with 50 pct methanol; centrifuge and decant.

3. Add 2-3 ml 70 pct methanol to tube; stir. Fill with 70 pct methanol; centrifuge and decant.

4. Add 2-3 ml 95 pct methanol to tube; stir. Fill with 95 pct methanol; centrifuge and decant.

5. Add 2-3 ml absolute methanol to tube; stir. Fill with absolute methanol; centrifuge and decant.

6. Add 2-3 ml of 50-50 mixture (by volume) of absolute methanol and xylene to tube; stir. Fill tube with 50-50 mixture; centrifuge and decant.

7. With a clean, dry dropper, mix sediment by expelling air through dropper in sediment.

8. Place 4-5 drops of canada balsam in clean, dry, porcelain evaporating dish on slide warmer.

9. With dropper place 2-3 drops of sediment in evaporating dish.

10. Mix well by stirring with dropper.

11. Using dropper place one drop of balsam-sediment mixture on clean, temporarily labeled slide on slide warmer.

12. Place cover glass directly on drop.

13. Transfer remaining residue from centrifuge tube to poly-seal storage vial. Rinse tube with a 50-50 ethanol-xylene mixture and put this in vial also.

14. Leave slides on slide warmer or in warming oven until mount is set.

15. After curing, remove slides from warmer and label.

(1) If residue has been stained (see "Staining" p.20) steps 1 and 2 are omitted.

(8) Have slide warmer set at about 40°C. A disposable aluminum weighing dish may be used instead of porcelain.

(9) Amount of sediment transferred to evaporating dish with balsam depends upon whether sediment is concentrated or sparse. (If sediment is concentrated use less.)

(11) Amount of balsam-sediment mixture depends on size of cover glass. For a smaller cover glass (22 x 22 mm) use less mixture.

(12) Mixture will spread evenly to edges of cover glass. Practice will determine exactly how much is needed to spread exactly to edges of cover glass.

(13) If a porcelain evaporating dish was used in slide preparation it must be rinsed, before washing, with a 50-50 ethanol-xylene mixture to remove canada balsam. The dropper which was used to mix canada balsam and the sediment must also be rinsed with 50-50 ethanol-xylene mixture.

(14) Curing time commonly ranges from 1-2 weeks.

(15) Canada balsam slides need not be sealed.

TRANSLUCENT MEGASPORES

1. Put >65-mesh residue in petri dish with water enough to float palynomorphs.

2. With a flattened teasing needle, pick megaspores from residue and place in a second petri dish with sufficient 50 pct methanol to cover.

3. When a representative number of megaspores have been picked, decant the 50 pct methanol from dish and add 95 pct methanol.

4. Allow to settle and decant.

5. Add 100 pct ethanol. Allow to settle and decant.

6. Add 50-50 mixture of ethanol-xylene.

7. Place small dots of canada balsam on a slide.

(7) Make one dot for each megaspore to be mounted.

8. With flattened teasing needle pick megaspores from ethanol-xylene mixture and place within the canada balsam dots.

9. Allow to cure briefly on the warming plate (40°C).

(9) Thirty to sixty seconds is commonly enough time.

10. Add additional canada balsam (1-2 drops) between the megaspores and place cover glass directly over preparation.

(10) The megaspores will remain in position when the cover glass is applied; thus, an orderly arrangement of mounted megaspores is possible.

Mounting in Glycerin Jelly

Preparation of glycerin jelly: 2 grams Phenol, 66 grams glycerin liquid, 14 grams gelatin, colorless. Add gelatin to 38 ml distilled warm water. Then add glycerin. Heat very gently in hot water bath for 15-20 minutes (or until liquid starts to clear and white bubbly foam rises to top). Add phenol and heat and stir until dissolved. CAUTION: Avoid heating glycerin jelly for long periods at the boiling point. Gelatin will polymerize and the mixture will be useless.

MOST SAMPLES

1. Place 2 drops of hot glycerin jelly on a clean, warm slide (40°C) which has been temporarily labeled.

(1) If too much glycerin jelly is used the mixture will spread out from all sides of the cover glass and much of the preparation will be lost. If too little glycerin jelly is used, extensive air pockets will form during the curing process.

2. Using a clean dropper remove a small amount of aqueous residue from 15-ml centrifuge tube and drop directly on jelly.

3. Mix thoroughly with dropper tip.

(3) Keep slide warm to reduce formation of bubbles.

4. Place cover glass over mixture holding one edge up and allowing it to drop slowly until mixture spreads evenly to all edges of cover glass.

(4) A teasing needle or a toothpick may be used to hold one end of the cover glass and allow it to drop slowly.

5. Invert slide on the slide warmer on parallel strips of wood designed to hold the slide about 4-5 mm from warmer so that the cover glass does not touch the warmer. The fossils will settle to the top of the cover glass.

(5) Allow to cure for 4-5 days.

6. Clean edges of cover glass with water.

7. Seal edges of cover glass with Glyptal clear varnish No. 1202. Allow to dry 2-3 hours.

(7) Slide is now ready for permanent labeling.

DINOFLAGELLATES

If palynomorphs have been stained they must be returned to water before mounting in glycerin jelly. Follow procedure below from step 1. If palynomorphs have not been stained begin procedure below at step 6.

1. After staining is completed, add 75 pct methanol to fill centrifuge tube; centrifuge and decant.

2. Add 2-3 ml 50 pct methanol to tube; stir. Fill with 50 pct methanol; centrifuge and decant.

3. Add 2-3 ml 25 pct methanol to tube; stir. Fill with 25 pct methanol; centrifuge and decant.

4. Add 2-3 ml 10 pct. methanol to tube; stir. Fill with distilled water; centrifuge and decant.

5. Add 2-3 ml distilled water to tube; stir. Fill with distilled water; centrifuge and decant.

6. Place a clean 76x25 mm (3x1 in.) glass slide on slide warmer (40°C).

(6) Slide should be temporarily labeled.

7. Place a clean 22x30 mm cover glass on slide.

(7) A small residue yield may necessitate using a smaller cover glass.

8. Pick up cover glass and put 2 drops of warm, dilute glycerin jelly directly on cover glass.

(8) To prepare dilute glycerin jelly: Mix one part glycerin jelly with one part distilled water.

9. Using a dropper, place one drop (or adequate amount) of residue on the dilute glycerin jelly and mix well, spreading mixture to edges of cover glass.

(9) A toothpick, bent dissecting needle, or a small glass rod can be used to mix the residue and jelly.

10. Return cover glass to slide on warmer, prepared side up, and allow to dry for 30 minutes.

(10) This process allows for evaporation of all water from glycerin jelly, and also aids palynomorphs to settle in one plane, against the cover glass.

11. Remove cover glass from slide and place 2 drops of warm glycerin jelly on slide.

12. Turn cover glass over and place on the drops of glycerin jelly. The mixture should flow only to edges of cover glass.

(12) Number of drops of glycerin jelly used depends on size of cover glass. If a mixture does not flow completely to edges, add warm glycerin jelly to edges of cover glass where voids occur and the jelly will flow under the cover glass.

13. Slide should now be placed face down on the slide warmer on parallel strips of wood designed to hold the slide about 4-5 mm from the warmer so that the cover glass does not touch the warmer. The fossils will settle to the top of the cover glass.

Single Grain Mounts

In the absence of any special equipment to isolate and extract individual specimens the following procedure has been used to obtain single grain mounts of pollen and spore specimens.

1. Put a few drops of residue in a 15-ml centrifuge tube. If residue is in alcohol, wash with distilled water to remove alcohol. (See step 1, "Mounting in glycerin jelly: Dinoflagellates" (p.26).)

2. Heat a small hot plate to about 150°C. Place clean slide on hot plate.

3. Extract enough hardened glycerin jelly from bottle to make a small smear on slide and let melt.

4. Mix residue in tube with dropper. Extract a dilute representative sample from the tube with dropper and place one drop of residue on top of the melted glycerin jelly and mix immediately.

5. Remove from hot plate and allow to cool.

(5) Place a petri dish over slide to keep it from collecting dust.

6. Place slide on microscope and locate specimen to be isolated.

7. When specimen is located, turn slide over so that the glycerin jelly is facing downward and move the condensor of the microscope as close as possible to the slide without touching the jelly.

(7) In this manner a fine point of light is obtained and encircles the specimen.

8. Using ink and a quill pen, place a very small dot of ink on the top of the upside-down slide where the point of light shines through the slide.

(8) This allows you to mark the exact spot where the specimen lies on the slide.

9. Place the slide on the stereoscope, highest power. Cut away excess glycerin and spores on the slide, leaving the single specimen mounted in a bit of glycerin jelly.

(9) To cut away the excess use a new razor blade.

10. To mount a cover glass over the specimen place the slide on a warmer. While melting, put a minute amount of melted glycerin jelly on a cover glass and place it directly over the ink dot, thus sealing the single grain on the slide.

11. To completely seal the grain, melt some carnauba wax on the warmer; place the slide on warmer until warm (enough to melt wax but not warm enough to melt glycerin jelly); place a drop of the wax at the edge of the cover glass, allowing it to flow under the cover glass.

(11) It takes only a few seconds for the slide to reach the temperature at which the wax will flow under the cover glass.

12. Immediately remove the slide from the warmer and allow it to lie flat until cooled and set.

(12) If the slide is left on the warmer too long it will become too hot and destroy the specimen. Time needed for the slide to cool and set is at least 15 minutes.

STORAGE OF UNMOUNTED MATERIAL

Residues may be stored in any size bottle. The fossil preparations of the U.S. Geological Survey are stored either in 2-dram or 2-oz flint glass vials with poly-seal screw caps.

Storage in water

When mounting in glycerin jelly the residues are in water and are ready for storage when slide-making is completed.

1. Using a dropper, remove residues from 15-ml centrifuge tube and transfer to storage bottle.

2. Rinse tube with enough distilled water to reclaim all the residue.

(2) When completed, the storage bottle should be approximately half full.

3. Add 3 ml 25 pct methanol to prevent fungal growth; add 2-3 drops glycerin, and screw on the cap.

Storage in Vinylite AYAF

Residues which are already in AYAF (in storage bottles) are ready for storage when slide-making is completed. (See "Slide preparation: Mounting in Vinylite AYAF" step 8 (p.23).) In time, alcohol may evaporate, leaving the residues imbedded in AYAF. Slides may be prepared later by adding 95 pct alcohol to dissolve the AYAF.

Storage in ethanol and xylene

1. After mounting in canada balsam (see p. 24) transfer (with dropper) the remaining residue from the 15-ml centrifuge tube to 2-dram flint glass vial with polyethylene screw cap. Rinse tube with 50-50 mixture of absolute methanol and xylene and add this to the vial.

(1) Vial should be one-half to two-thirds full after transfer.

2. Screw on cap and store.

REFERENCES

Funkhouser, J. W., and Evitt, Wm. R., 1959, Preparation techniques for acid-insoluble microfossils: Micropaleontology, v. 5, no. 3, p. 369-375.

Kosanke, R. M., 1950, Pennsylvanian spores of Illinois and their use in correlation: Illinois State Geological Survey Bulletin, no. 74, p. 8-11.

Schulze, Franz, 1855, Über das Vorkommen wohlerhaltener Zellulose in Braunkohle und Steinkohle: Berlin, Berliner Königlichen Akademie Wissenschaften, v. 21, p. 676-678.

Tschudy, R. H., 1958, A modification of the Schulze digestion method of possible value in studying oxidized coals: Grana Palynologica (n.s.), v. 1, no. 3, p. 34-38.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1980—677-129/11