Microstructure Physics and Metal Forming
Prof. Dr. D. Raabe

Structure and texture analysis of chitin-bio-nanocomposites using synchrotron radiation

A. Al-Sawalmih, P. Romano, C. Sachs, D. Raabe

Max-Planck-Institut für Eisenforschung GmbH
Düsseldorf/Germany

The exoskeleton (shell) of arthropods typically consists of a biological chitin-based nano-composite associated with inorganic fraction. Chitin which is a linear polysaccharide is the second most abundant polysaccharide in the world after cellulose. It has basically the same structure as cellulose; the chains in both polymers are (beta)1-4 linked, but the difference is that chitin has an acetyl-amide group NH-CH_3CO instead of the hydroxyl group (alcohol) in cellulose. Chitin occurs in the form of fibres in the shells of arthropods like crustacea and insects (α-chitin), and also in the loligo pen of the squids (β-chitin).
In arthropod exoskeletons α-chitin fibrils of about 3 nm diameter are surrounded by a protein helix of about 2 nm thickness. These chitin-protein composite ligaments which form a crystalline lattice structure act as the main structural element of the arthropod exoskeleton (see fig.1).
Depending on the type of arthropods the exoskeleton contains different fractions of biomineral precipitates such as calcite ($CaCO_3$) and magnesian calcite ($Mg_{0.1}Ca_{0.9}CO_3$). The crystalline particles control the hardness of the material. Chitin-based nano-composites with precipitate biominerals provide both considerable mechanical strength at a very small weight and functional flexibility such as required for living species.
Chitin is available in huge amounts as a natural resource from arthropods such as shrimps and related crustacea. It has a high biodegradability and biocompatibility, so that it is gradually gaining importance as a natural polymer which might face substantial future applications as an alternative to some synthesized polymers.

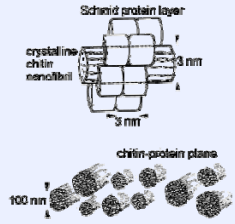


Fig.1: Nanofibril and fibers of Chitin in the exoskeleton

Structural data of Chitin and Calcite:

Chitin is a crystalline biopolymer where the c-lattice direction lies along in the fibril axis. Its density is 1.41 gm/cm3. Earlier works have revealed the crystallinity of chitin, the lattice parameters, and the space group. Studies reported two structures of chitin, depending on the directional order of the polymer chains, α-chitin and β-chitin. In α-chitin the chains are antiparallel, but in β-chitin, they are parallel, so that the unit cell in α-chitin is bigger. It was recorded that β-chitin has an orthorhombic unit cell with lattice parameters a=4.74 Å, b=18.86 Å, and c=10.32 Å. β-chitin has monoclinic unit cell with lattice parameters a=4.85 Å, b=9.62 Å, and c=10.38 Å. α-chitin is the most stable and abundant form of chitin with excellent mechanical properties; it is hard, and has rigid crystalline structure. β-chitin is less stable, flexible, and has loose crystalline structure. α-chitin existed in the shells of Arthropods like crab, lobster, and shrimps, but β-chitin existed in skeletal pen and stomach cuticle of squid (Mollusc).

Calcite ($CaCo_3$) is a crystalline biomineral, which has the hexagonal unit cell. Its lattice parameters are: a=b=4,989 Å, c=17.062 Å and its space group is [R-3 2/c]. Its density is 2.71 gm/cm3. Calcite is a most amazing and yet, most common mineral. It is one of the most common minerals on the face of the earth, comprising about 4% by weight of the earth's crust and is formed in many different geological environments. Calcite is the most stable form of the six known phases of calcium carbonate: aragonite, vaterite, monohydrocalcite, calcium carbonate hexahydrate, and amorphous calcium carbonate (ACC) which is the least stable.

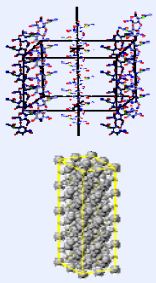
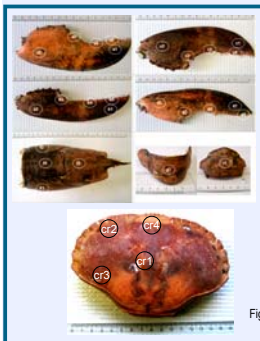


Fig. 2: Unit cell of α-chitin (above) and calcite (down)

Specimens and samples' size:



In our project, we deal with the nano-composites of α-chitin. We use three similar types of crustaceans' shells as specimens, namely, crab shell, lobster shell, and horseshoe crab shell which serves as a reference material for the other crustaceans since it is the only arthropod species whose shell is made out of pure chitin without any embedded biominerals.
The samples have small dimensions of a few centimetres length and a few millimetres width each. Its thickness is related to the shell thickness; in lobster, the shell thickness ranges between few millimetres (in the claws) to half a millimetre (in the abdomen), but in crab shell is rather regular (~ 2mm), while horseshoe crab has a very thin shell with thickness less than one millimetre. Due to the absorption change during sample rotation and our background correction in data processing later; we prepare sample with equal thickness and width.

Fig. 3: Lobster shell and crab shell. The marked are the original locations of the samples.

Beamline, sample geometry:

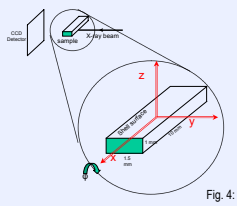


Fig. 4: Sample geometry

Protien Crystallography beamline:
3-circle goniometer (chi [constant]=45.7°)
CCD detector (60X60 mm2, 1024X1024 pixels)
SMART collecting data software (Bruker)

- X-ray @ 12.3 KeV (0.99955) Å
- direct beam spots on the centre of the detector)
- Beam size: 500 µm
- Sample-to-detector distance: 45 mm.
- Sample size: 1.5x1,0x10 mm3.

Diffraction profiles:

The sample was rotated (phi) 180° in 60 steps, and a CCD frame was collected in each step. For every sample, 60 frames were collected, to be used in creating the pole figure for a specific reflection.

In figure 5, the CCD frames of crab shell and lobster shell and their corresponding integrations, shows in addition to the strong texture, the amorphous calcium carbonate as a base-line in the diffraction pattern of the lobster shell, whereas the diffraction from calcite was absent because it was not created yet in our lobster. A powder diffraction for different lobster shell showed a presence of a fraction of crystalline calcite was already formed, and some ACC was detected, and in different powder diffraction study, even from part to another part in the lobster, the calcite content changes.

For arthropods, frequent replacement of the mineral is required, so most of the crustaceans have mineralized exoskeletons (or cuticles) that are cyclically renewed with exogenous or endogenous calcium carbonate. The use of an amorphous phase as the mineral composing the cuticle, also called carapace, can therefore be advantageous [2].

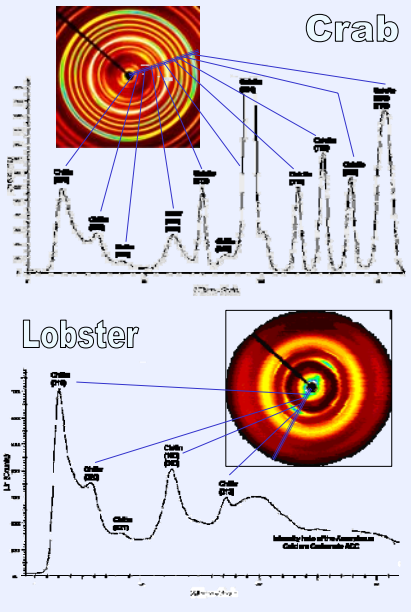


Fig. 5: CCD frames with the corresponding integration (diff. pattern) of crab shell and lobster shell

Pole figures:

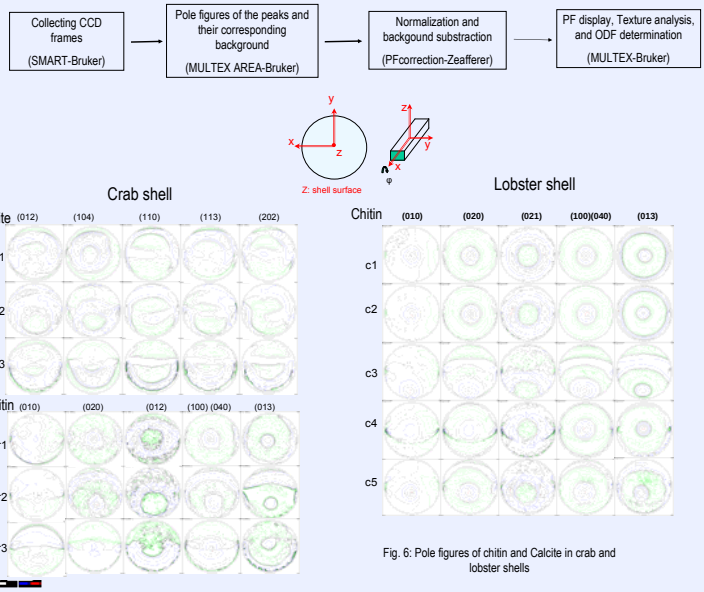


Fig. 6: Pole figures of chitin and Calcite in crab and lobster shells

Texture analysis: Chitin

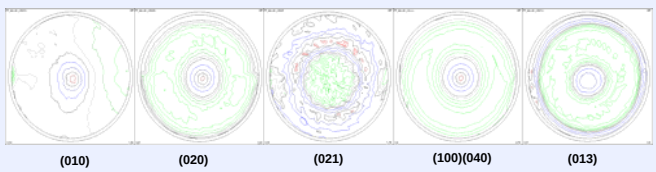


Fig. 6: Pole figures chitin in lobster

The crystallographic orientation distribution of the chitin network is characterized by the following feature:

- It reveals a pronounced 2 fiber texture axes perpendicular to each other with a very strong crystallographic <010> and <100> fiber axes normal (or perpendicular) to the surface of the lobster cuticle (see (010), (020), and (100) pole figure in figure). This means that the long b- and a- axes of the elementary cell of the a-chitin crystals points parallel to or towards the surface of the exoskeleton (in the crusher claw region from where the sample was taken).
- The chitin polymer-chain (c-direction) is distributed in different directions parallel to the surface as small grains.

Further studies:

- Layer-by-layer investigation of lobster, crab, and horseshoe crab shells in the micrometer scale using microbeam diffraction at the microfocus beamline -ESRF synchrotron facility, France.
- Small angle x-ray scattering measurements.
- Determination of the residual strain stresses and the grain size.
- The correlation between the texture and the mechanical properties of different parts of the shell.
- Texture analysis of the shells under different conditions: temperature, pressure and water content.
- Electrical, Magnetic, and optical properties of the shells and correlate the results with calcite and chitin contents, to be done with collaboration with Max-Planck-Institute for Metal Research and Max-Planck-Institute for Solid State Research-Stuttgart, Germany.

Literature:

1- Minke and Blackwell, J. Mol. Biol. (1978) **120**, 167-181.
2- Raz, Testeniere, Hecker, Weiner, and Luquet, Biol. Bull. (December 2002) **203**, 269-274.
3- Takai , Shimizu , Hayashi , Tokura , Ogawa , Kohriyama , Satake , and Fujita, ACS symposium series (1992), **489**: 38-52
4- Saito, Okano, Chanzy, and Sugiyama, J. Struc. Biol. (1995) **114**, 218-228

Acknowledgments

The authors thank the Deutsche Forschungsgemeinschaft (German Research Foundation) which, under the framework of the Gottfried Wilhelm Leibniz award, supports this project. We also acknowledge ANKA Synchrotron Radiation Facility (Karlsruhe-Germany) for provision of synchrotron radiation facilities and we would like to thank Dr. Buth for assistance in using XPbeamline .