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Microstructure and Thermal Expansion Properties of Ostrich Eggshell

A. Heredia, L. Lozano, C.A. Martinez-Matias, M.A. Peña-Rico, A.Rodriguez-Hernández, E. Villarreal[4], A. Martínez, M.V. García-Garduño[1,2], V.A. Basiuk[3,5], L. Bucio and E. Orozco

Instituto de Física UNAM, Apdo. Postal 20-364, 01000 México D.F., México
[1]Facultad de Ciencias UNAM, 04510 México D.F. México
[2]Div. de Posgr. e Invest., Facultad de Odontologia UNAM, 04510 México D.F. México
[3]Instituto de Ciencias Nucleares UNAM, 04510 México D.F. México
[5]Faculty of Engineering, Yokohama National University, Japan. Hodogaya-Ku, Yokohama 240-8501.
[4]Instituto de Investigaciones en Materiales, UNAM, 04510 México D.F. México

ABSTRACT

Textures of calcite crystals from ostrich (*Struthio camelus*) eggshells were examined with X ray diffractometry (XRD), transmission and scanning electron microscopy (TEM, SEM), and the thermal stability by thermal expansion analysis (TEA). Results showed that textures vary through the thickness of the eggshell and that expansion properties and thermal behaviour are unusual. Crystals from ostrich eggshell are arranged in two main configurations or layers; the outer layer with the *c*-axis of crystals oriented perpendicular to the eggshell surface and the inner layer with the *c*-axes of the crystals arranged almost parallel to eggshell surface; thermal expansion analysis show high stability through a wide range of temperatures until a steep growth near 450-460 °C. These results show that the manipulation of crystal texture and properties is under biological control and a better understanding of this biological phenomenon will provide more and better data for improving new synthetic composite materials.

INTRODUCTION

Biominerals (or biocrystals) has long been the object of biomimetic study because of its characteristic structural arrangements and interesting mechanical properties [1, 2]. Determining the structures of biological materials is the most important challenge to understand all this properties and mechanisms to design a new generation of biomimetic materials. The avian eggshell is an unusual kind of natural biomaterial because its formation process is extremely rapid (≈ 5.0 g of $CaCO_3$ deposited in 24 h [4]) and the structure features arrangement of calcium carbonate crystals (calcite) in different layers relative to a biological macromolecule. In turn among avian eggshells, ostrich is of particular interest because of its fine assembly and therefore excellent structural and mechanical properties [4]. However, information on the crystal distribution is not enough. Some authors have done microstructural analysis to determine qualitatively the crystal orientation relationship between the neighbouring crystals in ostrich eggshell, obtaining randomly distribution of crystals in inner layer. This information should be especially valuable for a complete understanding of biomineralization and particularly in controlling the organic framework role of crystal nucleation and orientation, assembly as well as preferred crystal growth [3]. As we know it is not the first description of crystals orientation and their physico-chemical processes in biological nature [1, 3-8] and in order to extend the understanding of biominerals properties and biomineralization as well, here we present the microstructural characterization concerning to morphology, texture and structures of the two surfaces (inner and outer) by scanning and transmission electron microscopy and X-ray diffraction and inner membrane by XRD. To our knowledge, it is the first report of a property (thermal expansion) related to crystal orientation and the first description of the organic amorphous related to a crystalline phase.

EXPERIMENTAL DETAILS

Ostrich eggshell samples (OE), obtained from Veterinary and Zootechnical Faculty, National Autonomous University of Mexico, were analyzed by powder X-ray diffraction method. Samples were prepared by cutting pieces of 1.0 cm^2 area. Both inner and outer surfaces were analysed putting the piece in the sample holder like a specimen having flat geometry and powdered eggshell was analysed as well. X-ray powder diffraction pattern was recorded with a step size of 0.02° over 22-90° 2θ-range. The diffraction data were collected with a counting time of 10*s* each step using a Siemens D5000 Diffractometer Cu$K\alpha$ radiation (35 kV, 25 mA), vertical goniometer, fixed diffracted beam graphite monochromator and scintillation counter. The slits configuration was an aperture slit of 2 mm, a scattered radiation slit of 2 mm, a monochromator slit of 0.6 mm and detector slit of 0.6 mm. For SEM and TEM studies OE samples were sliced with a diamond wheel producing 0.3 x 0.3 cm slices 250 to 500 μm thick. The slices were subsequently polished down to a thickness of approximately 100 μm with a No. 1000 silicon carbide paper and water. Afterwards they were ground to the thickness of 10 μm using a Fischione dimpler, with periodical reversals to make both surfaces as homogeneous as possible. The specimen was thinned in a Dual 600 Gatan Ion Mill machine until a small hole was observed at its center with edges thin enough for TEM observation. Finally, the specimens were covered with a carbon film 20 nm thick in order to minimize electron beam damage and electrical charging resulting from ion bombardment. For thermal expansion analysis, OE were cut in pieces of 8 x 3 mm parallel to longitudinal axis of eggshell and 8 x 3 mm for the perpendicular to longitudinal axis of eggshell, other sample was cut to expand in the thickness of eggshell and the data corresponding to thickness of samples is the thickness of ostrich eggshell (2 mm). These samples were analysed in a home-made thermal expansion device in accord with the international standard ASTM E 228-95 [10]; heating them from room temperature to 1000°C.

DISCUSSION

The outer layer of ostrich eggshell is the most crystalline and preferred orientation region comparing with inner layer, which is oriented in *a, b*- axis (see figure 1) what seems in some areas a monocrystalline layer (outer layer) by SEM (see figure 2).

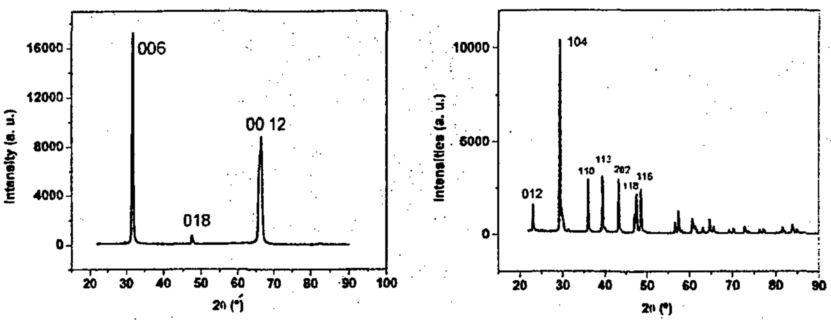

Figure 1. Powder XRD pattern of outer (left) and inner (right) layer of the ostrich eggshell. Due to the peaks and its intensities we have determined a preferred orientation of crystallites. This orientation regards c -axis perpendicular to ostrich eggshell surface (left) and crystals oriented parallel to surface (inner). The figure shows the only mineral phase is calcite.

Although some authors [4, 11] identified minerals of apatite group in the outer eggshell's layer, we did not find them in our samples. The results shown by other authors agree with ours and we include the crystallite average size in preferred orientation as well (See table 1).

Table I. Average size of crystallites related to ostrich eggshell surface.

	Outer	Inner	membrane
Crystallite average size	558.77 Å* $_{(006)}$	1000-3000 Å**	
Preferential orientation vector	001	Perpendicular to 001	None

By Sherrer's eqution and **By SEM

Figure 2. SEM image of outer layer of ostrich eggshell showing a region of a gaseous interchange pore. The smooth surface is due to compact arrangement of calcite nanocrystals. We regard this arrangement is a biological design directed by biopolymeric phase. Scale bar = 1.0 µm

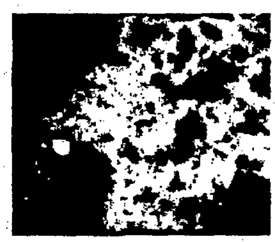

Figure 3. SEM (left) and dark field TEM (right) microphotographs of inner layer of ostrich eggshell showing biopolymer fibres bound to a grains of calcite crystals. This unidentified biopolymer we consider is the responsible for the thermal properties. Scale bar in SEM= 1.0 μm. Bigger grain measure in TEM is = 0.13 μm diameter.

Calcite crystals orientation resemble results obtained by other authors [4] but with some differences: those authors describe a non-preferred orientation in the inner crystal layer, whereas we found a preferred orientation with respect to the *a* -crystal axis (see XRD pattern shown in figure 1) and a large amount of unidentified fibrous biopolymer (Seen in figure 3).

This biopolymer cluster is associated with calcite crystals (Figure 3 and 4) to biomineralizate and although it is not well known some authors have found signs of type I, V and X collagen [11]. The biopolymer-crystal interaction is not well understood as well.
In this work the crystals are regarded as needles due to the XRD spectra where only some peaks are seen. In other words, the *c*- axis of needle-like crystals is preferentially arranged in parallel to the eggshell surface, and through the equivalence in *a* an *b* axes in calcite we distinguish a longitudinal arrangement to eggshell surface of *c*- axis in inner layer.

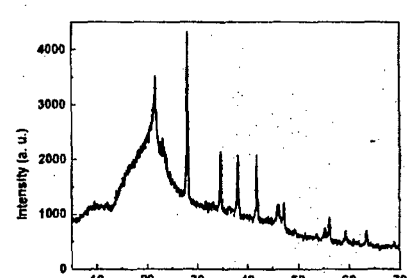

Figure 4. Powder XRD pattern of inner membrane of the ostrich eggshell. The peaks are more diverse than the presented in figure 1 what seems randomly oriented crystals and by its intensities we have determined a biggest size of crystallites than figure 1 cases. This figure is an approach to crystal-biopolymer interaction.

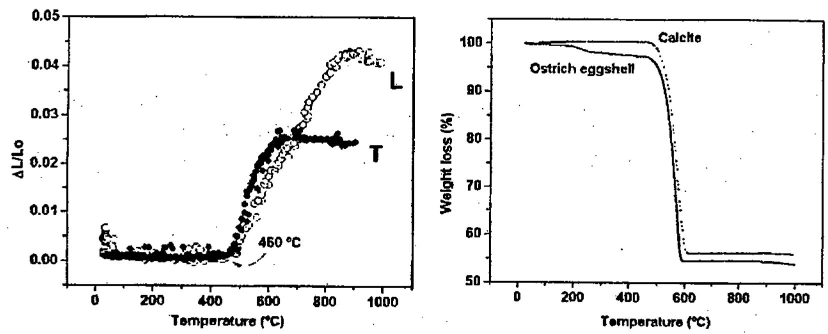

Figure 5. Anisotropic thermal expansion of OE (left) and OE TG (right) where mass loss initiate near 460°C what is related to chemical reactions in TG. In left graph L means a slice parallel to longitudinal OE axis while T is transversal direction (or side view) to longitudinal axis of OE.

The average size of crystallite was measured through Scherrer's equation with full width at half maximum FWHM of a distinctive peak (006) and obtaining values presented in table I. Scherrer's equation was useful only for outer layer because are colloidal crystals.

On the basis of the experimental data the ostrich eggshell has an evident quantitative expansion starting at high temperatures near 450-460 °C (As presented in figure 5). Preferred expansion is in longitudinal axis of OE This temperature is related by a TGA with a reaction of mass loss of calcite phase in OE.

We think the anisotropical thermal expansion of OE is due to amorphous biopolymer-calcite interactions producing a phenomenon that resemble monocristalization and is possible the rearrangement from *a* to *c*-axis by heating samples on OE samples. The rearrangement of crystals can be inferred by anisotropic thermal expansion of calcite [11], changing the orientations it would change the expansion behaviour as well. Any way a detailed analysis is required to elucidate this behaviour.

CONCLUSIONS

The results reported here directly show a complex structure on OE which is related to anisotropical physical properties (*i.e.* thermal expansion) independent of calcite properties what seems is related to biopolymer-crystal relationship.
How the bioplymeric matrix is forming calcite crystals is until now an unresolved problem. However, it is tempting to speculate that the multiple interactions of amorphous biopolymer and calcite is the responsible of nucleation, growth control and orientation assembly, and preferred crystal growth performing ion-ion reactions that withdraw properties of biomineral from mineral structure. The knowledge of interactions and properties of biological structures will provide the opportunity to mimic these highly complex structures by the manipulation of the molecules to synthesize nanostructural materials or composites with special anisotropic physical properties.

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