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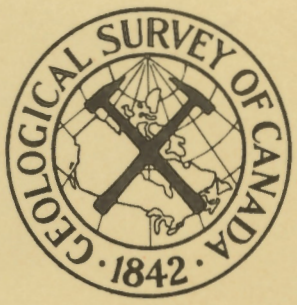

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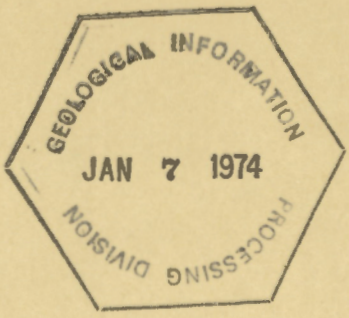
PAPER 73-26

PALYNOLOGY AND NANNOFOSSIL PROCESSING TECHNIQUES

M. S. Barss and G. L. Williams



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ABSTRACT

Techniques developed at the Atlantic Geoscience Centre are described. The procedures described permit the production of a large number of high quality preparations and should be useful to those responsible for establishing or maintaining palynological laboratory facilities.

RÉSUMÉ

L'auteur décrit les techniques mises au point au Centre géoscientifique de l'Atlantique. Elles rendent possible la production d'un nombre considérable de préparations de haute qualité et seront sans doute un atout précieux pour ceux qui doivent établir ou entretenir les installations d'un laboratoire palynologique.

PALYNOLOGY AND NANNOFFOSSIL PROCESSING TECHNIQUES

INTRODUCTION

The science, or perhaps more accurately the art of palynology and nannofossil processing, has made tremendous strides in the last 20 years. No longer should a palynologist or nannoplanktologist be excused for publishing plates in which the specimens are largely obscured by unwanted material. Unfortunately, many of the skills required to produce clean preparations are closely guarded personal secrets. In palynology processing most technicians are conversant with the use of acids and heavy liquid to concentrate the organic fraction, but each believes he or she knows best as to whether the sample should remain in hydrochloric acid (HCL) 1, 2, 3, or 4 hours. To alleviate some of these problems and to assist the palynologists faced with the task of establishing an operational laboratory with untrained help, the processing techniques developed at Eastern Petroleum Geology Section, Atlantic Geoscience Centre, are presented. The techniques for the concentration of palynomorphs (Pt. I; Pl. II, Figs. 1-4, 6, 8 and 9) and nannofossils (Pt. II; Pl. II, Figs. 5 and 7) are summarized in Figures 1 and 8 respectively. Acetylation, a concentration technique used by palynologists studying Recent and Pleistocene palynomorphs is not discussed in this paper. The reader is referred to Traverse (1965) for a detailed description of acetylation.

The procedures described are for a system to produce large numbers of high quality preparations. Quantity and quality must be constantly assessed in any processing procedures. It is a futile exercise to turn out upwards of 400 samples a month per technician if the majority of these contain a high percentage of unwanted material.

Acknowledgments

This paper has evolved from a description of palynological techniques by Miss Sheila Clyburne. The authors are deeply indebted to Miss Clyburne for providing the framework, and for testing many of the authors hypotheses in the laboratory. Mr. David Clark aided in the development of the concentration techniques for nannofossils. Thanks are also extended to Mr. William MacMillan who modified and improved some of the procedures. Mr. Edward MacDonald, an untrained technician, successfully processed a number of samples for palynomorphs and nannofossils following the techniques described in this paper. The authors are grateful to Mr. MacDonald for his assistance in clarifying some of the descriptions.

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PREPARATION OF SAMPLES FOR PROCESSING

Samples can be categorized into one of four types depending on origin. These are well cuttings, sidewall cores, conventional cores and outcrop samples (including subterranean sections and dredge samples).

Contamination from cavings, drilling lubricants (including seawater) and additives pose special problems when processing samples from well cuttings. The washing technique described below has evolved from the need to nullify as much as possible these contaminants, whilst providing a sample suitable for micropaleontology, palynology and nannoplankton processing.

Well cuttings are washed through a series of nesting screens of the following sizes: 10 mesh - 1.63 mm, 60 mesh - 0.250 mm, and 150 mesh - 0.106 mm. A portion of the fraction remaining on the 60 mesh - 0.250 mm screen (particle size 0.250 mm to 1.63 mm) is taken for palynology and nannoplankton processing. The fractionon the 10 mesh (1.63 mm) screen is considered to contain most of the caving. The fraction on the 150 mesh screen (0.250 mm to 0.106 mm) and the material washed away is considered to contain a high proportion of fossils that may be present in the drilling lubricants and additives.

Sidewall core samples from wells are usually needed for other studies and are small, so amounts available are minimal. Such samples must be carefully cleaned of drilling mud, which can even penetrate the core. These small samples are crushed to 1/16 inch (1.6 mm) between two aluminum trays or pieces of aluminum foil folded in several thicknesses, which are discarded after each sample. Conventional cores and outcrop samples are crushed in the same way.

Samples with oil staining or impregnation should be washed in cellosolve prior to processing.

Coal fragments can readily be separated from other lithologies by placing the sample in a beaker with carbon tetrachloride (always use gloves and carry this out in fumehood). Stir the sample and scoop or pour off the carbon tetrachloride and floating coal into a filter paper in a funnel and collect the filtered carbon tetrachloride in a flask. Refilter the carbon tetrachloride for re-use. The coal and the sample remaining in the beaker are dried before processing. Coal samples are crushed to 1/16 inch (1.6 mm) as previously described.

Portions of samples to be processed for nannofossils must be further crushed to a fine powder with particle size below 50 microns (Pt. II, Step 3).

PART I: PROCESSING PROCEDURES FOR PALYNOLOGY

Safety in the Laboratory

Palynological processing of samples should only be undertaken in a laboratory which meets or exceeds the local bylaws concerning safety and disposal of waste chemicals. Equally important is the disposal of the chemical containers, especially those used to store the acids. These containers should be thoroughly washed out with a neutralizer before discarding. It is important that the pipes used in the plumbing system in the laboratory and those leading from the laboratory should be of a material which is able to withstand these corrosive chemicals particularly hydrofluoric acid. The ventilation pipes from the fumehoods should also be able to withstand these chemicals.

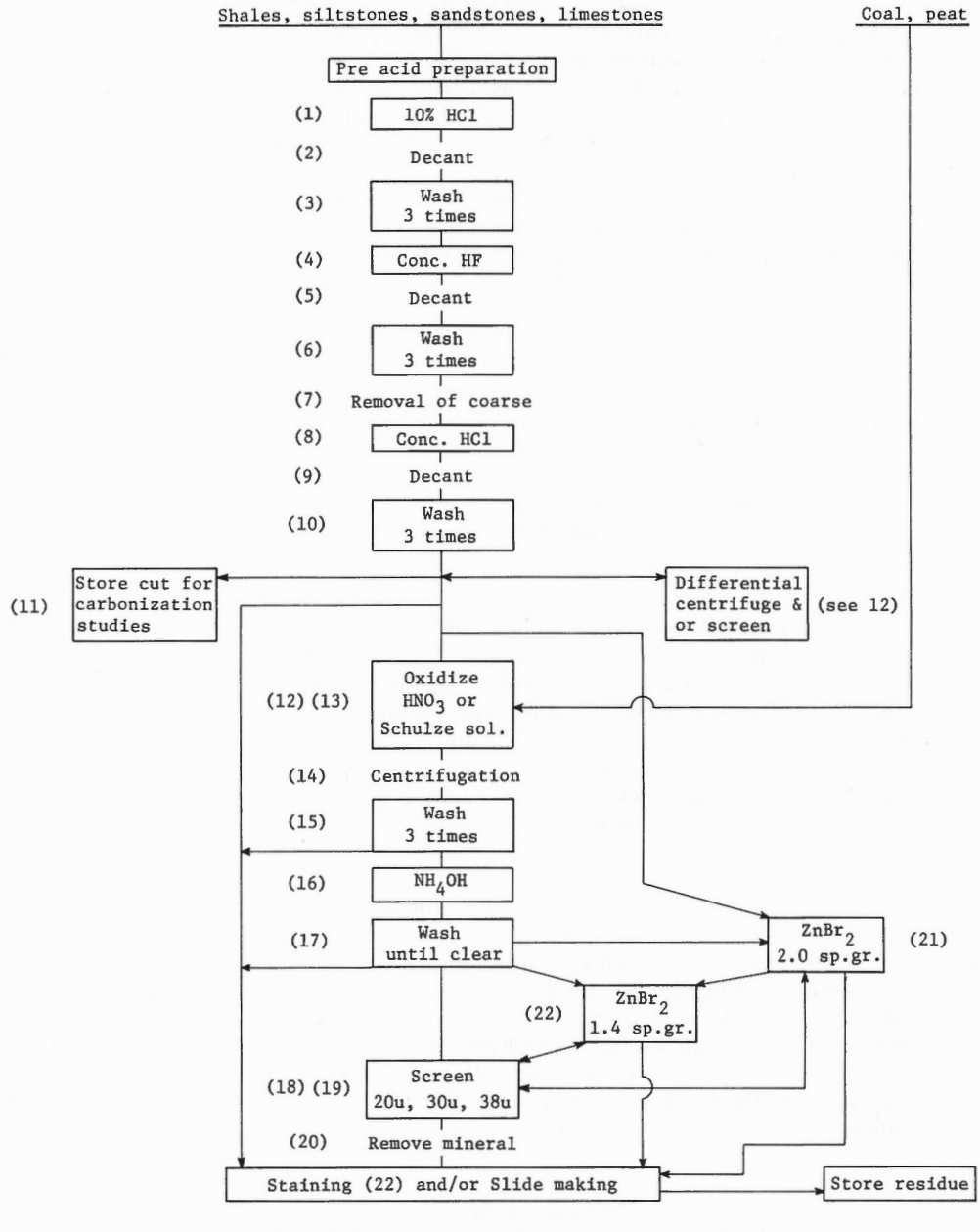


Figure 1. Flow chart for palynological processing.

It cannot be stressed too strongly that when using acids, as well as other chemicals involved in these processing procedures, protective clothing, gloves, facemask, etc., should be worn. A fire extinguisher, eye washes, and shower, should be part of the standard laboratory equipment (see appendix). A medical kit containing chemical neutralizers and antidotes for treatment of chemical burns or inhalation of fumes should be easily accessible in the laboratory. The technicians should make themselves fully aware of the safety requirements for handling the various chemicals used in the laboratory and the antidotes for burns caused by these chemicals.

Since acetone is a good dampener for most violent reactions with acids, a squeeze bottle full of acetone should be readily available at all times in the fumehood. A squeeze bottle of distilled water should also be available.

Amounts of Sample Required

Amount of sample needed is dependent on lithology. The following amounts have proved satisfactory:

5 - 15 grams ... shales, coals.

25 - 30 grams ... calcareous or siliceous shales, argillaceous limestones and argillaceous sandstones.

35 - 50 grams ... sandstones, limestones.

For processing chalks the reader is referred to Wilson, 1971, who described a suitable technique for processing. He suggested 100 - 150 grams of sample. Klaus (1953) and Leschik (1956) described methods for recovery of palynomorphs from salt and salt clay. According to these authors quantities vary from 50 g to a large lump (taken to be approximately 400 g).

To avoid confusion or mixing of samples when processing, it has been found advisable to assign each sample a unique processing number which should be marked clearly on every beaker or tube used.

Production Techniques

The centrifuges used are equipped with 12 and 6 place heads. Consequently samples are worked in groups of twelve. Shelves built into the fumehood allow up to 48 samples in the fumehood at one time, at various stages of preparations, e.g. 12 samples in hydrochloric acid, 12 in hydrofluoric acid, 12 being oxidized, 12 being washed (Fig. 2). To assure a smooth production system, samples must enter the fumehood as other samples are removed for screening techniques, heavy-liquid separation, etc.

Processing Procedures

Step 1: Non-coal samples are placed in 600 ml polypropylene beakers. Processing of coal samples begins at Step 12. Smaller beakers or disposable styrofoam cups can be used, but with the amounts of sample used in the following procedures the 600-ml size is considered primarily for the safety it provides when violent reactions occur. This larger size also allows a faster washing of the residues, and the larger

volume of water added after the oxidation treatment stops the reaction more quickly than a smaller volume would. If the sample contains unwanted organic material such as drilling mud additives (sawdust, coconut fibres, hay, wood) or fibres from cloth sample bags, 250 ml of distilled water should be added to the beaker as the bulk of these materials will float and can be easily removed at this stage. The water is decanted before proceeding to Step 2.

Step 2: From 250 - 300 ml 10% hydrochloric acid (see appendix) is added to remove any carbonates present. Occasional agitation with a polypropylene stirring rod will allow the acid to penetrate the entire sample. If the reaction does not stop before several hours have elapsed, the depleted acid is replaced with fresh acid every hour or so until all reaction has ceased. Heating the sample can speed the reaction but only low heat should be used (95°C). The polypropylene beakers can be used directly on the hotplate at this temperature. Styrofoam cups should be placed in a water bath. Never boil the sample as this may alter the organic material for carbonization studies. Violent reactions in the polypropylene beakers can be controlled by spraying the mixture with acetone from a squeeze bottle. Acetone must not be used to dampen reactions in styrofoam cups since it dissolves the styrofoam.

Step 3: When the residue has settled the acid is decanted or siphoned off according to preference. The stirring rod should be held so that the residue is not disturbed while decanting. This applies to decanting during Steps 3 to 7. Some fine material remaining in suspension may be poured off, but this should be kept to a minimum. Avoid splashing, since the decantant is not neutral and can cause burns.

Step 4: The residues are washed three times with distilled water, allowing the residue to settle at least one half hour each time, and decanting as much water as possible. These washings generally assure that the residue is in a neutral medium, which can be determined with calibrated litmus paper. Some fine material that remains in suspension will be poured off during these washings. The beaker should be filled to maximum each wash in order to neutralize the acid most effectively. The mixture should be thoroughly agitated with the stirring rod when the water is added. This washing procedure should be adhered to throughout the processing. Before addition of HF in the next step, the sample must be neutral. Otherwise calcium fluoride crystals will form in the sample on addition of the HF and these are extremely difficult to remove.

Step 5: Cautiously add 250 ml concentrated hydrofluoric acid (HF, technical grade), stirring constantly with a polypropylene rod. (Do not use anything made of glass with HF.) Maximum protective clothing must be worn when handling HF. This cannot be stressed too strongly because burns from HF are always severe and require immediate medical attention. The HF further breaks down the residues by removing the silicates. Since some residues react violently at this stage the acid may be added in 50-ml portions. Acetone can be sprayed over the mixture to control any violent reaction. The mixture is left at least 18 hours. Residues treated with HF in the morning would be left until the next morning (24 hours). Residues are stirred at least every hour where practical. This does not mean that one works all night.

Samples needed urgently are heated to 95°C on an oscillating hotplate which is fitted with a rack or pan so that the beakers or cups cannot tip or be dislodged by agitation (Fig. 2). If styrofoam cups are used a water bath is needed.

To disperse the residue within the acid without increasing the degree of agitation, the mixture should be stirred in a counter-oscillation direction.

If a more vigorous agitation is desired the mixture is stirred in the direction of oscillation. Extreme care should be exercised at all times to prevent serious accidents. For vigorous agitation minimal amounts of acid should be used. Under no circumstances should the beaker be more than one half full.

After the residue appears to have broken down, the acid is poured off and the sludge stirred. If much material remains unbroken the acid treatment is repeated, agitating the mixture on the oscillating hotplate with low heat (95°C).

Step 6: Allow the residue to settle for at least one hour and decant or siphon off the acid, obeying instructions given in Step 3.

Step 7: The residues are washed as described in Step 4.

Step 8: After the last decanting any coarse particles remaining in the residue are removed as follows: approximately 100 ml of distilled water is added to the residue which is swirled and allowed to settle for 5 - 10 seconds before being decanted into a clean beaker. The coarse material which has settled out is discarded by rinsing from the beaker with a spray of water. The residue is returned to the original beaker, allowed to settle, and the water is then decanted. If one beaker is used repeatedly for decanting the residues it must be thoroughly rinsed out after each residue.

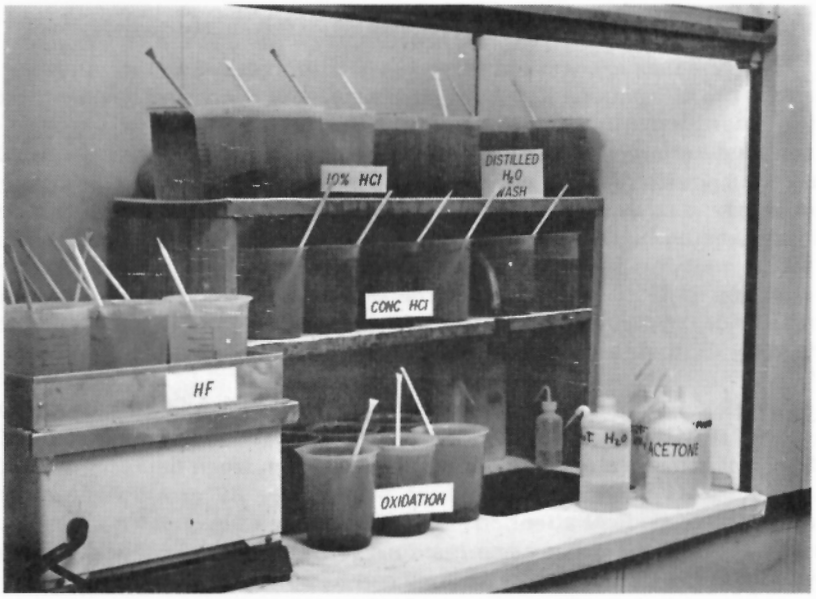


Figure 2. Illustration of fumehood shelving and safety rack on oscillating hotplate.

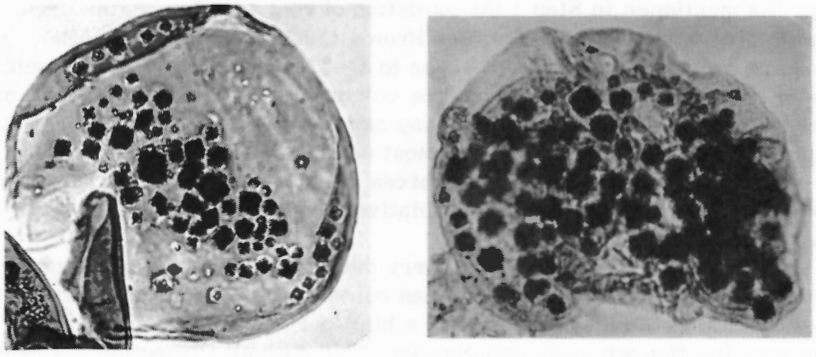


Figure 3. Palynomorphs with mineral attached.

Figure 4. Screen and funnel arrangement.

Step 9: Approximately 200 ml of concentrated hydrochloric acid (HCL, technical grade) is added to the residue to remove any fluorides that may have been precipitated in the post HF water washes. The mixture is heated (95°C) and agitated on an oscillating hotplate for at least 30 minutes. After settling the acid is decanted.

Step 10: The residue is washed as described in Step 4.

Step 11: At this stage a portion of each well mixed residue is taken and stored for carbonization studies. A one dram vial (4 ml) with a screw cap is a suitable container.

Step 12: As mentioned in Step 1 the oxidation of coal samples begins here. For processing coal, peat, lignite etc., see Brown (1960); and Gray (1965b). The residues are checked under a microscope to see if oxidation of the organic fraction is necessary (Pl. I, fig. 1). From the colour of the fossils and other organic material the amount of oxidation necessary can be determined. If spores are present their appearance (Pl. II, fig. 1) is the most useful criterion. For example, light yellow colour indicates no oxidation whereas dark brown colour indicates fairly long oxidation. Experience permits a relatively astute estimate of the length of oxidation time needed.

Some technicians prefer to carry out a heavy liquid separation prior to oxidation. We have found that in residues containing pyrite, (Fe_2S) which is commonly embedded in the fossils (Fig. 3), a high percentage of palynomorphs go down with the sink fraction which is usually discarded without inspection. A more representative assemblage is obtained if the pyrite is removed during oxidation before the heavy liquid separation.

The use of various oxidants has been fully described elsewhere, e.g. Brown (1960); Gray (1965b).

Oxidation is a relative technique which ultimately removes all the organic material. It is therefore better to underoxidize because oxidation is irreversible. The type of oxidant and length of oxidation chosen, is dependent on the degree of metamorphism of the palynomorphs, which is either a factor of depth of burial, present or pre-erosional, or tectonic activity. The degree of metamorphism is reflected in the colour of the organic material. The darker the colour the greater the metamorphism. However specimens that appear black can very often be made transparent by oxidation. In wells, the deeper the sample in the well the greater the oxidation necessary.

For those residues that are judged to need only slight oxidation, 10% nitric acid (see appendix) is used. All other residues are oxidized with concentrated nitric acid (HNO_3, technical grade). However, if a residue is found to require a long oxidation period in nitric acid (longer than 30 minutes), potassium chlorate ($KC10_3$), is added in the required amounts to make Schulze solution (see appendix). This speeds up the reaction. With increasing skill a technician will be able to place residues directly into Schulze solution.

Oxidation is carried out using 50 ml of oxidant per residue. The residues are agitated during this stage and the degree of oxidation is checked periodically, depending on the strength and length of time decided for each residue, e.g. a residue judged to require 30 to 40 minutes of concentrated nitric acid oxidation should be checked about every 10 minutes.

Residues requiring mild oxidation are processed in groups of six so as to control precisely the oxidation time. Residues requiring long oxidation can be treated in larger groups.

Residues containing pyrite (Pl. I, fig. 1) will react violently when the nitric acid is added. Great care must therefore be exercised when adding the acid so that a serious accident does not occur. In these residues the pyrite will have been detected when the residue is checked, so the acid should be added in small quantities.

If the residue contains large quantities of fine material, oxidation may have little effect. The fines can be screened off using a 20μ screen (see Step 18), or the residue can be differentially centrifuged, decanting the fines. For this procedure a floor model centrifuge with twelve 50-ml tube head is used (see appendix). The residues are well mixed in distilled water, transferred to the 50-ml polypropylene centrifuge tubes and spun at 1500 rpm for 20 seconds (500 gravities), braked

to a stop, and the fines decanted. A small amount (10 ml) of alconox solution (see appendix) added to the residue facilitates the removal of the fines. Bond (1964), described a method using alcojet. The procedure can be repeated if required, depending on the quantity of fines. Also the time and rpm can be adjusted to the various types of residues to acquire the desired separation.

Step 13: When oxidation is complete as much acid as possible is decanted and the beakers are filled with distilled water. Although the addition of the water all but stops the reaction, allowance should be made for some additional mild oxidation. The residues are allowed to stand undisturbed for one hour. Residues that require very short oxidation can be transferred without decanting the acid to centrifuge tubes as in Step 14 and the acid removed by washing with centrifuging as in Step 15.

Step 14: The diluted acid is decanted and the residue transferred to a 50-ml polypropylene centrifuge tube. Centrifugation is carried out on up to twelve residues for 2 minutes at 2000 rpm (1000 gravities), in the floor model centrifuge. This step may have to be repeated with any remaining residue in the beaker so that all residue is collected in one 50-ml tube. At this stage the tube should not be more than 1/5 to 1/4 full of residue. Amounts larger than this make the subsequent steps in the procedure more difficult to complete. Residue in excess of this amount is stored for future use in one dram vials with a screw cap.

Step 15: The residue is washed three times and centrifuged at 2000 rpm for 2 minutes each time. Some water is added to the tube and the tube agitated on a Vortex mixer to mix thoroughly the compacted residue with the water. Additional water is added from a squeeze bottle with sufficient force to mix thoroughly with the residue. The tube is filled to one half inch from the top for each wash.

Step 16: Ten ml of 5% ammonium hydroxide (NH_4OH) (see appendix) is added to each of the twelve tubes in turn and mixed on a Vortex mixer. (If after the third washing of Step 15 a small amount of water is left in the tube which is agitated on the Vortex mixer, the NH_4OH added from a squeeze bottle sufficiently mixes with the residue.) The NH_4OH serves to remove the oxidized humic compounds in solution. The twelve tubes are then in turn filled with water, centrifuged 2 minutes at 2000 rpm, and the supernatent liquid decanted. The procedure should be a continuous operation, taking approximately 3 - 4 minutes to add NH_4OH, mix, and add water to the twelve tubes. Thus the NH_4OH remains at full strength for only 2 - 3 minutes. When a residue has required long oxidation it is advisable to first add a few drops of NH_4OH to a portion of the residue to determine the effects on the palynomorphs. This is because in some residues NH_4OH or any other base can destroy all the organic material, or the reaction can stain the palynomorphs and make them more opaque for examination.

Step 17: The residues are washed three times as in Step 15. If the supernatent is not clear after three washings, continue until clear. If only a small amount of residue remains which might be lost if processing is continued, a slide should be made.

Step 18: The residue (Pl. I, fig. 2) is then screened. The usefulness of screens in palynology procedures was demonstrated by Kidson and Williams (1969). Screening is carried out using two 3-inch diameter screens, a 180μ (80 mesh) over a 30μ (see appendix). Sometimes a 20μ is used in place of the 30μ. This depends

on the palynologist's requirements. Some might prefer to have the fossils between 20 and 30 microns remain in the coarse fraction. If residues are very difficult to screen through a 30μ screen, a 38μ (400 mesh) screen can be used. After wetting both sides of the screens with water, so that the residue in suspension does not stay on the screen because of capilliary action, the screens are placed above a suitable funnel with a short spout, which in turn stands in a 250-ml beaker. Some residues may require a larger beaker because of the volume of water needed to complete the screening. There are however many instances when all the fines can be collected in a 50-ml centrifuge tube (Fig. 4).

The residue is rinsed from the tube onto the 180μ screen with the spray from a squeeze bottle, (500-ml size, with tip widened to 1.5 mm opening). The residue passes quickly through the 180μ screen onto the 30μ (or other, 38μ or 20μ). The 180μ screen is removed and set aside. The 30μ screen will probably be full of residue and water. The spray from the squeeze bottle is directed into the mixture in a continuous rotating or figure-of-eight motion whilst tilting the screen from side to side and swirling the residue until no fines, the minus 30μ fraction (Pl. I, fig. 3), remain on the screen. The idea is to keep the residue in suspension as this prevents the screen from becoming clogged by settled residue which would stop the fines from passing through. The beaker containing the fines is left undisturbed until processing of the coarse fraction is completed and slides are made of both fractions.

The coarse fraction from the screening (Pl. I, fig. 4) should be checked under the microscope the first few times but a technician will soon develop the ability to tell by the appearance of the residue whether the screening is complete or not. If any drilling additives (e.g. sawdust, fibres) are present on the screen they should be removed before the coarse fraction is rinsed from the screen into a funnel set into a 15-ml centrifuge tube. Frequently mineral (mostly quartz, which looks greyish) which has remained in the residue, can be removed at this stage by carefully washing the lighter organic fraction from the screen into the 15-ml tube. The residue is then centrifuged at 2000 rpm (600 gravities) for one minute, in a table model centrifuge with 6-place, 15-ml head and the water is decanted.

Screens are cleaned by immersing them in an alconox solution in an ultrasonic bath (see appendix) for 5 to 10 minutes, then rinsing with distilled water.

In developing these procedures the screens were frequently checked under a microscope after the ultrasonic cleaning and were found to be devoid of material that would contaminate other residues. The ultrasonic treatment appears to destroy any fossil material that may have been left on the screens. Kidson and Williams (1969), suggested staining can be used to detect contamination. The alconox solution is frequently changed (after 12 residues are screened or more often if residues from different wells or localities are being processed at the same time). If several screens are available one can be cleaned as the next residue is being screened.

Step 19: A portion of the coarse fraction from Step 18 is checked under a microscope to determine if further processing is required. Techniques for further concentrating the palynomorphs are described in Steps 20, 21 and 22. It is not possible to formulate strict guide lines in the subsequent stages (20-22). Only the experience of the technician can determine the application or alteration of the subsequent steps to produce the best results. Steps 20-22 are carried out only if sufficient amounts of the coarse fractions are available after the screening. If further processing is not necessary the coarse fraction is ready for staining, if desired, and slide making.

Step 20: A coarse fraction containing small quantities of mineral (e.g. quartz) remaining after oxidation and not removed during screening, may be treated as follows: the fraction is well mixed in the 15-ml tube, allowed to settle long enough (not more than 15 seconds) for the mineral to sink to the bottom and then decanted into another tube. This can be repeated as many times as required to remove this material. This is often the best way to recover the fossils if the residues are very small and could be lost in a heavy liquid separation. The residue is centrifuged at 2000 rpm for 2 minutes and the water decanted. Stain if desired, as in Step 23, before making slides.

Step 21: Coarse fractions having a high mineral content are placed in a zinc bromide ($ZnBr_2$) solution with a specific gravity of 2.0 (see appendix). This heavy liquid effects a separation into two fractions. The material with a specific gravity greater than 2.0 settles to the bottom of the tube and is referred to as the "sink". The material with a specific gravity of less than 2.0 rises to the top of the tube and is referred to as the "float". As explained below the procedure is accelerated by centrifuging.

The coarse fraction from Step 19 is washed in an alconox solution, centrifuged at 2000 rpm for one minute and as much solution as possible is decanted. A small amount of $ZnBr_2$ solution is first added from a squeeze bottle. The mixture is agitated on a Vortex mixer and the tube is then filled to ½ inch from the top with $ZnBr_2$. This usually thoroughly mixes in with the coarse fraction. Up to six tubes are centrifuged in a table model centrifuge at 1000 rpm (170 gravities) for 20 minutes. The tubes are removed from the centrifuge and placed in a tube rack. The "float" is stirred gently with a toothpick to free any material adhering to the tube and poured into one or two 15-ml tubes. If a clear separation between the "float" and "sink" is evident, one tube is used. If any doubt exists that a separation is not clear cut, then two tubes should hold all that is poured off and when water is added the specific gravity is reduced allowing the "float" to settle. The "sink" fraction is discarded by washing from the tube. The tubes containing the "float" are filled with distilled water from a squeeze bottle with enough force to mix thoroughly, and centrifuged at 2000 rpm for 2 minutes. The supernatent liquid is decanted. After combining the two portions (if two tubes were used) and thoroughly washing (three times as in Step 15) the fraction is ready for staining as in Step 23, if desired, and slide making as described later; alternatively further treatment as described in Step 22 may be carried out.

Step 22: If the coarse fraction from the screening or the fraction from Step 21 contains large quantities of carbonaceous material a heavy liquid separation using a $ZnBr_2$ solution with a specific gravity of 1.4 (see appendix) is carried out. This procedure is as follows: the coarse fraction is washed in an alconox solution centrifuged at 2000 rpm for 2 minutes and the solution is decanted. (With the float fraction from Step 21 an alconox wash is not necessary.) The tube is filled to ½ inch from the top with the $ZnBr_2$ solution from a squeeze bottle using the technique as described in Step 21. Up to six tubes are centrifuged at 1000 rpm for 2 minutes and carefully braked to a stop. Two fractions separate out.

The "float" and "suspension" (Fig. 5, and Pl. I, fig. 5) are stirred with a toothpick to loosen particles from the "float" which may adhere to the tube. The "float" and "suspension" are decanted, dividing equally into two 15-ml centrifuge tubes, care being taken not to disturb the "sink" (Fig. 5 and Pl. I, fig. 6). The tubes are filled with distilled water, mixed, and centrifuged at 2000 rpm for 2 minutes as in Step 21. The two "float" plus "suspension" containing tubes

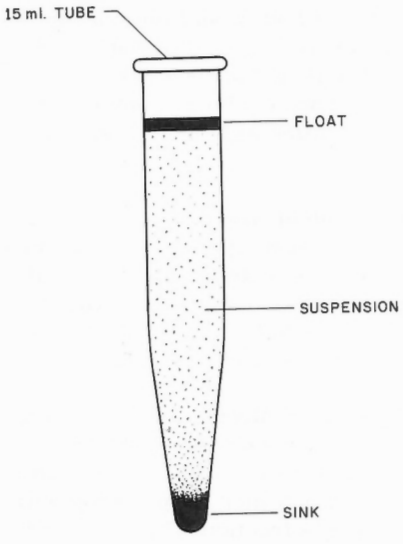


Figure 5. Fractions resulting from 1.4 sp. gr. $ZnBr_2$ separation.

are combined and washed twice to remove any traces of the zinc bromide. (Any zinc bromide not removed may cause the mounting medium to remain liquid.) The "sink" is washed in the same way. Check the two fractions at this stage as it is sometimes necessary to repeat the separation. If many palynomorphs have gone down with the "sink" a shorter centrifugation is used. Similarly if many carbonaceous particles remain in the "float" plus "suspension", the time may be lengthened. Occasionally rescreening is necessary. This is only carried out if there is sufficient residue available. The residues are now ready for staining, if required, and slide making.

A series of tests have been carried out using specific gravities of 1.3 to 1.6 and the 1.4 sp. gr. solution appears to provide the best results for a wide variety of residues. Other specific gravities may be more effective on certain types of residues. To separate the "floats", "suspensions" and "sinks" produced by the different specific gravities used, we prefix the fraction with the specific gravity value for that particular separation, e.g. a 1.4 sink, a 2.0 float, etc.

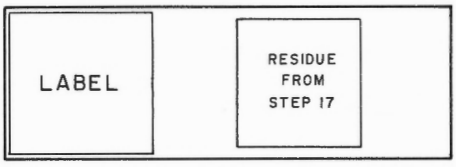
Step 23: When the palynomorphs lack body colour, microscopic examination and photography is much easier if the specimens are stained. Satisfactory results are obtained with Safranin Y (see appendix). For the palynomorphs to most effectively take up the stain the residues should be slightly alkaline. A small quantity of 1% ammonium hydroxide (see appendix) is added to the residue followed by as much Safranin Y solution as is needed to obtain the desired density of staining of the palynomorphs. If the density of staining is too great it can be reduced or removed by addition of hydrochloric acid after washing the residue. The residue should be neutralized by washing with distilled water (three times as previously described).

Slide Making for Palynomorphs

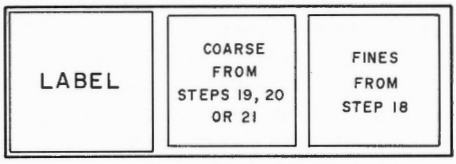
Slides should be made as soon as possible after processing, since most residues will tend to clump or break down if left unattended in the tubes or vials. Residues which show a tendency to clump are given 3-5 seconds treatment in the ultrasonic bath (see appendix) before slides are made. Such short periods of ultrasonic treatment do not damage the palynomorphs.

All fractions are mounted on 22 x 22 mm coverslips (we use no. 1 thickness), which are in turn mounted on 25 x 75 mm glass slides. The individual can devise a method of marking or identifying the various fractions and samples. Some identifying mark should be scratched on the glass slide in case any label deteriorates or is removed.

Residue from STEP 17 produces one slide type.



Residues from STEPS 18 and 19, 20 or 21 produces one slide type.



Residues from STEPS 18 and 22 produces two slide types.

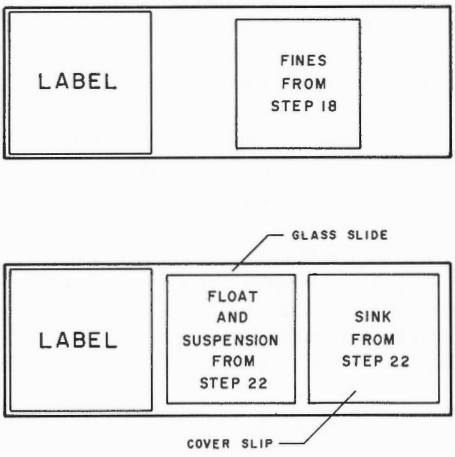


Figure 6. Arrangement of coverslips on slides.

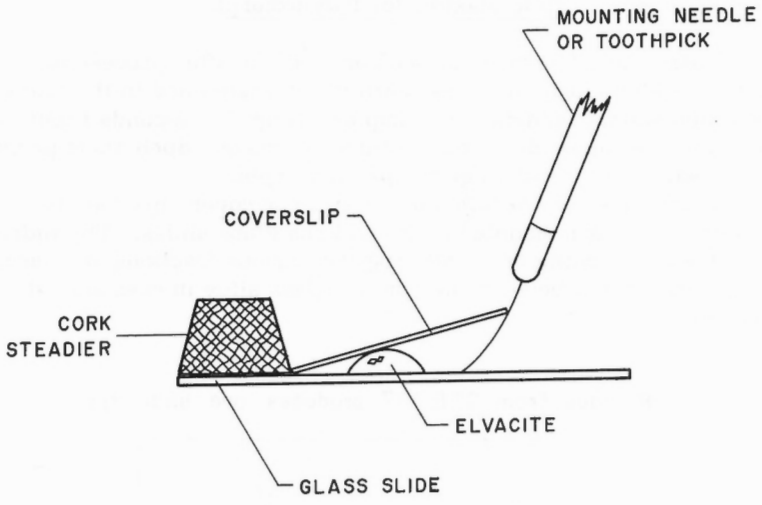


Figure 7. Mounting of a coverslip.

The residue from Step 17 or the coarse fraction from screening (Step 18), the 2.0 float, the 1.4 sink or the 1.4 float plus suspension, are mounted on the coverslips with Clearcol mounting medium (see L.R. Wilson, 1959, and appendix). Cellosize (see Jeffords and Jones, 1959, and appendix) is used for the fine fraction because it more effectively disperses the fine material. Coarse material is more effectively dispersed by Clearcol. There is also less tendency for air bubbles to be trapped within or without the palynomorphs, especially those with spines or processes, than with Cellosize. The major disadvantage of Clearcol is that because it is acidic it removes the stain from specimens over a period of time. Clarke (1963), described a method to eliminate this disadvantage using a small amount of bleach when making the slides. Whether the Clearcol or the addition of the bleach causes damage through oxidation is not known.

Step 1: From each fraction from processing there will be varying amounts of residue remaining in the centrifuge tubes and/or vials. Water should be removed from or added to the tubes and/or vials to adjust the mixture to the density desired when one drop of mixture is added to the mounting medium on a coverslip. The total amounts of material placed on the coverslip should be kept to a minimum.

Step 2: Place one drop of mounting medium on one or more coverslips as desired. If Clearcol is used, only a small drop is required. The drop of Clearcol should be one half the size of the drop of residue.

Step 3: Mix the residue in the tube or vial thoroughly by agitation on a Vortex mixer or by blowing through a disposable pipette with 1.0 mm opening, and add one drop of the residue to the mounting medium. Mix the residue with the mounting medium using a toothpick, spreading the mixture over the coverslip which is held in place with a second toothpick. The toothpicks are always discarded after use.

Step 4: The coverslips are air dried in a dust-proof case or in an oven at $35^{\circ}C$ or less. The coverslips are not left in the oven any longer than is necessary to dry them. Clearcol will lift and crack if left too long at this temperature.

Step 5: The coverslips are inverted and permanently mounted with Elvacite (see appendix) on suitably marked and labelled 25 mm x 75 mm glass slides. The coverslips are arranged as shown in Figure 6.

One method for dropping the coverslips onto the Elvacite medium is shown in Figure 7. Another method is to place one or two drops of Elvacite onto the coverslip, invert the coverslip and carefully drop on the slide. Two drops of Elvacite is a sufficient amount to use and will flow to all edges of the coverslip.

Step 6: The slides are placed in an oven at 35°C for a day or two to cure the Elvacite. Cured surplus Elvacite that has flowed past the edges of the coverslips can be removed if desired by cutting away with a razor blade knife.

Step 7: After slide making the residues are stored in one dram (4 ml) vials with screw caps in a 10: 10: 1 solution of glycerine-water-phenol (see appendix). If desired the caps can be sealed with wax. Do not put any phenol in the fraction saved for carbonization studies. On no account should the residues be stored in acetic acid since this will oxidize the palynomorphs. Stored residues must be periodically checked to see if they have dried out. To wash a stored residue or reclaim a dried one, place the residue in a 50-ml tube and fill with an alconox solution. Allow to soak for a few hours, then centrifuge at 2000 rpm for 2 minutes. Wash two times using method described in Step 15 and proceed with further processing or slide making.

PART II: PROCESSING PROCEDURES FOR CALCAREOUS NANNOFOSSILS

The processing techniques for nannofossils are entirely mechanical, the particles size range within which the nannofossils fall (0.25μ-30μ) being concentrated by centrifuging. Because of the short time of the technique it was found most convenient to process samples in groups of six. A table model centrifuge, with a 6-place 50-ml capacity head, is used. An alconox solution is used for dispersing the samples. Distilled water is used in all procedures and should have a pH 6.7-7 because these fossils will be destroyed if the water is acidic. Procedures for concentrating nannofossils have been described by others (Edwards, 1963; Hay, 1965), but no mention is made of the size or type of centrifuge used. Since the radius of the centrifuge head and the speed of rotation used can be translated into "gravities", the "gravities" and time of centrifugation therefore determine the particle size concentrated.

The "gravities" and time for the centrifuge used in these procedures are provided so that the reader may correlate these to the model of centrifuge they have available. The reader is also advised to consult the nomograph supplied with the centrifuge.

Amounts of Sample Required

0.5 gram ..... chalks

0.5 - 2.0 grams ..... shales, limestones

2.0 - 3.0 grams ..... siltstones

3.0 - 5.0 grams ..... sandstones

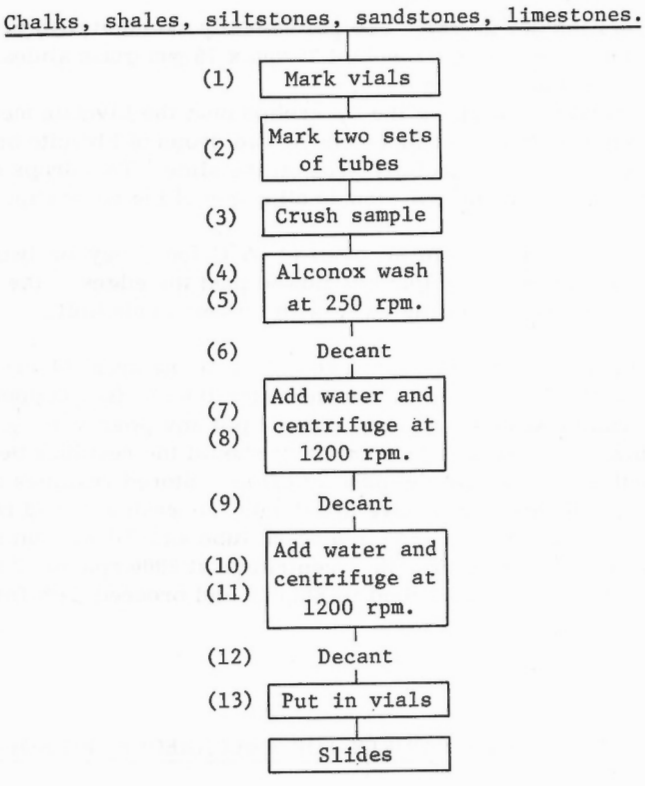


Figure 8. Flow chart for nannofossil processing.

Processing Procedures

Step 1: Six one-dram screw top vials are marked with sample and/or preparation numbers and set aside until Step 13.

Step 2: Two 50-ml centrifuge tubes are marked with numbers for each sample and placed as two sets of six in parallel rows in a tube rack. Designate the first row as "set one" and the other as "set two".

Step 3: Approximately 0.5 to 5.0 grams (depending on lithology) of the sample which was prepared for palynology processing is crushed to a powder (50 microns or less) between double thicknesses of aluminum foil, approximately 3 inches square on a steel plate. A flat faced hammer is used to crush the sample. The aluminum foil is discarded. The powdered sample is put in its corresponding tube of "set one". A polypropylene stirring rod is placed in the tube. Six samples are prepared in this way.

Step 4: The six tubes of "set one" are filled to within one half inch from the top with an alconox solution (see appendix) and placed in the centrifuge. The solution is thoroughly mixed with the stirring rod to put the sample in suspension. The mixing has to be done quickly so that a minimum amount of settling of the samples takes place before centrifuging. Remove the stirring rods and place in the matching tubes of "set two".

Step 5: The tubes of "set one" are centrifuged at 250 rpm (110 gravities) for 15 seconds and braked carefully to a stop.

Step 6: Quickly decant each tube of "set one" in turn into the matching tube of "set two", being careful not to allow any sink to be decanted. Set aside the tubes with the sink for washing.

Step 7: Place tubes with decantant ("set two") in the centrifuge, add water to ½ inch from top, and mix thoroughly with stirring rod as described in Step 4. Remove stirring rods and set aside to be used again in Step 10.

Step 8: Centrifuge decantant at 1200 rpm (260 gravities) for 3 minutes and brake to a stop.

Step 9: Decant the liquid being careful to keep all the sink.

Step 10: Fill the centrifuge tubes with water to ½ inch from top, place in centrifuge, mix thoroughly with stirring rod as described in Step 4, and set aside the stirring rods for washing.

Step 11: Centrifuge mixture at 1200 rpm for 3 minutes and carefully brake to a stop.

Step 12: Decant the liquid being careful to keep all the sink.

Step 13: Add 2-3 ml of water to each tube, agitate on Vortex mixer and decant the residue into the previously marked 1 dram vials (Step 1). When the residues have settled in the vials slides can be made.

A piece of wood ¾ inch thick with holes about ½ inch deep makes a good holder for the vials until they are stored. Usually thirty-six samples are prepared as described above before making slides. Following is the method for making slides for transmitted light examination. For methods to prepare slide for reflected light and/or S.E.M. examination, see Clark (in press).

Slide Making for Calcareous Nannofossils

Cellosize without phenol (see appendix) is used as a mounting medium. The phenol, if included, will dissolve the nannofossils. Clearcol which is acidic, also dissolves the nannofossils.

Step 1: Adjust the density of the residue as described in Step 1 of palynomorph slide making procedures.

Step 2: Mix the residue thoroughly using a disposable pipette by blowing through the pipette and place one drop of mixture on a no. 1 coverslip. Do not stir the residue, as any quartz grains present can mark the glass vial and a slight tap on the vial is sometimes sufficient for the bottom to break off, resulting in loss or drying up of the residue.

Step 3: Add one drop of Cellosize (without phenol) and mix with residue using a toothpick, as described in Step 3 of palynomorph slide making.

Step 4: Place coverslips in a dust free box or cabinet and allow to dry. Drying can be done in an oven at 35° C.

Step 5: Mount the coverslip on a 25 x 75 mm slide with Elvacite as described in Step 5 of slide making procedures for palynomorphs.

Step 6: Cure the Elvacite as in Step 6 of palynomorph procedures.

Step 7: Residues should be stored in water only. Do not use phenol as this will dissolve nannofossils. Vials can be sealed with wax to further help in preventing drying out of residues.

APPENDIX

The reader is referred to Gray (1965a) and Brown (1960) for a list of equipment and supplies for a palynological (including nannofossils) laboratory. The need has been stressed for adequate ventilation, acid resistant plumbing and safety approved protective clothing (gloves, aprons, facemask, etc.). An overhead shower with floor drain (floor of laboratory should slope to drain), eyewash, and fully equipped medical kit to treat any chemical burns should be part of any well equipped laboratory. A safety chart on the various chemicals used and antidotes for treatment of chemical burns and inhalation of fumes, should be displayed in the laboratory. These are available from most chemical firms.

Following are listed specifications for our centrifuges and ultrasonic, both of which should help the reader to correlate available equipment re: gravities or cycles per second, and recipes for solutions and mounting media used in our procedures.

Centrifuges

Two International, table top, Model HN-S (1EC3472). One with 6-place 15-ml head (1EC221). At 2000 rpm the relative centrifugal force is 600 x gravity. One with 6-place 50-ml head (1EC958). At 2000 rpm the relative centrifugal force is 700 x gravity. The rotating radius is taken from centre post to tip of tube when horizontal.

One International, floor model, Model UV (1EC3425). With 12-place 50-ml head (1EC279). At 2000 rpm the relative centrifugal force is 1000 x gravity.

Screens

20μ and 30μ screens are available from Buckbee Mears Company of St. Paul, Minnesota.

Ultrasonic Bath

Blackstone Heated Tank Ultrasonic Cleaner. Model HT-1.9, 200 watts, ST-2D generator, nominal frequency 23 Khz. The bath can be enclosed in an insulated box to reduce the noise to a tolerable level.

SOLUTIONS

Alconox Solution

7 grams of Alconox to 1 litre of distilled water.

Ammonium Hydroxide (NH_4OH) 5%

1 part NH_4OH (30%) to 5 parts distilled water.

Ammonium Hydroxide (NH_4OH) 1%

1 part NH_4OH (30%) to 29 parts distilled water.

Hydrochloric Acid (HCl) 10%

1 part concentrated HCl (37% to 2.7 parts distilled water.
Always add water to acid.

Nitric Acid (HNO_3) 10%

1 part concentrated HNO_3 (70%) to 6 parts distilled water.
Always add water to acid.

Safranin Y Solution

1 part Safranin Y to 500 parts distilled water.

Schulze Solution

1 cc Potassium chlorate ($KClO_3$, crystalline)
50 ml Nitric acid (HNO_3, technical grade).

Mix in as large a quantity as desired. Store in well marked dark glass bottle, with airtight cap.

Zinc Bromide ($ZnBr_2$) sp. gr. 2.0

1 pound Zinc Bromide ($ZnBr_2$, technical grade)
50 ml Hydrochloric acid (HCl)
± 225 ml distilled water

Stir the HCl into the $ZnBr_2$. There will be heat generated when mixing this solution. Since the specific gravity should be measured at room temperature, not all of the water should be added at one time. Add about 190-200 ml, mix until all $ZnBr_2$ has

gone into solution and allow to cool to room temperature (72°F). Use remaining water to adjust specific gravity to 2.0. Filter through glass fibre filter paper. Store in a dark coloured bottle.

$ZnBr_2$ sp. gr. 1.4

1 pound $ZnBr_2$
50 ml HCl
± 550 ml distilled water

Mix and store as above.

MOUNTING MEDIA

Clearcol

Clearcol mounting medium is only available from H.W. Clark, 33 South High Street, Melrose, Mass. U.S.A. 02176.

Cellosize

1.5 grams Cellosize (hydroxyethyl cellulose)
Methanol to cover Cellosize
100 ml distilled water

Add methanol to powder and allow to soak until all powder is wet. Add distilled water and thoroughly mix with stirring rod. Heat at low temperature to drive off the methanol. (The solution will drop 1/4 inch in a 250 ml beaker when methanol is gone.) If used for palynomorphs add 1 ml of liquified phenol. Cellosize (hydroxyethyl cellulose WP-09) is available from Union Carbide Chemicals Co.

Elvacite

65 grams Elvacite 2044 (from Dupont)
100 ml Xylene

Add Xylene to Elvacite and allow to dissolve in an airtight bottle. (This takes a day or two.) Make sure the tiny bubbles formed by pouring the solution into a dropping bottle have disappeared before using. Use in small quantities (small dropping bottles) and discard when thickening of the Elvacite prevents a smooth spreading under the coverslip.

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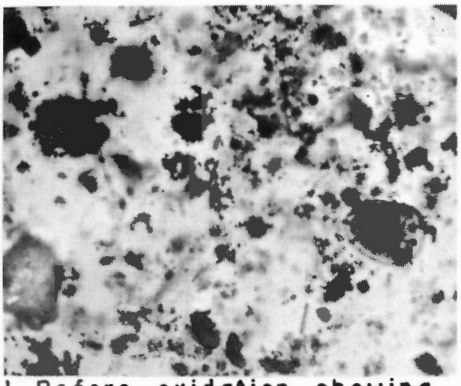
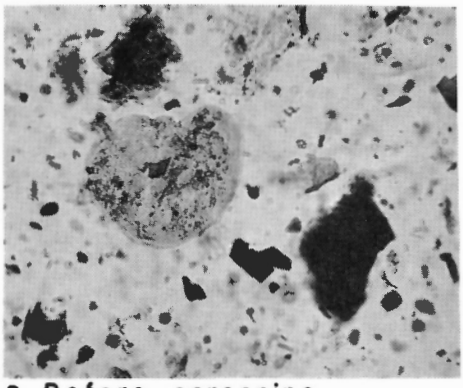
Plates I and II

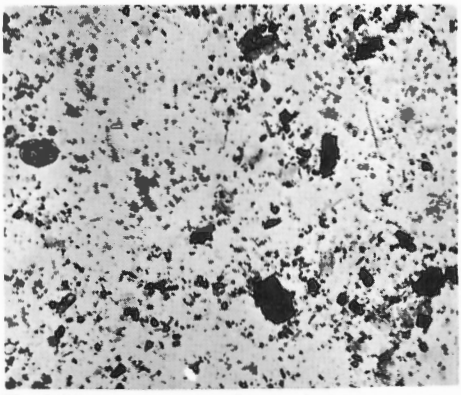
PLATE I



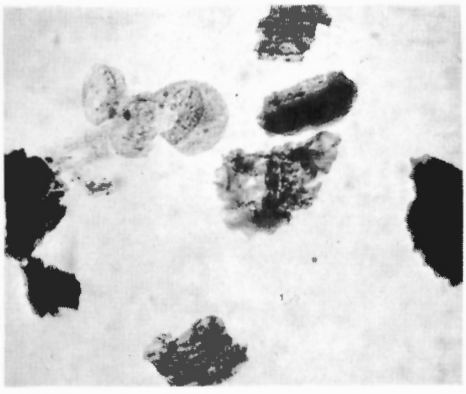
1. Before oxidation showing pyrite.



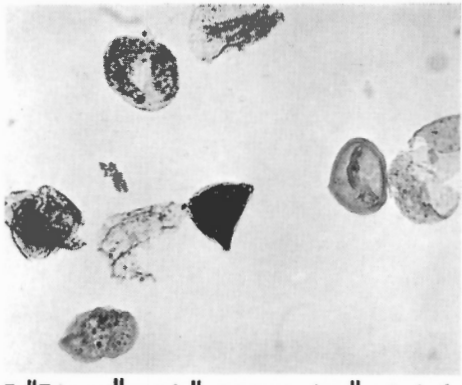
2. Before screening



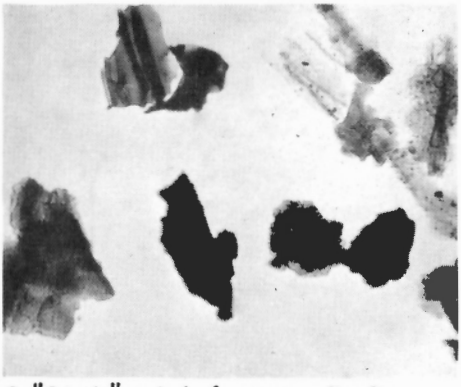
3. After screening < 30 μ.



4. After screening > 30 μ.

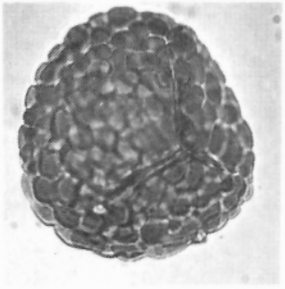


5. "Float" and "suspension" at 1.4 sp. gr. $ZnBr_2$.



6. "Sink" at 1.4 sp. gr. $ZnBr_2$.

PLATE II



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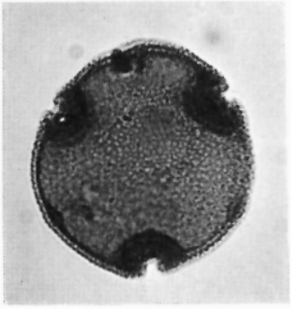

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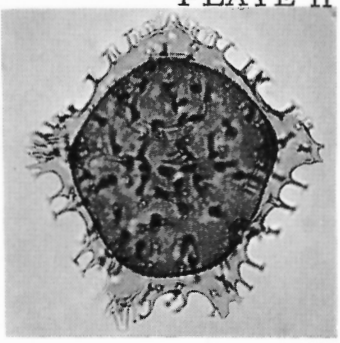

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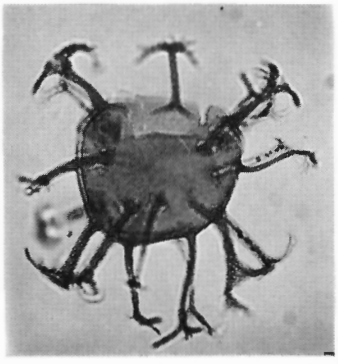

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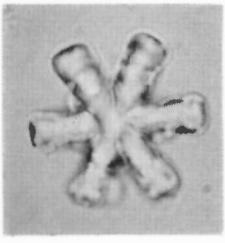

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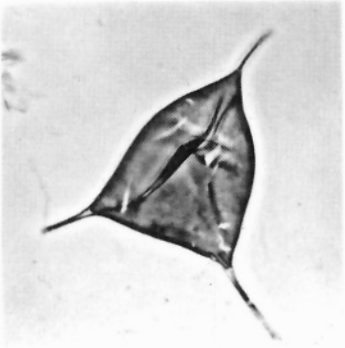

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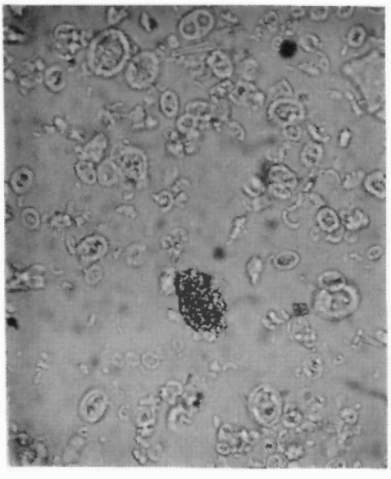
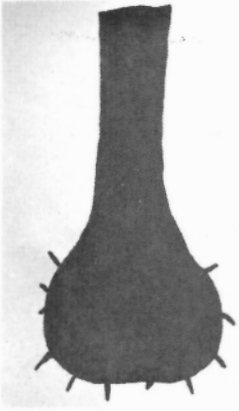


7. NANNOPLANKTON



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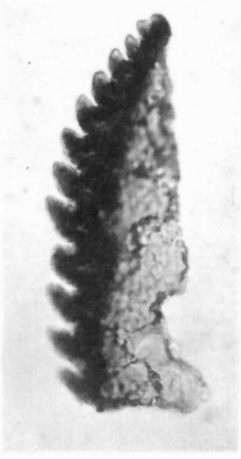

9. SCOLECODONT