Spectroscopic studies on irradiated Crab carapace

Saleh M Abdou*1, S EBRAHEEM1 and R I Mohamed2

1 National Centre for Radiation Research & Technology, Atomic Energy Authority, Cairo, Egypt
2 Physics department, college of science, El-azhar university, Cairo, Egypt

*Corresponding Author E-Mail: Salehabdou03@yahoo.com

Abstract. The crab carapace is the shell covering the body. The present work provides an overview of the use of electron paramagnetic resonance (EPR) after reviewing the status of the structure and composition of the crab carapace shell using XRD and FTIR techniques. Crab samples were cleaned from soft tissue, dried and irradiated with Co-60, with doses up to 30 kGy. EPR spectra of native as well as irradiated crab carapace samples were recorded and analyzed. FTIR results confirm the XRD results of the analyses done on the crab carapace shell. They show that, the main composite of the shell is calcium carbonate. The EPR results show that, the amplitude of the CO3 signals increased with increasing the absorbed dose. Identification of the irradiated carapace samples was concerned with the detection of the EPR spectra for g-values which are attributed to those in the coaxial CO3 free radical.

The aim of the present work is studying the effect of radiation and investigation of some physicochemical properties of crab carapace shells using XRD, FTIR spectroscopy. Also, evaluation of the induced free radical in the crab carapace due to irradiation using the EPR technique.

1. Introduction.

The carapace is the shell on the back of the crab that is constructed of a hard bone called chitin [1]. Crab exoskeletons were analyzed with structural and chemical methods. The composition varies among different parts of the skeleton, the main composites of the carapace are calcium, carbon and inorganic minerals [2]. The attribute of the crab carapace are related to the scientific investigation properties of fine nanostructures. This biomaterial has high toughness and fracture strength. The thickness of calcite crystal sheet is among the nanometer scale that organized in parallel distribution [3].

International food stuff trade, particularly seafood is spreading up that will increase the risk of the transfer of some diseases. This passed to legalize radiation treatment of food by the World Health Organization [4, 5]. International health and safety authorities have supported the safety of irradiation of all foods up to a dose level of 10 kGy. Nevertheless, a recent evaluation of an international expert study group specified by WHO, IAEA, and FAO showed that food treated underlying to good producing practices (GMPs) at a dose above 10 kGy is also rather safe for consumption [6].

Seafood is often contaminated with infective organisms such as Shigella, parahaemolyticus, Vibrio, and Salmonella. Treatment of seafood with combinations of irradiation with low-dose irradiation and refrigeration that don't alter texture or flavor may extend the shelf-life. Irradiation with a dose of 1.0:7.0 kGy causes the removal of spoilage and pathogenic microorganisms, while radiation dose of 30–50 kGy is taken into account as industrial sterilization in combination with mild heat [6].
Irradiated food could be identified using different methods such as thermoluminescence, mass spectrometry, electron paramagnetic resonance and gas chromatography [7]. EPR spectroscopy is characterized by its non-destructive detection of radiation-induced radicals [8, 9]. Hence, it is the topmost technique for the realization of irradiated food and also the accurate determination of the dose delivered, which tends to accepting or rejecting of food transfer.

EPR detects free radicals induced by ionizing radiation and trapped in the irradiated food. Their stability is linked up to the crystallinity of the solid part and the moisture content of nutrient. In principle, EPR signals evoked by ionizing radiation should be absent from nonirradiated food. Hydroxyapatite, Ca10(PO4)6(OH)2, is the bone main mineral content. Upon irradiation, it can accommodate different free radicals, but the most common are those of oxygen and carbonate. CO2 is the most stable among these carbonate radical and its EPR signal dominates the spectrum. It has a good radiation sensitivity so that its signal can be used in the radical identification, dating of fossils, dosimetry and detection of irradiated food containing bone [10].

2. Material and Methods.

2.1. Material.
The carapace is the part of the exoskeleton that lid the head and thorax of the crab, it is a hard substance made of chitin. Crabs exhibit solid calcified exoskeleton representative of crustaceans. Male Hermit crabs obtained from the red sea were used in the current investigation. Samples were washed and the flesh and soft tissues attached to carapace were eliminated as possible.

2.2. Irradiation:
Irradiations were carried out using a cobalt-60 source of gamma radiation “4000 A, Indian gamma chamber”, with a dose rate of 2.2 kGy/h. The unit consists of an annular source permanently enclosed inside a lead shield and cylindrical drawer that can go upwards and downwards along the fundamental seam. The drawer has a chamber to carry the samples to be irradiated. The irradiation was carried out at room temperature with radiation doses of 0.25 kGy, 1.0 kGy, 2 kGy, 5 kGy, 10 kGy, 15 kGy, 20 kGy, and 30 kGy.

2.3. X-Ray Diffraction (XRD).
X-ray diffraction (XRD) is an analytical technique used for identifying whether the material is a crystalline or amorphous material. X-ray diffraction (XRD) patterns were recorded using Shimadzu diffractometer XRD-6000. The x-ray diffraction spectrometer with a copper target (λ = 0.1542 nm) at operating voltage of 40 kV and an electric current of 30 mA. The pattern was recorded at a scanning rate of 2°/min and the (2θ) scan range of 20° to 80°. These operating conditions were sustained all over the examination.

2.4. Fourier-transform infrared (FTIR) spectroscopy.
FTIR spectroscopy was performed using a Bruker vertex 70/70v infrared spectrometer. All the measurements were done in the range of 400 to 4000 cm\(^{-1}\). Molecular vibration modes of bands were characterized by FTIR transmission bands as a function of wavenumber.
2.5. Electron paramagnetic resonance (EPR).
EPR signals of the carapace samples were recorded at room temperature using a Bruker–ER4102, X-band spectrometer. The used conditions of EPR spectrometer were: Microwave power 1.008 mW, modulation frequency 100 kHz.
The EPR spectrum of empty quartz tube of 3 mm diameter was detected before recording the sample spectra to detect any undesirable signals. Readings were corrected to the peak-to-peak height of the reference standard material, which was measured before and afterwards every single spectrum of the crab samples to ensure the stability of the spectrometer (change in sensitivity). The spectrum of every single sample was received five or more consecutive times, each spectrum is a single scan.

3. Results and Discussions.
Crab shell (carapace) is shown in Figure 1. X-ray powder diffraction (XRD) and Fourier transform infrared (FTIR) Spectroscopy are powerful tools for investigating mineral phases. EPR spectroscopy is used in broad scale to detect and identify the induced paramagnetic pieces (free radicals) under the action of radiation.

Figure 1. Crab shell (carapace).

3.1. X-Ray Diffraction (XRD).
The XRD pattern of the crab carapace shell is shown in figure 2. The position of each diffraction peak was obtained and compared with the corresponding peak in the database. This pattern is similar to the pattern of the calcium carbonate (calcite). The obtained characteristic peaks of the analyzed samples of the crab carapace are very close to the corresponding calcium carbonate (calcite) peaks at the same 2θ degrees. The angular positions in the XRD results of calcium carbonate in carapace shell and the angular positions represented in 5-586 ICDD database principal diffraction pattern for calcium carbonate are shown in table 1.
In calcite, the carbon atoms are in the planar CO$_3$ group, and the Ca atoms are in an octahedral coordination environment of the oxygen atoms. The spectrum of the XRD analysis of the carapace samples show also other peaks which may be attributed to traces of other minerals.

Table 1. The comparison of angular positions in XRD results of calcium carbonate in carapace shell and the angular positions (*) represented in 5-586 ICDD database principal diffraction pattern for calcium carbonate.

| No. | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
|-----|------|------|------|------|------|------|------|
| 2 theta | 22.87 | 29.5 | 31.58 | 36.06 | 39.32 | 43.06 | 47.62 |
| * 2 theta | 23.04 | 29.43 | 31.45 | 36  | 39.44 | 43.18 | 47.53 |
| No. | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
| 2 theta | 48.5 | 57.44 | 60.96 | 61.34 | 65.86 | 77.32 | 81.63 |
| * 2 theta | 48.56 | 57.45 | 60.73 | 61.4 | 65.66 | 77.25 | 81.63 |

3.2 Fourier-transform infrared (FTIR) spectroscopy.

Fourier-transform infrared spectroscopy is utilized, for identifying types of chemical bonds in a molecule. The chemical bonds can be either organic or inorganic. FTIR was utilized in identifying the compounds and investigate the carapace sample composition.

The infrared spectrum for the carapace samples gamma irradiated (30 kGy) is quite similar to the spectrum of native samples, which indicates that no chemical groups were created by gamma irradiation. Therefore, irradiation doesn’t induce the discernible crosslinking between carapace molecules. Despite the high energy gamma rays able to react with some chemical groups to arrange free high energy chemical groups (for example, high energy radicals).

The spectral details of the carapace samples are shown in figure 3, reveals broad absorption peaks of CO$_2$ at about 709 cm$^{-1}$, 855 cm$^{-1}$, 1082 cm$^{-1}$, 1455 cm$^{-1}$, 1786 cm$^{-1}$. It has been reported [12] to be the
common characteristic options of the carbonate ions in calcium carbonate and are the basic modes of vibration for this molecule.

![FTIR spectrum](image)

**Figure 3.** FTIR spectrum. Relation between the wavenumbers and the corresponding transmission intensity of the infrared in the crab carapace.

Carbonate ions have four normal modes of vibration peaks: $V_1$, symmetric stretching; $V_2$, out-of-plane bending; $V_3$, doubly degenerate planar asymmetric stretching; and $V_4$, doubly degenerate planar bending [11]. However, the observed bands at about 1082 cm$^{-1}$ and 855 cm$^{-1}$ were carefully assigned as $V_1$ symmetric stretching, and $V_2$ out-of-plane bending modes of CO$_3^-$, respectively. The peak at 1082 cm$^{-1}$ was observed in calcium carbonate, whose CO$_3^-$ ions are inactive in the infrared region. This observation was verified by alternative reports within the literature on characteristic infrared bands, that are by experimentation, not ascertained within the spectrum of calcite [12]. In distinction, the doubly degenerate peak that seems at 709 cm$^{-1}$ is attributed to the $V_4$ in-plane bending mode of CO$_3^-$ ions, that indicates an amendment within the structure of the Ca ions from the symmetry of the calcite phase A similar result was also reported by [13]. The bands observed at 3271 cm$^{-1}$ correspond to vibrations stretching of hydroxyl groups.

The results obtained from the FTIR-analysis of the carapace shell shows a transmission band according to be the common characteristic options of the carbonate ions in calcium carbonate. The results are relevant to the data obtained via XRD analyses.

### 3.3. Electron paramagnetic resonance (EPR):

In proportion to the absorbed dose, ionizing radiation generates free radicals (unpaired electrons). EPR can select and sensitively detect and quantify the number of unpaired electrons. For the irradiated crab carapace, these free radicals were measured via EPR.

#### 3.3.1. Analyses of EPR spectrum

The examined samples of unirradiated (native) crab carapace shells only offered a very weak signal may be from a residual organic radical. For the irradiated samples, the major signal can be referred the existence of coaxial radical with $g$-value of: $g^{\perp} = 2.00668$, and $g^{\parallel} = 2.0026$ see Figure 4(a) & 4(b). The most probable of these values are attributed to those in the coaxial CO$_3^-$ free radical [14,15,16], which represents natural carbonates of marine origin.

Reversely, this signal was attributed [17] to orthorhombic CO$_2$ radical with the $g$-values of: $g_x = 1.9973$, $g_y = 2.0016$, and $g_z = 2.0027$, with $g_{av} = 2.0005$. 

Figure 4(a) & 4(b) represents EPR spectra of the crab samples irradiated by Co\(^{60}\) with gamma radiation at various dose levels up to 30.0 kGy.

There are four other isotropic signals appears in Figure 4. First, at g= 1.9857 with \(\Delta H_{pp}\) of 7.43 mT, this signal accredited to that of Na free radicals in the zeolite [18]. Second and thired, at g = 2.01384 with \(\Delta H_{pp}\) of 3.482 mT and at g = 2.02037 with \(\Delta H_{pp}\) of 1.173 mT. These signals can be referred to those in [19,20] which is comparable to the g of the axial CO\(_3\) radical, and may be assigned to axial CaCO\(_3\). Fourth, at g= 2.0408 with \(\Delta H_{pp}\) of 8.21 mT attributed to hydroxyl ions [21].

3.3.2. Concentration of Free Radicals.

We evaluate the concentration of free radicals \(N_s\) using the relation:

\[
N_s = K \cdot \frac{[H_0 \cdot (\Delta H)^2 \cdot A / 2]}{[H_{m} \cdot G_r \cdot (P)^{0.5}]}
\]

Where: \(K = 10^3\), \(H_0\): magnetic field in Gauss, \(\Delta H\): peak width, \(A\): amplitude/weight, \(H_m\): modulation amplitude, \(P\): microwave power in mW and \(G_r\): receiver gain.

Table 2: represents the carapace irradiation doses in kGy, the corresponding EPR peak height per gram and their induced free radical concentration.

| DOSE kGy | Peak Height/ Weight, gm | Free radical concentration |
|----------|-------------------------|---------------------------|
| 0.0kGy   | 933.6823735             | 2.53315E+16               |
| 0.25KGy  | 2653.606411             | 8.37105E+16               |
| 1KGy     | 8813.559322             | 9.91079E+16               |
| 3KGy     | 38892.82551             | 4.37398E+17               |
| 5KGy     | 43720.72853             | 5.12986E+17               |
| 10KGy    | 70311.95841             | 7.5965E+17                |
| 15KGy    | 87519.44685             | 9.07741E+17               |
| 20KGy    | 95945.94595             | 9.9514E+17                |
| 30KGy    | 111288.0766             | 1.15427E+18               |
The concentration of the induced radical is proportional representation to the absorbed dose transferred to the irradiated samples and it reflected by the EPR peak signal height. It was found that, the amplitude of the CO$_3$ signals increased with increasing the absorbed dose.

4. Conclusion.
XRD & FTIR results distinguished that, crab exoskeleton combines mainly calcite minerals. Identification of the irradiated carapace samples was concerned with the detection of the EPR spectra for g-values which are attributed to those in the coaxial CO$_3$ free radical. The results confirm the applicability of EPR spectroscopy in studying the induced radical concentration. It is proportional to the EPR peak height signal. It depends also on the absorbed dose delivered to the irradiated samples.

References
[1] Cornwall J 2014. Edible Crab Measuring Guide”. http://helfordmarineconservation.co.uk/os/regulations/edible-crab.php
[2] Boßelmann F, Romano P, Fabritius H, Raabe D and Epple M 2007, *Thermochimica Acta* **463** 65. 
[3] Kushwaha R K et al 2018, Recent Developments in Bionanocomposites, Res. J. Nanosc. Eng. **2** 1.2, 
[4] WHO1988. Food irradiation: a technique for preserving and improving safety of food. World Health Organization, Geneva, Switzerland. 
[5] WHO1994. Safety and nutritional adequacy of irradiated food. World Health Organization, Geneva, Switzerland. 
[6] ICGFI, 1999. Facts About Food Irradiation. International Consultative Group on Food Irradiation. 
[7] Ziegelmann B., Bögl K, Schreiber G 1999. *Radiat. Phys. Chem.* **54** 413. 
[8] Desrosiers M., 1990, *Nature* **345** 6275. 
[9] Sünnetioglu M, Dadayli D, Celik S and Koksel H 1999. *Appl. Radiat. Isot.* **50** 557. 
[10] Callens F, Vanhaelewyn G, Matthias P and Boesman E 1998. *Appl. Magn. Reson.* **14** 235. 
[11] Wang Y, Moo Y X, Chen C, Gunawan P and Xu, R 2010. Journal of Colloid and Interface Science **352** 393. 
[12] Linga Raju C, Narasimhulu K V, Gopal N O, Rao J L and Reddy B C V 2002. *Journal of Molecular Structure* **608** 201. 
[13] Cheng B and Cai W., Yu J 2010, *Journal of Colloid and Interface Science* **352** 43. 
[14] Callens F J, Verbeeck R M H, Naessens D E, Matthys P F A and Boesman E R 1989b, *Calcif.Tissue Int.* **44** 114. 
[15] Callens F J , Verbeeck R M H, Naessens D E, Matthys P F A and Boesman E R 1991. *Calcif. Tissue Int* **48**, 249. 
[16] Vanhaelewyn G, Goovaerts E and Callens, F 2002, *Radiat. Eff. Defects Solids* **157** 1127. 
[17] Maghraby A 2007, *Radiation Measurements* **42** 220. 
[18] Weckhuysen Bert M, Heidler Ralf and Schoonheydt Robert A, 2004, *Mol. Sieves* **4** 295. 
[19] Serway R A and Marshall S A 1967, *J.Chem.Phys.* **47** 868. 
[20] Fattibene P and Callens F 2010, *Appl. Radiat. Isot.* **68** 2033. 
[21] Meriaudeau P, Vedrine J C, Taarit Y B and Naccache C 1975, *J. Chem.Soc.Faraday Trans. II* **71** 736.