IDENTIFICATION OF IRRADIATED SOUTH SEA CULTURED PEARLS USING ELECTRON SPIN RESONANCE SPECTROSCOPY

Youngchool Kim, Hyunmin Choi, Bohyun Lee, and Ahmadjan Abduriyim

Irradiated South Sea cultured pearls (SSCPs) from the *Pinctada maxima* mollusk typically show colors from light gray to silver. It is difficult to identify gamma-ray irradiation of SSCPs using standard gemological methods because of their thick nacre. Therefore, an advanced analytical technique such as electron spin resonance (ESR) spectroscopy is needed to detect the treatment. ESR measurements of minute amounts of SSCP powders revealed the formation of CO_2^- radicals, and the parameter known as the *g*-factor was measured at 2.0015 ± 0.0005. Higher levels of CO_2^- radicals were detected in the pearl nacre than in the nucleus. Therefore, the existence of CO_2^- radicals is an indicator of irradiated SSCPs.

Irradiation, dyeing, bleaching, and heat treatment are widely used methods to alter pearl color. Although most artificial colors are easily recognized, some resemble attractive colors that occur in nature (Elen, 2001; Li and Chen, 2001; Zachovay, 2005; Wang et al., 2006; "Better techniques improve brown pearls," 2006; McClure et al., 2010).

Lower-quality freshwater and saltwater cultured pearls are regularly exposed to ^{60}Co gamma-ray radiation in an attempt to simulate black pearls or enhance orient (Crowningshield, 1988; Li and Chen, 2002; O'Donoghue, 2006). In recent years, the irradiation process has been applied to not only Akoya cultured pearls and freshwater cultured pearls (FWCPs), but also to South Sea cultured pearls (SSCPs) (Choi et al., 2012). The irradiation-induced color change results from the darkening of the nucleus, caused by $MnCO_3$ oxidation, as well as denatured damage to the pearl's conchiolin (Matsuda and Miyoshi, 1988). FWCPs have a higher abundance of proteinous components and manganese than saltwater pearls (Hatano and Ganno, 1962).

Gamma-ray irradiated SSCPs (figure 1) were first discovered in the Korean market in April 2011. At the March 2011 Hong Kong Jewelry Show, a Japanese trader reportedly sold a Korean counterpart irradiated SSCPs without disclosing the treatment. They were light gray or silver loose cultured pearls and beads 10–16 mm in size. While a cream, yellow, or black color is produced by a protein pigment in the nacre, a blue or silver color is caused by organic material between the nacre and nucleus (Komatsu, 1999; O'-

Figure 1. This necklace contains gamma-ray irradiated silver South Sea cultured pearls (12.0–14.0 mm). Electron spin resonance (ESR) spectroscopy proved effective in identifying the gamma irradiation. Photo by Jae Hak Ko.

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Donoghue, 2006). Korean consumers typically prefer SSCPs with a silver color created by organic material.

According to the research of Choi et al. (2012), gamma-ray irradiated SSCPs with colors ranging from white to cream turned light gray to silver, with the depth of color correlating with increasing irradiation dose. A dose of 0.5–1 kGy caused a light gray color, while a dose above 5 kGy produced a silver color.

For Akoya cultured pearls, with a typical nacre thickness of 0.2–0.6 mm, irradiation can be identified through standard gemological tests (Komatsu, 1999; O'Donoghue, 2006). But for SSCPs, which have a nacre thickness of roughly 1.5–3.0 mm, detecting irradiation is difficult with methods such as transmitted light, magnification, fluorescence reaction, and UV-Vis spectrometry (Choi et al., 2012).

This study attempted to identify irradiated SSCPs using electron spin resonance (ESR) spectroscopy. This method, also known as electron paramagnetic resonance (EPR) spectroscopy, identifies the presence of unpaired electrons. Moreover, the study sought to minimize damage during examination by obtaining a minimal sample of powder from each cultured pearl.

MATERIALS AND METHODS

For the study, some 300 SSCPs weighing 6.55–18.05 ct (8.0–16.6 mm in diameter) with white to cream color were exposed to gamma-ray irradiation at room temperature. The irradiation was conducted at the ^{60}Co facility of the KAERI (Korea Atomic Energy Research Institute) in Jeongeup, South Korea. The absorbed doses were set at 0.2, 0.4, 0.6, 0.8, 1, 5, and 100 kGy.

Inductively Coupled Plasma-Atomic Emission Spectrometer. Chemical composition analyses of the SSCPs were performed with an inductively coupled plasma–atomic emission spectrometer (ICP-AES, Varian Vista-PRO). The nacre, nucleus (bead), and conchiolin were separated and powdered, and 0.2 g of each powder was dissolved in a solution of 37% HCl (6 ml) and 65% HNO_3 (2 ml). We tested the samples after 20 minutes at 200°C and after 10 minutes at the same temperature to obtain an average value.

Electron Spin Resonance Spectroscopy. This study relied on electron spin resonance analysis to observe radicals produced by the irradiation process. The ESR spectrometer gauges the absorbed dose corresponding to the splitting energy of unpaired electrons in a magnetic field. The technique can rapidly identify an irradiation-related signal from a small amount of sample in a few minutes. For this study, we collected at least 10 mg of SSCP powder from both the nacre and the nuclei of each cultured pearl. To determine if the ESR signals correlated with Mn^{2+}, solid samples of FWCP, which contain more manganese than SSCPs, were irradiated with a 100 kGy dose.

Figure 2. This JEOL FA-300 spectrometer with a manganese marker is the ESR instrument used in the study. Photo by Y. C. Kim.

Room-temperature ESR spectra were recorded using a JEOL FA-300 spectrometer with a manganese marker (MgO: Mn^{2+}), using 9.8 GHz microwave frequency, 1 mW microwave power, a 1–2 G modulation amplitude, a 2 min sweep time, and a 0.03 s response time (figure 2).

Mn marker for ESR analysis. The *g*-factors of free radicals created by irradiation are approximately 2.00. For comparison, the "free electron" *g*-factor is 2.0023. Standard reference samples can be used to correct for any systematic errors in the measured magnetic field values and to verify the sensitivity of the system. Standard samples include DPPH (2.2-diphenyl-1-picryl-hydrazyl), TCNQ-Li (tetracyanoquino-dimethane Li

BOX A: WHAT IS ESR?

Electron spin resonance (ESR), alternatively known as electron paramagnetic resonance (EPR), is a spectroscopic method for observing the resonance absorption of microwave power by paramagnetic molecules, defects or free radicals (characterized by at least one unpaired electron) which are simultaneously subjected to an applied magnetic field. For most materials the electrons are "paired," and are thus invisible to ESR. ESR can be used to detect paramagnetic defects or free radicals introduced by irradiation in some materials, and has been applied to the study of irradiated food (Chauhan et al., 2008). Oftentimes, its high sensitivity allows the detection of irradiation-related defects in small sample volumes in just a few minutes, making it an effective and minimally invasive technique.

In a typical ESR experiment the sample is subjected to microwaves of a fixed energy (i.e., fixed frequency ν), while the magnitude of the magnetic field is varied. A property called *electron spin* is attributed to each unpaired electron, where a single unpaired electron has only two allowed energy states. ESR is used to probe the energy differences between those states. In the absence of a magnetic field the two states have the same energy, yet when a magnetic field is applied the energy separation of the states will increase. The dominant interaction governing the splitting is known as the *Zeeman effect*, whereby the energy difference increases linearly with increasing magnetic field according to the equation $\Delta E = g\beta H$. Here ΔE denotes the energy difference, g is the spectroscopic splitting factor known as the *g-factor*, β is a constant called the Bohr magneton, and H is the magnetic field. The *g*-factor is influenced by the characteristic environment of the unpaired electron(s) of a free radical, providing a "fingerprint" to be used for identification. The *g*-factor values of known paramagnetic molecules, defects and free radicals are tabulated in the literature. There are additional interactions which can lead to more complicated spectra and a wealth of additional information, but those will not be discussed here.

Figure A-1 shows the energy diagram for the electron spins of two different radical species with differing *g*-factors, one given by g_1 and the other by g_2. As the magnetic field is increased it is apparent that the energy levels for the two radical species split at different rates. The ESR resonance condition is met when the energy separation is equal to the energy of the applied microwave radiation,

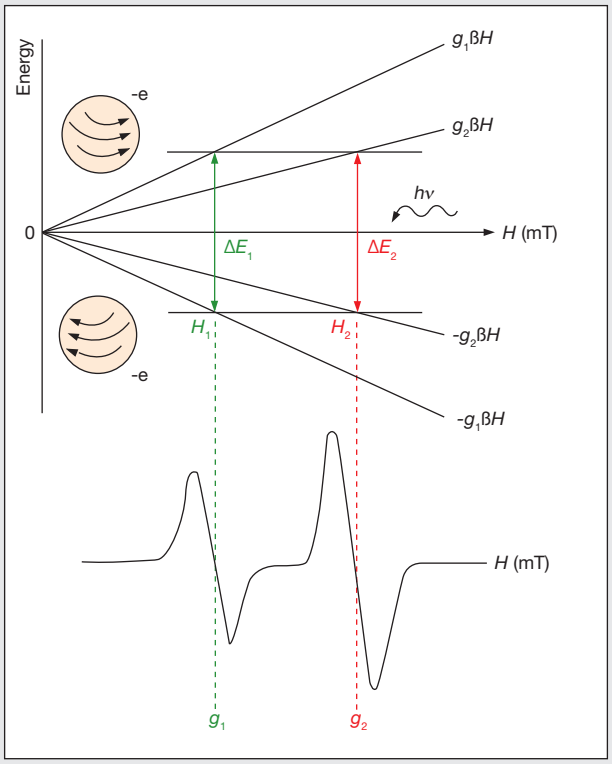


Figure A-1. Energy levels of two paramagnetic species with g-factors g_1 and g_2. As the magnetic field H is increased the energy levels for the two species split at different rates, according to the Zeeman effect. Resonance occurs, and signals with derivative lineshapes are detected, when the energy separations (ΔE_1 and ΔE_2) are equal to the energy of the applied microwaves (hν). From Ikeya (1993).

$\Delta E = g\beta H = h\nu$ (where h is Planck's constant), leading to an absorption of the microwaves and the detection of an ESR signal. Thus, the signals for the radical species with *g*-factors g_1 and g_2 will occur at H_1 and H_2, respectively. Hence, the identity of the radical(s) producing the ESR spectrum can be determined by careful analysis of the magnetic field values at which the resonance signals are detected. Furthermore, the intensity of the ESR signal is proportional to the number of radicals present, allowing quantitative analysis.

saly), CaO:Mn^{2+}, and MgO:Mn^{2+}. The choice of standard sample used depends on what the user wants to determine. For example, DPPH is used to calculate *g*-factors, to monitor the sensitivity of the equipment, and to quantify spin concentrations. TCNQ-Li is used to find the *g*-factor. CaO:Mn^{2+}, MgO:Mn^{2+}, and Mn^{2+} are used to measure the *g*-factor and to correct magnetic field variations.

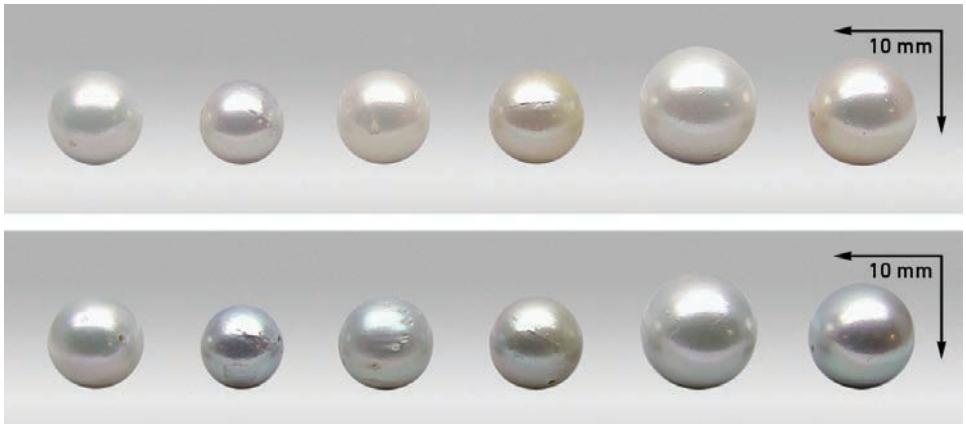


Figure 3. Before and after photos of SSCPs exposed to gamma irradiation at a dose of 5 kGy. The irradiated pearls turned gray to silver, slightly different from their original colors. Photos by H. M. Choi.

The *g*-factor of most standard samples is also located around 2.00. The Mn marker is shown with six Mn^{2+} signals; the third (2.034) and fourth (1.981) signals are used to correct magnetic field variations. Each signal has a regular interval from 2.00. From this property, the MgO:Mn^{2+} marker could be more suitable to measuring the *g*-factor than the alternative standard samples. The MgO:Mn^{2+} marker was supplied with the Jeol X-band spectrometer in the shape of a small rod that can be electromechanically inserted externally into the microwave cavity. When a sample and a Mn marker are measured simultaneously, the resulting ESR spectrum will contain signal contributions from both. It is easy to distinguish the ESR spectra of one from the other, since the Mn^{2+} signals have the opposite phase to that of the sample's signal (i.e., the signal's lineshape will appear to have been flipped across the baseline).

RESULTS AND DISCUSSION

The major element of a pearl is calcium. Chemical composition analysis of bead-cultured pearls using ICP-AES demonstrates that the nacre and the freshwater nucleus contain similar trace elements but vary in their composition. The nacre contains more Na, Mg, and Sr, while the nucleus has higher Mn and P contents (table 1).

After ^{60}Co gamma-ray irradiation at a dose of 5 kGy, the SSCPs exhibited gray to silver coloration (figure 3). The interior of one of the irradiated pearls revealed a grayish brown to dark gray nucleus, along with an altered nacre color (figure 4). The irradiation-induced color change is chiefly attributed to the darkening of the nucleus (bead), which in turn darkens the nacre—especially in the thinner-skinned Akoya cultured pearls (Komatsu, 1999). As shown in this experiment, color change took place in the nacre as well.

Figure 4. These photos show the interior (left) and exterior (right) of a light yellow South Sea cultured pearl before and after irradiation at a dose of 5 kGy. Left: The nucleus (bead) became grayish brown to dark gray, and the nacre color was similarly altered. Right: The pearl's surface turned a silver color. Photos by B. H. Lee.

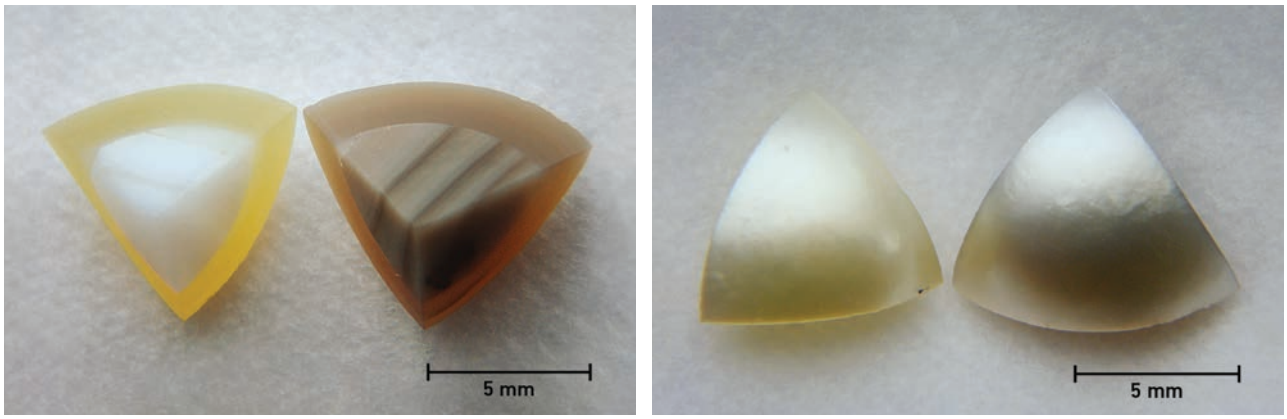


TABLE 1. Representative composition of the nacre and nucleus in South Sea cultured pearls in ppm (parts per million), determined by ICP-AES.

Sample	Ca	Na	Mg	P	Mn	Fe	Sr
Nacre	293099.06	4717.01	100.27	12.47	19.43	30.29	1086.80
Nucleus	287998.86	1704.52	26.24	94.42	431.43	26.80	249.21

Figure 5 shows that the concentration of radicals produced by irradiation exposure increases with the absorbed dose. Formerly undetected free radicals were observed after a low-dose radiation of 0.2 kGy. The *g*-factor was 2.0015 ± 0.0005, which agrees with that of CO_2^- radicals (Wieser et al., 1985; Ikeya, 1993; Seletchi and Duliu, 2007). With higher absorbed doses, the CO_2^- radical signal intensity further intensified. The identification of CO_2^- radicals through ESR analysis thus serves as a way to distinguish irradiated cultured pearls.

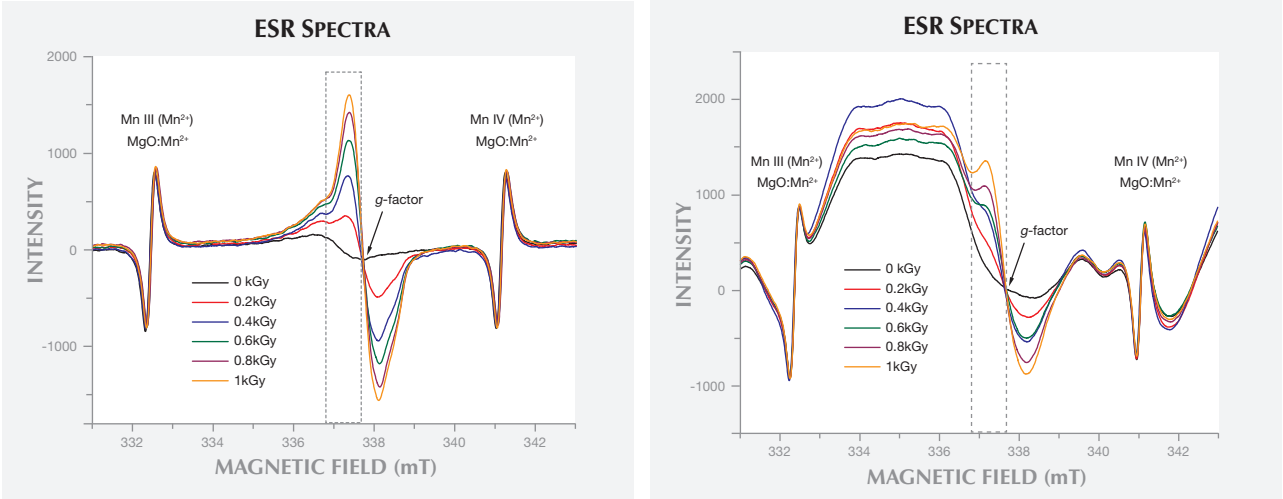
Matsuda and Miyoshi (1988) reported that the irradiation-induced change of color is caused by manganese (Mn). They noted that $MnCO_3$ in the nucleus (bead) turned into oxidations such as Mn_3O_4, Mn_2O_3, and Mn_2O after irradiation. Their results are still cited in literature related to color change in irradiated pearls (e.g., Komatsu, 1999; Wada, 1999; McClure, 2010).

Yet existing mechanisms are insufficient to explain the alteration of pearl color by irradiation (Li and Chen, 2002). Based on the results of gamma-ray irradiation tests in this study, the authors believe that post-irradiation color change cannot solely be attributed to $MnCO_3$ oxidation. Two factors support this hypothesis:

1. After irradiation, the pearl nacre blackened to a similar extent as the nucleus (bead), even though it contains approximately 20 times less Mn (see figure 4 and table 1).

   Figure 6 is an ESR spectrum comparing untreated FWCP, irradiated (100 kGy) FWCP, and a Mn marker (MgO: Mn^{2+}) attached to the JEOL equipment. The Mn marker consists of Mn^{2+} and shows six sharp peaks in the ESR spectrum (figure 6b). Before (figure 6a) and after (figure 6c) irradiation spectra of FWCPs (typical in the carbonate spectrum) do not match the positions of the Mn^{2+} signals. Nevertheless, a change was observed in the spectra before and after irradiation: the formation of CO_2^- radicals between the third and fourth Mn^{2+} peaks (highlighted by the green circle in figure 6c). Because these results were the same

Figure 5. These ESR spectra show a South Sea cultured pearl's nacre (left) and nucleus (right) before and after irradiation up to 1 kGy. These spectra were obtained for a single cultured pearl irradiated with different doses. CO_2^- radicals appeared as irradiation doses increased in both the nacre (left) and nucleus (right). Nucleus spectra (right) show a large unassigned signal in the 333–337 mT range, both before and after irradiation.



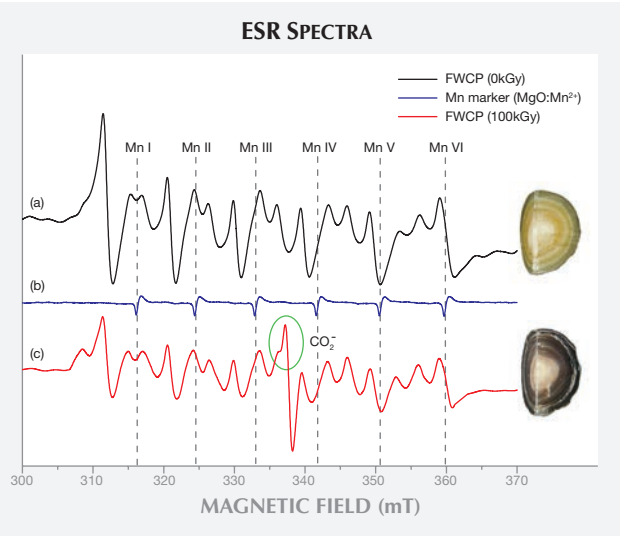


Figure 6. This graph demonstrates the spectra acquired for solid samples of FWCPs, which contain more Mn than saltwater cultured pearls. Shown are the spectra of untreated FWCPs (a), irradiated FWCPs (c), and a Mn marker (b). The Mn marker has six Mn^{2+} signals, and their positions are highlighted by the dotted vertical lines. After irradiation, CO_2^- radicals are observed only between the third and fourth Mn^{2+} resonance peaks. The signals before (a) and after (c) irradiation do not match the positions of the Mn^{2+} signals (b).

among all SSCPs investigated in this study, peaks in the ESR spectrum are unrelated to Mn.

2. CO_2^- radicals appeared as irradiation doses increased and multiplied in proportion to the dose (figure 7). The intensity of CO_2^- radicals was also proportional to the blackening of the pearl nucleus (bead). The CO_3^{2-} molecular ion in $CaCO_3$ is easily ionized by radiation. Elementary defects induced by ionizing radiation are an electron center (CO_3^{3-}) and a hole center (CO_3^-). While the CO_3^{3-} and CO_3^- centers are stable at low temperatures, the electron center CO_2^-, formed by irradiation, is an electron center similar but more stable than CO_3^{3-} (Ikeya, 1993). Additionally, we found that the color of nacre and nucleus had been bleached under incandescent light (approximately 50°C) for 30 days. The color changed by irradiation and heat (by light) is related to the color center. Therefore, the color change of the nacre and the blackening of the nucleus (bead) are believed to be related to color centers formed by CO_2^- radicals.

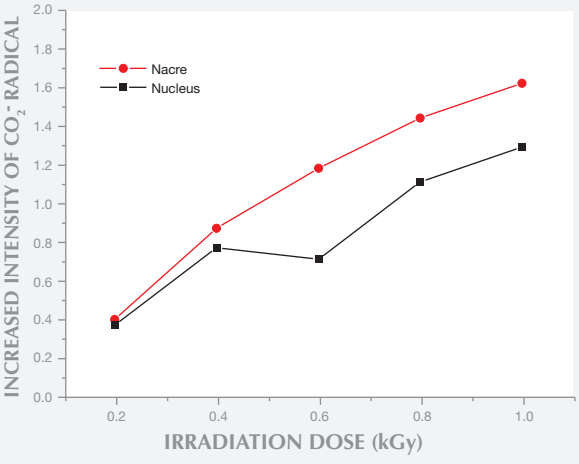
Choi et al. (2012) found that after irradiation, glutamic acid decreased 11.43% (from 3.5% to 3.1%), alanin 21.8% (from 22.5% to 21.8%), and histidine 43.75% (from 1.6% to 0.9%), according to amino acid analysis to examine the change of protein between aragonite platelets in pearl nacre. Hatano and Ganno (1962) found that gamma-ray irradiation destroyed 32% of the histidine, 16.6% of the methionine, 11% of the glutamic acid, and 9.3% of the proline in the protein of the FWCPs. The destruction of protein caused by irradiation can also alter the color of SSCPs.

CO_2^- radicals at the absorbed irradiation dose of 0.2 kGy are barely visible in the nucleus sample but far more intense at doses above 0.4 kGy (figure 5, right). In particular, CO_2^- radicals emerging after irradiation were better observed in the nacre than in the nucleus at the same absorbed dose (figure 5, left). After normalizing the results of figure 5 to a nonir-

In Brief

- Gamma-ray irradiation is routinely applied to South Sea cultured pearls (SSCPs), typically producing a light gray to silver color.
- For SSCPs, which have a particularly thick nacre, detecting irradiation is difficult using methods such as transmitted light, magnification, fluorescence reaction, and UV-Vis spectrometry.
- Electron spin resonance (ESR) spectroscopy rapidly identifies the presence of CO_2^- radicals, whose concentration is proportional to the absorbed irradiation dose.

Figure 7. Normalizing the CO_2^- radical intensity of figure 5 shows that the radicals' intensity increases depending on irradiation dose, even though the CO_2^- radical of the nucleus (bead) decreased at 0.6 kGy.



radiated spectrum (0 kGy, black line), the increased intensity of radicals was calculated by peak-to-peak height. The intensity of the CO_2^- radical is stronger in the nacre than in the nucleus when irradiated with a dose above 0.4 kGy (figure 7).

Ikeya (1993) reported that Mg^{2+} ions might be accompanied by H_2O molecules, leading to a rapid reduction in hydrated radicals. The saturation level of isotropic CO_2^- also increases with the Mg/Ca ratio. Barabas et al. (1989) studied synthetic carbonate crystals doped with Mg^{2+} and observed the following: (1) ESR spectra that displayed signals at the same spectroscopic properties as natural carbonates; and (2) an increase of the *g*-factor signal with Mg concentration in the carbonate crystals.

Mg also plays an important role in the formation of the crystal lattice of carbonates (Katz, 1973) and may enhance the formation of specific defects (Barabas et al., 1992). Lattice distortions caused by the incorporation of Mg^{2+} ions (Goldsmith and Graf, 1958) may lead to CO_2^- by creating larger interatomic distances (Barabas et al., 1992). In this context, the higher abundance of CO_2^- radicals in the nacre is thought to be related to the Mg/Ca ratio.

Considering the combined published observations on Mg^{2+} and CO_2^- (Ikeya, 1993; Barabas et al., 1989, 1992; and Katz, 1973) it is likely that the saturation level of CO_2^- rises proportionally with the Mg/Ca ratio in pearls of this study. As shown in table 1, the nacre and the nucleus (bead) contain 100 and 26 ppm of Mg, respectively. The nacre's Mg/Ca ratio is approximately four times greater than that of the nucleus (bead). Mg, which is more abundant in the nacre, therefore results in the preferential formation of CO_2^- in the nacre rather than in the nucleus when exposed to the same absorbed radiation dose. This is consistent with the higher CO_2^- ESR signal intensity observed in the nacre than in the nucleus (again, see figure 5). This suggests it is possible to identify an irradiated SSCP using ESR spectroscopy.

CONCLUSIONS

Identifying irradiated SSCPs through traditional gemological methods has been difficult, as their nacre is usually quite thick. But as this ESR study demonstrates, the separation of untreated pearls from irradiated pearls is possible. In doing so, an infinitesimal amount of sample was taken from the nacre in the form of powder. After irradiation, CO_2^- radicals were formed, and their presence was confirmed using ESR spectroscopy. The amount of CO_2^- radicals increased in proportion to the irradiation dose, and they were more observable in the nacre than in the nucleus (bead). Until now, irradiation-induced color changes in pearls were thought to be due to the change of the $MnCO_3$ oxidation number. But as this study notes, such color alteration is apparently related to an alteration caused by protein destruction rather than Mn, as well as color centers created by CO_2^- radicals.

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