Electron spin resonance dating and XRD analysis of archaeological bone samples recently excavated in Karnataka, India

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ABSTRACT
The study of radiation defects created in biomaterials, such as bones can be used in dating with importance to paleontology and archaeology. A preliminary attempt has been made to date the bone samples recently excavated from the archaeological site Gudnapur in Karnataka state, India. Each sample was divided into five sets which were given an artificial dose (AD) by using γ irradiation of 50 Gy, 300 Gy, 800 Gy, 1500 Gy and 3200 Gy. All the samples show similar EPR spectra having g-values 2.0026, 2.0025 and 2.0013 corresponding to orthorhombic CO$_2^-$ and axial CO$_2^-$ respectively. These signals have been used for the age estimation of the archaeological bone samples assuming the dose rate to be 6.8 mGy/a. The calculated ages of the samples are 36 ± 24 ka, 12 ± 87 ka and 19 ± 83 ka. These samples correspond to the upper stage of the Pleistocene epoch respectively and in good agreement with age predicted by archaeological Department. X-ray diffraction studies revealed the transformation of the mineral components partially into fluoroapatite form with addition of goethite, quartz phases and with preserved collagen remains. The aim was to establish their degree of preservation and possibilities of inferring the life conditions from them.

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agate mortar then the samples were given to the artificial γ irradiation (from 60Co) in different dose rates labeled 50, 300, 800, 1500 and 3200 Gy at the rate of 810 Gy/hr and ESR spectra were recorded on a JEOL TES100 ESR spectrometer, having 100 kHz field modulation and phase sensitive detection. DPPH with g value of 2.0036 was used as an internal standard for g factor calculations. The ESR defects, i.e., unpaired electrons are created in the samples by α-, β- or γ-rays. The concentration of the unpaired electrons can be determined directly by microwave absorption giving the EPR signal intensity. These unpaired electrons are trapped by an impurity or left as a hole, either of which is simply called “defects” [6]. All the EPR spectra were recorded at room temperature with an optimum microwave power (1.0 mW), sweep time (2 min) and time constant (0.03 s) to get good resolution. The g factor was determined using the equation

\[ g = 71.44836 \times (\nu/B) \]  

(1)

Here \( \nu \) is the microwave frequency in GHz and \( B \) is the magnetic field in mT.

**Fig. 1 Photograph of the Archaeological bone samples**

Additive dose method and age calculation:

If one could know the defect concentration at a certain future time, it would be possible to estimate past passage of time from the present concentration. In conventional TL dating [7] and ordinary EPR dating [1] known additive doses of artificial irradiation are used to produce additional defects which can be used to calibrate the concentration within a sample and to determine the production yield of defects.

In this work the peak-to-peak method was used. This method consists on taking the amplitude between the top to the bottom of a chosen ESR line. The sample irradiated with known doses (Q) yields a growth curve. For each aliquot measurement the ESR signal intensity is taken and plotted against the received dose. Later, the experimental data points are fitted to a mathematical expression. The choice of the fitting model has long been a source of discussion [8, 9] and the data appear to be in very good agreement with the saturation fit. The following equation was used to relate the natural dose or archaeological dose (AD) received by the sample up to the present:

\[ I(Q) = I_s [1 - \exp(-Q+AD)/SD] / SD \]  

(2)

Where \( I_s \) is the saturation intensity, SD is the saturation dose in a laboratory irradiation. Thus, by fitting the experimental data (dose response) to the above equation, the equivalent/archaeological dose (AD) can be found out. The negative interception of the linear line gives the value of equivalent dose (ED). The annual dose rate has been determined [10] using methods described in [1] according to the uranium-disequilibrium model [11] as 6.8 mGy/y (external annual dose rate has been measured as 0.063 mGy/y). The age of EPR may be expressed (in ka) using the relation:

\[ T_{EPR} [ka] = ED [Gy]/D [mGy/ka]. \]  

(3)

The ED and D estimation for geological and archaeological samples constitutes the main part of EPR dating.

**XRD analysis:**

The bones are the remains of our distant ancestors which are always transformed during a long process of the interactions with surrounding sediments after burial. The phase analysis of the chemical structure of the powdered bone was carried with the use of Mini Flex II XRD manufactured by the Rigaku Corporation with the operating voltage 30 KV and the current 15 mA on X-ray tube. The data were compared with the JCPDS database. Alternately, the comparison was done with the Match (Crystal Impact) program. Bone is supported on a template which mainly consists of collagen I. That kind of natural polymer is built of the triple helix of essentially three polypeptides (two α1 – peptides and one α2 –peptide) which are tightly bound with each other. These tightly bound polypeptides decide on the bone resistance to fractures and about the elasticity. Due to the fossilization process the changes as a rule start with the decay of the organic components. Primarily, the degradation of collagen was expected to have taken place and next the process of demineralization within the hydroxyapatite crystal [12]. The magnitude of changes is largely dependent on the specific environmental conditions which interact with the fossil material [12, 13, 14].

**Result and discussion:**

The ESR spectra of these samples show a strong signal with spectroscopic factors g at 2.0026, 2.0025 and 2.0013 related to CO\(_2\) radical in hydroxyapatite. Hydroxyapatite is a microcrystalline hexagonal mineral. The structure of apatite changes by the replacement of each constituent cation and anion by other impurity ions. The physics of defects in apatite is rather complicated due to large unit cell structure and symmetry involved. The ESR signal at g= 2.0026 and 2.0025 were belongs to the orthorhombic CO\(_2\) and it is known as the carbonated apatite where the CO\(_2\)\(^-\) has been substituted at OH ion. This orthorhombic CO\(_2\)\(^-\) is presumably due to hydrogen bonding [1].

**Fig. 2:** ESR Spectra of sample GDB1

**Fig. 3, 4 and 5:** showing the ESR spectra recorded for GDB1, GDB2 and GDB3 after subjecting the sample to different radiation exposures. The equivalent doses of the samples were identified by the additive dose method. The g values have been calculated for all three samples and are given in Tables 1 for GDB1, GDB2 and GDB3. The intensity values of the peaks for the signals are calculated from the ESR spectra shown in Table 2. These intensities were plotted against artificial dose shown in the Fig 5, 6 and 7. The predominant signal only considered for the age calculation for each doses as its linear variation with the artificial doses. However, the values of the equivalent doses for these three samples are 246.44 Gy, 87.18 Gy and 134.89 Gy. These signals have been used for the calculation of Equivalent Dose (ED). The calculated ages are 36 ± 24 ka, 12 ± 87 ka and 19 ± 83 ka for the samples GDB1, GDB2 and GDB3 respectively. These three samples were corresponding to upper stage of the Pleistocene epoch respectively [15].

**Fig. 2 ESR Spectra of sample GDB1**
X-ray diffraction analysis of the sample showed that the apatite with a significant admixture of fluoroapatite was the major component of analyzed bone. Fig 8 shows the XRD patterns of the samples GDB1, GDB2 and GDB3 respectively. The presence of fluoroapatite [211] confirmed the typical partial transformation of hydroxylapatite into fluoroapatite characteristic for the fossilized bones. Quartz [100] and goethite [130] are the additional mineral components present within the sample matrix. These minerals have been conveyed by water from the sand and minerals surrounding the analyzed bone. Such composition is sometimes characteristic also for the desert environment [16]. In the X-ray diffraction pattern we have also found some residuary amounts of organic matter present as collagen. The presence of collagen was confirmed by a broad scattering band focused around the value $2\theta = 11^\circ$ [17, 18]. Emergent changes in the elemental composition of the fossilized bone are often much different from the composition of contemporary bones even though they still may have many common components. Changes as mentioned above can be represented by the increased presence of the fluoroapatite in the fossilized bones. Fluorides from residues, as well as those from the aquiferous layer of the earth crust which were percolating the bones, substituted the hydroxyl groups (OH$_{-}$).

**Conclusion:**

The ESR dating has been carried out for the mammalian bones from the archaeological site Gudnapur in Karnataka state, India. Based on knowledge of the physics of stable radiation defects created in hydroxylapatite, present in the bones, it has been possible to determine the accumulated dose by plotting intensity of ESR lines against artificial irradiation and ages of the bone were calculated. The estimated ages were mentioned in table 3. These results indicate dates that are coherent with other archaeological and paleontological findings in specified site. The ages obtained by ESR suggest that these animals had lived in the upper (Tarantian) stage of the Pleistocene epoch [15]. From the XRD studies, it is noted that the preservation of collagen and the minerals goethite and quartz. The presence of Fluoroapatite confirms the transformation of Hydroxylapatite into Fluoroapatite which characteristic for the fossilized bones in these three samples. Therefore the mineralization pattern of bones may change a lot after stopping the living functions of the organism. The recrystallization of the bone material leading from the relatively soluble bone apatite to the insoluble mineral-type remains is another post-mortem change of the bones [19].
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References:

Research Highlights
- We attempted to date Archaeological shell with electron spin resonance spectroscopic technique
- Radicals produced by natural radiation in the mineral part CO₂ present in the shell utilized for dating.
- We examined the gamma irradiated samples in different dose rates with ESR. Equivalent dose identified by additive dose method.
- The equivalent doses to gamma irradiation used to estimate the ages of the sample as 232 ± 74 ka in good agreement with archaeological data.
- XRD studies confirm the transformation of aragonite into secondary calcite and confirm the preservation of calcite and the minerals such as Mg and Sr.

Table 1. The estimated ‘g’ values for irradiated samples

<table>
<thead>
<tr>
<th>Artificial dose (Gy)</th>
<th>GDB1</th>
<th>GDB2</th>
<th>GDB3</th>
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<tbody>
<tr>
<td>50</td>
<td>2.0005</td>
<td>2.0040</td>
<td>2.0008</td>
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<td>300</td>
<td>2.0004</td>
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<td>2.0050</td>
<td>2.0004</td>
<td>2.0005</td>
</tr>
<tr>
<td>3200</td>
<td>2.0037</td>
<td>2.0006</td>
<td>2.0007</td>
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</table>
### Table 2. The Intensity values for corresponding artificial dose for the samples GDB1, GDB2 and GDB3

<table>
<thead>
<tr>
<th>Artificial dose (Gy)</th>
<th>ESR signal Intensity (cm)</th>
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<tbody>
<tr>
<td></td>
<td>GDB1</td>
</tr>
<tr>
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<td>1.0</td>
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<tr>
<td>300</td>
<td>1.3</td>
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<td>800</td>
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<td>1500</td>
<td>3.5</td>
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<td>3200</td>
<td>5.1</td>
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</table>

### Table 3. The estimated ages of the samples

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Age (Ka)</th>
<th>Error (a)</th>
<th>Geological period</th>
</tr>
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<tbody>
<tr>
<td>GDB1</td>
<td>36</td>
<td>±24</td>
<td>In Pleistocene epoch</td>
</tr>
<tr>
<td>GDB2</td>
<td>12</td>
<td>±87</td>
<td>Upper (Tarantian) stages</td>
</tr>
<tr>
<td>GDB3</td>
<td>19</td>
<td>±83</td>
<td></td>
</tr>
</tbody>
</table>