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## Recovering polymerized fossil palynomorph residues

### ABSTRACT

Fossil palynomorph residues stored in polyvinyl acetate resin (such as Vinylite AYAF) can be freed after polymerization by dissolving in DMSO (dimethyl sulfoxide). This provides an undamaged residue which then can be mounted or stored again in liquid form. DMSO can also be used to dissolve any alcohol-based mounting or to remove residues from the surfaces of palynomorphs to facilitate examination.

### INTRODUCTION

Procedures for processing fossil palynomorphs are well known, derived from basic techniques (Funkhouser and Evitt, 1959; Brown, 1960; Kremp, 1963; Doher, 1980). Refinements and variations of these techniques have done much to improve palynological recovery. The procedure described here represents a technique derived from the solidification of palynomorph residues stored in AYAF. The solubilization of these residues has freed the otherwise inaccessible palynomorphs.

A study by the first author involved fossil residues\*\* stored in AYAF. A few of these residues had become polymerized, possibly due to the fact that the palynomorphs had not been completely dehydrated during preparation. A similar phenomenon was reported earlier by Tschudy (in Kremp, 1963, p. 21); his residues became cloudy and white if not completely dehydrated.

AYAF is used by laboratories because it provides a thin, evenly distributed film of pollen and spores in a mounting medium of favorable refractive index (Tschudy, 1970).

AYAF is usually prepared by adding 100 ml of 95% ethyl alcohol or methyl alcohol to 20 g of Vinylite AYAF and 4 g of dibutyl phthalate, a plasticizer. Evaporation of the alcohol from the AYAF mixture may cause the residues to solidify, but this is corrected easily by simply adding more 95% ethanol or methanol.

Polymerizing of a small percentage of stored residues poses a more severe problem to all labs which currently use, or formerly used, AYAF. The technique described here has been successful in dealing with this problem, and should be useful for depolymerizing other alcohol-based plastic media as well.

### MATERIALS AND METHODS

DMSO (dimethyl sulfoxide) was chosen for its proven solvent qualities and high boiling point (189°C). The high boiling point of DMSO greatly reduces the possibility of explosion or flaming during heating. This is a particularly useful character when compared with boiling points of other commonly used solvents, e.g., acetone at 56.6°C and methanol at 64.7°C. Yet another property which makes DMSO particularly well-suited for palynological work is that it is miscible with many other solvents such as water, acetone, alcohol and benzene.

A sample vial containing the polymerized residue was carefully broken open to extract the solid but pliable plug which had formed. The plug was trimmed with a razor blade to reduce the volume of solvent re-

\*Currently employed by the U.S. Geological Survey, Oil and Gas Branch, as a Graduate Student Appointee.

\*\*Fossils kindly provided by the U.S. Geological Survey, Paleontology and Stratigraphy Branch, Denver.

quired to dissolve the residue. The excess was then halved and both portions treated with DMSO.\* This procedure was designed to act as a control before the residues were processed. One-half of the trimmings was dissolved at room temperature, and the other half was heated in a ceramic crucible. Favorable results were obtained in both instances. The portion of plug that was heated dissolved within 30–45 seconds without agitation. The unheated portion was gently agitated with dissolution becoming complete within approximately 45 minutes. The fossil-bearing residues were then treated using the same two procedures. The unheated portion was gently teased with a 7.0 mm o.d. disposable pipet until no agglutination of the residue was observed under a dissection stereomicroscope. The heated portion was allowed to cool to room temperature before viewing.

The separate and dissolved residues were then mixed with distilled water (1 part residue to 10 parts of water) and centrifuged in 15 ml glass tubes. Three to five complete washes were sufficient for the removal of DMSO and other products of the dissolution. The residues were then dehydrated by rinsing successively in 70%, 90% and anhydrous alcohol. It is important that the anhydrous alcohol used be kept over a molecular sieve to prevent water contamination if an alcohol-based plastic is to be used later in storage or mounting. The residues, in anhydrous ethanol (or methanol), were then stored in vials and examined with visual spectrum and scanning electron microscopy techniques.

\*Available from VWR Scientific Inc. for about \$18.00 a pint. Caution should be exercised when handling DMSO because it is readily absorbed through the skin.

## RESULTS

The palynomorphs showed no damage from the treatment of DMSO in either the light or SEM observations. The exines of the pollen and spores examined were extraordinarily clean and free of extraneous debris.

The treatment of the polymerized fossil residues with DMSO provides a quick and easy procedure for making these residues usable again. Because of the unique properties of DMSO, we suggest that it has excellent prospects for palynological use.

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## REFERENCES

- BROWN, CLAIR A., 1960. Palynological techniques. Baton Rouge, 188 pp.
- DOHER, L. IMOGENE, 1980. Palynomorph preparation procedures currently used in the paleontology and stratigraphy laboratories, U.S. Geological Survey. U.S. Geol. Surv., circular 830:29 pp.
- FUNKHOUSER, JOHN W., and EVITT, WILLIAM R., 1959. Preparation techniques for acid-insoluble microfossils. *Micropaleontology*, 5(3):369–375.
- KREMP, GERHARD O. W., 1963. Processing samples for palynological study. *Geochron. Labs., Univ. Arizona, Tucson*, 27 pp.
- TSCHUDY, R. H., 1970. Two new pollen genera (Late Cretaceous and Paleocene) with possible affinity to the Illicaceae. *U.S. Geol. Surv., Prof. Paper 643-F*, 13 pp.

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