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Use of UV-curable acrylates gels as mounting media for palynological samples

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Abstract: UV-curable acrylates are used as an easy, economic and rapid mounting media to mount palynological samples. The aqueous palynological residue is dehydrated with ethanol in order to be set in UV-curable acrylates such as Trabasil ® NR2 and Acrysoft ® urethane acrylates. These mounting medias have advantages over other ones: specimens remain in fixed position, and/or they are not attacked by any organism or modified by any environmental conditions such as humidity and heat. In addition refraction index is similar to that of sporopollenin and glass which provides an excellent interfase to discern fine morphological features.

Key words: palynomorphs, microscope slides, mounting media, acrylates.

Resumen: Acrilatos inducidos por la luz UV son usados como un medio simple, fácil y económico para el montaje de muestras palinológicas. El residuo palinológico en medio acuoso es deshidratado con etanol para luego ser fijado en acrilatos inducidos por luz UV, comercializados como, por ejemplo, Trabasil ® NR2 y Acrysoft ®. Estos medios de montaje son más ventajosos que los tradicionalmente conocidos: los especímenes quedan en una posición fija, el medio no es atacado por organismos o modificado por ninguna condición climática, tal como humedad y/o calor. Por otra parte el índice de refracción es similar al de la esporopolenina y al del vidrio, lo que confiere una excelente interfase para discernir los caracteres morfológicos más delicados.

Palabras clave: palinomorfos, portaobjetos, medio de montaje, acrilatos

INTRODUCTION

Microscope slides are the principle form of archive for both extant and fossil palynomorphs sustaining published material and holotypes worldwide. Samples are usually mounted in different media (e.g. Canada balsam, acrylic resin such as Elvacite ®, polyvinyl alcohol (PVA), glycerin jelly), which are selected depending upon the final use of the sample. Generally, a good mounting media (m.m.) should have an index of refraction close from that of sporopollenin (1.48) and glass (1.52) (Traverse, 2007) and have the longest sustainable life.

A new simple protocol using m.m. based on UV-curable acrylates to prepare a microscope slide, for both extant and fossil palynological organic matter is presented herein. The most common problems of the most popular media are also commented on.

MATERIAL

The m.m. used are UV-curable acrylates constituted by acrylic oligomers or urethane acrylic oligomers with polymerization initiators activated by UV light (direct sunlight or UV lamp) and diluent monomers that regulate viscosity. Oxygenated organic solvents (e.g. ketones, esters, certain alcohols) and polar aromatic compounds are good diluents before polymerization occurs. Ethanol is a good diluent in low amounts. Acrylates have a weak yellow tone, hardly noticeable, which do not interfere with the observations.

UV-curable acrylates are frequently used as printing resins, for cosmetic and dental applications and as an adhesive for glass, aluminium, steel and plastic bonding. These polymers are locally commercialized as Trabasil ® NR2 and Acrysoft ® UV m.m. T and Acrysoft ® m.m. S (http://www.acrilab.com/) (Table 1). However, these kind of acrylates can be purchased in numerous countries under different denominations in hardware stores. Silverman (1986) used similar acrylates to prepare histological slides dehydrating with alcohols and xylene.

Table 1. Acrylates used as mounting media.

	Component	Component	Component
Trabasil ® NR2	Acrylic esthers	very low	RI:1.507
Acrysoft ® UV mounting media T	urethane Acrylic	high	
Acrysoft ® UV mounting media S	urethane Acrylic	low	

METHODS

I- Fossil material

For fossil samples, remains of organic material are extracted from an inorganic matrix following standard methods (e.g. Traverse, 2007 and references therein) and are usually neutralized and kept in water. Since water is not miscible with this m.m., the residue needs to be dehydrated.

1. Transfer the suspended organic residue to a falcon tube and centrifuge for 10' at high speed or decant for as long as it takes in order to concentrate the organic material in the bottom. Discard the supernatant.
2. Fill the tube with ethanol 96% (commercial medicinal alcohol) and shake it to homogenize the sample.
3. Centrifuge for 10' at high speed and decant.
4. Repeat steps 2 and 3 two more times. This works well for volumes of suspended residues up to approximately 0.5 ml. Larger volumes may require more repetitions of steps 2 and 3.
5. Once the residue is dehydrated, place a drop of the m.m. directly on the slide and then mix it with a drop of the residue using a disposable stick. Cover with a coverslip, press gently and expose to UV light. It takes around two minutes of exposure to either direct sunlight or a UV lamp to harden the m.m. Overexposure to UV does not produce any modification to the final result (Fig. 1).

II- Extant material

Extant dry pollen samples may be put directly "raw" on the slide with a drop of the m.m. If the subject of study is the exine morphology, the samples should be by acetolysis, introduced by Erdtman (1943) and later modified by Traverse (1955), or similar method. The resulting residues, suspended in water, should follow the steps accounted for fossil material (see above) in order to dehydrate the material for its subsequent fixation on the microscope slide.

Comments

Ethanol can be replaced with organic solvents such as acetone or xylene. Do not mount under direct sunlight to prevent the m.m. from hardening before ending the process.

DISCUSSION

In order to analyze the performance of the tested m.m. several parameters were taken in consideration, such as size alteration of palynomorphs, refraction index and ease of handling, listed below.

Size

Measurement of the major diameters of different palynomorph specimens, extant and fossil pollen grains and fossil spores were carried out. Slides were both prepared from material stored in alcohol or directly from dry residue. A number of specimens from each slide were selected, measured, photographed and exact coordinates of each specimen were recorded, the same day the slides were mounted. A year later some of the same specimens were measured and photographed. Table 2 presents the results of those measurements, indicating that there is no significant difference in the size of specimens.

Refractive indices

The refraction index of Trabasil NR2 is 1.507 which is similar to that of glass and sporopollenin. Although the refraction index is not provided for the other two m.m. (Acrysoft ® acrylates) according to our observations they do not show appreciable variations from that of glass and sporopollenin.

Table 2. Measurements made with light microscope at 100X. Error ± 1 μm.

Specimen	Suspension	Control measurement [μm]	Measurement after one year [μm]
Extant pollen grain	Dry	69 ± 1	68 ± 1
Extant pollen grain	Ethanol	74 ± 1	74 ± 1
Fossil specimen	Dry	82 ± 1	82 ± 1
Fossil specimen	Ethanol	36 ± 1	35 ± 1

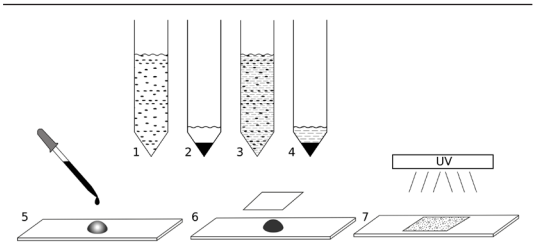

Fig. 1. Steps for preparing a palynological slide with UV-curable acrylates. Steps 2-3 should be repeated one or two times. 1. Aqueous palynological suspension. 2. Decanted organic matter. 3. Palynological residue suspended in ethanol. 4. Decanted organic matter. 5. Mounting media resting on the microscope slide. 6. Organic matter mixed with the mounting media. 7. Exposure to U.V.

A significant difference of the refractive indices between the sporopollenin and m.m. leads to high contrast, which may obscure specimen features; a refraction index too close between them leads to low contrast, which may be insufficient to discern fine details (Bennet & Willis, 2002; Traverse, 2007).

Comparisons with other mounting media

Although each different m.m. has different requirements and purposes, UV-curable acrylates have many advantages over other m.m.

For those who are looking for a m.m. that provides some mobility, silicone oil (Andersen, 1960) or even a highly hydrated glycerin jelly might be a good choice. The most popular m.m. is the latter, since its components are widely available, inexpensive and the recipe is easy to prepare. Nonetheless it has serious disadvantages. It absorbs water from the atmosphere causing grains to degrade (Moore *et al.*, 1991). There is also controversy as to whether this m.m. results in a change of grain diameter, resulting in swollen components (e.g. Andersen, 1960; Moore *et al.*, 1991; Sluyter, 1997). There are other investigations, which affirm that despite the m.m. used, the grains have a tendency to shrink (Faegri & Deuse, 1960). Furthermore, glycerin jelly slides should be stored flat and in a temperature controlled room (Wood *et al.*, 1996), to prevent specimens to get displaced. Aside from the issues detailed above, the most important problem is the longevity of the samples. If the slide is not provided with a proper seal (e.g. paraffin), which demands extra processing time and monetary resources, the sample may experience unwanted air bubbles and voids within the m.m. which spoils the appearance of the organic material (Fig. 2A).

Silicon oil keeps the pollen grains size stable (Andersen, 1960; Faegri & Iversen, 1989) but there is evidence that despite the sealant used (e.g. nail polish, adhesive, paints and paraffin) pollen grains may still get corroded (Cushing, 2011).

Canada balsam and Elvacite ® are known both to be good, but the organic residue has to be dehydrated beforehand with alcohol and xylene. The manipulation of the latter solvent has well documented hazards to human health (Kandyala *et al.*, 2010) and, therefore it is recommended the utilization of a fume hood to work with it. It is not until the dehydration is complete that the organic residue can be mixed with either of the m.m. quoted above. Additionally, the refraction indices are either too close to the sporopollenin, as in the case with Elvacite ® (1.49) or too different as in the case with Canada balsam (1.53).

The m.m., presented herein, has several important advantages, e.g. slides with more than 10 years look exactly the same. When the location stability of the specimens is required, the UV-curable acrylates provide an excellent interface for the palynomorphs, keeping the specimens exactly in the same place. Conveniently, the m.m. has low or high viscosity depending on which one is required. This method also negates exposure of

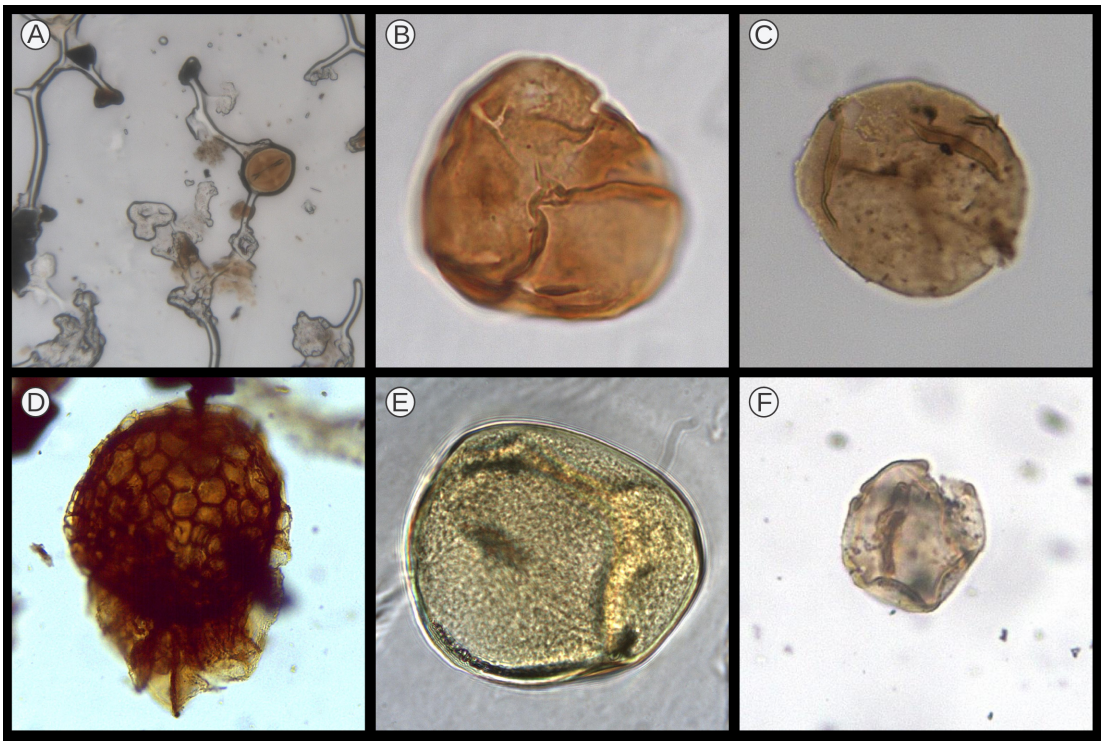

Fig. 2. Samples of palynological specimens mounted with UV-curable acrylates. A. Palynological slide mounted with glycerin jelly altered by air dendrites after ten years. B. Fossil spore mounted with Trabasil ® NR2 (photographed with a 100x objective). C. Fossil spore mounted with Acrysoft ® UV mounting media T (photographed with a 100x objective). D. Fossil spore mounted with Trabasil ® NR2 (photographed with a 40x objective). E. Extant pollen (without acetolysis) mounted with Trabasil ® NR2 (photographed with a 100x objective). F. Fossil pollen mounted with Acrysoft ® UV mounting media S (photographed with a 40x objective).

the m.m. to higher temperatures (e.g. glycerin jelly) in order to achieve a manageable consistency.

When the location stability of the specimens is required the UV-curable acrylates succeeds by a large amount over other m.m. such as glycerin jelly. The polymerization that develops around the specimens would prevent them to enlarge or to displace.

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