OBTAINING THE SUITABLE TECHNIQUES FOR PALYNOLOGICAL PREPARATION

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ABSTRACT

The term of "standard techniques" in preparation is no longer accepted since every sample has discrete characteristic which may require different treatment. Experiments prove that the modifications are needed to process the Eocene samples from Central Java and South Sulawesi. There are four key points emphasised in the Eocene preparation such as oxidation, alkali treatment, the choice of 5 micron mesh and the use of ultrasonic treatment. The most critical points are oxidation and alkali treatment because they will determine the number of palynomorphs remaining in the residue. These might be achieved by obtaining the suitable combination between the strength of the solution and the time interval for the reaction.

This paper provides the alternative techniques to process the Eocene and most likely Palaeogene samples which can gain the reliable interpretation.

I. INTRODUCTION

The aim of preparation is to separate or release any palynomorphs from minerals or sediments which enclose them, or obscure them, in order to make them clearly visible and concentrated enough for microscope study and photography. Samples used in this study consist of fine grained sediments and coals and they were collected from Central Java and South Sulawesi. Apparently, the use of some "standard methods" is inadequate to extract sufficient palynomorphs from these samples which was indicated by the poor palynomorph recovery as seen in the slides. This encouraged the author to find the suitable techniques by modifying the existing methods which can gain the reliable results. In order to judge the both methods, samples are processed using "standard" and modified methods. Each sample is split into two parts. One part is treated on the basis of the "standard" preparation whilst another part is processed using the modified method. The slides produced by two different methods are compared to get the fair judgment in term of palynomorph concentration.

Each technique in the "standard" method is evaluated based on its effect to the residue. The techniques producing poor palynomorph recovery are modified by refining those existing techniques such as finding the suitable concentration for chemical solution and defining time interval for reaction. Apparently, this means "try and error" processes because the suitable techniques are only established based on experiments.

II. "STANDARD" METHODS

The "standard" methods mean preparation techniques which have been published in many publications. These existing techniques generally consist of the following steps: cleaning, crushing, chemical extraction, separation technique, mounting/slide preparation and storage (Figure 1).

Sample is cleaned to remove any loose contamination because it may cause bias interpretation. There are two different approaches which depend on sample condition. For consolidated sediments the following methods were proposed by Gray (1965). The first method is to wash the sample with water or acetone or alcohol; second, the sample surface is scraped or cut off; third, the sample is brushed with a wire brush; fourth, the sample is burned to remove the combustible contaminant; fifth, the sample is blown with compressed air and treated in an ultrasonic generator. In the case of unconsolidated sediments, the dry surface may be scrubbed or scratched or the exposed surfaces may be cut away with a sharp knife.

The clean sample is then crushed into small particles using a porcelain pestle and mortar. Crushing increases sample surface area thereby reducing time consumed for chemical reaction. However, powdering the sample may damage palynomorphs and should be avoided. Rock particles are transferred into the polypropylene container to total the appropriate weight of sample. The next step is to extract palynomorph from the sample using various chemical solutions. This extraction includes carbonate removal, silicate and clay mineral removal, fluoride removal, oxidation and alkali treatment. Hydrochloric acid (HCl) is added into the container to remove carbonate from the sample. The most common minerals within the carbonate suite in the rock sample are *Calcite* ($CaCO_3$) and dolomite

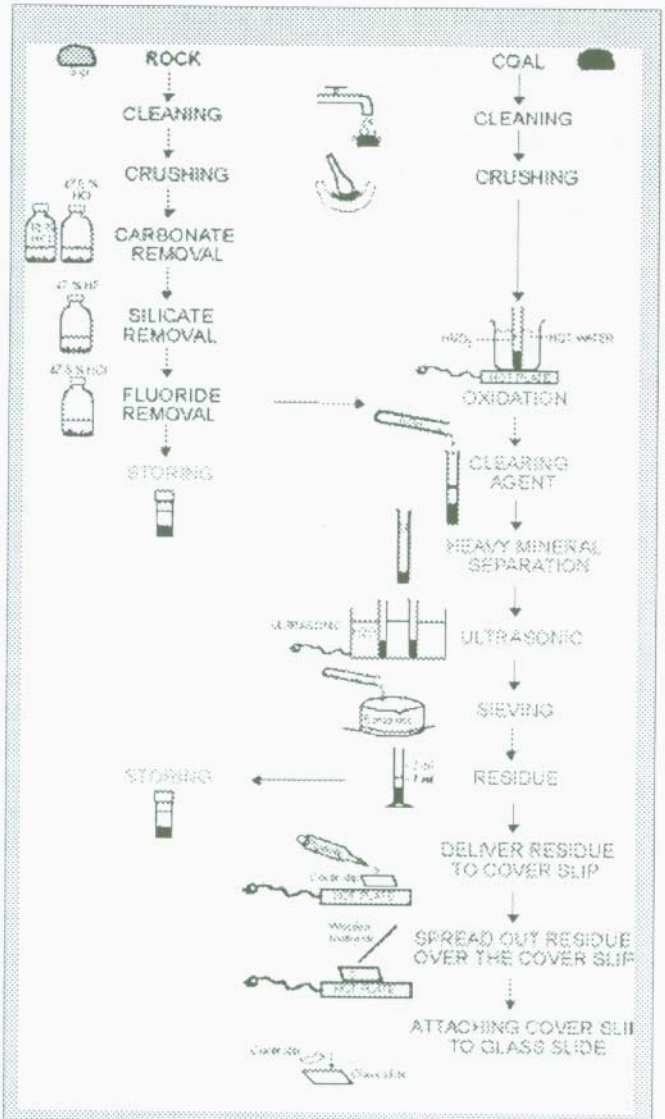

Figure 1
Flow chart of the preparation techniques which is applied for the Palaeogene samples

($CaMg(CO_3)_2$). These minerals must be removed from the sample, otherwise they will form calcium fluoride (CaF) when the sample is digested by hydrofluoric acid (HF^3). CaF_2 is a fine precipitate which is difficult to remove from the sample (Walton, 1923 and Sittler, 1955 in Gray, 1965). Phipps and Playford (1984) suggest to initially use the low concentration of HCl (10%) to prevent destruction of palynomorphs since prolonged exposure to strong HCl may cause a corrosive effect. 37% HCl is then added into the solution. HCl treatment ceases when all carbonate is exhausted which is indicated by no further reaction upon mixing when new HCl is added. Following HCl treatment 40% hydrofluoric acid (HF) is added to the container to remove silicate and clay minerals from the sample. After settling for at least 24 hours the acid solution is disposed of into a plastic bowl containing calcium carbonate, following the procedure for the HCl above. The addition of HF into solution often causes the formation of insoluble calcium fluoride (CaF_2) which subsequently obscures the palynomorphs under the microscope (Gray, 1965). Therefore, it is important to remove this insoluble compound from solution, after the HF treatment, by adding 37% HCl since the fluoride compound is soluble in HCl. The solution is then left to settle for a night. Basically it is assumed that all fluoride salts have been removed from the residue if the color of the solution becomes yellow or green.

Nitric acid (HNO_3) is added into the residue to reduce the level of amorphous organic material (including undecomposed lignin) and sulphide minerals such as pyrite (FeS_2) and marcasite (FeS_2). This treatment (oxidation) transforms any organic debris into alkali-soluble humic acids, thereby enabling undesirable organic material to be removed from the residue (Phipps and Playford, 1984). In order to remove humic material, hence clearing the residue, KOH is added, bringing humic material into solution. Many authors suggest to apply further techniques if these treatments are not completely successful in removing undesirable black debris (non-organic debris) from the residue. Heavy liquid is used in an attempt to remove pyrite and other debris from the residue. By mixing residue and heavy liquid, it is expected that pyrite and other heavy minerals sink down whilst organic particles which are normally less dense than 2 g/cm^3 will remain floating. The organic particles are pippeted and collected in another clean tube. In case debris still exist after heavy liquid treatment residue is sieved to remove them. In order to avoid clumping among palynomorphs or between palynomorphs and other coarse organic materials the residue has to be ultrasonicated just before sieving. The residue retained on sieve is called the final residue and ready for slide preparation. The mounting/ slide preparation technique varies depending on the type of the mounting media which is used in the slide preparation. Some mounting medias require simple technique (for example Canada balsam) but others need longer step of preparation (for example Petropoxy resin 154). Mounting is intended to transfer residue onto slide to make it available for microscopic viewing. Generally, residue is transferred onto cover slip and spread out evenly. The cover slip is subsequently attached to the glass slide using mounting media. At this stage slide is ready for observation under the microscope.

Microscopic observation showed poor palynomorph recovery. Slides contain lots of undesirable debris which indicate that the above methods were ineffective to remove

them from the residue. This situation encourage the author to reevaluate those methods, and hence reprocessing the remaining samples.

III. MODIFICATION OF THE "STANDARD" METHODS

Modification is intended to refine the existing techniques through experiments in order to find suitable methods for producing reliable slide. The experiments showed four key point within preparation including oxidation, alkali treatment, sieving and ultrasonic treatment. Oxidation and alkali treatment are the most critical techniques which determine the number of palynomorphs in the residue. A part from those points, other "standard" techniques are suitable for Eocene preparation. These four key points are discussed in detail below:

A. *Oxidation*

Basically, oxidation transforms any organic debris into alkali-soluble humic acids to reduce the level of amorphous organic material. Therefore this technique must be followed by alkali treatment to remove humic acids from the residue by decanting the supernatant. Oxidation is also intended to remove sulphide minerals such as pyrite (FeS_2) and marcasite (FeS_2) which are in the sample and may appear in the slide as small nodules, black flecks or pale to dark debris which frequently clumps with palynomorphs causing uneven distribution and obscuring their form. In addition, this treatment lightens palynomorphs so that they can be viewed more clearly under the microscope. Here, modification is aimed to find suitable combination between the strength of the solution and the time interval for the reaction. A very strong solution combined with a long reaction time will remove all palynomorphs together with the organic materials and sulphide compounds, whilst a weak solution and short reaction may not produce any increase in palynomorph concentration.

It is found that 69% nitric acid (HNO_3) with one minute reaction in hot water is suitable for removing undesirable materials as mentioned above from Eocene samples. The oxidation is conducted according to the following technique: 69% HNO_3 is added to the centrifuge tube containing the untreated residue. The tube is stood in hot water for up to one minute. This may result in a violent reaction due to pyrite and other sulphide compounds. The tube is removed if such a reaction begins and it is allowed to subside. When the requisite time is achieved, the contents of the tube are immediately poured into another tube containing distilled water in order to terminate the reaction. The residue is neutralised by centrifuging it four times in distilled water. After each centrifugation, the residue is mixed thoroughly using a mixer. Indicator paper can be used to ensure the neutrality of the residue.

B. *Alkali treatment*

It is often found that lots of humic material in the residue reduces palynomorph concentration and obstructs microscope viewing of palynomorphs in the slide. In addition, this material possesses an acidic character because of previous oxidation treatment. Here, alkali is added to remove humic material and neutralise the residue. This treatment also clarifies detailed structure of palynomorphs facilitating observations under the microscope by the palynologist. It is selected potassium hydroxide (KOH) for alkali treatment because it is more vigorous than other alkalis. It often causes grains to swell and if it is used too much it may disintegrate the grains. However, some degree of expansion is useful since it allows the internal structure of grains to be interpreted easily (Morley, pers. com.).

After intensive experiments, it is concluded that 10% KOH with 5 seconds reaction is the best combination for clearing the residue. The treatment is as follow: 10% KOH is poured onto the oxidized residue. The tube is shaken to mix the solution then left for about 5 seconds. To terminate the reaction after the requisite time, the concentrate is poured into another 100 mls tube containing distilled water. The tube is centrifuged at 3000 rpm for about 3 minutes. Centrifuging in distilled water is normally repeated 3 or 4 times until the concentrate is neutral and colourless.

In fact, oxidation and alkali treatment are the most crucial techniques within palynological preparation. The appropriate technique for these two treatments will determine the number of palynomorphs which remain in the residue. The important points within these techniques are to obtain the most suitable combination between the strength of the solution and the time interval for the reaction.

C. *Sieving method*

Although oxidation combined with alkali treatment will remove some non-organic materials, some debris (particles less than 5 microns such as pyrites particles) tends to remain in the residue in significant amounts. Following alkali treatment a sieving method is used to separate undesirable fine debris from coarser particles. This method is a physical fractionation based on differential sizes of particles, without involving any chemical solutions. Experiences have shown that the smallest size of palynomorphs of interest in Eocene preparation is somewhat greater than 5 microns. Therefore, in theory, all important palynomorphs are retained on the 5 microns sieves whilst the fine debris is passed through the meshes. On the other hand, the more widely used 10 micron mesh would be too large since some important pollen might disappear from the residue such as *Zonocostites ramonae*, *Florschuetzia trilobata* and *Triorites minutipori*.

The sieve apparatus such as a 5 microns disposable nylon sieve mesh held in a holder are wetted using distilled water and the sieve mesh is clamped tightly into the holder. A treated residue is poured into the sieve and small particles pass through the mesh. Due to the small mesh size, the sieve blocks easily and separation may take a long period of time. In order to speed up the slow separation, the sieve can be sprayed with distilled water evenly using a squeezable wash bottle. The underside of the sieve may be rubbed gently or, alternatively, shaken by sharp up and down movements in water. These methods help to prevent the mesh clogging with fine particles. This separation is terminated when all or most fine particles have been removed from the residue leaving those greater than 5 microns on the sieve.

D. *Ultrasonic treatment*

Lots of palynomorphs were clumped, either together or with other coarse organic materials making observation difficult. In order to solve this problem it was decided to ultrasonicate the residue just before sieving. The ultrasonic device vibrates the residue for a certain time interval causing palynomorphs to separate. The determination of the time interval is the most crucial factor within this method since long vibration may damage the palynomorphs (Dodson, 1983).

The tube containing the residue is placed on the tray and the tray is put into the ultrasonic bath. The bath is filled with water to three quarters of the bath volume. Distilled water is added into the tube until its surface is level with that of water in ultrasonic bath. Residue is vibrated for about 15 seconds using time controller, then transferred to a centrifuge tube. The tube is centrifuged at 3000 rpm for 3 minutes. The liquid is decanted and the residue is settled for further treatment.

Observations on processed slides using modified method show an increase in palynomorph recovery. On the contrary, undesirable black debris reduces significantly. This result indicates that the modified method is suitable for palynomorph extraction. However, it is realised that this method may not be suitable to process samples which derive from different age.

IV. STORAGE OF THE RESIDUES

The method of storing the residue is adopted from that introduced by Chapman (1985). She used glycerol as a storage medium to which was added a few drops of phenol in order to prevent fungal growth. She observed 100 residues which were stored over various periods and found that after 3 years storage 53% were in good condition, 33% dried out and the remaining 14% were very viscous. Regarding this situation, she suggested to keep the residue in the sufficient storage medium to avoid drying out and the sufficient fungicide to prevent destruction by fungi. In addition, an annual check is needed to observe the actual condition of the residue. Glycerol and phenol may be added to keep them in a sufficient volume for residue preservation.

V. DISADVANTAGE

There is a possibility that palynomorphs are lost during decanting of supernatant as observed in this study. This fact is important since it will affect the diversity and concentration of palynomorphs. Therefore, decanting with minimal disturbance was taken to reduce this possibility.

VI. SUMMARY

The observation shows that the modified method as described above seems to be suitable for processing Eocene and most likely Palaeogene samples. The application of standard preparation techniques such as introduced in many publications would not have been successful. In particular, different sediments need different treatment. This is caused by different depositional, diagenetic and katagenic factors and effects can be seen clearly by observing the reaction of the sample to certain treatments.

There are 4 key points in the sample preparation used here. They are: oxidation; alkali treatment; the choice of 5 micron mesh and the use of ultrasonic treatment. Various experiments with oxidation and alkali treatment were tried to find a suitable combination between the strength of the solution and the time interval for the reaction in order to obtain a reliable palynomorph assemblage. A strong solution combined with a long reaction time could result in the loss of palynomorphs. On the contrary, weak solution and short reaction might not increase palynomorph concentration in the residue. The most suitable combinations for the Eocene materials are 69% HNO_3 for one minute for oxidation and 10% KOH for 5 seconds for alkali treatment. Here, it can be emphasised that oxidation and alkali treatment are the most crucial stages within this preparation technique which determine the number of palynomorphs remaining in the residue. In addition, the use of 5 micron mesh increased the concentration of palynomorphs by reducing the level of undesirable fine debris significantly. The use of 5 micron mesh in preparing Eocene samples is recommended since the smallest size of palynomorph of interest is rather greater than 5 microns but smaller than 10 microns. Ultrasonic treatment is needed to prevent clumping between palynomorphs or palynomorphs with other materials because clumping obstructs the microscope view. Although ultrasonic is useful, over ultrasonicating the residue will damage the palynomorphs. The appropriate time interval for ultrasonic is 15 seconds.

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