Terra Antartica
1998, 5(3), 549-552

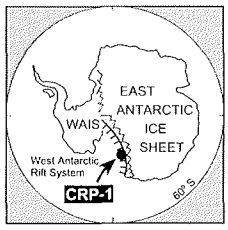
Palynologic Processing in Antarctica

J. SIMES[1] & J. WRENN[2]

[1]Institute of Geological and Nuclear Sciences, PO Box 30368, Lower Hutt - New Zealand
[2]Department of Geology and Geophysics, Center for Excellence in Palynology, Louisiana State University, Baton Rouge, Louisiana 70803 - USA

Received 28 July 1998; *accepted in revised form* 10 November 1998

Abstract - On site palynological processing has been completed for the first season of a drilling programme in Antarctica. Microwave digestion was used to dissagregate samples using Hydrofluoric acid in a situation where speed, safety and environmental considerations were critical. The methods described contribute to rapid down-hole analysis for this project, allowing palynology to provide results in as timely a way as other biostratigraphic disciplines.



INTRODUCTION

Microwave technology has opened a new chapter in the history of palynology by permitting sample preparation and on-site palynostratigraphic analyses to be conducted in Antarctica for the first time. Successful palynostratigraphic support of the first drilling season of the Cape Roberts Project (CRP) McMurdo Sound, Ross Sea, Antarctica was only possible due to the unique characteristics of this new technology. A palynology processing laboratory, the most southerly in the world to date, was established within the Crary Science and Engineering Center (CSEC) at McMurdo Station, Ross Island. The CRP drill site was located at 77.008°S, 163.755°E, approximately 125 km northwest of McMurdo Station.

Helicopters ferried cores from the drill site to McMurdo Station twice a day. Two sampling streams were submitted to the laboratory for processing. Fast-track samples were taken at regular intervals and dispatched to CSEC where they were divided between various biostratigraphic disciplines and processed as a matter of priority to provide timely biostratigraphic input to the drilling programme. As full core arrived at CSEC, subsamples were taken, processed and analysed. An initial report detailing the activies of the drilling season was completed in Antarctica and published within three months after drilling was terminated (Cape Roberts Science Team, 1998).

Traditional palynologic processing takes significantly longer than for other biostratigraphic disciplines, consequently, palynology has contributed less to on site drilling decisions. Lengthy processing times as well as the chemical hazard, the need for laboratory space and fume cupboards are some of the reasons why offshore oil rig and shipboard drilling programmes do not generally include palynology processing facilities onboard. In the sensitive Antarctic environment, processing was possible for the first time because of the use of this new technology for the fast and safe acid digestion of samples. Processing times in Antarctica compared favourably to that of other biostratigraphic diciplines employed on this project.

TRADITIONAL PALYNOLOGICAL PROCESSING

Laboratories extract pollen from consolidated rock using variations on the following basic scheme:

- physically disagregate rock material by crushing;
- remove carbonates using hot hydrochloric acid (10%) and wash to neutrality using distilled water;
- remove silicates using hydrofluoric acid (50%) and wash to neutrality using distilled water;
- remove insoluble fluorides using hydrochloric acid (36%?) and wash to neutrality using distilled water;
- oxidise organic residue to separate palynomorphs from other, less resistant, organic material. This stage will also remove sulphide mineral matter. Wash to neutrality using distilled water;
- separate organic matter from mineral matter using a heavy liquid and wash. Selectively isolate preferred size fractions using a combination of sieving and or filtering;
- mount preferred size residue on a glass slide and cover;
- detailed summaries of the method and variations are outlined in Wood et. al., (1996).

PALYNOLOGICAL PROCESSING IN ANTARCTICA

In Antarctica, environmental considerations required that processing methods avoid production of large volumes of waste acid and contamination of air with acid fumes. Traditional methods involving relatively large volumes of fuming acids in open beakers did not meet a suitable

environmental standard. Health and safety considerations also suggested that handling of large volumes of open acids should be avoided. Finally, if processing was to keep pace with the drilling programme and contribute to the biostratigraphic information in a timely manner, along with the other biostratigraphic techniques, then a faster processing technique would be necessary. To satisfy all these requirements the project purchased a ProLabo M401 microwave digestion system. The use of microwave digestion in palynology was pioneered in the Geology Department at Sheffield University, Sheffield, England (Ellin & Maclean, 1994; Jones, 1994; Jones & Ellin, 1998). During the past 5 years, they have experimented with several different types of microwave units and found that focused microwave digestors, such as the Prolabo M401, are the best type for processing palynologic samples (Jones, 1995; Jones & Ellin, 1998). Microwave digestion theory and its importance in palynology is detailed in the aforementioned papers.

Processing proceeded as per the schematic flow chart (Fig. 1).

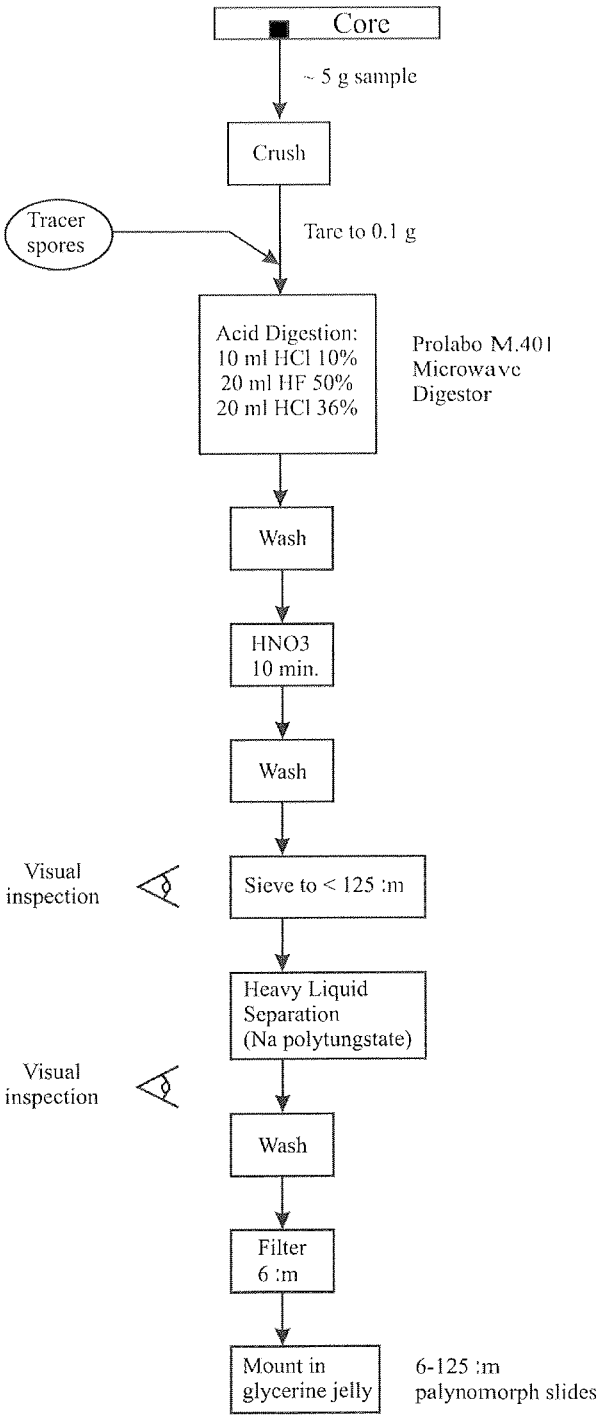


Fig. 1 - Flow-chart of palynological processing procedures used on CRP-1.

ACID DIGESTION USING THE PROLABO M401 MICROWAVE DIGESTER

The Prolabo M401 digestion unit consists of four main components:

1 - an electronic control pad that can be programmed to control the operation of the M401;
2 - the digestion unit that heats the sample and reagents contained in a reaction vessel;
3 - a bank of three peristaltic pumps that are connected directly to reagent containers and the reaction vessel by plastic tubing;
4 - a scrubber unit to clean fumes extracted from the digestion vessel.

Processing with the ProLabo (Fig. 2) was according to the procedure followed at the Center for Excellence in Palynology, Louisiana State University, where the system has been in place for two years. The crushed sample is placed in a removable, slender teflon processing vessel or reaction chamber with a screw lid. The lid is covered by a swing-out unit during operation. This moveable unit is attached to peristaltic reagent pumps and reagent bottles by a series of very fine, plastic tubes. For this project the three reagents used were dilute HCl (10%), concentrated HF (50%) and concentrated HCl (36%). The internal shape of the screw lid resembles a funnel at its apex and is connected by a 10 mm diameter tube to the fume scavenging and neutralizing system. This exerts a gentle suction action to the reaction chamber, removing acid vapours and bubbling them through a sequence of boric acid solution and mild caustic soda solution. The neutralized vapour is returned by a tube to the fume cupboard where clean, scrubbed air is vented. The application of acids and heat to the sample is controlled by the programmable control unit, which activates the peristaltic pumps and the heat generating magnetron in a predetermined sequence.

Approximately 5 grams of sediment were used for each sample. The microwave unit was set to dispense 10 ml of reagent A (HCl) followed by twenty minutes of heating. This was followed successively by 20 ml of reagent B (HF) and 20ml of C (HCl) with a further 20 minutes of heating for each. These parameters may be easily reset each time a sample is to be run. Any variation is possible so long as the required factors are entered into a programme that is then selected. Acid digestion time for each sample was one hour. As there were a number of teflon sample containers, it was normal procedure to have the next sample ready to place in the microwave immediately after a sample was finished, thus a number of

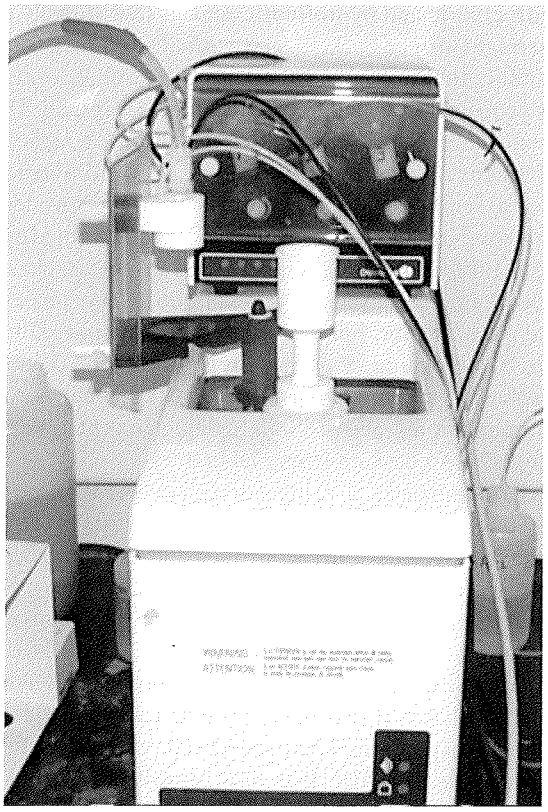


Fig. 2 - The Prolabo M401 in operation.

samples could be processed in quick succession. From this point samples were transferred to 50 ml screw lid centrifuge tubes for neutralizing prior to the next procedure. Sample volume remaining at this stage is considerably greater than that normally experienced with traditional beaker digestion of carbonates, silicates and fluorides. Using the ProLabo microwave digester, there was no attempt to dissolve all siliceous material away, as this would not be possible using such small volumes of reagent. Acid digestion is intended only to dissolve cements and chemically desegregate all sediment and rock material to its separate constituent grains, be they mineral or plant material. Later sieving and heavy liquid separation procedures focus on removing the considerable remaining mineral matter from the organic residue by physical rather than chemical means. All material could be dissolved chemically if so desired, but there seemed little point in dissolving quartz grains and volcanic minerals in an effort to extract palynomorphs. Subsequent repeat processing of 5 samples by traditional beaker methods in the IGNS laboratory in Lower Hutt New Zealand showed no significant difference to samples processed in Antarctica using the ProLabo microwave.

OXIDATION

Organic residues from CRP-1 contained sparse kerogen and palynomorph assemblages. Residues were not initially oxidized out of fear that the few palynomorphs present would be lost during the washing and centrifuging required for neutralising. However, some dark-grey-to-black palynomorphs, were found in the heavy fraction indicating probable fine pyrite coatings. All residues were then treated in nitric acid for 10 minutes and then neutralised, thereby lightening these palynomorphs both in colour and density.

SIEVING

All samples were sieved at this stage through a 125 micron stainless steel sieve to remove coarse undissolved material.

HEAVY LIQUID SEPARATION

On this project, heavy liquid separation compensated for the partial nature of the acid digestion of mineral material. While in traditional processing, heavy liquid separation is used to remove usually rare, small undissolved mineral grains from the target palynomorphs, for CRP-1 it served to separate a large volume of mineral grain residue from a very sparse organic residue. It should be remembered that the sample at this stage is not an appreciably smaller volume than the original crushed sample. It is, therefore, a different matter to use heavy liquids on this sort of sample than on one with little mineral matter present. Washing and centrifuging these water laden and bulky samples leaves a great deal of water in the pore spaces. This pore space water has a marked effect on the specific gravity of the heavy liquid.

Two strategies were adopted to assure the optimum specific gravity of 2.2 for palynomorph separation. Heavy liquid specific gravity was adjusted to 2.4 before addition to the sample, and the separation was carried out in a 50 ml tube rather than a 15 ml tube,. These precautions counteracted the considerable dilution effect of pore space water on heavy liquid. The heavy fraction of each sample was monitored carefully to be certain that a good separation had been achieved. On occasions, heavy liquid separation was repeated to float more organic material from the sunken mineral residue.

While this technique used relatively large volumes of heavy liquid, it was possible to simply and efficiently retrieve most used liquid and readjust it to the required specific gravity. Sodium polytungstate (SPT) was used on all separations. This water soluble relatively non-toxic product was well suited to Antarctic environmental requirements. SPT is best used with deionised water to reduce the formation of insoluble precipitates. Recovery of SPT washings for reuse was a simple matter in the dry Antarctic atmosphere by placing in a beaker on a low temperature hot plate to aid evaporation. Reused SPT was filtered through 3 micron paper before reuse.

FILTERING

All samples were filtered through a synthetic 6 micron filter cloth to remove unwanted fine-grained material using the Vidal Filter Apparatus (Fig. 3) described by Raine and Tremain (1992). This system, which is a form of the Reissinger technique, draws the sample through the filter cloth using gentle suction. To reduce filter clogging,

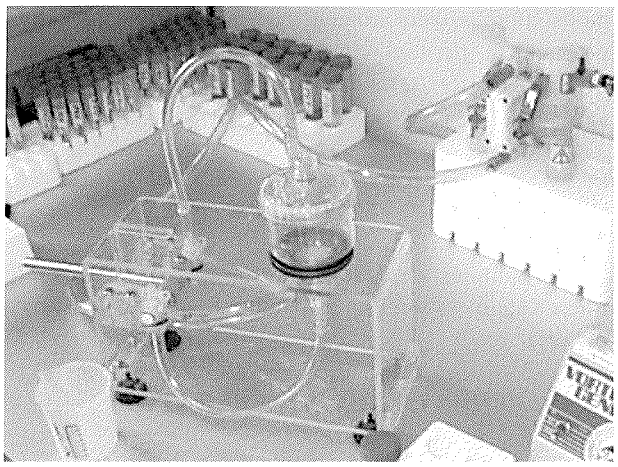


Fig. 3 - The Vidal Filter Apparatus and double diaphragm pump in oblique view.

a reverse suction is applied briefly to the cloth. This resuspends the sample instantly and can be repeated as many times as necessary. This system is used routinely at IGNS using a tap aspirator pump to provide the suction. In Antarctica, water supply is too precious to operate the filter unit with this style of pump, so alternatives were sought. The requirement is for a self priming pump that will suck air and water equally well. A Wilden M.025 double diaphragm pump operated off compressed air, was inexpensive and performed the task admirably. This pump proved to be an advance over a tap aspirator pump because it provides greater vacuum and thus speeds up the filtering process. Filtering apparatus such as this, with numerous "O" ring seals and clip on plastic connections, has many opportunities for minor leaks of air into the system. These leaks do no harm except to reduce the vacuum and slow the filtration process. With the potential for greater vacuum of the Wilden pump, there was no need to spend valuable time to search for and eliminate minor leaks in the apparatus. Vacuum recall when back-flushing is almost instantaneous compared to an aspirator pump. The combination of the IGNS filtering apparatus and double-diaphragm Wilden pump proved to be a very satisfactory and rapid method of achieving clean sample preparations.

SLIDE MAKING

All samples were mounted in glycerine jelly. These preparations were done by placing the slide with a drop of sample on a low temperature hot plate. As the water content reached a minimum, a drop of jelly was added and homogenised. Finally a cover slip was added. Sparse residues meant that in most cases there was only enough sample for two or three slides. The slides were sealed with finger nail varnish.

SUMMARY OF TIME COMPONENT OF PALYNOLOGIC PROCESSING

To crush a sample, time taken did not differ from any other palynology laboratory. Mineral solution using hydrochloric and hydrofluoric acids is traditionally done overnight in open beakers. This normally requires not only standing time for the three successive acids to work, but also considerable technician time in acid removal. This is done either by settling and decantation, or siphoning. Alternatively, centrifuging followed by decantation is used to remove acid. Both options involve a considerable time investment, most of which was circumvented by use of the ProLabo microwave digestor. Subsequent phases of oxidation and heavy liquid separation do involve processing and washing time, but only minor standing time. Filtering was faster using a compressed air operated, double diaphragm vacuum pump rather than a tap water aspirator pump, as noted above. Slide making in Antarctica was similar to processing in any laboratory.

To summarize, acid digestion phase and filtering were completed much faster than in usual laboratory processing, whereas the other operations took the usual amounts of time to complete. Overall, sample processing time was greatly improved by the use of microwave technology for acid digestion of the sediment samples.

The use of microwave digestion to process palynologic samples makes it feasible to provide palynostratigraphic support in situations where speed, safety or environmental concerns are critical. It is now feasible to process and analyse samples safely and swiftly on offshore drilling rigs, on board ship, or in areas as remote and environmentally sensitive as Antarctica.

ACKNOWLEDGMENTS

Crary Lab staff deserve thanks, for they diligently applied themselves to our need to quickly establish a laboratory. Ian Raine has been a valuable scrutineer of our techniques, and Roger Tremain has kindly drawn the complex filtering apparatus.

REFERENCES

Cape Roberts Science Team, 1998. Background to CRP-1, Cape Roberts Project, Antarctica. *Terra Antartica*, **5**(1), 1-30.

Ellin S.J. & Mclean D., 1994. The use of microwave heating in hydrofluoric acid digestions for palynological preparations. *Palynology*, **18**, 23-31.

Jones R.A., 1994. The application of microwave technology to the oxidation of kerogen for use in palynology. *Review of Paleobotany and Palynology*, **80**, 333-338.

Jones R.A., Dorning K.J., Ellin S.J. & Brooks I.P., 1995. Microwave preparation techniques for the palynological analysis of flint and chert. *Abstract, 7th International Flint Symposium, Institute of Archaeology and Ethnology, Polish Academy of Science*, Al Solidarnosd, Warsaw, Poland, **105**, 140.

Jones R.A. & Ellin S.J., 1998. Improved palynological sample preparation using an automated focused microwave digestion system. In: Bryant Jr. V.M. & Wrenn J.H. (eds.), *New developments in palynomorph sampling, extraction, and analysis*, American Association of Stratigraphic Palynologists, Inc. Contributions series 33, AASP, Dallas, TX, 23-28.

Raine J.I. & Tremain R., 1992. Apparatus for rapid sieving of palynological residues. *New Zealand Geological Survey Report Pal.*, **151**, 1-14.

Wood G.D., Gabriel A.M. & Lawson J.C., 1996. Palynological Techniques-Processing and Microscopy. In: Jansonius J. & McGregor D.C., (eds.), *Palynology: Principles and Applications*, American Association of Stratigraphic Palynologists Foundation, **1**, 29-50.