PHOTOTHERMAL AND STRUCTURAL COMPARATIVE ANALYSIS OF CHITINOUS EXOSKELETONS OF MARINE INVERTEBRATES

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Abstract

Chitinous materials are common in nature and provide different functions including protection and support of many invertebrate animals. Exoskeletons in these organisms constitute the boundary regulating interaction between the animal and the external environment. For this reason it is important to study the different physical properties of these skeletons, in particular thermal properties. The objective of this study is to investigate the thermal diffusivity of the skeletons of four species of marine invertebrates, *Antipathes caribbeana* (black coral), *Panulinus argus* (lobster), *Litopenaeus vannamei* (shrimp), *Callinectes sapidus* (crab) and *Limulus polyphemus* (xiphosure).The characterization is performed using photothermal techniques, such as photothermal radiometry, and open photoacoustic cell. Our measurements are complemented with structural characterization using X-ray diffraction. The results indicate that the thermal properties recorded are strongly dependent on the studied species.

Introduction

Chitin is a linear polysaccharide, made of β-(1→4)-2-deoxy-2-acetamido-D-glucopyranose repeating units. It is similar to cellulose from the point of view of its structure and abundance in nature. In its native state, this biopolymer occurs in association with proteins, pigments, lipids and inorganic substances and is chiefly found as a fibrillar semicrystalline material.

Chitin has protective and supporting biological functions. It is also an important component of the organic matrix of exoskeleton of many invertebrate animals (insects, crustaceans, mollusks, corals, annelids, ectoprocts, brachiopods), fungi and bacteria. It is therefore important to study the crystalline structure, physical properties and quantitative proportion of chitin available in different species, in particular the marine ones (Fig.1).

In this work we present the thermal characterization of biogenic chitin. The study has been performed in the skeletons of five species of marine invertebrate organisms that develop their structures based on chitin: *Antipathes caribbeana* (black coral), *Panulinus argus* (lobster), *Litopenaeus vannamei* (shrimp), *Callinectes sapidus* (crab) and *Limulus polyphemus* (xiphosure). The thermal analysis was performed at room temperature using thermal techniques (open photoacoustic cell technique and photothermal radiometry) and the structural characterization was made with X-ray diffraction.

Materials and Methods

Source of material

The crab (*Callinectes sapidus*), xiphosure (*Limulus polyphemus*), shrimp (*Litopenaeus vannamei*), and lobster (*Panulinus argus*) specimens were collected from the sandy coastal area of the Yucatan Peninsula in Mexico. The black coral colony (*Antipathes caribbeana*) is native from the deep reefs of Cozumel Island, Quintana Roo Mexico (Western Caribbean).

Sample preparation

For the photoacoustic (PA), photothermal radiometry (PTR) and the X-ray diffraction measurements flat samples of 1 cm^2 were cut from dorsal crab, xiphosure, lobster and shrimp carapaces, and black coral was cut transversally in the perpendicular direction to the main axis of the colony coral. After that, the flat samples were polished with sand paper up to reaching thicknesses of around 150–260 μm. For the X-ray diffraction, the samples were ground until a powder was obtained.

Photothermal measurements

Thermal diffusivity was measured in the configuration of transmission using the technique photothermal radiometry. In order to fulfill the requirement of opacity implicit in the photothermal models used, a thin aluminum foil (25 μm) was attached to the samples using thermal paste. Figure 2 shows the experimental setup. A 659 nm Mitsubishi ML 1016R-01 maximum power: 100 mW semiconductor laser was modulated by the laser diode controller (Thorlabs SP500) and the built-in function generator of the lock-in amplifier (Stanford Research SR830). The laser beam was focused onto the aluminum foil. The heat transmitted generates a modulated heating in the sample that generates infrared modulated infrared radiation. This radiation is collected and focused by two off-axis paraboloidal mirrors onto a HgCdTe detector. Before being sent to the lock-in amplifier, the PTR signal was amplified by a preamplifier (EG&G Judson PA-300). The experiments were performed varying the frequency in order to generate a depth profile of the material.

The measurement of very thin samples (shrimp) was performed using the open photoacoustic cell (OPC) technique. The samples were mounted directly on top of the front sound inlet of an electret microphone and fixed with vacuum grease. The samples were illuminated with a modulated light beam of an 80mW Argon Laser (Omnichrome 545A), whose beam was mechanically modulated with a chopper (Stanford Research Systems 540). As a result of the periodic heating of the sample, the pressure in the chamber oscillates at the modulation frequency and can be detected by the electret microphone. The signal from the microphone was sent to a lock-in amplifier (Stanford Research Systems 830) in which the signal amplitude and phase were both recorded as a function of the modulation frequency.

X-ray diffraction

The flat thin samples and powders were characterized by X-ray diffraction with a Bragg-Brentano geometry (Siemens D5000) using a monochromatic CuK α radiation (λ =1.5418 Å), operating at 34 kV, 25 mA. The diffractograms were registered in a range of $5^\circ < 2\theta < 70^\circ$ in a step scan mode of 0.02° (2θ) with a counting time of 12 s per step.

Results

Photothermal measurements

Figure 3 shows the heat diffusion of five species of marine invertebrates. The sample thickness and thermal diffusivity (α) are summarized in Table 1. The heat diffusion is higher in crab and lobster than black coral, even though this sample is thicker. However, the lowest thermal diffusivity belongs to xiphosure sample (Fig. 4). The shrimp sample was very thin (45 μm) therefore, the measurements made with photothermal radiometry the observed signal belongs the aluminum foil. An open photoacoustic cell was used in order to obtain the shrimp thermal diffusivity data.

Structural characterization

The X-ray diffractograms for the thin samples (Fig. 5a) and powders (Fig. 5b) exhibit two peaks at 12º and 19° (2θ), except for the shrimp that also the reflection at 9.6º was observed, corresponding to the chitin structure (Minke 1978). However, the relative intensities are rather different, whereas the wide and strongest peak at 19° (2θ) is associated with the plane (110). Xiphosure sample showed the highest intensity X-ray pattern, followed by black coral and shrimp, although, the shrimp sample showed the highest crystalline X-ray pattern on the flat sample (Figure 5a).

Discussion

The strong differences observed in thermal diffusivity are related to the array of the mesostructure of the different species. These results could suggest that organisms as crab and lobster are more susceptible to external temperature changes of the surrounding water [4]. These results could also be worth in modelling the growth process, adaptability and success of these species and in understanding the process of skeletogenesis.

The strong and well defined X-ray peaks in the crab and lobster samples is related to a highly crystalline calcite, however they showed a preferred orientation in the (hk0) reflections. The highest values of thermal diffusivity corresponding to crab and lobster could be due to the presence of highly orientated calcite on the skeleton.

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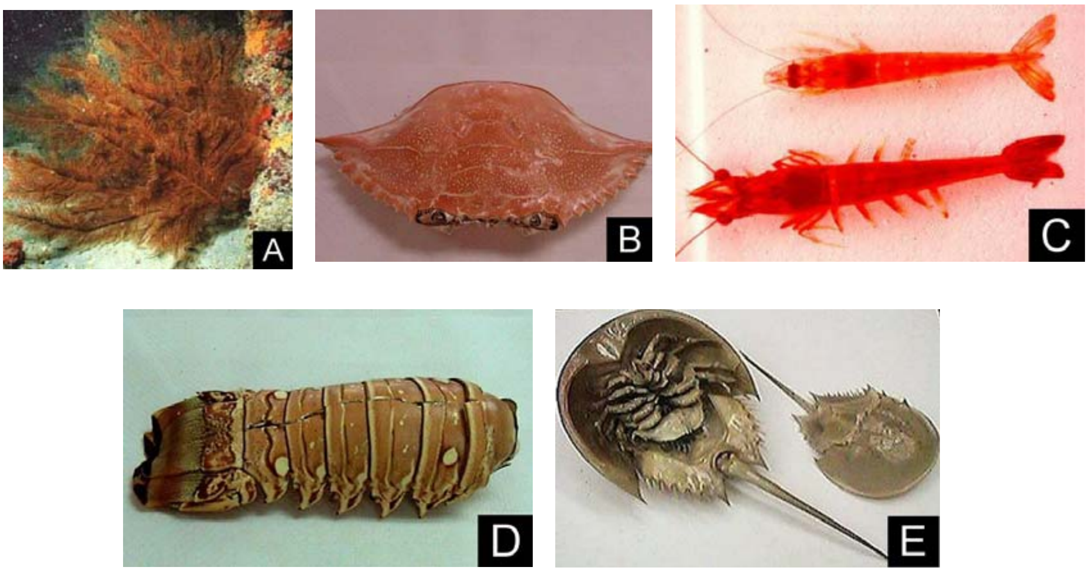


Figure 1 : Figure 1.Chitinous exoskeletons of marine invertebrates: A) *Antipathes caribbeana* (black coral), B) *Callinectes sapidus* (crab), C) *Litopenaeus vannamei* (shrimp), D) *Panulinus argus* (lobster) and E) *Limulus polyphemus* (xiphosure).

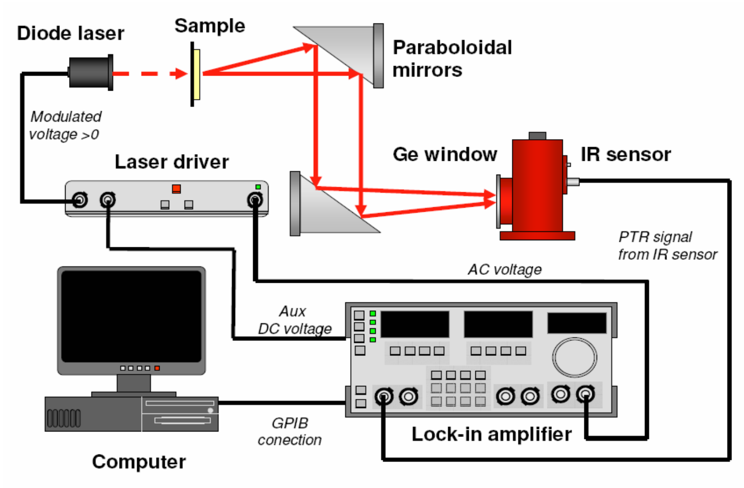


Figure 2 : Figure 2. Experimental setup for (PTR) measurements.

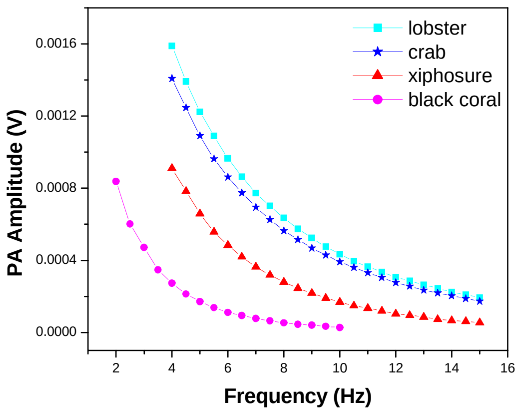


Figure 3 : Figure 3. PTR response using the amplitude (V) of the PT signal as a function of the frequency (Hz) for species of marine invertebrates.

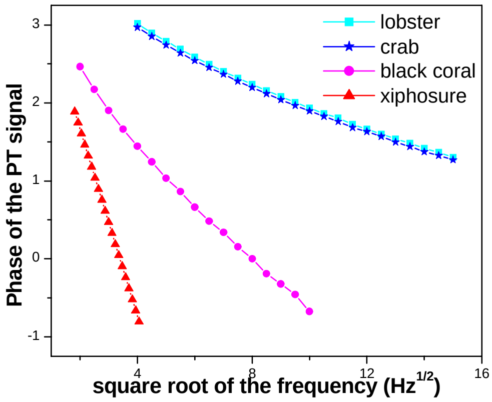
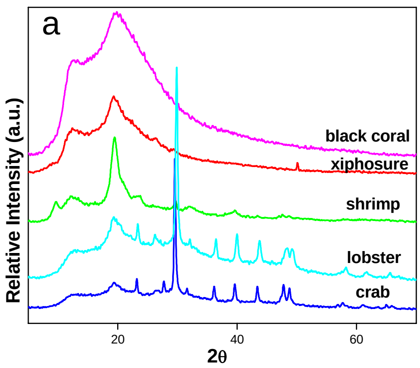


Figure 4 : Figure 4. Phase of the PT signal as a function of square root of the frequency ($Hz^{1/2}$) and line of best fit for obtaining the thermal diffusivity ($\alpha = \pi L/m2$, where L=sample thickness and m=slope of the line).



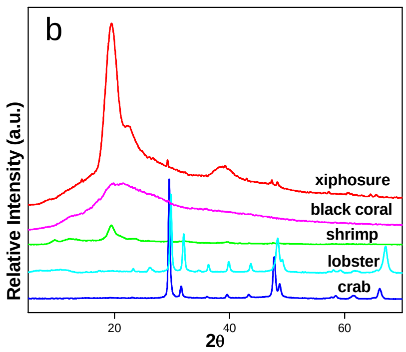


Figure 5 : Figure 5 .Diffractograms of the marine invertebrate skeletons. a) The flat sample; b) The powder sample.

Figure 6 : Table 1. Thermal diffusivity (α) values of five species of marine invertebrates.

Specimen	Sample thickness L (μm)	α (cm^2/s)
Xiphosure	197	0.00083
Black coral	385	0.00151
Shrimp	45	0.00152
Lobster	220	0.00184
Crab	182	0.00185