

**Experiment title:**

The role of organic pigments in biogenic and synthetic calcite lattice distortions.

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**Report:**

Here we report on our HR- X-ray diffraction experiments at ID22 on coloured calcite synthesised with organic pigments (naphthazarin, lawsone, Echinochrome A, Spinochrome (B, D, E) and juglone). HR-XRD measurements were carried out to study the effect of the pigment molecules on the crystalline structure of calcite. To do so the HR-XRD profiles were refined with the Rietvelt procedure to evidence possible lattice distortions within the colored calcite samples as respect with pure calcite. In order to enhance eventual and small lattice distortions due to the presence of pigment we also performed HR-XRD measurements upon *in situ* heating up to temperatures at which the pigments are degraded.

**Experimental Procedure**

**Samples:** Calcite samples were prepared by four different methods in the presence of the different organic pigments (at three different concentrations):

A, B) precipitation in water at pH=10.5 and 12.5, respectively through the precipitation of ACC particles by the addition of CaCl<sub>2</sub> to a Na<sub>2</sub>CO<sub>3</sub> solution and its complete crystallization into calcite after 48 hours under stirring in the absence of NaOH (pH=10.5) and after 5 hours stirring in presence of NaOH (pH=12.5);

C) physical mixing by grinding in an agate mortar for 10 min the CaCO<sub>3</sub> powder previously synthesized by A) or B) and the pigment powder (0.5wt.%); and

D) vapour diffusion in ethanol leading to the formation of ACC particles further filtered, rinsed with cold ethanol and dried for a few minutes in a desiccator under a vacuum. ACC powders were divided into two parts: One part was converted to calcite by heating and the other by stirring it in water for 48 hours.

All powder samples were filled in capillaries and mounted on the magnetic spinner for measurement.

**Methods:** HR-XRD measurements were performed at ID22 beamline, ESRF. For our measurements, the beam energy was set to 35KeV and the beam size was 1.0x1.0 mm<sup>2</sup> in cross-section. Si standard NIST 640c was used for calibration. The heating of the samples was carried out by a heat blower available at the beamline and the temperature was increased from 25°C to 450°C at a rate of 10°C/min and stopped for 20min at each relevant temperatures during HR-XRD acquisition.

**Data acquisition and evaluation:** X-ray scattering data were acquired using the flat panel Perkin Elmer XRD 1611CP3 detector, which is suited for total scattering measurements at high energies. Recorded 2D patterns were processed using 2D powder diffraction data reduction software available for calibration and data integration. The data integrated was plotted using Origin software. The Rietveld refinement was carried out using a script provided by ID22 beamline scientist for the TOPAS software.

## Results

Here, we report the results obtained for the syntheses A), B) and C) performed in the presence of naphthazarin molecules for two different concentrations (20 and 100 µg/mL) (Figure1) as these data are being summarized for a first publication, the data obtained for the others pigments will be analyzed in details in a second step.

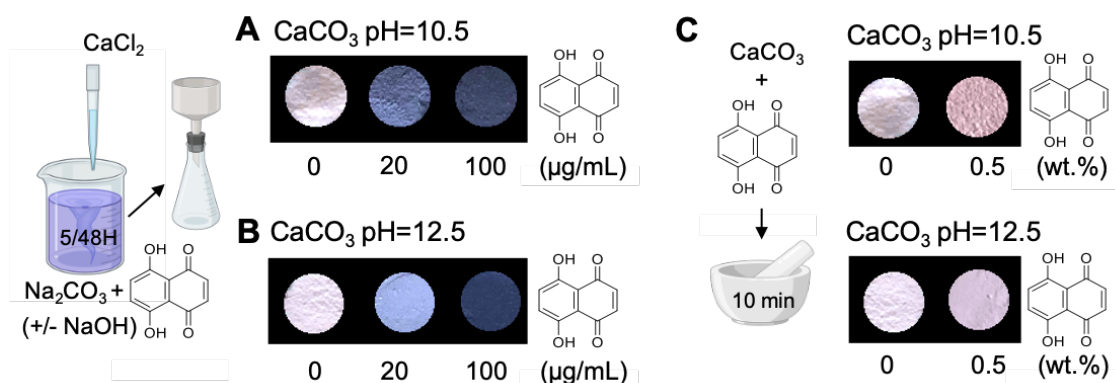


Figure 1. Naphthazarin-calcite powders obtained after A) precipitation in water at pH 10.5, B) precipitation in water at pH 12.5 and C) physical mixing of naphthazarin with CaCO<sub>3</sub> previously synthesized at pH=10.5 and pH=12.5.

Figure 2 A) shows the HR-XRD profiles of pure and naphthazarin-calcite powders confirming that all samples were indeed calcitic. Figure 2 B) displays the (104), (006) and (110) reflection of geological calcite and pure calcite synthesized by precipitation in water at pH 10.5 and 12.5 evidencing the difference in structure between the three samples. Calcite synthesized at pH=12.5 is less crystalline whereas once synthesized at pH=10.5 it is as crystalline as geological calcite but present peak shift to lower 2Theta, suggesting higher lattice parameters as confirmed by the results of Rietveld refinement (Table 1). Lattice distortions were calculated as compared to geological calcite. For pH=10.5, calcite lattice distortions are isotropic with relatively low  $\Delta c/c$  and  $\Delta a/a$  (0.05%) while for pH=12.5, lattice distortions are anisotropic with  $\Delta a/a$  quite important (0.24%) and  $\Delta c/c$  very low (0.01%).

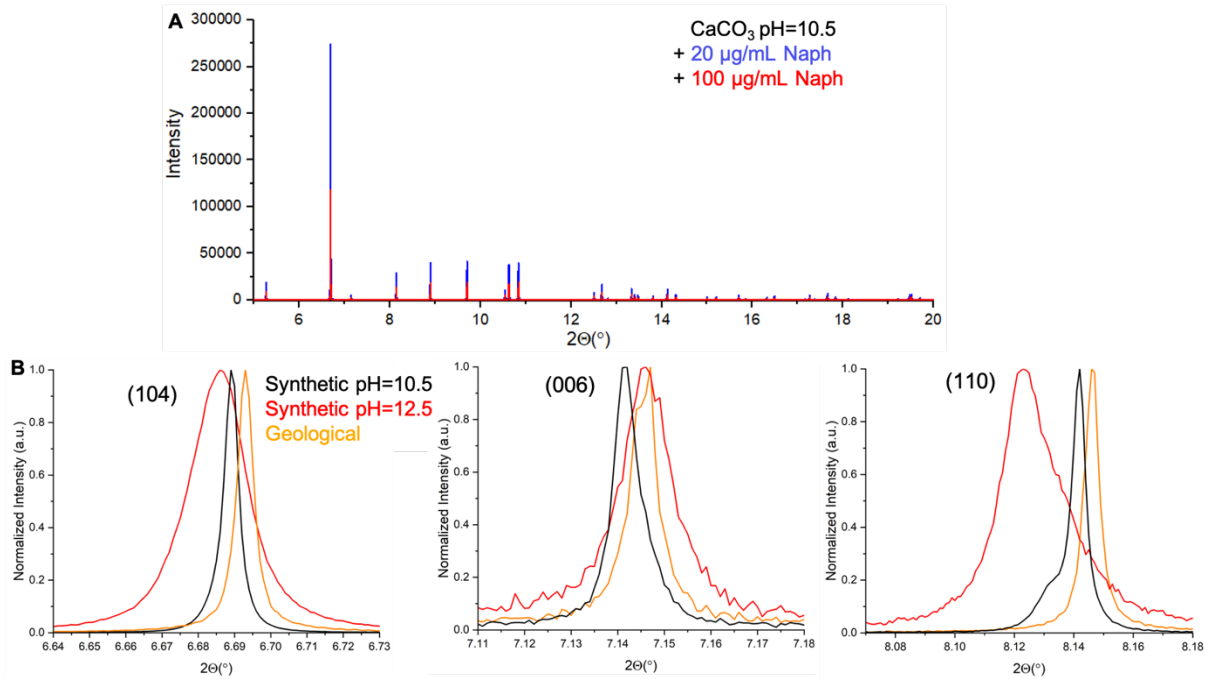


Figure 2. A) X-rays diffractograms of pure calcite and naphthazarin-calcite powders synthesized at pH=10.5 and B) (104), (006) and (110) reflection of geological calcite as compared to pure calcite synthesized at pH 10.5 and 12.5.

Table1: Calcite lattice parameters extracted by applying Rietveld refinements to the HR-XRD profiles of the different samples with one calcite phase (in red) and two calcite phases (in black).

Samples	pH	[Naphtha] (ug/mL or wt%)	$a$ (Å)	$c$ (Å)	$\Delta a/a$ (%)	$\Delta c/c$ (%)	Phase1/ Phase2 (%)
			$a_1$ (Å)	$c_1$ (Å)	$\Delta a_1/a_1$ (%)	$\Delta c_1/c_1$ (%)	
			$a_2$ (Å)	$c_2$ (Å)	$\Delta a_2/a_2$ (%)	$\Delta c_2/c_2$ (%)	
Geological calcite	$\approx 8$	0	4.98850	17.05740	-	-	
Synthetic calcite precipitation	10.5	0	4.99110	17.06580	0.052	0.049	
			4.99583	17.05922			30/70
			4.99095	17.06772			
		20	4.99118	17.06560	0.002	-0.001	
			4.99543	17.05860	-0.008	-0.004	30/70
			4.99080	17.06700	-0.003	-0.004	
		100	4.99103	17.06600	-0.001	0.001	
			4.99545	17.05870	-0.008	-0.003	20/80
			4.99086	17.06670	-0.002	-0.006	
		12.5	0	5.00062	17.05640	0.24	-0.01
		20	4.99349	17.06140	-0.14	0.03	
		100	4.99485	17.05890	-0.12	0.01	
Synthetic calcite physical mixing	10.5	0.5	4.99196	17.06450	0.017	-0.008	
			4.99700	17.05700	0.023	-0.013	35/65
			4.99100	17.06720	0.001	-0.003	
	12.5	0.5	4.99909	17.05620	-0.03	0.00	

The comparison of (104), (006), and (110) peaks of HR-XRD profiles of calcite synthesized at pH=12.5 without and with naphthazarin molecules suggests that the presence of pigment introduces a significant peak shift only for the (110) (Figure 3 A)). This is reflected in the calculated anisotropic lattice distortions with  $|\Delta a/a|=0.12\%$  and negligible  $|\Delta c/c|$ .

The comparison of (104), (006), and (110) peaks of HR-XRD profiles of calcite synthesized at pH=10.5 without and with naphthazarin molecules suggests that the presence of pigment does not introduce significant peak shift (Figure 3 B)). Indeed  $|\Delta c/c|=0.001-0.008\%$  indicates that the lattice distortion in the  $c$  axis direction is negligible when compared to sea urchins spines (0.03%) or mollusk shells (0.1%). The addition of naphthazarin however only introduces a broadening of the peaks.

In addition, it can be observed a peak splitting of the (110) peak suggesting the possibility of another phase in our system. Therefore, Rietveld refinement has been also performed with two different calcite phases (Table 1). This secondary peak is much broader than the main peak suggesting smaller crystallites. However, this peak is also present in the pure synthetic calcite and is the most intense in the sample formed by physical mixing therefore, its origin must still be investigated.

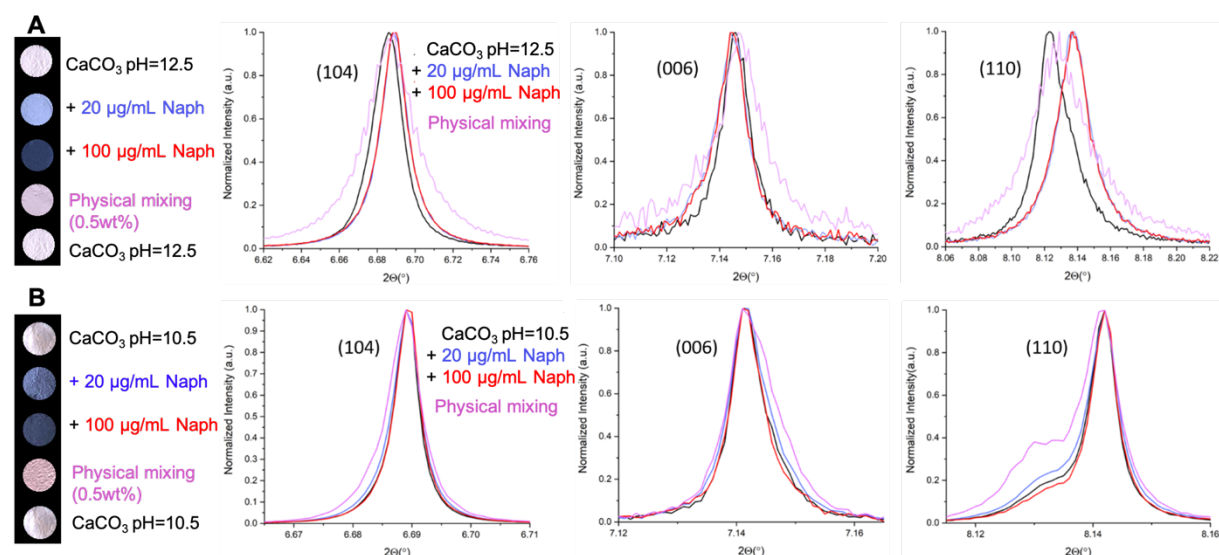


Figure 3. (104), (006) and (110) reflections of HR-XRD profiles once normalized of pure calcite, 20 and 100 µg/mL-naphthazarin-calcite synthesized at A) pH=12.5 and B) pH=10.5.

One clue is its disappearing after heating (Figure 4). *In situ* heating experiments were conducted to reveal small calcite lattice distortions induced by the presence of pigments before and after heating. In biogenic samples, 400°C is sufficient to guarantee most of the organic destruction and colors were observed to fade away at 200°C in sea urchin spines. Here, we observe that at 400°C the color is still present even if changed from bleu to purple. However, despite the disappearing of the peak splitting at 200°C no significant peak shift and slight peak broadening are observed. At 200°C structural water associated (or not) with the pigment molecules might be the origin of this change of structure but further studies are needed.

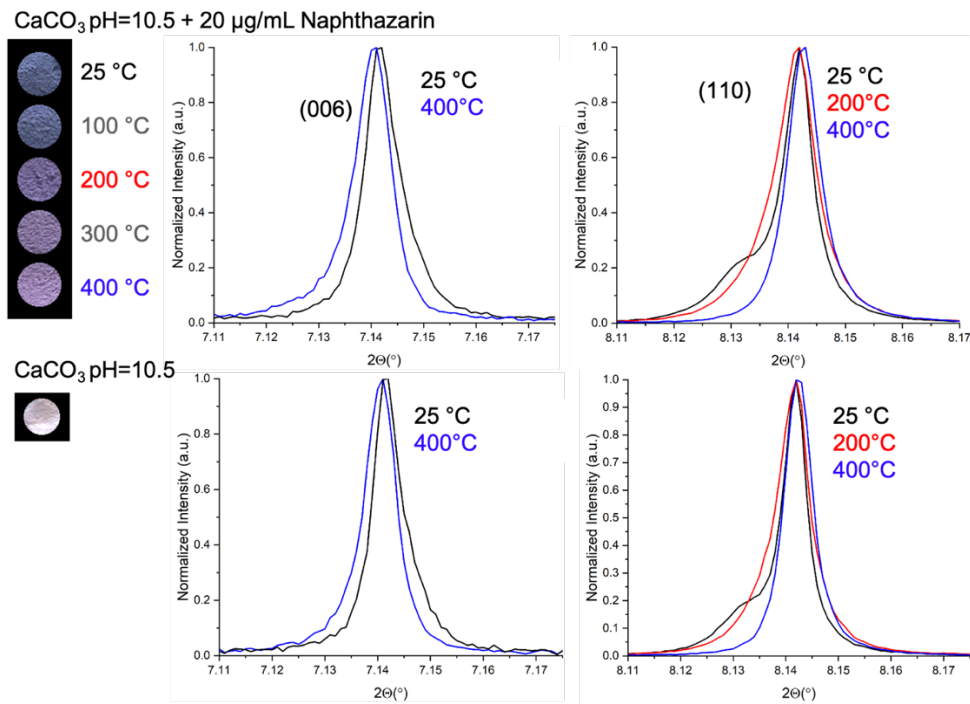


Figure 4: Photographs of the color changes according to the temperature with the same ramp (10°C/min + 20 min at each temperature) used for the HR-XRD experiments. HR-XRD profiles with normalized intensity after calcite thermal expansion correction of the (006) and (110) peaks for pure calcite and calcite + 0.02mg/ml naphthazarin at 25°C, 200°C and 400°C.