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Mechanical properties of top neck mollusks shell nano composite in different environmental conditions

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Abstract. The mechanism of biological materials structure is very complex and has optimal properties compared to engineering materials. Top Neck mollusks shells, as an example of biological materials, have hierarchical structure, which 95 percent of its structure is Aragonite and 5 percent organic materials. This article detected mechanical properties of the Top Neck mollusks shell as a Nano composite using Nano-indentation method in different situations. Research findings indicate that mechanical properties of the Top Neck mollusks shell including elastic modulus and hardness are higher than a fresh one preserved in -50 centigrade and also a Top Neck mollusks shell preserved in environmental conditions. Nano-indentation test results are so close in range, overall, that hardness degree is 3900 to 5200 MPa and elastic modulus is 70 to 85 GPa.

Keywords: nano indentation; top neck mollusks shell; mechanical properties; elastic modulus; hardness

1. Introduction

There are many composite biomaterials which have been introduced and commercialized for various applications due to their advantages over conventional materials (Salernitano and Migliaresi 2013, Bigi and Boanini 2017, Mohammadimehr *et al*. 2018). Oysters are sea creatures which their shell acts as a defensive shield against external shocks, so that they conform their living conditions, protect against external attacks from hunters, rocks or rubbles displaced by sea waves. Oyster shells are composed of a three -layer structure that outer layer is periostracum, middle layer is Prismatic and the inner layer is nacre (Lopes-Lima *et al*. 2010, Zaremba *et al*. 1996). These layers are consisted of organic and inorganic materials, 95 weight percent of which are inorganic materials (Aragonite $CaCO_3$) and 5 percent are organic materials (protein-polysaccharide) (Zaremba *et al*. 1996, Parhizkar *et al*. 2016). The inner layer structures of oysters, depending on their type, are different. But the main structure of oyster inner shells is like brick-cement structure in which Aragonite tablets are like brick, and organic materials in shells act as cement (Barthelat and Espinosa 2007, Khater 2016). Aragonite tablets are firmly glued together with soft organic materials to create an organized structure.

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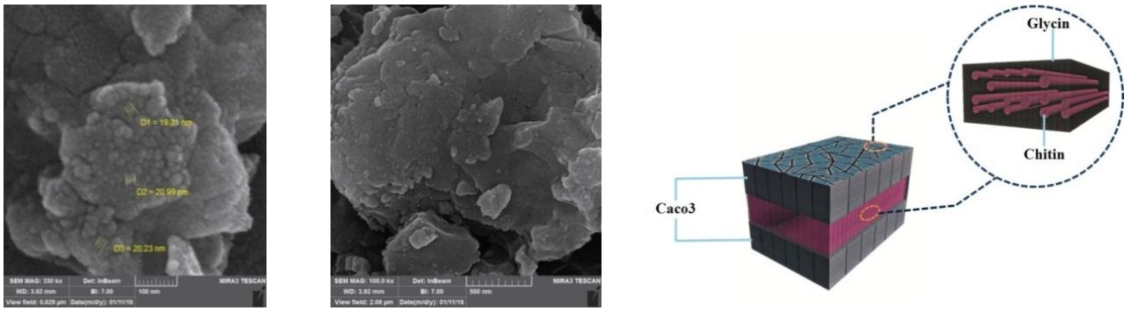


(a) SEM-FE images of top neck mollusks shell in different scales

(b) Shells sandwich structure in nano scale

Fig. 1 structure in bio nanocomposite

Variety of oyster shells' general structure types are (1) column structure; (2) plate structure; (3) horn structure; (4) prismatic structure; (5) layer structure; (6) composite layer structure; (7) homogeneous structure (Barthelat *et al*. 2006, Lopes-Lima *et al*. 2010, Meyers *et al*. 2008). The Top neck mollusks shell structure type in this study is hierarchical layer. (Fig. 1) Therefore, the studied shell in Nano scale has sandwich structure which is indicated in Fig. 1(b). As shown in Fig. 1(b), it's obvious that upper and lower layers are consisted of inorganic materials (Aragonite $CaCO_3$) formed of a large number of irregular Aragonite nanoparticles combination cemented by organic materials. The longest irregular Aragonite particles are 15-20 nanometers. Regular Aragonite tablet length and diameter are 5 micron and 0.3-0.5 micrometer in order. Between these two layers, there is engineering materials of organic materials (Chitin-Glycine) with diameter of 205 nanometer (Meyers *et al*. 2008, Sumitomo *et al*. 2007). Oyster shells have a high flexibility due to their unique structure which attracted researchers' attention and is called the nature magic. So oyster shells created a way to inspire new ideas to form engineering materials and new structures, such as composite materials, as an inspirational resource. Because of interest in nanotechnology, inner shells of Top Neck mollusks shell mechanical properties are investigated recently (Barthelat and Zhu 2011, Kakisawa and Sumitomo 2011, Kobayashi 1964, Padture 1995). In general, studies on oysters began in 1911. The properties of Top Neck mollusks shell had been identified since the Maya Indians used it as a dental implant, and in 1930, Boggild analyzed Top Neck mollusks shell structure and determined the Aragonite and calcite matters (Kobayashi 1964, Padture 1995). In 1984, Tang and Kotov obtained a new model using B-Chitin and cluster macro molecules for organic layer of pearl oyster shell (Tang *et al*. 2003). Some researches were conducted on Abalone shell by Wang in 2001 and Barth in2007 (Stokowski 1951). Jackson acquired mechanical properties (Young's modulus) of Abalone in wet and dry situations in 1998 (Jackson *et al*. 1988). Sari kaya obtained how to get shell fracture by deflection experiment in1990 (Sarikaya *et al*. 1990). And Tong and Luz in 2003 and 2009, respectively, attempted to make artificial oysters (Luz and Mano 2009, Sarikaya *et al*. 1990). A number of researches are conducted by scientists on shells, which were mainly abalone and conch, to gain their mechanical properties by Nano-indentation tests. In 2004, Zhi Dong Lee investigated the mechanical properties of red abalone, tablet rotation and Aragonite transformation (Li *et al*. 2004). Barth studied mechanical properties of an Aragonite tablet on conchs in2007 (Barthelat 2007). Lung calculated oysters' mechanical properties in2007 (Long *et al*. 2007). Roma Krishna studied fracture on conchs in2013 (Krishna *et al*. 2015). Rodriguez presented a technique and model for engineering material construction named Layer By Layer in 2017 (Chokshi 2018). In 2018, Chokshi conducted a study on the mechanical behavior of nacre across length scales. This study probes the role of different hierarchical length scales in governing the strength and modulus of nacre using a combination of bulk compression tests, micro indentation and Nano indentation tests. The results show a minimal influence of length scales on elastic-plastic transitions, suggesting that initiation of plasticity occurs through a common bio mineral sliding mechanism across length scales (Chokshi 2018). In 2018, Lee and Sinha at all conducted a study on the Nacre-inspired composite prepared by rolling method .In this study, they prepared a perfectly ordered nacre-mimetic composite from plate-like alumina particles and epoxy resin using a rolling method. A specific feature of this method is that it progressively aligns the component particles along a single direction regardless of the product thickness at resin contents as low as 25wt%. The obtained composite exhibited strength as high as 200 MPa during bending. Their research focused on the orientation effect produced by the plate-like particles and deformation behavior of the composites observed at different orientation degrees. It was found that the obtained composite material with perfectly ordered particles exhibited pull-out behavior similar to that of natural nacre. (Lee 2017).

Regarding the structural differences in various types of shellfish, it is clear that researchers have focused their attention on the red abalone and conchs, and in none of the previous studies, mechanical properties of oysters have been investigated. The main focus of this paper is to calculate the mechanical properties (elastic modulus and hardness) at Nano-scale at ambient temperatures and conditions using Nano-indentation test and comparing these properties.

2. Materials and methods

In this paper, the mechanical properties of oysters, including the elastic modulus (E) and hardness (H), are obtained by using a Nano-indentation test in different conditions. In the first case, oysters are fresh, in the second case, studied oysters were kept in cool storages for 40 days at -55℃, and in the third case, and oysters were kept under environmental conditions for 5 years (provided from Bandar Abbas Marine Aquatic Research Institute).

2.1 Sample preparation

The preparation method of oysters in different situations for the Nano-indentation test is identical. For this, primarily we prepared shell, then removed a section at a size of 1.0 × 5.0 × 1 mm with a diamond knife, and then insert it into a resin filled container (1 cm in diameter) for 18 hours to be firm and stable. (Fig. 2(b))

(a) The exterior view of oysters

(b) Sample preparation for nano-indentation test

Fig. 2 Image of shells and preparation it

Then the prepared section is polished so its surface get smooth and level. To avoid any scratches on the surface of the sample to be examined, they are polished by different roughness sandpapers and finally, an optical microscope is used to control the surface quality. To ensure that there is no scratch on the sample, after the completion of the preparation, the sample is delivered to the lab for Nano-indentation test.

2.2 Nano-indentation test

Indentation in Nano scale has been widely popular to describe mechanical methods in recent decades, and its high popularity is due to its increased attention to thin films and low volume samples as modern applied stimulant such as thin films, MEMS (Micro-Electro Mechanical Systems), bio materials and so on. On the other hand, it is non-destructive. One of the important applications of the Nano-indentation method is measuring the hardness and elastic modulus of the tested sample. One of the main parts of the Nano-indentation device is its geometry and indenter substance. Here, the Berkovich indenter is made of a diamond, is applied to obtain material properties given in Table 1 (Oliver and Pharr 1992).

There are three important parameters in the Nano-indentation test: (1) Maximum loading volume (F_{max}); (2) maximum Indentation depth (H_{max}); (3) slope of the unloading area, where the force and depth of indentation during loading is recorded from zero to a maximum and then the form maximum to zero, and if plastic deformation occurs, an effect remains on the sample surface. The output of the Nano-indentation test is the force-movement diagram (Fig. 3), from which the mechanical properties of the test sample are extracted.

To calculate the sample hardness, use the following relation

$$H = \frac{F_{max}}{A_c} \tag{1}$$

Table 1 The Berkovich properties nano-indentation

Material	Mohs hardness	Density	Young's modulus (E)	Thermal conductivity
Diamond	10 Mohs-scale	3.51 g/cc	1050 Gpa	2050 W(m˚k)

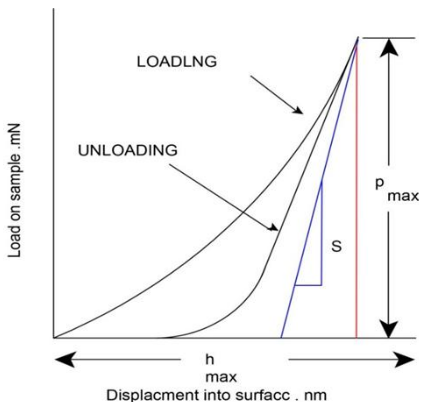


Fig. 3 Adaptation curves (loading and unloading) from a nano-indentation test

F_{max}, A_c represents the maximum load and illustrated contact surface, respectively. To calculate Ac, the relation (2) is used to associate the illustrated area with the distance from the indenter tip (Fig. 7)

$$A_c = kh_c \tag{2}$$

Because the indenter tip is not sharp enough and cause deviations from the ideal geometry, Oliver-Far uses Eq. (3) as a region function, which is obtained as follows (Oliver and Pharr 1992)

$$A_c = C_0 {h_c}^2 + C_0 h_c + C_0 {h_c}^{\frac{1}{2}} + C_0 {h_c}^{\frac{1}{4}} \tag{3}$$

This equation usually has a good fit with experimental data (in a wide range of contact depths, as far as empirical data are taken on the reference sample surface), in equation (3), C_1, C_2, C_3 are geometric parameters of the indenter tip (Barthelat and Zhu 2011); For Berkovich indenter, we have

$$A_c = 3\sqrt{3}{h_c}^2 \tan^2 \theta \tag{4}$$

θ For Berkovich indenter is 63.27 degrees. If this angle is given in Eq. (4), the relation (5) is obtained as follows

$$A_c = 24.5{h_c}^2 \tag{5}$$

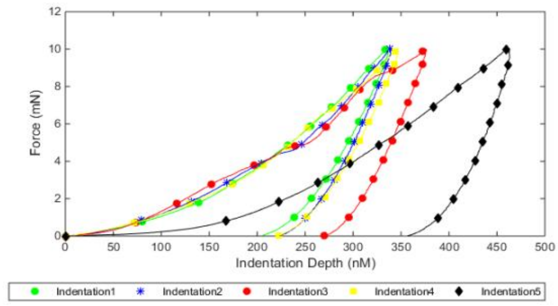
H_c indicates elastic deflection of the surface in a particular contact. Therefore, by obtaining the equations from (2) to (5) and putting them in Eq. (1), sample hardness comes to hand. However, elastic modulus determination requires more consideration, although the indenter tip is preferably made of diamond, but not completely hard, simultaneously with an elastic deformation in the sample, elastic deformation in indenter also occurs. Therefore, effect of indenter deformation in the elastic modulus should be considered. This elastic modulus is known as a reduced elastic modulus and is expressed by

$$E_r = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i} \tag{6}$$

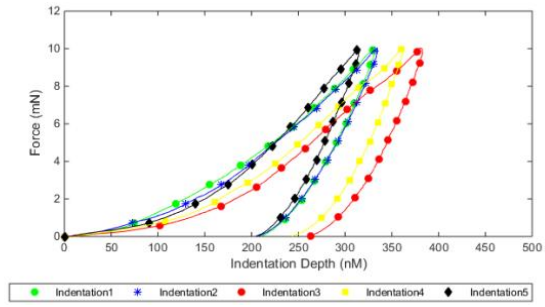
E_r is the reduced Elastic modulus, and, respectively, v and E are the Poisson coefficient and the experimented elastic modulus sample, and ν_i and E_i are respectively, of the Poisson coefficient and the indenting elastic modulus. The reduced elastic modulus according to content stiffness is as follows

$$E_r = \left(\frac{\sqrt{\pi}}{2B}\right)\left(\frac{s}{\sqrt{A_c}}\right) \tag{7}$$

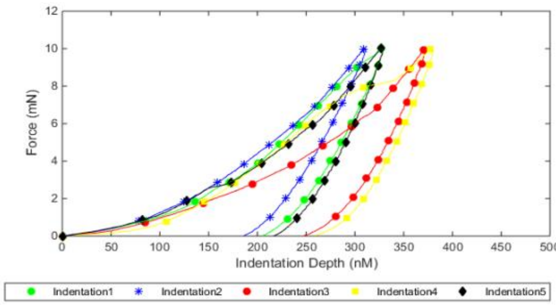
In the above relation, the contact stiffness (s) is defined as the slope of the force-movement in the initial stage of the loading stage (Fig. 6). The correction factor B, for Berkovich indentation, is 1.034, and varies depending on the type of indenter geometry. By determining of the value of relation 5 and replace in relation (7), E_r value is obtained; which by placement of E_r, ν_i and E_i (relevant to indenter values) in relation (6), the value of sample elastic modulus can be calculated.



(a) Lept in environmental conditions for 5 years



(b) Kept at temperature of -50℃



(c) Sample alive (Fresh shells)

Fig. 4 Force-Indentation depth diagram of top neck mollusks shell samples

Table 2 Calculation of effective parameters

Sample	No. Indentation	h_{max} (nM)	h_c (nM)	h_r (nM)	h_p (nM)	A_p(nM^2)	S (mN/nM)
5 years *	Indent1	336.17	178.64	258.57	210.18	2098888.75	0.1495
	Indent2	338.37	286.37	268.38	227.85	2208976.25	0.1423
	Indent3	375.08	324.47	307.68	275.17	2795265.25	0.1486
	Indent4	346.81	289.91	270.42	226.43	2260405.50	0.1311
	Indent5	461.23	421.61	406.90	326.76	4642016.00	0.1847
-50℃	Indent1	330.95	273.25	253.78	213.74	2023842.50	0.1299
	Indent2	338.84	273.13	252.70	211.33	2022267.88	0.1236
	Indent3	380.61	328.62	310.32	266.82	2863497.75	0.1427
	Indent4	360.99	312.62	295.31	248.16	2605009.00	0.1529
	Indent5	314.74	262.88	245.93	209.16	1883699.25	0.1449
Fresh	Indent1	327.77	269.02	249.25	208.53	1966143.38	0.1279
	Indent2	310.20	248.30	228.01	189.33	1695788.13	0.1222
	Indent3	372.38	320.55	302.16	252.36	2731472.00	0.1431
	Indent4	379.06	328.19	310.21	264.47	2856375.50	0.1460
	Indent5	327.68	275.00	257.22	220.31	2048058.75	0.1425

* Sample 5 years: Top neck mollusks shell kept in environmental conditions for 5 years; sample -50℃: top neck mollusks shell sample kept at temperature of -50℃; sample fresh: top neck mollusks shell sample fresh

3. Results and discussion

After preparation, the sample is placed inside the Nano-indenter NHTX S/N 01-03119 test device, then a marking on the sample is done with the aid of the AFM device, after marking the sample under indenter, till unload and loading operation is done. Unloading and loading for each of the three listed modes, linearly from zero to 10 mN (loading mode) and from 10 mN to zero (unloading mode) was carried out at 20 mN/min, with a frequency of 10 HZ, and progressive speed of 100 mN/min. Loading is carried out using diamond indenter; diamond properties in Table 1 indicate which indenter has indented the sample five times per test.

The output of the Nano-indenter device is reported as a force-movement diagram, in which there is a graph for each indenter. Fig. 4 illustrates the force diagrams by indenter depths in different test conditions.

Also, data of force- indentation depth for the Fig. 4 have been gathered in Table 2. In these data,

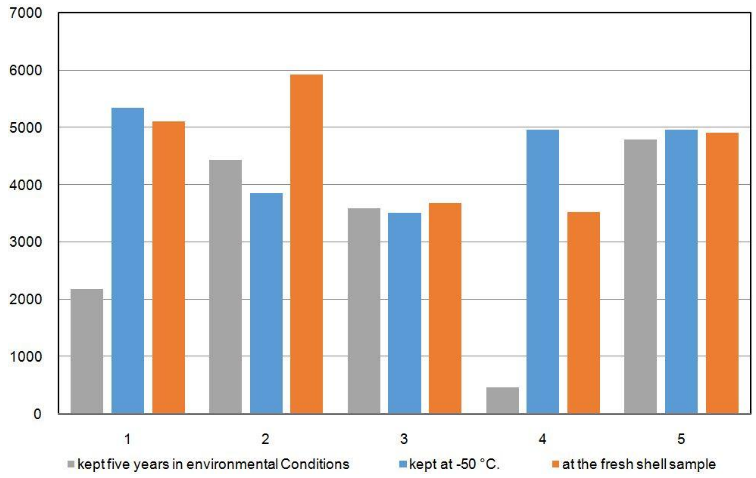


Fig. 5 Hardness of top neck mollusks shell samples

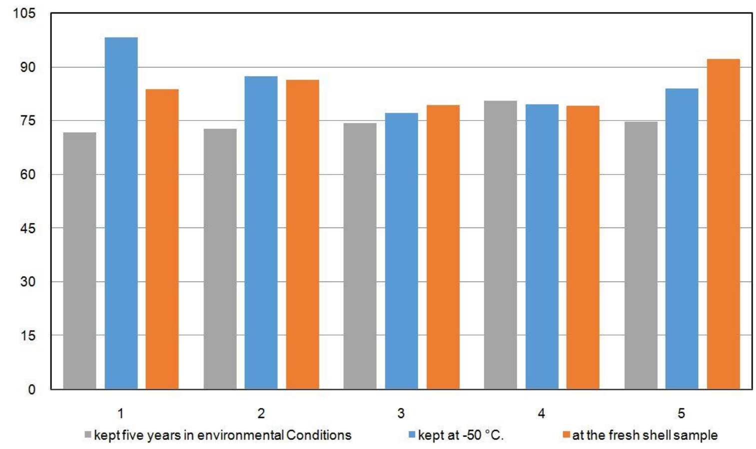
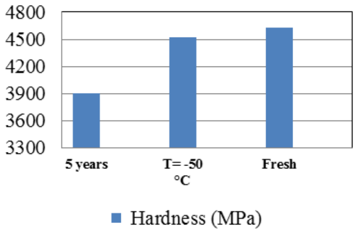
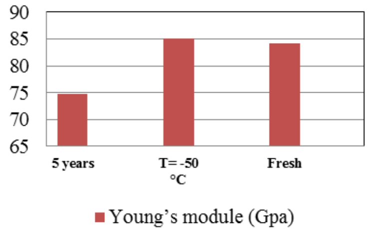


Fig. 6 The young’s modulus of top neck mollusks shell samples



(a) Hardness of top neck mollusks shell samples in different situations and directions



(b) The young's modulus of top neck mollusk shell samples in different situations and directions

Fig. 7 Mechanical properties of top neck mollusks shell samples in different situations

hardness and elastic modulus of the sample are calculated by using relations (2) and (7). Therefore,for each tested sample, five elastic modulus and hardness are obtained (Figs. 5-6).

To calculate the hardness and elastic modulus of each individual sample in general, we must calculate the mean of the elastic modulus and the obtained hardness and consider it as the mechanical properties of the sample. (Fig. 7).

4. Conclusions

This paper is an empirical study of Nano-mechanical behavior of oysters in various environmental conditions and different indentation situations using the Nano-indentation method. Based on the interpretations and results of this research, the following main results can be obtained.

- This study shows that in different environmental conditions, different elastic modulus and hardness are obtained so that the elastic modulus and hardness of Top Neck mollusks shell stored in environmental conditions are equal to 74.758 (GPa) and 3904.136 (MPa), as well as the elastic modulus and the hardness of stored oysters at -50℃ are 85.180 (GPa) and 4520.929 (MPa), and the elastic modulus and hardness of fresh oysters are equal to 84.110 (GPa) and 4625.749 (MPa), respectively.
- The mechanical properties obtained for all the different environmental conditions for the elastic modulus are in the range of 70 to 85 (GPa) and the hardness range between 3900 and 520046 (MPa). By comparing these values with Jackson studies of abalone, they are compatible

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