Role of polyenes in the coloration of cultured freshwater pearls

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Abstract: The exact nature of pigments present in cultured freshwater pearls is still not well known. We examined 21 untreated cultured freshwater pearls from *Hyriopsis* of typical colors by diffuse reflectance UV-Vis-NIR and Raman scattering measurements, at high resolution. The objective was to establish the relation between color and the nature of the pigment mixture in pearls, using strictly non-destructive methods. All natural color samples show the two major Raman resonance features of unmethylated (unsubstituted) polyenes, not carotenoids. Their general chemical formulae are $R-(-CH=CH)_N-R'$ with $N = 6$ to 14 and they give absorptions from violet to yellow–green. Each color is due to a mixture of pigments, not a single pigment. Different colors are explained by different mixtures. Each pigment identified by Raman spectroscopy can be related to a specific absorption with apparent maximum in the range 405–568 nm, thus absorbing from violet to yellow–green. This is the first study of the precise relation between Raman and diffuse reflectance spectra of cultured freshwater pearls.

Key-words: pearl, non-destructive spectroscopy, pigments, unmethylated polyenes.

1. Introduction

The aim of this work is to establish the relation between color and the nature of the pigment mixture in cultured freshwater pearls using non-destructive methods, such as diffuse reflectance UV-Vis-NIR spectroscopy and Raman resonant or (near-resonant) scattering (RRS), at high resolution. This subject rises in importance as the demand for colored pearls has grown over the past three decades. Pearls are composites of calcium carbonate, chiefly aragonite, and organic material, deposited in concentric layers. However, the exact nature of pigments present in such gem materials is still not well known. Previous studies have established that Raman scattering is useful to detect pigments in natural saltwater (Urmos *et al.*, 1991) and cultured freshwater pearls (Li & Chen, 2001; Fengming *et al.*, 2003). The pigments have been identified as carotenoids. More recent studies have shown that the color of pearls is due to a mixture of short unsubstituted polyenes, not carotenoids (Karampelas *et al.*, 2006 and 2007).

2. Background

Pearls are biominerals secreted in the interior of living mollusks (phylum Mollusca) with or without human

intervention. However, not all pearls are desirable but a few species of mollusks have the ability to produce pearls which are iridescent, and represent the typical commercial pearl product (Hänni, 1999). Pearls are natural or cultured. Natural pearls are secreted accidentally in a mollusk without any human intervention. Cultured pearls are formed in mollusks by human intervention, either in freshwater or saltwater. The vast majority of pearls found today in the market are cultured. Moreover, only a small number of mollusk species are used for large scale commercial cultivation. For cultured saltwater pearls these are the bivalves *Pinctada margaritifera*, *Pinctada fucata (martensii)* (producing so-called akoya cultured pearls), *Pinctada maxima* (producing so-called South Sea cultured pearls), *Pteria sterna*, and more rarely the gastropod *Haliois iris* (producing so-called abalone cultured pearls). For freshwater pearls, the following bivalves are used for cultivation: *Hyriopsis schlegeli* (*H. schlegeli*) (still known in the market as Biwa cultured pearls even if they are not all cultured at Lake Biwa in Japan), *Hyriopsis cumingi* (*H. cumingi*) and the hybrid *Hyriopsis schlegeli* x *cumingi*.

Raman spectroscopy is useful to identify the pigments in cultured freshwater pearls. However, in other cases Raman spectroscopy is not always useful for pearl pigment detection, for example no pigment signal is obtained for the yellow “golden” colored pearls from the bivalve *Pinctada maxima*.

The present study is done on cultured freshwater pearls. They occur in five main hues, *i.e.* white, grey, pink, orange and purple. However, various combinations of hue, tone and saturation yield a broad range of color appearances.

Polyenes (polyacetylenes) are polyunsaturated organic compounds that contain one or more sequences of alternating single and double carbon-carbon bonds, named a polyenic chain. Carotenoid molecules contain a polyenic chain, however there are four additional methyl groups substituted on this chain. Pigments containing polyenic chains are widespread in nature, for example in phytoplankton (Hoskins & Alexander, 1977), coral polyps and mollusk shells (Fox & Wilkie, 1970; Merlin & Delé-Dubois, 1983; Merlin, 1985; Merlin & Delé-Dubois, 1986; Vershinin, 1996; Barnard & De Waal, 2006; Hedegaard *et al.*, 2006), vegetables (tomatoes, carrots *etc.*; Withnall *et al.*, 2003), bird feathers (Stradi *et al.*, 1995, 1996, 2001a and b; Veronelli *et al.*, 1995), human retina (Ermakov *et al.*, 2005), *etc.* The vast majority of these pigments belong to the carotenoid family. It is therefore understandable that, when the pigment is not a carotenoid, it may still be reported as such. This is the case also for coloring agents in natural saltwater (Urmos *et al.*, 1991) and cultured freshwater pearls of various colors (Li & Chen, 2001; Fengming *et al.*, 2003), in which a signal due to unsubstituted polyenes was detected, but was interpreted as due to carotenoids.

As early as 1882, Krukenberg recognized that parrot feathers contain pigments other than carotenoids, and named them psittacofulvins. These pigments differed from the widely distributed carotenoids in their chemical reactivity and solubility (Stradi *et al.*, 2001b). More recently, Raman spectroscopy has shown that these pigments are unsubstituted (unmethylated) polyenes (Veronelli *et al.*, 1995). Similar pigments have been known to color also some coral polyps (*i.e.*, *Corallium rubrum*; Merlin & Delé-Dubois, 1986), some molluscan shells (Barnard & De Waal, 2006; Hedegaard *et al.*, 2006) and cultured freshwater pearls (Karampelas *et al.*, 2006 and 2007). Stradi *et al.* (2001b) observed that the color of parrot feathers of *Ara macao* is due to a mixture of at least four unsubstituted (unmethylated) polyenes. Recent Raman studies have shown that the color of cultured freshwater pearls of the *Hyriopsis* genus is also due to a mixture of short unsubstituted polyenes (Karampelas *et al.*, 2006 and 2007).

It has been shown that the Raman spectra of polyenes exhibit strikingly intense features because of the existence of delocalized π -electrons, which may induce a resonance under visible wavelength excitation (Veronelli *et al.*, 1995; Withnall *et al.*, 2003). Two strong Raman resonant (RR) bands, due to polyenic chains, occur in the 1100–1200 and 1450–1600 cm^{-1} ranges. These bands correspond respectively to the carbon-carbon stretching vibration (ν_2) of a C-C single bond (coupled to C-H in-plane bending modes), and to that of a C=C double bond (ν_1) in a polyenic chain (Saito *et al.*, 1983; Okamoto *et al.*, 1984). The exact position of these bands is dependent on the length of the chain (Schügerl & Kuzmany, 1981; Schaffer *et al.*, 1991; Barnard & De Waal, 2006). For carotenoids, which contain methyl groups in their polyenic

chain, the position of the ν_2 vibration is shifted about 20 cm^{-1} towards higher energy compared to polyenic chains without CH_3 (Okamoto *et al.*, 1984). The intensity of these bands is strongly enhanced over those not due to the polyenic chain. This enhancement is due to the coupling of electronic and vibrational transitions. Thus, polyenes can be easily studied without interference from other, non-resonant products, even in systems as complex as biological materials (Merlin & Delé-Dubois, 1986). In solutions this kind of pigments may be detected at very low concentrations (detection limit of about 10^{-8} M; Hoskins & Alexander, 1977).

Ermakov *et al.* (2005) attribute C_{2h} point group symmetry to polyenes. Their absorption is caused by electric dipole-allowed transitions of the molecule's conjugated π -electrons from the 1^1A_g singlet ground state to the 1^1B_u singlet excited state. Optical excitation within the absorption bands leads to weak luminescence. By analogy with the well studied β -carotene, the extremely low quantum efficiency of polyenes' luminescence (peaking at 530 nm) is caused by the existence of a second singlet state, a 2^1A_g state, which lies below the 1^1B_u state. Following optical excitation of the 1^1B_u state, the energy relaxes rapidly (within approximately 200–250 fs in β -carotene) by non-radiative transitions to the lower 2^1A_g state, from which electronic emission is symmetry forbidden. Their extremely weak $1^1B_u \rightarrow 2^1A_g$ luminescence and the absence of the $2^1A_g \rightarrow 1^1A_g$ transitions allow one to measure the RR spectra response of the molecules without interfering with luminescence signals. This combination of properties makes these molecules exceptionally easy to detect by Raman scattering. Moreover, the position of the maximum absorption (λ_{max}) is mainly a function of the length of the conjugated double-bond chromophore. Their energy gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) is generally small (*e.g.*, for a polyene in a solution with $N > 3$ the energy gap is < 4.5 eV; Schaffer *et al.*, 1991; Heller *et al.*, 1995). The first allowed electronic transition may occur in the visible region and give rise to color (Salares *et al.*, 1977; Schaffer *et al.*, 1991; Heller *et al.*, 1995; Veronelli *et al.*, 1995).

3. Materials and methods

This study was carried out on 19 colored untreated cultured freshwater pearls from *H. cumingi* and *H. schlegeli* covering a wide range of typical colors for this material and two white colored pearls from *H. cumingi* (Table 1). The samples are borrowed from reputed commercial sources (see Acknowledgements). As for all gems, the methods used to study the samples must be non-destructive. For this study, Raman scattering and UV-Vis-NIR diffuse reflectance spectra were acquired.

Diffuse reflectance spectra of all the samples were obtained at the IMN (Institut des Matériaux Jean Rouxel, France), for the 250–1600 nm range with a Cary 5G ultraviolet/visible/near-infrared (UV-Vis-NIR) spectrometer

Table 1. Color range and homogeneity as well as mollusk species of naturally colored cultured freshwater pearls studied.

Sample number	Color description and distribution	Mollusk
SK56	White-cream (homogeneous)	<i>H. cumingi</i>
SK57	White-cream (homogeneous)	<i>H. cumingi</i>
SK58	Orange-slight grey (homogeneous)	<i>H. cumingi</i>
SK59	Orange-slight grey (homogeneous)	<i>H. cumingi</i>
SK60	Pink-grey "light pink" (homogeneous)	<i>H. cumingi</i>
SK61	Pink-grey "light pink" (homogeneous)	<i>H. cumingi</i>
SK83	Orange (inhomogeneous)	<i>H. cumingi</i>
SK84	Violet-grey (inhomogeneous)	<i>H. cumingi</i>
SK85	Grey-little violet (inhomogeneous)	<i>H. cumingi</i>
SK86	Pink-grey "light pink" (inhomogeneous)	<i>H. cumingi</i>
SK87	Violet-grey (inhomogeneous)	<i>H. cumingi</i>
SK88	Violet-grey (inhomogeneous)	<i>H. cumingi</i>
SK89	Violet-grey (homogeneous)	<i>H. cumingi</i>
SK90	Violet-grey (homogeneous)	<i>H. cumingi</i>
SK91	Grey (homogeneous)	<i>H. cumingi</i>
SK92	Grey (homogeneous)	<i>H. cumingi</i>
SK116	Slight yellow (homogeneous)	<i>H. schlegeli</i>
SK117	Slight purple-grey "light purple" (homogeneous)	<i>H. schlegeli</i>
SK118	Pink-grey "light pink" (homogeneous)	<i>H. schlegeli</i>
SK119	Pink-grey "light pink" (homogeneous)	<i>H. schlegeli</i>
SK120	White-very light grey (homogeneous)	<i>H. schlegeli</i>

fitted with a Varian diffuse reflectance accessory. The data sampling interval (D.I.) and spectral bandwidth (SBW) of every measurement were 0.7 nm, to have good resolution, and the scan rate was relatively slow, 60 nm/min, to obtain a good signal/noise ratio. For the different pearl sizes, different black mat supports were used. Sample holders had different hole sizes (with a diameter from 2 to 10 mm), to position the samples with the maximum possible exposed surface to measure, producing a more intense signal. For pearls with inhomogeneous color distribution (see Table 1), spectra were taken in differently colored areas. Before each measurement, background spectra were taken using a white MgO powder (for 100 %) and a black mat card (for 0 %) reference. All measurements were repeated at least twice, in order to confirm the repeatability of the results. Each measurement took about 25 min.

The acquisition conditions for Raman scattering spectra are almost identical to those described previously in detail (Karampelas *et al.*, 2007; see also Table 2). The main changes are the following:

- (1) The acquisition time is increased from 20 to 60 s in order to improve the signal to noise ratio. This better reveals some weak components of the Raman bands, otherwise lost in noise.

Table 2. Excitation conditions, resolution and acquisition times for Raman measurements.

Excitation wavelength (nm)	Power on the sample (mW)	Acquisition time (s)	Laser	Resolution (cm^{-1})
364	10	1200	Ar ⁺	6
458	2	60	Ar ⁺	1.5
488	2	60	Ar ⁺	1.5
514	2	60	Ar ⁺	1
561	1	60	solid semiconductor	0.8
647	3	60	Kr ⁺	2.5
1064	100	2000	Nd:YAG	4

- (2) We obtained measurements with yellow excitation using a semiconductor laser that emits at 561 nm with 10^{-5} nm spectral bandwidth, the backscattered light being then dispersed on a 1800 grooves/mm holographic grating. This confirms the presence of certain pigments, harder to detect with only red and green excitation, and helps to demonstrate that they absorb (*i.e.*, are Raman resonant) in this spectral region.

Raman spectra were all taken in the same area of the same sample by simply changing the excitation wavelength. This experimental area was always in the area where the UV-Vis-NIR diffuse reflectance spectrum was taken. All measurements were repeated at least twice, in order to ensure reproducibility. Note that the Raman spectra are normalized to the major Raman peak of aragonite (at 1085 cm^{-1}), which is by far the major constituent of this kind of pearls. It can therefore be considered an internal standard. This allows a better comparison concerning relative intensity of the pigments signals. All spectra are vertically offset for clarity in the figures.

Overlapping Raman peaks at about 1500 cm^{-1} were decomposed for a finer analysis using Peak Fit 4.11 (Systat Software, Richmond, California). All bands were considered to have Gaussian shape. Band position (with an accuracy of 2 cm^{-1}) and full width at half maximum (FWHM) were constrained for a given peak, so that only the intensity could be adjusted. In contrast to previous work (Karampelas *et al.*, 2007), the FWHM used for the bands, whatever the excitation, was fixed at 10 cm^{-1} .

4. Results

Compared to our previous study (Karampelas *et al.*, 2007) we have added 5 samples from *H. schlegeli* (so-called Biwa cultured pearls) which show similar results (see Table 3). Moreover, with the new excitation used (at 561 nm) often a new pigment is observed in some samples (*e.g.*, at 1486 cm^{-1} for sample SK92, see again Table 3). The measurements of the samples common to our two

Table 3. Absorptions observed in the visible spectral region (after visual decomposition; see text) as well as Raman bands observed using different excitation wavelengths (after decomposition with Peak Fit and constraints; see text) and estimated pigment composition (after Schaffer *et al.*, 1991 and Barnard & De Waal, 2006; Table 4). When the color is inhomogeneous, we report data for several measurement points. In bold are the peaks with the highest relative intensity (-: no other peaks than those of aragonite are observed).

Sample number/ Color of measurement point		Absorptions observed in the visible range (nm)	Excitation wavelengths (nm) and peaks observed (cm ⁻¹)					Number of double bonds (N) after Barnard & De Waal, 2006
			458	488	514	561	647	
SK56	White-cream	625, 670	—	—	—	—	—	—
SK57	White-cream	575, 625, 670, 740	—	—	—	—	—	—
SK58	Orange-slight grey	432, 455 , 478 , 498, 518, 575, 625, 670, 740	1556 1544 1530 1521 1513	1543 1542 1530 1523 1513	1542 1542 1530 1522 1511	1542 1530 1522 1522 1511 1506		7–12
SK59	Orange-slight grey	430, 452, 477 , 500 , 520, 535, 575, 625, 670, 740	1556 1541 1530 1521 1513	1542 1542 1530 1523 1513	1542 1542 1530 1522 1513	1542 1530 1522 1522 1513 1506		7–12
SK60	Pink grey “light pink”	430, 453 , 480 , 500 , 517 , 535 , 555, 572, 625, 670, 740	1557 1543 1530 1522 1513 1504	1542 1543 1529 1521 1512 1504	1543 1531 1530 1523 1511 1504	1540 1530 1529 1521 1512 1506 1503 1495 1496 1488		7–14
SK61	Pink grey “light pink”	430, 458 , 477 , 502 , 520 , 540 , 555, 575, 625, 670, 740	1554 1541 1530 1522 1512 1503	1543 1543 1529 1521 1512 1506	1543 1543 1529 1522 1511 1504	1543 1529 1522 1522 1511 1502 1496 1496		7–13
SK83	Orange (upper part)	408 , 433 , 458 , 477 , 497, 514, 537, 670	1554 1543 1529 1522 1513	1540 1542 1530 1523 1512 1504	1542 1531 1530 1522 1512 1505	1531 1531 1522 1522 1512 1505		7–12
	More orange than the 1st (lower part)	408 , 433 , 458 , 477 , 497, 514, 540, 625, 740	1557 1541 1529 1522 1512	1556 1541 1529 1522 1512	1541 1541 1530 1523 1512 1512	1541 1541 1530 1523 1522 1513 1503 1505		7–12
SK84	Violet-grey (upper part)	408 , 433 , 458 , 477 , 497, 514, 540, 555, 568, 575, 625	1556 1542 1531 1523 1513 1504	1543 1540 1529 1522 1514 1505	1540 1530 1530 1522 1513 1506 1495 1495 1488	1532 1532 1522 1512 1512 1505 1505 1495 1488		7–14
	More violet than the 1st (lower part)	405, 430, 460, 480, 500 , 515 , 535 , 555, 570, 575, 625, 670, 740	1557 1542 1530 1522 1511 1504	1557 1542 1531 1522 1513 1505	1542 1531 1531 1522 1512 1505	1531 1530 1520 1511 1506 1504		7–14

Table 3. Continued

Sample number/ Color of measurement point		Absorptions observed in the visible range (nm)	Excitation wavelengths (nm) and peaks observed (cm ⁻¹)				Number of double bonds (N) after Barnard & De Waal, 2006
				1496	1496	1496 1488 1487	
SK85	Grey–little violet (upper part)	405, 435, 456 , 478 , 495 , 518 , 535, 555, 570, 575, 625, 670, 740	1554 1541 1530 1521 1511 1504	1543 1530 1523 1511 1505	1542 1530 1521 1513 1506 1504	1531 1520 1511 1506 1497 1488	7–14
	More grey than the 1st (lower part)	405, 432, 455, 480 , 498 , 518 , 537 , 555, 570, 575, 625, 670, 740	1557 1543 1529 1521 1511 1504	1555 1541 1529 1521 1510 1503	1556 1542 1531 1522 1512 1503 1494 1494 1484	1520 1511 1510 1504 1497 1487	7–14
SK86	Pink–grey “light pink” (upper part)	405, 432 , 457 , 477 , 498 , 518, 535, 670	1556 1542 1529 1522 1511	1553 1541 1529 1522 1512 1504	1541 1540 1529 1529 1521 1512 1504 1504 1494 1484	1540 1540 1529 1529 1521 1511 1505	7–12
	More pink than the 1st (lower part)	405, 435, 458 , 477 , 498 , 518, 535, 555, 570, 575, 625	1568 1556 1541 1529 1522 1511 1502	1556 1542 1529 1522 1512 1503	1556 1541 1529 1522 1512 1504 1494 1494 1486	1541 1531 1522 1512 1512 1504 1494 1484	6–14
SK87	Violet–grey (upper part)	405, 434, 455 , 477 , 498, 517, 535, 553, 568, 595, 625	1569 1556 1542 1530 1522 1511 1502	1543 1530 1522 1511 1502	1541 1529 1522 1512 1504 1495 1485	1530 1520 1510 1504 1504 1496	6–14
	More violet than the 1st (lower part)	405, 435 , 455 , 478, 498, 518, 575, 595, 625	1568 1556 1542 1529 1522 1512 1504	1557 1542 1529 1522 1512 1502	1541 1530 1530 1521 1513 1504 1494 1496	1529 1521 1512 1505 1505	6–13
SK88	Violet–grey (upper part)	405, 435 , 455 , 478, 498, 518, 538, 555, 570, 595, 625, 670	1556 1541 1529 1522 1511 1504	1555 1542 1529 1522 1513 1505	1541 1530 1529 1522 1511 1503 1494 1497 1486	1530 1520 1511 1505 1505	7–14
	More violet than the 1st (lower part)	408, 430 , 455 , 477, 498, 518, 540, 558, 570, 595, 625, 670	1568 1557 1541	1556 1544	1542 1541		6–14

Table 3. Continued

Sample number/ Color of measurement point	Absorptions observed in the visible range (nm)	Excitation wavelengths (nm) and peaks observed (cm ⁻¹)	Number of double bonds (N) after Barnard & De Waal, 2006
		1530 1530 1530 1530 1531 1523 1522 1522 1522 1522 1513 1512 1511 1511 1513 1504 1504 1503 1503 1504 1494 1492 1495 1484	
SK89	Violet–grey	408, 430, 455, 477, 498 , 518 , 540 , 555, 570, 595, 625, 670, 740 1569 1555 1555 1555 1541 1540 1541 1530 1529 1529 1529 1532 1521 1521 1521 1521 1523 1512 1512 1511 1511 1513 1503 1503 1504 1504 1504 1495 1495 1495	6–13
SK90	Violet–grey	405, 430, 455, 480 , 498 , 516 , 540, 555, 570, 595, 625, 670, 740 1569 1568 1569 1554 1555 1556 1554 1541 1541 1541 1540 1531 1530 1530 1531 1530 1523 1522 1523 1522 1522 1511 1511 1512 1511 1513 1503 1503 1503 1502 1505 1495 1495 1495 1495 1496 1485 1487 1485 1485 1485	6–14
SK91	Grey	407, 430, 457 , 478 , 498 , 517 , 540, 558, 570, 595, 625, 670, 740 1567 1555 1555 1542 1542 1541 1543 1530 1529 1530 1530 1530 1522 1521 1521 1522 1521 1512 1512 1512 1510 1511 1504 1502 1504 1505 1503 1494 1496 1497 1487 1484	6–14
SK92	Grey	405, 432, 459, 477 , 498 , 517 , 542, 555, 570, 595, 625, 670, 720, 740 1569 1555 1557 1556 1543 1541 1543 1530 1530 1529 1530 1530 1523 1522 1522 1521 1521 1512 1510 1512 1511 1512 1503 1504 1503 1505 1504 1496 1494 1494 1494 1485 1486 1485	6–14
SK116	Slight yellow	408, 434 , 453 , 478, 497, 517, 542, 555, 575, 595, 625, 670, 740 1555 1542 1543 1542 1531 1531 1542 1530 1522 1522 1530 1521 1510 1511 1522 1511 1510 1502 1496	7–13
SK117	Slight purple–grey “light purple”	455, 478, 498, 517, 542, 555 , 572 , 595, 625, 670, 695 1542 1542 1531 1531 1530 1530 1521 1522 1521 1520 1511 1511 1511 1511 1505 1504 1504 1504 1496	8–13
SK118	Pink–grey “light pink”	432, 455, 478, 498, 517, 541 , 555 , 572, 595, 625, 670, 720 1555 1542 1543 1543 1543 1531 1531 1531 1531 1520 1520 1521 1520 1511 1511 1511 1512 1504 1504 1501 1502 1497 1497	7–13

Table 3. Continued

Sample number/ Color of measurement point		Absorptions observed in the visible range (nm)	Excitation wavelengths (nm) and peaks observed (cm ⁻¹)				Number of double bonds (N) after Barnard & De Waal, 2006
SK119	Pink–grey “light pink”	435, 455, 478, 498, 518, 542 , 555 , 572, 595, 625, 670, 720	1556 1543 1531 1522 1511	1556 1543 1531 1521 1511	1543 1530 1521 1521 1511	1531 1511 1502 1497	7–13
SK120	White (very light grey)	435, 458, 477 , 497 , 518, 542, 555, 572, 575, 595, 625, 670, 695, 720	1555 1541 1530 1521 1512	1542 1542 1531 1521 1511	1542 1531 1521 1511		7–11

investigations are updated from this previous study, taking into account slight differences in method. There are actually very few differences (see also Table 3), so details are not repeated here and can be found in the previous publication.

The main points are that, in the spectra of the white cultured freshwater pearls, we observe a band at about 1085 cm^{-1} and a doublet at about 702 and 706 cm^{-1} (Fig. 1) which correspond respectively to the ν_1 symmetric stretching and the ν_4 in-plane bending modes of the carbonate ion (CO_3^{2-}) in aragonite (Urmos *et al.*, 1991). Moreover, all colored samples present two intense additional bands in the 1000 – 1600 cm^{-1} region (*e.g.*, Fig. 2). These are characteristic of polyenic chains (Salares *et al.*, 1977). Some slight changes in shape and relative intensities of the two more intense bands are visible from one sample to the next. Such changes occur for the same area of

the same sample by simply changing the excitation wavelength (see Fig. 2 and Table 3). The main changes are observed at about 1500 cm^{-1} (approximate position of the peak corresponding to the double carbon–carbon stretching vibration, ν_1). This is a very "sensitive" region to the length of the polyenic chain (Schügerl & Kuzmany, 1981; Schaffer *et al.*, 1991; Barnard & De Waal, 2006). Positions of the band at about 1500 cm^{-1} are summarized in Table 4 as a function of the effective number of carbon double bonds in the polyenic chain, using equations previously established (Schaffer *et al.*, 1991; Barnard & De Waal, 2006) slightly extrapolated for the range $N = 12$ to 14 (Karampelas *et al.*, 2007). After decomposition of the spectra, it is estimated that up to 9 different bands may be found in the same pearl, at about 1486 , 1495 , 1504 , 1512 , 1521 , 1531 , 1542 , 1555 and 1569 cm^{-1} (positions $\pm 2 \text{ cm}^{-1}$) (Table 3). Each pearl is colored by a mixture of unsubstituted polyenes, with a general formula $\text{R}-(\text{CH}=\text{CH})_N\text{R}'$, with $N = 6$ to 14 . All our colored samples contained at least five pigments ($N = 7$ – 11) and up to nine. Shorter excitation wavelengths excite pigments with Raman peaks at higher wavenumber position (Table 3). Unmethylated polyenes which absorb in the ultraviolet (with $N < 6$) may also be present in these pearls.

The ν_2 bands are dependent upon inverse conjugation or chain length of polyene chains too (Schaffer *et al.*, 1991; Table 4). In our samples, the position of the single carbon–carbon stretching vibration (ν_2) is shifted as well as a function of sample color and excitation wavelength, with apparent maxima in the range from about 1120 to 1140 cm^{-1} , thus comprising component bands from about 1115 to 1160 cm^{-1} . Moreover, weak bands related to polyenes are revealed in all our spectra (Fig. 2) at about 1010 , 1190 and 1295 cm^{-1} , but they are not useful to provide further details on the pigments. No changes in shape and full width at half maximum (FWHM: $2.1 \pm 0.1 \text{ cm}^{-1}$) of the ν_1 aragonite band are noted from one sample to the other. In addition, no peaks linked to other carbonate polymorphs (*e.g.*, calcite and vaterite) were observed.

Fig. 1. Raman spectra of a white-cream pearl (SK56; diffuse reflectance spectrum is presented in Fig. 3), using five different excitation wavelengths. Only Raman peaks due to aragonite are present, at 1085 cm^{-1} (ν_1 symmetric stretching mode of carbonate), and a doublet at about 702 – 706 cm^{-1} (ν_4 in-plane bending of carbonate).

Fig. 2. Raman spectra of the lower part of a pink freshwater cultured pearl (SK86; diffuse reflectance spectrum is presented in Fig. 5) using five excitation wavelengths. The ν_1 band decomposition (with Peak Fit and constraints; see text) for the spectrum obtained with excitation at 561 nm is shown as inset.

Table 4. Positions of ν_1 and ν_2 bands as a function of the number of carbon double-bonds in the polyenic chain (as calculated from Schaffer *et al.*, 1991, and Barnard & De Waal, 2006) as well as correlation of decomposed Raman peaks and visible absorption features of samples measured in this study.

Number of carbon double bonds (N)	ν_2 (cm^{-1}) Schaffer <i>et al.</i> , 1991	ν_1 (cm^{-1}) Schaffer <i>et al.</i> , 1991	ν_1 (cm^{-1}) Barnard & De Waal, 2006	ν_1 (cm^{-1}) Karampelas <i>et al.</i> , 2007 and this study	Absorption in the visible (nm), this study
3			1638.4		
4			1610.4		
5			1588.8	1587	
6			1571.1	1569	405
7		1555.6	1556.1	1555	432
8	1140.1	1542.2	1543.2	1542	455
9	1134.9	1530.9	1531.7	1531	478
10	1129.8	1520.9	1521.5	1521	498
11	1125.1	1514.0	1512.2	1512	518
12	1121.8	1505.9	1503.8	1504	535
13				1495	552
14				1486	568

In Figs. 3–5 the UV-Vis-NIR diffuse reflectance spectra of different natural color cultured freshwater pearls in the 250–900 nm range are presented. All spectra revealed a decrease in diffuse reflectance due to absorption in the ultraviolet range at 280 nm. This peak is found in all pearls examined for this study, regardless of their color. Figure 3 shows that all the light of the visible part of the spectrum (390–780 nm) is transmitted, in agreement with the white color. We observe also two small absorptions at 620 and 670 nm.

Figure 4 shows the UV-Vis-NIR spectra of the upper parts of a natural orange (SK83 up) and a violet grey (SK87 up) cultured freshwater pearl. In both spectra we

observe absorptions mainly in the blue and green range (450–540 nm). The pearl SK83 absorbs also the violet (390–450 nm) with a transmission window in the orange range. The pearl SK87 exhibits absorption at approximately the same level throughout the visible spectrum, thus the overall grey color, but additionally has small transmission windows in the yellow to red range, apparently responsible for the violet hue. Moreover, absorptions in the violet (390–450 nm) are less intense compared to those in the blue and green part (450–540 nm), another factor which plays a role for the violet hue.

In Fig. 5, UV-Vis-NIR spectra of the natural color cultured freshwater pearl (SK86), of the upper (light

Fig. 3. Diffuse reflectance spectrum in the 250–900 nm range of a white-cream cultured freshwater pearl (SK56). A decrease in reflectance due to absorption in the ultraviolet range at 280 nm is observed. All wavelengths of the visible part of the spectrum (390–780 nm) are transmitted. Two weak absorptions at 620 and 670 nm are marked by arrows.

Fig. 5. Diffuse reflectance spectra in the 250–900 nm range of the upper and lower part of the cultured freshwater pearl SK86. The upper part is light pink (pinkish grey) and the lower part pink. The two spectra are different in the visible region. The spectrum of SK86 lo is shifted down by 20 % for clarity.

Fig. 4. Diffuse reflectance spectra in the 250–900 nm range of the upper part of a natural orange (SK83 up) and violet-grey (SK87 up) cultured freshwater pearl. In both spectra we observe that the most intense absorptions are in the violet, blue and green part of the spectrum (390–540 nm). However, the spectra are different in the visible region, as expected. The spectrum of SK87 up is shifted down by 5 % for clarity.

pink; pinkish grey) and the lower part (pink) are given. As expected, the two spectra are different in the visible range (see again Fig. 5). In the upper part, weaker absorptions spread mostly over the violet and the blue range, whereas the lower part absorbs more strongly, even in the green part, giving the more saturated pink color.

It has been previously established from Raman spectroscopy that the color results from the presence of nine pigments at the most. The visible diffuse reflectance data have to be consistent with the Raman scattering ones.

Hence, according to a tentative simplified approach, there should be as many absorptions bands as there are pigments for any given pearl. Therefore, one would expect in a given sample the same number of components in the diffuse reflectance spectrum as the number of pigments previously established via Raman scattering. Some diffuse reflectance spectra appear relatively continuous and smooth, suggesting that they are composed of one single very wide Gaussian band (Fig. 6 bottom, SK87up). However, other

Fig. 6. Diffuse reflectance spectra in the 390–580 nm range for samples presented in Fig. 4 (SK83 up and SK87 up). Bands observed (after visual decomposition; see text) at about (± 5 nm) 405 (408 for SK83 up), 432 (433 for SK83 up and 434 for SK87 up), 457 (458 for SK83 up and 455 for SK87 up), 477, 498 (497 for SK83 up), 516 (514 for SK83 up and 517 for SK87 up), 535 (537 for SK83 up), 552 (not observed for SK83 up and 553 for SK87 up) and 568 (not observed for SK83 up) nm. The spectrum of SK87 up is shifted down by 5 % for clarity.

spectra clearly show the presence of many components, as is particularly apparent in Fig. 6 top (SK83up). Those are used to visually estimate the apparent maxima of at least some of the components. Looking at all the diffuse reflectance spectra, a list of all the maxima (*i.e.*, components) observed is established. Nine major ones are observed, as well as a series of weaker absorptions that may not play a significant role in the color. Hence, the spectra in Fig. 6 result from linear combinations of Gaussian bands with maxima at about (± 5 nm): 405, 432, 457, 477, 498, 516, 535, 552 and 568 nm (see also Table 3). In other words, to compose the absorption pattern, one needs a combination of these elementary bands.

Absorptions, always weaker than those cited above, are revealed in some spectra from yellow to red at about 575, 595, 625 (*e.g.*, SK87 up; Fig. 4), 670 (*e.g.*, SK83 up; Fig. 4), 695, 720 and 740 nm (see Table 3). Some samples exhibit absorptions features also in the ultraviolet at about 320, 345 and 370 nm (*e.g.*, SK87 up; Fig. 4 and SK 86 up and lo; Fig. 5). Other bands are observed in the near-infrared region. Because of their position or weak intensity, these bands do not play a role in the color of the samples.

5. Discussion

The major absorption features for all the natural color samples studied are found from violet to green–yellow, from 390 to 570 nm. Moreover, the two Raman resonance features of polyenes, assigned to the C=C (ν_1 near 1500 cm^{-1}) and C–C stretching vibrations (ν_2 near 1130 cm^{-1}), are observed. These Raman bands can be assigned to a series of unsubstituted (unmethylated) polyenes (Okamoto *et al.*, 1984; Merlin & Delé-Dubois, 1986; Veronelli *et al.*, 1995; Barnard & De Waal, 2006; Hedegaard *et al.*, 2006; Karampelas *et al.*, 2006 and 2007).

The fact that these Raman bands are observed in naturally colored cultured freshwater pearls and not in white ones suggests that these compounds play the main role for the color of these samples. If we assume that the polyenic chains contained in pearl pigments are free of any perturbation, then it appears after spectral decomposition that a cultured freshwater pearl may contain different pigments which have 6 to 14 double bonds in their polyenic chains (see Tables 3 and 4). The nature of the terminal groups cannot be determined from our spectra. As these terminal groups are not associated with the resonance phenomenon, the respective bands are certainly too weak to be observed (Merlin & Delé-Dubois, 1986). Thus, we can only determine a general formula for the pigments present in cultured freshwater pearls as $\text{R}-(\text{CH}=\text{CH})_N\text{R}'$ with $N = 6$ to 14. Unmethylated polyenes which absorb in the ultraviolet (with $N < 6$) may also be present in these pearls. However, it is difficult to detect them because of experimental difficulties (the microscope coupled to the Raman spectrometer is very poorly transparent to UV light). These bands would not play a role in the coloration of the pearls anyway.

A relation between the positions of the Raman peaks and the absorption features can be established. The absorption maximum for the lowest energy optical transition of molecules containing a polyenic chain has a linear dependence with inverse conjugation (Schaffer *et al.*, 1991). Longer polyenic chains give absorption maxima at lower energies (higher wavelengths). Thus, tuning the laser for excitation wavelength selects conjugated chains of a specific range of conjugation length. The fact that in our samples different peak intensities are obtained for different excitation wavelengths proves that each pigment has a different absorption spectrum in the visible part of the spectrum. For shorter excitation wavelengths Raman resonant spectra reveal Raman bands having higher Raman shifts (thus, shorter chain length). This is in agreement with observations in Table 3 and Fig. 2. Peaks with lower and higher Raman shifts are due to respectively longer or shorter chain lengths, pigments with 13 and 14 double bonds (peaks at about 1495 and 1486 cm^{-1} respectively) are revealed clearly with yellow excitation (561 nm). With red excitation the same bands are also revealed with lower intensity. Thus, no additional bands are observed in the orange and the red spectral range due to polyenes. Pigments with less than 9 double bonds are best excited at 458 nm. This is in agreement with previous publications, which also stated that longer polyenic chains have a lower Raman shifts and visible absorption at shorter wavelengths (Salares *et al.*, 1977; Schaffer *et al.*, 1991).

The pigments with ν_1 peaks below 1504 cm^{-1} have polyenic chains with $N > 12$. These peaks are absent (or barely seen) for pearls with pink, orange and yellow colors (*e.g.*, Table 3 and Fig. 2 with excitation at 561 and 647 nm). Thus, pigments with $6 \leq N \leq 11$ absorb from the yellow to the violet part of the visible range. Hence they induce pink (absorption typically around the yellow–green), orange (absorption typically from the violet to blue) and yellow colors when they are relatively concentrated. In colored samples the main absorptions are observed from violet to yellow–green (405 to 568 nm). Absorption at lower energy (568 nm) seems to correspond to the Raman peak at 1486 cm^{-1} . In the same way, absorption at higher energy in the visible range (405 nm) is linked with the peak at 1569 cm^{-1} . This first approach of the relation between absorption features in the visible range and Raman peaks is summarized in Table 4. Absorptions with $N = 9\text{--}11$ are the most common.

Pigments which absorb from yellow–orange to red (575, 595, 625, 670, 695, 720 and 740 nm) do not present Raman resonance as those absorbing from violet to yellow–green, thus they are not polyenes. In the diffuse reflectance spectra, the absorptions cited above are not more intense than those due to polyenes. These absorptions are probably linked with undetermined impurities that may be contained in pearls or with other organic molecules that do not give measurable Raman peaks. Further research is needed in order to establish the origin of these minor absorptions. Absorption features in the UV region (at about 320, 345 and 370 nm) may be observed in cultured freshwater pearls. It is likely that these peaks are caused by

polyenes with $N < 6$ (Raman shifts above 1569 cm^{-1} ; see Table 4).

The nature and relative proportions of the pigments listed in Table 4 define a pearl's color. Thus, different mixtures of purple, orange and pink are due to different mixtures of pigments in varying relative proportions. However, similar colors can be caused by different mixtures of pigments (e.g., SK89 and SK90). Moreover, grey colors are due to mixtures of pigments absorbing in the visible range with similar efficiency. Also a light color is due to a slightly dominating absorption by the group of pigments giving this specific color. Because of the sensitivity of resonant Raman scattering, pigments can be detected at very low concentrations, that is, even when they do not play an appreciable role in color. Hence in some cases, Raman peaks due to pigments may be detected with low intensity in white or very light-colored pearls (e.g., Table 3; SK120).

In our spectra, ν_2 bands are shifted for different pigments as well. However, the superposition of several bands is not as easily seen as for the ν_1 band, and doublets are barely resolved. This is because the bands of the different polyenes are very close to one another, e.g. at about 1125 and 1122 cm^{-1} for polyenes containing respectively 11 and 12 double bonds. Moreover, ν_2 bands above 1150 cm^{-1} may be observed too. These bands should not be linked to carotenoids (peaks at $>1150\text{ cm}^{-1}$), as stated by Soldati *et al.* (2008), but to unmethylated polyenes with very short polyenic chain ($N \leq 6$) (see also Table 4).

In polyacetylenic molecules, weak bands are revealed in all of our Raman spectra at about 1010 , 1190 and 1295 cm^{-1} . The band at about 1295 cm^{-1} is assigned to the $\text{CH}=\text{CH}$ in-plane rocking mode (ν_3), whereas the band at about 1010 cm^{-1} is assigned to the deformation-activated $\text{CH}=\text{CH}$ wagging mode (ν_4), rather than the methyl rocking mode (Fujimori *et al.*, 2004; Barnard & De Waal, 2006). In some cases a weak band at about 1190 cm^{-1} is also found. Bands in this region are attributed to the C-C stretching and the C-H in-plane bending modes in β -carotene (Saito & Tasumi, 1983). In our case, no methyl groups are attached to the polyenic chain, thus only the C-C stretching seems to be responsible for this peak.

Variations in the positions and FWHM of Raman bands of carbonates may indicate rotational disordering of the carbonate ion in carbonates (Urmos *et al.*, 1991). However, spectra of all measured samples do not show any change in position and FWHM of the major (ν_1) aragonite peak. This is probably because of the low degree of trace element substitution in aragonite ($<1\%$ for Sr^{2+} ; Urmos *et al.*, 1991). The fact that no difference is observed in Raman bands of carbonates for white and colored samples is suggestive that a CaCO_3 -pigment complex is unlikely. Thus the most probable hypothesis is a complex with pearls' organic matrix. Pearls, similarly to the nacreous part of shells, do contain β -chitin as a major protein, as well as an assemblage of acidic glycoproteins (Levi-Kalishman *et al.*, 2001). Thus, a complex with a part of these matrices is the most likely at this point.

Our preliminary results on pearls and/or shells from different genus (other than *Hyriopsis*) suggest that the same pigment may not necessarily exhibit exactly the same absorption in different genus. This is probably because polyenes are attached in different ways, thus they offer slightly different absorption features.

Unmethylated polyenes have rarely been observed in nature, as stated above. Determination of the exact chemical structure of these pigments has hitherto been hindered because their extraction is difficult and the isolated products are extremely unstable (Merlin & Delé-Dubois, 1983; Veronelli *et al.*, 1995; Stradi *et al.*, 2001b). As a consequence, their synthesis process is still unknown. Recently, Stradi *et al.* (2001b) used advanced chemical methods (HPLC coupled with UV-Vis spectroscopy and mass spectrometry) on feathers of *Ara Macao* (a species of parrot) in order to find the exact chemical structure of the feather pigments. These authors proposed that it is actually a mixture of four pigments containing polyenic chains of general formula $\text{CH}_3-(\text{CH}=\text{CH})_N\text{-CHO}$ with $N = 6-9$. Recently, McGraw & Nogare's (2004) results concerning parrots' coloration systems suggest that these pigments cannot originate from dietary sources (as is the case for carotenoids). They are locally synthesized, likely within the mantle tissue (in the follicular tissue of growing feathers). Thus, possibly there is, also, no direct link between pearl color and food. Moreover, the fact that different colors are observed at different points of the same pearl suggests that different series of pigments are secreted at the same time.

6. Conclusions

All naturally colored cultured freshwater pearls investigated in this study are colored by a mixture of unsubstituted polyenes. These pigments absorb from the violet to the yellow-green spectral range and appear to be the major cause of their color. Their general formula is $\text{R}-(\text{CH}=\text{CH})_N\text{-R}'$ with $N = 6-14$. We have established a relation between N and the absorption maximum for each pigment in freshwater cultured pearls. Pigments with $N > 14$ are absent in the samples studied. Molecules with $N < 6$ may be observed in cultured freshwater pearls. However, they absorb in the ultraviolet, thus they do not play a role in the color. Other absorptions, always less intense compared to the polyene ones, are observed in the yellow-orange to red range. Extraction may shed further light on the origin of these absorptions. Understanding the nature of the pigments in natural-color freshwater pearls can help to separate them from their treated equivalents.

Similar series of pigments are found in pearls (either fresh- or salt-water) and/or their shells. Our preliminary results suggest that the same pigment does not cause the exact same absorption features in samples from different mollusk genus. A study of the precise relation between optical absorption, Raman peaks and mollusks' genus of the samples is ongoing.

Thus, pigments contained in pearls are not carotenoids, they rather belong to the same unique class of biochromes named psittacofulvins as the lipid-soluble pigments in the parrots' feather. The identification of the exact chemical formula of these pigments would be useful, even though experimentally challenging. This would provide a better understanding of the biosynthesis of unsubstituted (unmethylated) polyenes coloring naturally the cultured freshwater pearls as well as other mollusks.

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