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Selective colors reflection from stratified aragonite calcium carbonate plates of mollusk shells

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A B S T R A C T

An interaction between the incident light and the structural architecture within the shell of Asian green mussel (*Perna viridis*) induces observable pearlescent colors. In this paper, we investigate the influence of the structural architecture on the expressed colors. After a removal of the organic binder, small flakes from crushed shells show vivid rainbow reflection under an optical microscope. An individual flake expresses vivid color under a bright-field illumination while become transparent under a dark-field illumination. The expressed colors of the aragonite flakes are directly associated with its structural architecture. The flakes with aragonite thickness of 256, 310, and 353 nm, respectively, appear blue, green, and red under an optical microscope. The spectral simulation corroborates the experimentally observed optical effects as the flakes with thicker aragonite layers selectively reflected color with longer wavelengths. Flakes with multiple aragonite thicknesses expressed multi-color as the upper aragonite layers allow reflected colors from the lower layers to be observed.

1. Introduction

The structural colors in nature mainly originate from interference of light with periodic structure in some living organism such as butterfly wings, beetle wings, bird features, shells, fishes, plant leafs (Tan et al., 2004; Adachi, 2007; Schenk et al., 2013; Ding et al., 2009; Shawkey et al., 2009; Thomas et al., 2010; Kolle et al., 2010; Yoshioka et al., 2012; Kinoshita and Yoshioka, 2005; Meadows et al., 2009; Chung et al., 2012; Yoshioka and Kinoshita, 2004; Lertvachirapaiboon et al., 2014). The structural colors function as attractants to conspecific species, warning signs, and camouflage (Kinoshita and Yoshioka, 2005; Meadows et al., 2009). The expressed colors are attributed to refractive index of materials, angle of incidence, structural arrangement with respect to the incident light, and thickness of stratified layers within periodic structure (Kinoshita and Yoshioka, 2005; Meadows et al., 2009; Chung et al., 2012). A well-known example of structural colors in the nature is butterfly wings of the genus *Morpho* which selectively reflect brilliant blue color due to a multiple reflection of white light within an alternating multilayers of chitin and air (Ding et al., 2009; Chung et al., 2012; Yoshioka and Kinoshita, 2004). The elytra of many beetles express fascinating colors due to their structure. Yoshioka et al. found that the jewel beetle (*Chrysochroa fulgidissima*) exhibited a green color at the normal angle and the blue color under an oblique angle due to an optical interference within stacked layers of chitin (Adachi, 2007). The mollusk shell also express colors originated by an interference of light with periodic stratified layers of aragonite calcium carbonate layer and organic matrix. The unique structure of mollusk shells that provide the pearlescent colors is a stratified assembly of alternated 200–500 nm-thick aragonite layers bound by 20–30 nm-thick organic film (Lertvachirapaiboon et al., 2014; Jackson et al., 1988; Leung and Sinha, 2002; Lin and Meyers, 2005; Lopez et al., 2014; Meyers et al., 2008). Tan et al. corroborated that the uniform stacking of nacre induces the interference effects. The high groove density on the surface strongly contributed to the vivid pink and blue-green pearlescent colors in abalone (*Haliotis glabra*) shell (Tan et al., 2004). Recently, the pearlescent colors from Asian green mussel (*Perna viridis*) shell and the color enhancement induced by air gaps were reported (Lertvachirapaiboon et al., 2014).

Most of the structural colors study related to color-shift with respect to viewing angle. The structural colors of nacre layer from sea shells express extraordinary reflections as several colors are visible at the same time. After organic binder was eliminated, the shells were disintegrated to small fragments called ‘pearlescent flakes’. In this current investigation, the relationship between

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structure of pearlescent flakes and the color expressions were characterized to gain insight understanding of rainbow-colors reflection of nacre. The modified transfer matrix method enabled us to characterize the origin of rainbow colors reflected from stratified layer of aragonite calcium carbonate as only the organic binder was disintegrated without destroying the structural assembly of nacre of shells. Moreover, the calculation was used to confirm the relationship between structure and expressed colors.

2. Experimental procedures

The pearlescent flakes from Asian green mussel (AGM), abalone, and oyster shells were prepared by our previously developed methodology with a minor modification in order to obtain shell fragments with uniform and narrow thickness distribution of aragonite plates (Lertvachirapaiboon et al., 2014). Briefly, the shells were thoroughly cleaned to remove residual tissues and other contaminants before drying under an ambient air. The dried shells were baked at 300 °C for 2 h. The shells were then immersed in 30% hydrogen peroxide (H_2O_2, Merck, Thailand) for 24 h to dissolve the degraded organic binder. The treated shells were brittle and easily broken-down into small flakes. The flakes were cleaned with tap water several times and air dried before keeping in a desiccator for further investigation.

Scanning electron microscope (SEM, JSM-6510A, JEOL) and atomic force microscope (AFM, SPA 400, SII NanoTechnology Inc.) were employed to investigate the structural architecture of the flakes. To acquire SEM images, a flake was wrapped with aluminum foil before mounting on a modified stub with the cross-section face of the shell normal to the electron beam. Since the pearlescent flake is nonconductive, an electron charge-up effect occurs during SEM measurement. Aluminum foil was employed for decreasing charge-up effect by facilitation an electron discharge from the flake. SEM images were acquired under low acceleration voltage of 5 kV. The average thickness of aragonite plates was calculated from 100 aragonite plates selected from unique SEM images (Lopez et al., 2014). AFM images were recorded with a scan rate of 1 Hz in a non-contact mode using silicon tips with a rounding size of 20 nm, force constant of 17 N/m, and a resonance frequency of 139 kHz.

The expressed colors of pearlescent flakes were recorded by a CCD camera (AxioCam HRc, Carl Zeiss) attached on an optical microscope (OM, Carl Zeiss Axio Scope.A1). The reflection spectra of pearlescent flakes were collected by a fiber optic spectrometer (USB4000 portable UV–visible spectrometer, Ocean Optics) coupled to the OM. A reflectance standard from Ocean Optics was employed as a reference for spectral acquisition. Fig. 1 shows instrumental setup consisting of CCD camera and fiber optic spectrometer capable of recording OM images and spectroscopic data simultaneously.

3. Results and discussion

Calcium carbonate flakes of AGM, abalone, and oyster shells prepared by a consecutive thermal/chemical treatment appeared white. When viewing under an OM (10× objective), they showed vivid color (Fig. S1) not observed in shell fragments prepared by crushing technique (Fig. S2) and chemically precipitated calcium carbonate (Fig. S3). The flakes from AGM shells showed the most vivid colors covering the entire visible region. Therefore, it was selected as a model material for our study on color expression of the nacre structure. The pearlescent effect of a virgin AGM shell originated by an interaction between light with nacre is well-known, Fig. 2A. To acquire the structural information of the shell, we recorded SEM images across the thickness of the AGM

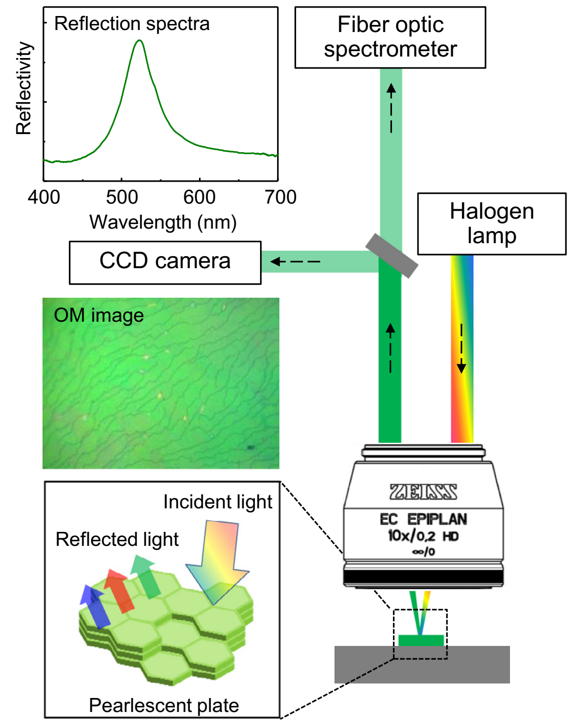

Fig. 1. An experimental setup capable of simultaneously acquires visible spectra and the corresponding OM images of a pearlescent flake.

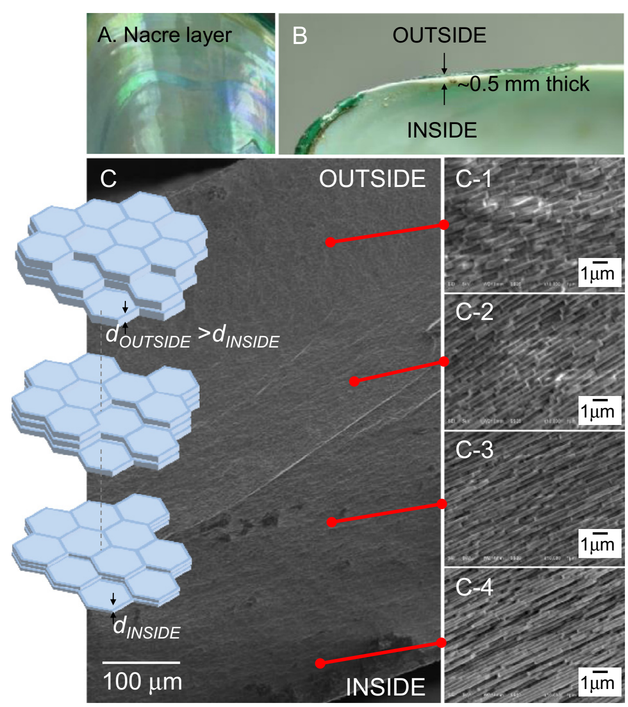

Fig. 2. (A) Pearlescent colors of AGM shell. (B) Thickness of AGM shell. (C) A cross-section SEM images of AGM shell. The detailed SEM images across the thickness (C1–C4) show a gradual thickness increment of the aragonite plates from the inner layers toward the outside layers.

shell. The cross-section SEM images in Fig. 2C show a gradual increment of the thickness of aragonite plates toward the outer layers.

After the removal of the organic binder, the treated shells showed stronger reflection with more vivid colors compared with