

Fluorescence from Pearls to Distinguish Mother Oysters Used in Pearl Culture

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The fluorescence spectra of pearls and shells of *Pteria penguin* (mabe), *Pinctada fucata* (Japan's Akoya oyster) and *Pinctada maxima* (yellow lip oyster) have been measured in order to distinguish species of the mother oyster which produce that pearl. A distinction is possible for these pearls using differences in the fluorescence spectra. The spectral difference has been attributable to colouring matter or scleroprotein in pearls and shells.

§1. Introduction

Several mother oysters are used in pearl culturing: seawater bivalves (*Pinctada fucata* (Japan's Akoya oyster), *Pinctada maxima* (yellow lip oyster), *Pinctada margaritifera* (black lip oyster) and *Pteria penguin* (mabe)), seawater gastropod (genus *Haliotis* (abalone)) and freshwater bivalves (*Hyriopsis shlegeli* et al.). Nondestructive methods for distinguishing mother oysters have been important from practical points of view since the commercial value of pearls depends on the mother oysters. In summary, freshwater pearls can be distinguished from seawater pearls by using X-ray fluorescence analysis, since freshwater pearls contain a large amount of manganese.¹⁾ Pearls of *P. margaritifera* show a peculiar absorption at 700 nm.²⁾ Pearls of genus *Haliotis* can be distinguished from pearls produced from any other mother oysters under a microscope since the surface of gastropod nacre shows a pyramid stack formation.³⁾ Here, nacre is the surface layer part of a pearl and a shell.

In a previous paper, we reported that a distinction is possible for pearls of *P. fucata* and *P. maxima*, using differences in the fluorescence spectra under N₂ laser excitation.⁴⁾ On the other hand, as far as we know, there has been no report on the distinction of mother oysters for *P. penguin*. In this work, we measured the fluorescence spectra of pearls and shells of *P. penguin*, *P. fucata* and *P. maxima* under N₂ laser and ordinary lamp excitation in order to apply the fluorescence method to the distinction of these pearls.

§2. Experimental Procedure

Laser-induced fluorescence has been measured at room temperature using the following apparatus. The excitation source was a pulsed N₂ laser (NDC JS-1000L: $\lambda = 337.1$ nm, pulse duration = 5 ns, repetition rate = 4 Hz). The laser beam was set at an angle of about 50° off the normal incidence to the plane of the sample and was focused on a spot about 1 mm² in area by a quartz lens (focal length $f = 150$ mm, diameter $d = 40$ mm). The peak power density of the laser light on a sample was regulated to be about 50 kW/cm² using neutral density filters. The sample was held between Cu and an Al plates with a hole 6 mm in diameter. The laser beam and fluorescence passed through the hole. Fluorescence was observed at 90° to the laser beam and was focused on the

entrance slit of a 50 cm monochromator (Oyo Bunko ASI-50S) by a glass lens ($f = 70$ mm, $d = 40$ mm). A glass filter (Toshiba L-39) was used in order to cut off any scattered light of the laser beam. Time-integrated and time-resolved fluorescence spectra were measured with the monochromator, a photomultiplier (Hamamatsu R955), a boxcar integrator (NF Circuit Design Block BX-531) and a recorder. The transient characteristics of fluorescence were measured using a storage oscilloscope (Iwatsu Electric TS-8123). Ordinary fluorescence has also been measured using a spectrofluorophotometer (Japan Spectroscopic FP-770) to examine the effect of the excitation source on the fluorescence spectra. The apparatus constant is not corrected for the ordinary fluorescence spectra.

The materials in this study were the shells of *P. penguin* as well as the shells and pearls of *P. fucata* and *P. maxima*. Mother oyster of *P. penguin* produces various pearl colours: reddish brown, pink and white. Reddish brown is caused by colouring matter in nacre and pink is caused by an interference effect in nacre. In this experiment, we adopt the nacre of shell instead of pearl for the following reasons: nacre of shell has the same chemical composition as a pearl itself and the pearl of *P. penguin* is hard to obtain commercially since the amount produced is very small. Although we could not obtain the pearl of *P. penguin*, nacre of shell would show a similar fluorescence spectrum to that of the pearl. The spectrum similarity was confirmed for *P. fucata* and *P. maxima* (Figs. 1 and 5).

Mother oysters of *P. fucata* produce various pearl colours: white, green, pink, yellow and blue. Green and pink are caused by an interference effect in nacre; while yellow and blue are considered to be due to organic substances in the pearls. Mother oyster of *P. maxima* produces white and yellow pearls. The yellow appearance is also considered to be due to organic substances. In this experiment, white and yellow pearls and shells of these two species have been investigated.

Conchiolin, which is a kind of scleroprotein in nacre for *P. fucata* and *P. maxima*, was investigated. Nacre was completely decalcified with an EDTA (ethylenediamine tetraacetic acid) solution; then, nacreous conchiolin was obtained as an insoluble residue. We adopt nacre of shell instead of pearl since it has the same chemical composition as a pearl itself (as described above).

§3. Results and Discussion

3.1 *P. penguin*

Figure 1 shows the time-integrated fluorescence spectra of nacre of shells of pearl oysters: reddish brown and white shells of *P. penguin*, a white shell of *P. maxima* and a yellow shell of *P. fucata*. Three peaks were observed at about 440 nm, 540 nm and 620 nm in the reddish-brown shell of *P. penguin*. Peaks at 540 and 620 nm were not observed in the shells of *P. maxima* and *P. fucata*. Thus, a distinction is possible for reddish-brown pearls of *P. penguin* from pearls of *P. fucata* and *P. maxma* using this difference in the fluorescence spectra. On the other hand, the 540 and 620 nm peaks were not observed in the white shell of *P. penguin*. The fluorescense spectrum of the white shell is similar to that of *P. maxima*.

Since the fluorescence intensity of the 620 nm peak is considered to depend on the wavelength of the excitation light, the excitation spectrum is measured by using a spectrofluorophotometer. Figure 2 shows the excitation spectra of the 620 and 540 nm peaks of the reddish-brown shell. In the excitation spectrum, the observed wavelength was fixed at 620 nm (upper curve) or 540 nm (lower curve) and the excitation wavelength was scanned. The abscissa represents the excitation wavelength. A peak was observed at about 400 nm. Thus, 400 nm light excitation may be most favourable for an observation of the 620 nm peak. Figure 3 shows ordinary fluorescence spectra of reddish-brown and white shells under 400 and 350 nm light excitation using the spectrofluorophotometer. The 620 nm peak was observed even in the white shell under 400 nm excitation. The pink shell of *P. penguin* shows a similar fluorescence spectrum to that of the white shell. On the other hand, the 620 nm peak was not observed in the nacre of shells and pearls of *P. fucata* and *P. maxima* under 400 nm excitation. Thus, a distinction is possible for pearls of *P. penguin* with any colours using the fluorescence spectra under 400 nm excitation.

The fluorescence intensity of the 620 nm peak depends on the colour of shells. This suggests that the 620 nm peak can be attributed to colouring matter, which causes a reddish-brown appearance of the shell. Time-resolved fluorescence spectra were measured in order to examine the properties of the colouring matter. Figure 4 shows the

time-resolved fluorescence spectra of the reddish-brown shell of *P. penguin* under N₂ laser excitation. While the relative intensity of the 620 nm peak is weak at short delay time, it increases with an increasing delay time. This result suggests that the 620 nm peak has a long decay time. The decay time was determined from the transient characteristics of the fluorescence measured with a storage oscilloscope. The decay time is about 4 ns for both the 440 nm peak and the 540 nm peak and about 12 ns for the 620 nm peak. This result is consistent with that of the time-resolved fluorescence spectra. It is reported that porphyrin is contained in shells of *P. penguin*;⁵⁾ it shows a fluorescence peak at about 620 nm and an absorption peak at about 400 nm.⁶⁾ The decay time of fluorescence is 11 ns.⁷⁾ These results indicate that the 620 nm peak is caused by porphyrin.

The fluorescence intensity of the 540 nm peak depends on both the colour of shells and on the wavelength of excitation light (Figs. 2 and 3). This behaviour is similar to that of the 620 nm peak. However, the 540 nm peak is not so intense as the 620 nm peak under the 400 nm excitation. Moreover, the decay time of the 540 nm peak is different from that of the 620 nm peak. Thus, the 540 nm peak is considered to be due to other colouring matter. It is reported that carotenoids show a fluorescence peak at about 550 nm and that they are in marine shells such as shell-cameos, *Chlamys nobilis*, *Pecten varius* et al.⁸⁾ On the basis of this result, we consider that the 540 nm peak is caused by carotenoids.

3.2 *P. fucata* and *P. maxima*

Time-integrated fluorescence spectra of pearls of *P. fucata* and *P. maxima* were reported in a previous paper.⁴⁾ Since pearls of *P. fucata* show a peak at a longer wavelength than that of *P. maxima* (Fig. 5), a distinction is possible by using this difference. It has been interpreted that the spectral difference observed in pearls of *P. fucata* and *P. maxima* is caused by the difference in conchiolin, which is a kind of scleroprotein in pearls and shells.

In this paper, fluorescence from conchiolin extracted from nacre was measured for *P. fucata* and *P. maxima* in order to examine the validity of the interpretation above. Nacre consists of aragonite (CaCO₃), conchiolin and other trace components. The ratio of

aragonite and conchiolin is about 95:5 (w/w). A single crystal of aragonite is enclosed with conchiolin sheets and thousands of these piles build the nacreous structure like a brick wall. Conchiolin is a binding material between aragonite crystals. Figure 6 shows the fluorescence spectra of conchiolin, aragonite and mixture of these materials for *P. fucata* (solid curves) and *P. maxima* (dashed curves). Aragonite is powdered ore, which is collected in the Aragon province of Spain. The peak wavelength of fluorescence from conchiolin for *P. fucata* is longer than that for *P. maxima* and the fluorescence spectra of a mixture of aragonite and conchiolin in the ratio of 93:7 are in qualitative agreement with that in Fig. 5. Thus, the result in Fig. 6 supports the interpretation that the spectral difference in Fig. 5 is due to a difference in conchiolin.

Time-resolved fluorescence spectra were measured in order to examine the properties of conchiolin. Figure 7 shows the time-resolved fluorescence spectra of the white pearls of *P. fucata* (solid curves) and *P. maxima* (dashed curves) under N₂ laser excitation. Fluorescence peaks for both pearls shift to longer wavelengths with an increasing delay time. The time-resolved fluorescence spectra of conchiolin also show similar results. These results suggest that there are two groups of components in pearls. Group A shows fluorescence with a shorter decay time and a shorter wavelength peak, while group B shows that with a longer decay time and a longer wavelength peak. The ratio A/B may be large in pearls of *P. maxima*, so that they show fluorescence with a shorter wavelength peak. On the other hand, the ratio A/B may be small in pearls of *P. fucata*, so that they show fluorescence with a longer wavelength peak. The decay time was determined from the transient characteristics of fluorescence measured with the storage oscilloscope. The decay time is about 4 ns for $\lambda \leq 470$ nm and about 5 ns for $\lambda \geq 500$ nm for both pearls. This result is consistent with the two group model above.

The fluorescence spectra of pearls of *P. fucata* have been measured using an Hg lamp as the excitation source.⁹⁾ The peak wavelength of the fluorescence depends on the colour appearances; it appears at $470 \text{ nm} \leq \lambda \leq 530 \text{ nm}$. This result is in qualitative agreement with the present result shown in Fig. 5, while the peak wavelength of the former is longer than that of the latter. This difference is considered to be due to the difference in the excitation wavelength.

In summary, the fluorescence spectra of pearls and shells of mother oysters have been

measured under N₂ laser and ordinary lamp excitation. A distinction is possible for pearls of *P. penguin* using the peculiar peak at 620 nm, which is attributable to porphyrin in nacre. A distinction is also possible for pearls of *P. fucata* and *P. maxima* using the difference in the fluorescence spectra. The spectral difference is considered to be attributable to the difference in conchiolin.

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Figure captions

Fig. 1. Time-integrated fluorescence spectra of nacre of shells of several mother oysters at 300 K under N₂ laser excitation. Peak intensities are normalized.

Fig. 2. Excitation spectra of the 620 nm peak and the 540 nm peak of the reddish-brown shell of *P. penguin*.

Fig. 3. Ordinary fluorescence spectra of reddish-brown and white shells of *P. penguin* under 400 and 350 nm light excitation. Peak intensities are normalized.

Fig. 4. Time-resolved fluorescence spectra of the reddish-brown shell of *P. penguin*. Intensity ratios of the 620 nm peak are as follows: 0.6 (delay time $t = -2$ ns), 1 (8 ns), 0.3 (18 ns). The inset shows transient characteristics of the laser pulse.

Fig. 5. Time-integrated fluorescence spectra of yellow and white pearls of *P. fucata* (solid curve) and *P. maxima* (dashed curve). Peak intensities are normalized.

Fig. 6. Time-integrated fluorescence spectra of conchiolin, aragonite and mixtures of these materials. The percentage of conchiolin is represented by weight percent. Conchiolin is extracted from nacre of *P. fucata* (solid curves) and *P. maxima* (dashed curves). Peak intensities are normalized.

Fig. 7. Time-resolved fluorescence spectra of the white pearls of *P. fucata* (solid curves) and *P. maxima* (dashed curves). Peak intensity ratios are as follows: 1 (delay time $t = -2$ ns), 1 (8 ns), 0.3 (18 ns).

Fig. 1

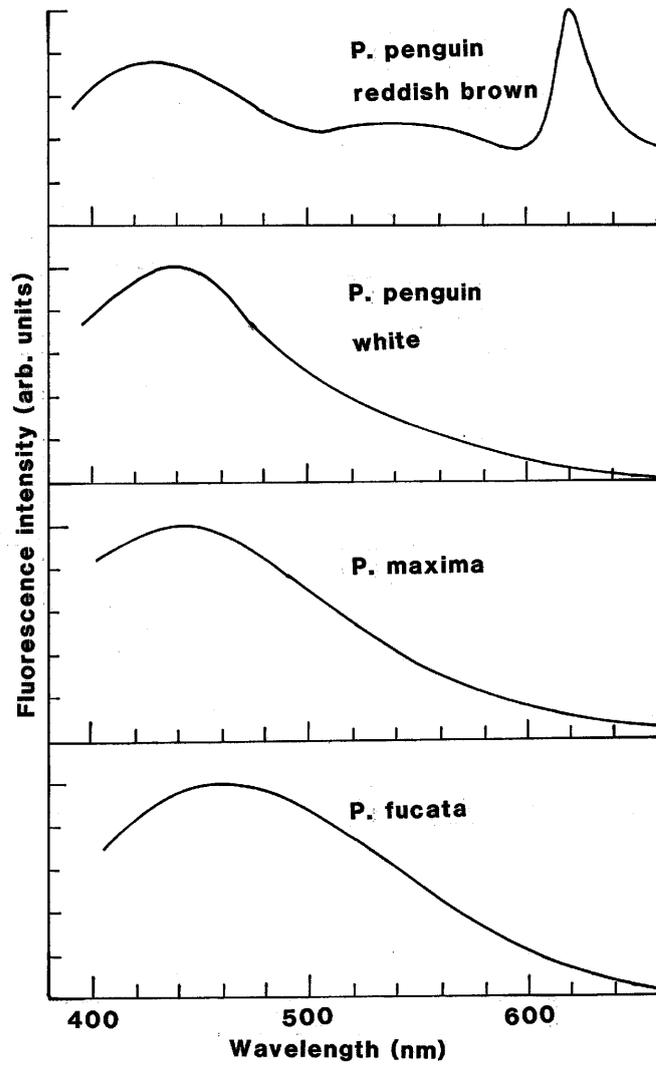


Fig. 2

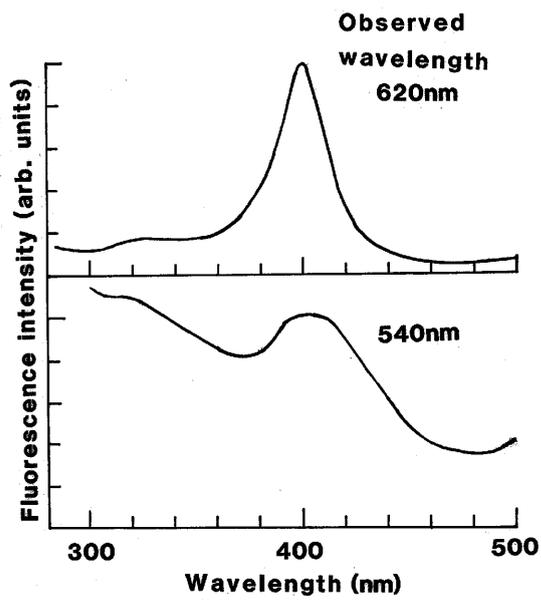


Fig. 3

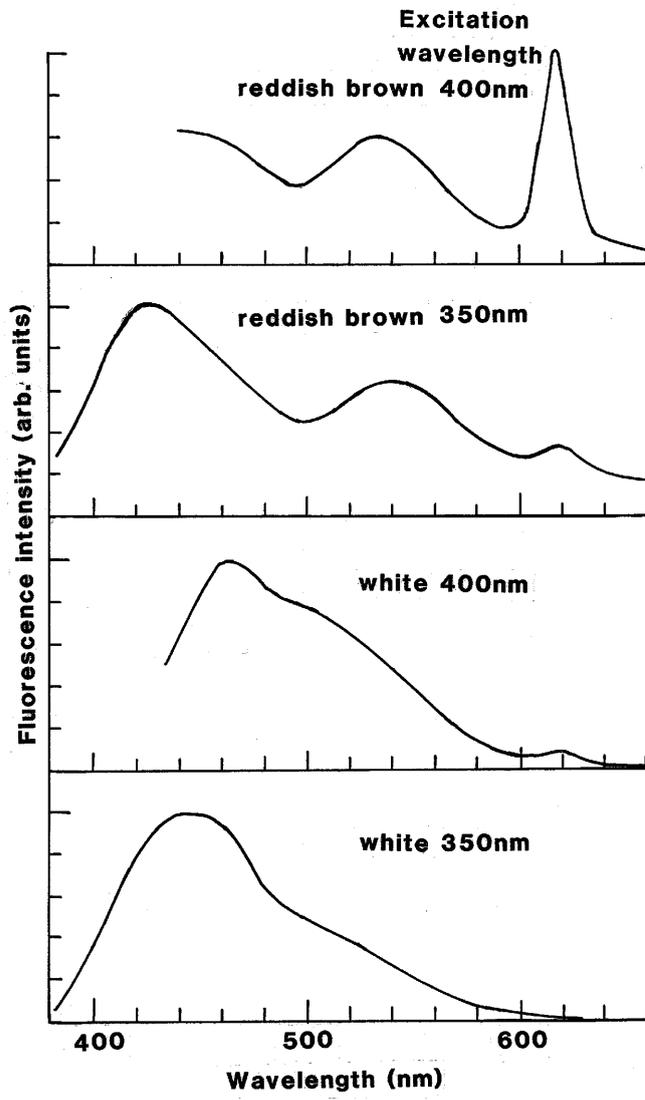


Fig. 4

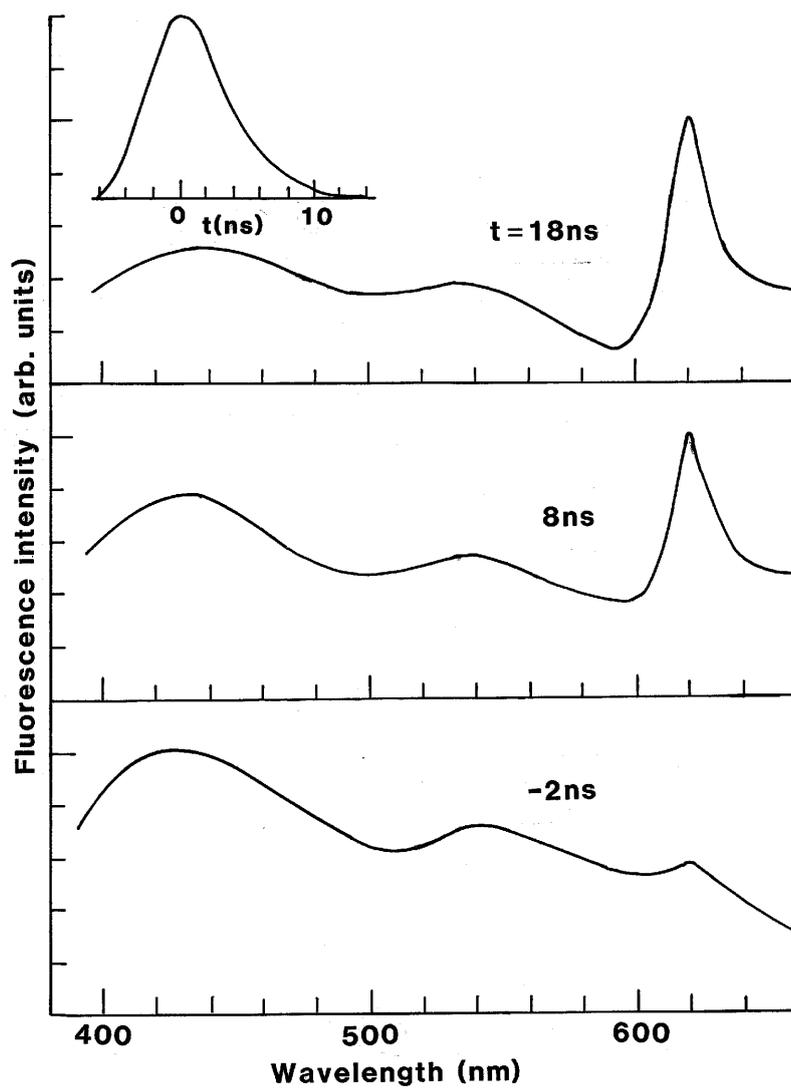


Fig. 5

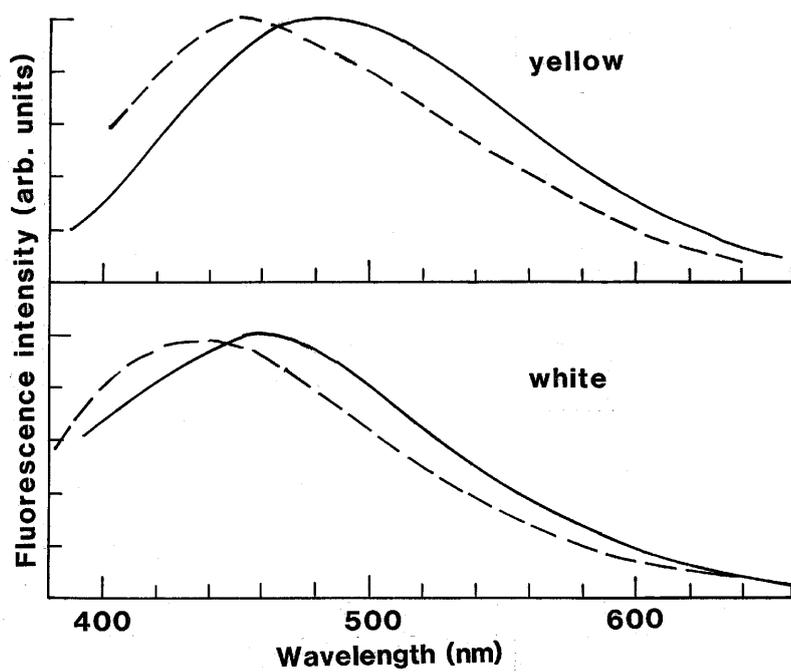


Fig. 6

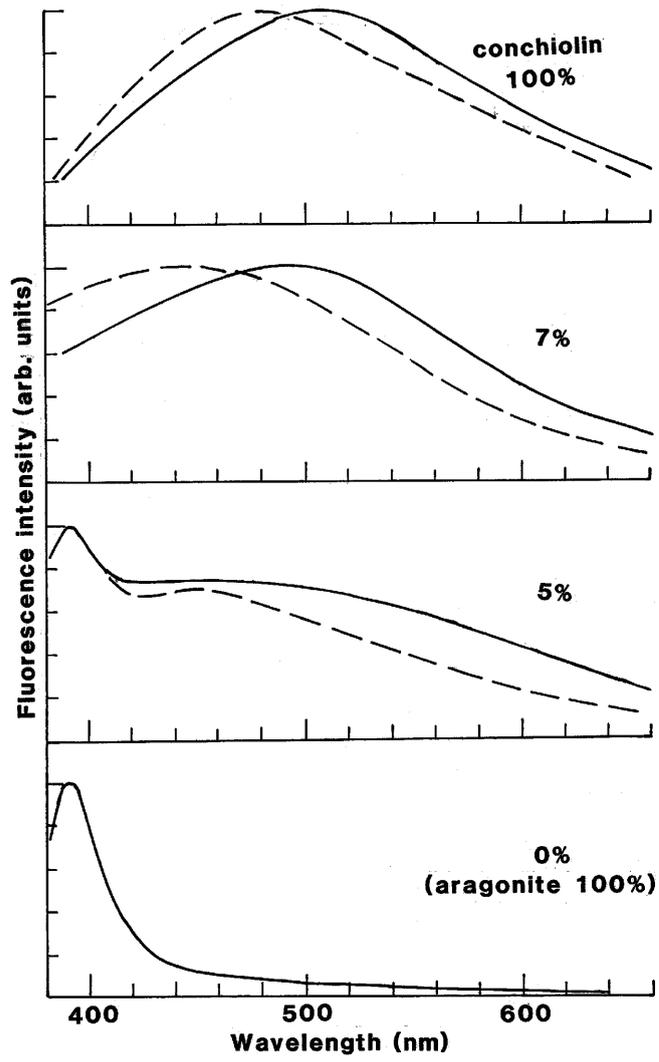


Fig. 7

