Molecular stratigraphic analysis with Raman spectroscopy of the shell of a mussel

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Abstract. *Two different cristallographical forms of calcium carbonate (aragonite and calcite) has been identified in mussels with Raman spectroscopy.*

Keywords: Raman spectroscopy, shells, pigments, calcium carbonate.

I. INTRODUCTION

The main objective of this work is to analyze the materials (colored or not) that compose a mussel´s shell. In order to get this objective a meticulous stratigraphic analysis has been carried out with Raman spectroscopy. The fundamental reason to use Raman spectroscopy is because this analytical technique provides, in a non-destructive and unambiguous way, information about the molecular composition and crystal structure of the compounds. This molecular knowledge of the materials that form this kind of sea shell can be useful to understand the formation and growth biologic mechanisms of the shells of molluscs.

II. RAMAN SPECTROSCOPY TECHNIQUE

Raman spectroscopy is a non-destructive photonic technique that provides both, molecular and structural information, starting from the inelastic scattered light for a material when a monochromatic light is focused on it.[1,2]

The application fields of Raman spectroscopy have increased enormously thanks to the technologic evolution of the instrumentation. The development of modular and compact equips (figure 1), with a high sensibility and spectral quality, and the use of lasers and optical fiber can facilitate the analysis of materials of difficult access as, for example, submarine objects independently of whether the sample is solid, liquid or gas. Furthermore, it is possible to discriminate among closed particles inside of the same material (the spatial resolution depends on the focus lens) and to obtain complete information about the molecular composition of the marine sample.

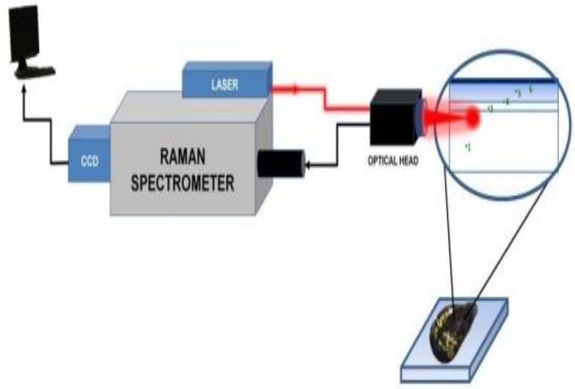

Fig. 1. Scheme of a fiber optic Raman system

The Raman system employed in this work is a dispersive equipment of new generation, iHR320 model, of Jobin Yvon (Horiba Group). The Raman spectra recovered of the mussel´s shell and the reference minerals have been obtained with a red laser of He/Ne that emits at 632.8 nm of wavelength with a power on sample of 2 mW approximately. The laser light is guided through a 10 m multimode optical fiber of 50µm of core diameter until the optical head and, here, the light is filtered with an interferential filter. A lens of 4.5x focuses the laser on the material and collects the scattered light, both the elastic (Rayleigh) and the inelastic (Raman).

Then, the scattered signal is filtered with an EDGE filter that allows to obtain only the Raman Stokes radiation. A second section of optical fiber with 100 µm of core diameter guides the scattered radiation to the spectrometer.

The monochromator consists of three interchangeable gratings of 950, 1200 and 1800 grooves/mm with a resolution of 1.76, 1.33 and 0.73 cm^{-1}/pixel respectively, at the wavelength of 636.8 nm. The focal lenght of the spectrometer is 320 mm and the detector is a CCD (Synapse 1024x256 pixels) with a termoelectric refrigeration system that achieves a work temperature of -70 ^{o}C.

III. REFERENCE MINERALS

Different minerals of calcium carbonate[3,4] have been characterized with Raman spectroscopy: calcite ($CaCO_3$, trigonal), aragonite ($CaCO_3$, orthorhombic) and dolomite ($CaMg(CO_3)_2$). Figure 2 shows the corresponding Raman spectra of each mineral. The spectra have been normalized with respect to the fundamental band for correct comparisons. In the case of aragonite, three spectra with differences in frequencies and intensities, which depending on the orientation of the mineral, have been obtained. In table 1 the frequencies of the Raman bands are shown for each mineral.

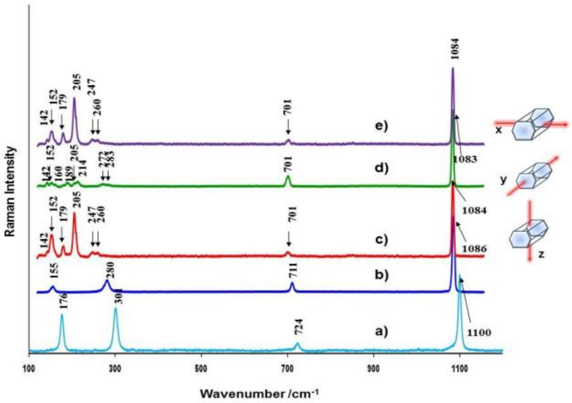

Fig. 2. Raman spectra of the reference minerals: a) dolomite ($CaMg(CO_3)_2$); b) calcite (trigonal $CaCO_3$); c),d) and e) are three different orientations (x,y,and z edges) of aragonite (orthorhombic $CaCO_3$).

Table 1. Raman shifts of the reference minerals.

Mineral	Wavenumbers (cm^{-1})
Dolomite	176 (m), 301 (m), 724 (w), 1100 (s)
Calcite (trigonal)	155 (w), 280 (w), 711 (w), 1086 (s)
Aragonite (orthorrombic, x+y+z)	142 (vw), 152 (w), 160 (vw), 179 (vw), 189 (vw), 205 (m), 214 (vw), 247 (vw), 260 (vw), 272 (vw), 283 (vw), 701 (w), 1084 (s)

IV. STRATIGRAPHIC ANALYSIS WITH RAMAN SPECTROSCOPY

The most remarkable of this investigation is the discrimination of two different crystal structures of calcium carbonate in a mussel, this is to say, aragonite in the substrate and calcite in the blue areas. In figure 3, the cross-section microphotography of this mussel´s shell is shown. The corresponding Raman spectra obtained in the areas (a) to (f) appear in figure 4.

The aragonite ($CaCO_3$, orthorrombic), with bands at 152, 179, 205, 701 and 1082 cm^{-1}, has been identified for comparison with the reference minerals (practically isolated or combined) as the principal compound of the biologic substrate in the mussel´s shell (spectra a, b and c). The Raman bands at 1013 and 1093 cm^{-1} in the spectra (b)-(f) can be attributed to a certain kind of blue pigment (polyene)[5,6]. Its concentration increases progressively at the same time that the concentration of aragonite decreases.

However, with this rise in concentration of the blue pigment, also a structural change (crystallographic) of the calcium carbonate in the substrate is observed. In the areas (d) and (e) of the cross-section (Fig. 3) the Raman spectra (d) and (e) were obtained (Fig. 4). It is possible to identify, in addition to aragonite, other crystallographic form of calcium carbonate, this is, calcite ($CaCO_3$, trigonal) which fundamental bands are located at 154, 281, 711 and 1084 cm^{-1}. The band at 1084 cm^{-1} is masked due to the more intense band at 1093 cm^{-1} corresponding to the blue pigment (spectra d, e and f). Finally, in the total blue layer, the obtained spectrum (f) shows how the calcite appears as the fundamental compound mixed with the blue pigment instead of the aragonite, which presence has disappeared.

In order to compare the biogenic carbonate of the mussel´s shell with other shell, a pink-brown colored conch has been also analyzed with Raman spectroscopy (figure 5).

In this case, only aragonite has been identified as substrate, in both non colored and colored areas, and it hasn´t been observed any structural change associated with the formation of the pink-brown polyene pigment.

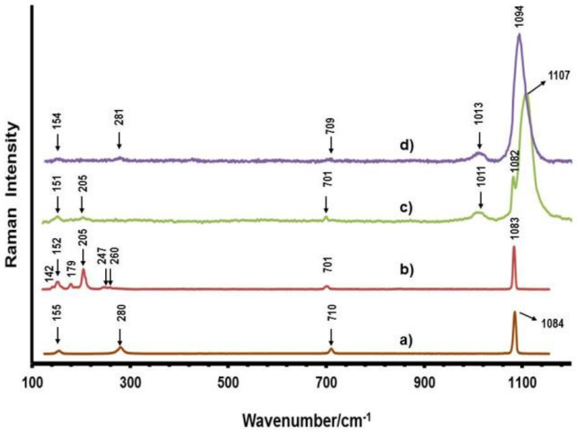

Fig.5. a) Raman spectrum of calcite mineral; b) informatic sum of the Raman spectra (x,y and z) of the aragonite mineral; c) and d) are the Raman spectra of the colored areas of the conch and the mussel shells respectively.

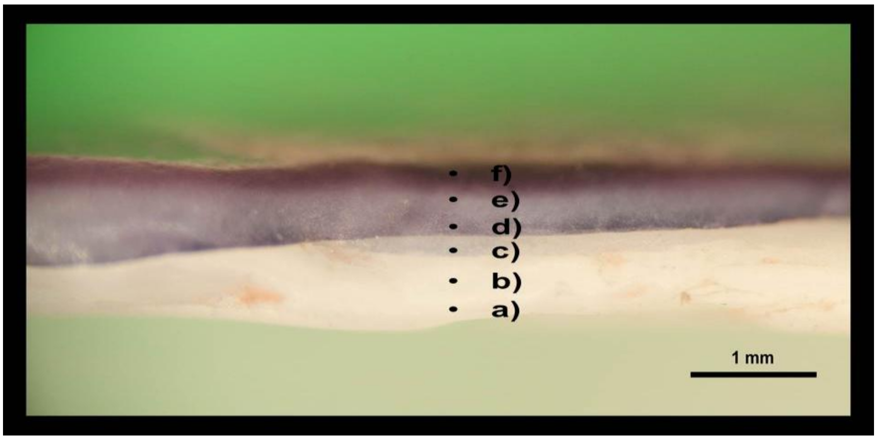

Fig. 3 Microphotography of a mussel´s shell cross-section

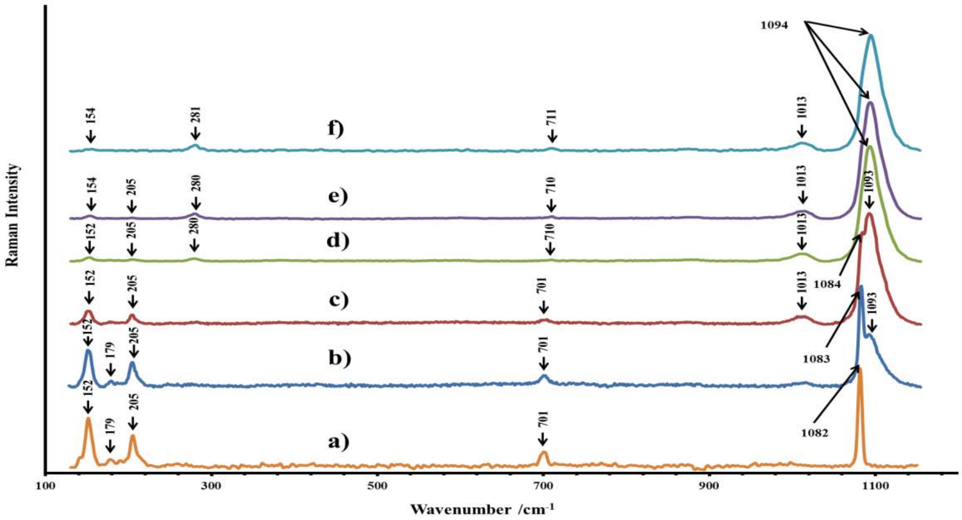

Fig. 4. Raman spectra obtained at different layers of the mollusk cross-section: a) aragonite; b) and c) different concentrations of aragonite mixed with the blue pigment; d) and e) different concentrations of aragonite mixed with the blue pigment and calcite; and f) calcite mixed with the blue pigment.

V. CONCLUSIONS

The fundamental compounds of a shell can easily be identified thanks to Raman spectroscopy. First, in the particular case of a mussel, note that the blue pigment is related with the organic family of polyenes. On the other hand, it seems relevant that two different crystallographic forms of calcium carbonate coexist together: aragonite in the general substrate and calcite in the blue areas. This fact, perhaps, helps to understand the progressive formation process of the shell of this mollusk.

ACKNOWLEDGEMENTS

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REFERENCES

[1] D.A.Long, *The Raman Effect*. Chichester: Wiley, 2002.

[2] R.L. McCreery. *Raman Spectroscopy for Chemical Analysis*. J.D.Winefordner, New York: Wiley, 2000.

[3] R.G.Herman, C.E. Bogdan, A.J. Sommer, and D.R.Simpson. (1987). Discrimination Among Carbonate Minerals by Raman Spectroscopy Using the Laser Microprobe. *Appl. Spectrosc. 41(3)*, pp. 437-440. Available: http://www.opticsinfobase.org

[4] J. Urmos, S. K. Sharma, F. T. Mackenzie. (1991). Characterization of some biogenic carbonates with Raman spectroscopy. *American Mineralogist*. *76*, pp. 641-646. Available: http://www.minsocam.org

[5] C. Hedegaard, J.F. Bardeau, D. Chateigner. (2006). Molluscan shell pigments: an in situ resonance Raman study. *J. of Mollusc. Stud. 72*, pp. 157-162. Available: http://mollus.oxfordjournals.org

[6] W. Barnard and D. de Waal. (2006). Raman investigation of pigmentary molecules in the molluscan biogenic matrix. *J. of Raman Spectrosc. 37*, pp. 342-352. Available: http://onlinelibrary.wiley.com