DEPARTMENT OF MINES AND ENERGY
SOUTH AUSTRALIA

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NOTES ON PALYNOLOGICAL AND PHOTOGRAPHIC TECHNIQUES, BIOSTRATIGRAPHY BRANCH

GEOLOGICAL SURVEY

by

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BIOSTRATIGRAPHY

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NOTES ON PALYNOLOGICAL AND PHOTOGRAPHIC TECHNIQUES, BIOSTRATIGRAPHY BRANCH

1. BASIC PALYNOLOGICAL PROCESSING TECHNIQUE

Reagents needed:
- 10% hydrochloric acid (HCl)
- Conc. hydrochloric acid (HCl)
- Distilled water
- Hydrofluoric acid (HF)
- Brij 35
- Zinc bromide ($ZnBr_2$)
- Potassium chlorate ($KClO_3$)
- Conc. nitric acid (HNO_3)
- Potassium carbonate (K_2CO_3)
- Methylated spirits
- Glycerine jelly
- Nail varnish

1. Clean off outer part of sample with knife to avoid contamination.
2. Place small amount of sample in a foil tray, and break up into tiny pieces with hammer; put this in a plastic beaker. Size of sample will vary according to sediment type; 5-10 grams may be required for inorganic sediments and only a gram or less for lignites.
3. Test for carbonates by squirting with 10% HCl, if no reaction go to step 4. If sample reacts add 25 ml of distilled water and 25 ml of concentrated HCl and place in waterbath for ½ hour. Centrifuge and wash twice.
4. Add just enough distilled water to wet sample and then 50 ml of HF. If reaction is vigorous, add HF slowly or in small portions. Heat in waterbath for at least 1 hour.
5. Transfer to 50 ml test tubes and centrifuge and wash three times. After third wash check that no reaction is occurring with sodium bicarbonate in sink.
6. Add approximately 15 ml conc HCl and fill test tube with water. Stir well and place in waterbath for ½ hour.

7. Centrifuge and wash at least 4 times - more if water is still reacting with the sodium bicarbonate when poured off. Stir well before each centrifuge.
8. Transfer samples to small glass beakers. Fill to 20 ml with distilled water. Add 1 drop of Brij 35, and ultrason at 70-80 for 30 seconds. Squirt nozzle with distilled water to wash all residue back into beaker. Clean nozzle after each sample to avoid contamination.
9. Pour samples into 15 ml conical test tubes (2 for each sample are needed) and centrifuge.
10. Add ½ inch of zinc bromide to each tube, mix well then fill to top and shake, to make sure zinc bromide is evenly dispersed.
11. Set centrifuge at 2000 for 5 minutes, after this a separation should have occurred. Scrape or pipette off top, and place in a clean test tube, continue process with this part.
12. Fill tube with HCl and shake very well. Centrifuge and wash 3-4 times.
13. Samples need to be checked under microscope at this stage to determine the most desirable processing technique.
14. Schulze Solution - with spatula place small amount of potassium chlorate in test tube, fill to 2/3 with conc. nitric acid and shake well, leave for a few minutes (usually between 3 and 7 minutes) shake after each minute, then centrifuge and wash 4-5 times.
15. Potassium carbonate (K_2CO_3) - Fill tubes with dilute (usually 1-2%) potassium carbonate solution, shake well, then centrifuge and wash until water poured off is clear (5-6 times).
16. Differential centrifuging - Where the use of K_2CO_3 is not desirable or only very small portion of sample remains, differential centrifuging may be required to reduce fine organic matter content. Fill tubes with distilled water and shake. Place in centrifuge, raise speed to 1000, hold for 30-40 seconds, tip off only top 1/3 of water.

17. Sieving - Place 10μ sieve cloth into filter, pour in sample and squirt with distilled water - use hand pump to pull water through if needed. Use about 100 ml of water (more or less may be needed depending on size of sample). If using 10μ sieve cloth retain what is left on top of sieve cloth, so using distilled water, carefully wash sample back into test tube. If using 129μ cloth retain what is in the bottom of the filter container, so pour into test tube and centrifuge.

18. Slides

Engrave glass slides with S. number, using the diamond pencil, clean them with methylated spirits and place on warm (4-5) slide warmer. Melt glycerine jelly in hot water. Take warm slide, and with a pipette add 2-3 drops of sample, and the same amount of glycerine jelly. Mix well, with a tooth pick or pointed spatula and place cover slip on. Turn slide upside down, and place on slide warmer for ½ hour. Remove from slide warmer and place upside down on cardboard trays until jelly has set. Clean slide with razor blade and seal edges with nail varnish. Label slides with S. number, locality and depth on a "permanent", not "removable" self-adhesive label.

ACETOLYSIS

This procedure is useful for processing lignites and more youthful sediments where cellulose is common.

Reagents needed: Acetic anhydride [$(CH_3CO)_2O$]
Concentrated Sulphuric acid (H_2SO_4)
Glacial acetic acid (CH_3COOH)

Acetylation reagent

The acetylation mixture consists of 9 parts acetic anhydride to 1 part sulphuric acid. The sulphuric acid must be added to the acetic anhydride. If mixed the other way an explosion is possible.

Always make up a fresh mixture immediately prior to use.

Procedure:

1. Wash samples thoroughly.
2. Add glacial acetic acid to the samples to dehydrate the cellulose. Mix well and centrifuge. Repeat if there is much residue.
3. Slowly add the acetylation mixture (put about 10 ml in a 15 ml test tube). Be careful as there may be a violent reaction if much cellulose is present.
4. Stir well.
5. Place samples in the waterbath at room temperature, heat up to 100°C. When boiling, turn waterbath off and leave samples standing in hot water for 15 minutes.
6. Centrifuge, and pour off the acetylation mixture into running water. Do not allow any water to splash into the test tube as it is potentially explosive.
7. Wash samples with glacial acetic acid to prevent precipitation of cellulose acetate. Stir well and centrifuge. Repeat if there is much residue.
8. Centrifuge and wash with distilled water 3-4 times.

PHENOL MIX FOR RESIDUE BOTTLES

Reagents needed: Phenol
Glycerol
Distilled water

To produce 5% phenol in 50:50 glycerol:water mix
Use 22.57 gm of phenol in 200 ml water and 200 ml glycerol
Use 28.2 gm of phenol in 250 ml water and 250 ml glycerol
Use 56.4 gm of phenol in 500 ml water and 500 ml glycerol

Place all ingredients in a beaker and stir until phenol is dissolved.

GLYCERINE JELLY FOR SLIDE MAKING

Reagents needed:

20 gm	Gelatin
1 gm	Phenol
120 gm	Distilled water
140 ml	Pure glycerol

Add gelatin to water and dissolve on waterbath. Add glycerol and phenol and stir well. Pour half into small plastic bottles. Dye the rest with a small amount of Safranin O - add slowly, stirring well until jelly is a bright pink colour (not red). Pour this into small plastic bottles also.

This makes approximately 12 bottles.

10% POTASSIUM CARBONATE

Reagents needed:

50 gm Potassium carbonate
500 ml Distilled water

Place potassium carbonate in the decanter, fill with distilled water and shake well. This can be further diluted with distilled water to any percentage needed.

ZINC BROMIDE

Usually zinc bromide is bought as a liquid with a specific gravity of 2.0. If it needs to be made from a powder, use the following:

$ZnBr_2$ 410 gm
Water 200 ml
HCl 5 ml conc. and 10 ml 10%

The specific gravity of this will be 2.0.

Zinc bromide : water ratios for different specific gravities

Specific Gravity	$ZnBr_2$: H_2O
2	1 : 0 (straight)
1.83	5 : 1
1.80	4 : 1
1.75	3 : 1
1.70	2.5: 1
1.67	2 : 1
1.60	1.5: 1
1.523	1.1: 1
1.50	1 : 1
1.40	1 : 1.5
1.33	1 : 2
1.25	1 : 3

PHOTOGRAPHY - DEVELOPING NEGATIVES

Loading the film must be done in total darkness.

1. Remove negative from holder and wind onto reel. Place reel in tank - making sure the lid is on correctly before turning on light.
2. Make up developer - mix 1:1 with warm water, it needs to be 20°C. 300 ml is needed for each reel.
3. Place developer in tank, tap sides to release any trapped air, agitate for 10 seconds, and then once every minute for 5-10 seconds. At 20°C the film needs 6 minutes in developer. If warmer it needs slightly less time and if cooler it needs slightly more time (see chart on darkroom wall).
4. Pour off developer and discard. Fill tank with water, shake for a few seconds and then pour this off and add fixer. The fixer is diluted with water - 1 part fixer to 4 parts water. The negative is left in this for 5 minutes.
5. Pour fixer into bottle or beaker - this can be used again.
6. Rinse negative in running water for 30 minutes, and then in distilled water for 5 minutes. Remove negative from reel and hang in darkroom with a heavy clip on the bottom. The film must dry for at least 4-5 hours before use.
7. Cut negative into strips, 6 frames long, and place in negative holders.

PHOTOGRAPHY - PRINTING

1. Three trays are required: in the first tray - put 400 ml water and 400 ml paper developer; in the second tray - fill with water and add a small amount of acetic acid; in the third tray - mix 800 ml water and 200 ml fixer.
2. Place negative in enlarger with the glossy side up.
3. Check focus for each print.
4. Set timer and expose paper - usually a grade 4 or 5 paper, for about 10-20 seconds.
5. Place paper in the developer, gently moving it all the time. Remove when photo has required clarity (30-60 sec.).
6. Move paper to wash for a few seconds.

7. Place in fixer; the light can be turned on after a few seconds, but leave paper in fixer for about 1 minute.
8. Put print into sink of water to wash for about 30 minutes.
9. Dry in dryer for 5-10 minutes.
10. Write negative number on the back of each print.

Note:

1. Developer and paper should be stored in the fridge at all times.
2. Only open box of paper in the dark room with lights off (except safe lights).
3. There are 6 grades of photographic paper: 0-5. 0 is very soft and low in contrast, this is used for very high contrast negatives. 5 is hard - high in contrast, this is used for low contrast negatives and when a sharp high contrast print is needed. Low contrast papers need less exposure time than the high ones.

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