

# Use of HMDS (hexamethyldisilazane) to dry organic microstructures in etched bivalve mollusk and barnacle shells

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## ABSTRACT

The organic framework of molluscan and barnacle shells yields clues to biocalcification processes. Slight demineralization of the shells reveals the fragile meshwork of insoluble organic fibers and membranes, which tend to collapse, wrinkle, and shrink when air-dried from water. Comparison of different drying techniques on etched bivalve mollusk (*Chione fluctifraga*) and barnacle shells (*Chthamalus* sp.) reveals that hexamethyldisilazane (HMDS) produced results qualitatively superior to critical point drying or drying from ethyl alcohol or water. HMDS dries structural details of the organic meshwork excellently and facilitates the recognition of faint growth increments for growth pattern analysis (sclerochronology). The HMDS method is cost-effective, saves time and can be used as a routine substitute for drying microstructures in slightly etched molluscan and barnacle shells.

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## INTRODUCTION

Organic matter plays an essential role in the formation of molluscan and barnacle shells. It has been realized that the organic matrix provides the structural framework (“template”; Clark, 1980) for biomineralization and influences the mineralogical and crystallographic properties (Mann, 1983; Simkiss and Wilbur, 1989; Crenshaw, 1990 and literature therein; Watabe et al., 1993). Demineralization of skeletal hard parts unveils the underlying three-dimensional organic microstructures, which may yield clues to biocalcification processes.

Organic matter is also a major constituent of some growth increments (e.g., Koike, 1986). Slight demineralization of cross-sectioned molluscan and barnacle shells reveals a three-dimensional relief of insoluble organic components and differentially dissolved crystals (as a result of different crystal sizes and orientation). In sclerochronological studies (growth analyses), superficial etching is commonly used to aid in the identification and measurement of internal growth increments in mollus-

can and barnacle skeletons (Rhoads and Lutz, 1980; Schöne et al., in press). The etching time varies for different species and depends on, for instance, the shell structure, mineralogy, and organic content. Although growth patterns in mollusk shells are the focus of numerous studies, only few papers deal with the growth patterns of barnacles. Unlike most crustaceans, barnacles do not replace their hardparts. Both mollusks (e.g., Davenport, 1938; Pannella and MacClintock, 1968) and barnacles (e.g., Bourget, 1980) grow by periodic accretion of skeletal material producing circadian growth increments (see sketches in Figure 1 and 2: direction of growth). In barnacles, the growth layers are best viewed in the sheath layer (Figure 1), and in most bivalve mollusks in the outer shell layer (Figure 2).

Like most soft tissues, the shell organic framework, including the organic-rich growth increments observed in cross-sections, is prone to collapse, shrinkage, and wrinkling when air-dried (e.g., Anderson, 1951; Nation, 1983; Clark, 1980, 1999). Preventing these unwanted effects requires special chemical treatment, which dehydrates and hardens the fragile organic structures. Several techniques are used to dry biological soft tissues. Although extremely time-consuming and quite dangerous (highly pressurized chamber), critical point drying (CPD) is by far the most common method (Anderson, 1951; using liquid CO<sub>2</sub>, e.g., Clark, 1980 or Freon 13 as a transitional fluid, e.g., Koike, 1986). On average, preparing one sample by CPD requires full attention over 1.5 hours. The basic CPD equipment costs several thousand dollars. Good results were also achieved with the sublimation dehydrant Peldri II (Kennedy et al., 1989). However, preparation following this technique takes more than twice the time as CPD, and Peldri II is no longer available because of environmental hazards. Fluids with low surface tension (acetone or propylene oxide, Boyde and Wood, 1969) sometimes produce reasonable, artifact-free results for biological soft tissues. Some workers prefer the extremely hazardous osmium tetroxide technique (Quattlebaum and Carner, 1980).

A reliable and simple drying technique, which produces results qualitatively comparable or superior to

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