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# Evaluation of ionizing radiation effects in bone tissue by FTIR Spectroscopy and Dynamic Mechanical analysis

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In many medical practices the bone tissue exposure to ionizing radiation is necessary. However, this radiation can interact with bone tissue in a molecular level, causing chemical and mechanical changes related with the dose used. The aim of this study was verify the changes promoted by different doses of ionizing radiation in bone tissue using spectroscopy technique of Attenuate Total Reflectance - Fourier Transforms Infrared (ATR-FTIR) and dynamic mechanical analysis. Samples of bovine bone were irradiated using irradiator of Cobalt-60 with five different doses between 0.01 kGy, 0.1 kGy, 1 kGy, 15 kGy and 75 kGy. To study the effects of ionizing irradiation on bone chemical structure the sub-bands of amide I and the crystallinity index were studied. The mechanical changes were evaluated using the elastic modulus and the damping value. To verify if the chemical changes and the bone mechanic characteristics were related, it was made one study about the correlation between the cristallinity index and the elastic modulus, between the sub-bands ratio and the damping value and between the sub-bands ratio and the elastic modulus. It was possible to evaluate the effects of different dose of ionizing radiation in bone tissue. With ATR-FTIR spectroscopy analysis, it was possible observe changes in the organic components and in the hidroxyapatite crystals organization. Changes were also observed in the mechanical properties. A good correlation between the techniques was found, however, it was not possible to establish a linear or exponential dependence between dose and effect.

## 1. INTRODUCTION

It is known that ionizing radiation is able to break polypeptide chains and cause release of free radicals by radiolysis of water molecules [1]. In particular for bone tissue, studies show that the interaction of ionizing radiation occurs in a molecular level, and it may cause dose-related chemical changes, as result of collagen degradation, decrease in density of intermolecular cross link and alterations in the mineral content of the bone. [2], [3], [4], [5], [6], [7], [8], [9], [10]. Nonetheless, the exposure of bone tissue to ionizing radiation is necessary in many medical practices, such as radiology, radiotherapy, brachytherapy, and allografts sterilization. So the knowledge of how different doses of ionizing radiation affect the bone tissue is essential.

The FTIR spectroscopy is a well-established technique in the characterization of organic materials that allows physiochemical characterization of materials. It allows identifying and quantifying components, and also provides information about the material structure [11]. It is a great tool to mineralized tissues analysis, since it requires a simple sample preparation, having already been used in the study of chemical and structural changes caused by temperature increase [12], [13], [14], in the study of several bone diseases [15], [16], [17], [18], studies of laser irradiation effects [14], [19], [20], and in the characterization of gamma radiation effects[4].

The chemical structure of bone tissue is highly correlated with its mechanical properties, such as tensile strength, elasticity and ductility. In this way, changes in chemical structure also promote changes in mechanical properties of bone tissue [4], [8]. In this context, the mechanical characterization of bone tissue is also an important tool to understand how different doses of ionizing radiation interfere in the mechanical properties of the tissue.

This study aims to evaluate the changes in bone tissue promoted by different doses of ionizing radiation. It was used FTIR spectroscopy to analyze the chemical effects, and a mechanical test to evaluate the consequences of these changes in bone mechanical properties

## 2. MATERIAL AND METHODS

Bone fragments were obtained from of bovine femur diaphysis, and the samples were prepared according to the method of analysis used.

### 2.1. Samples Irradiation

The irradiations were performed at Instituto de Pesquisas Energéticas e Nucleares (IPEN - CNEN/SP). Irradiations with doses of 0.01 kGy 0.1 kGy and 1 kGy were performed in a Cobalt-60 Gammacell irradiator, with dose rate of 1.43 kGy/h. Groups exposed to doses of 15kGy and 75kGy were irradiated in a multipurpose irradiator of Cobalt-60, with a dose rate of 6 kGy/h.

Before the first irradiation, each sample was analyzed its respective technique. Then the samples were irradiated and reanalyzed. This process was repeated until the samples were irradiated with all doses.

### 2.2. ATR-FTIR

To understand the effects of ionizing irradiation in collagen structure, the sub-bands of amide I bands (1660 cm-1 and 1690 cm-1) were analyzed. These bands are related with the collagen cross links pyridinoline (Pyr) and dihidroxinorleucine (DHLNL), and the ratio of this bands represent the proportion between mature and immature collagen crosslinks [21], [22], [23] To compare collagen changes promoted by the different irradiation doses, the ratio of the sub-bands of non-irradiated bone was used to normalize the results. It was applied the Kruskall-Wallis test with 5% of significance to evaluate the difference between the groups.

For ATR-FTIR analyses, five bone fragments were cut with approximately 4 mm×4 mm×1 mm and polished until have a flat surface. The infrared spectra acquisition was made using an ATR accessory (Smart Orbit, Thermo, EUA) coupled in a FTIR spectrometer (Nicolet 6700, Thermo, EUA). The range analyzed was from 4000 $cm^{-1}$ to 400 $cm^{-1}$, with a resolution of 2 cm-1 and 32 scans per spectra. In all steps, each sample was analyzed ten times, being repositioned on the equipment.

The Figure 1 shows three sub-bands that contribute to the amide I band: 1690 cm-1, 1660 cm-1, 1630 cm-1. These bands were chosen because they present highest intensity on second derivative of the spectra.

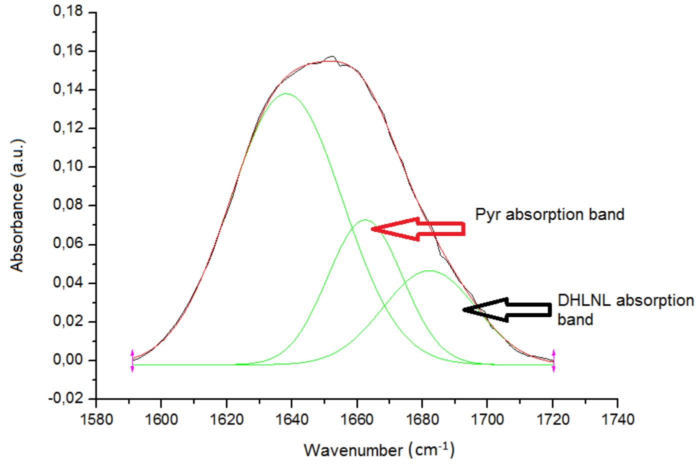


**Figure 1: Sub-bands of amide I, with highlight in Pyr and DHLNL bands.**

The inorganic structure of the tissue was evaluated with the study of crystallinity index. The index is reflects the combination of the relative sizes of the crystals and organization [24]. This index is calculated using the follow expression:

$$CI = \frac{I_{551cm^{-1}} + I_{587cm^{-1}}}{I_{588cm^{-1}}} \qquad (1)$$

where $I_x$ is the intensity of the band located in the wavelength $x$. The higher the value of CI, more organized are the crystals in the bone matrix [12], [24], [25], [26], [27].

### 2.3. Dynamic Mechanical Analysis

For the Dynamic Mechanical Analysis (DMA), bones fragments were cut with approximately 1 mm×50 mm×4 mm. They were storing in a humid environment, refrigerated at 4 °C. The Netzsch DMA 242 equipment was used. It was established a deformation of 60 µm with a frequency of 1 Hz, 2 Hz, 5 Hz, 10 Hz, with 10 minutes of break between them. All samples were analyzed using a 40 mm distance bracket, and they were measured four times at each step. It was determined the elastic modulus and the tangent of delta of each group for the different frequencies used. The tangent of delta is related to the damping of the material.

To verify how chemical changes affected the bone mechanic characteristics, it was conducted a study about the correlation the cristallinity with an elastic modulus, and between the sub-bands ratio with the damping value.

## 3. RESULTS AND DISCUSSION

In Figure 2, a typical spectrum of bone tissue is showed. The main bands are identified, with a highlight in bands used in this study. It was possible see all typical bone bands in all groups, indicating that the doses used have not caused total degradation of any bone component.

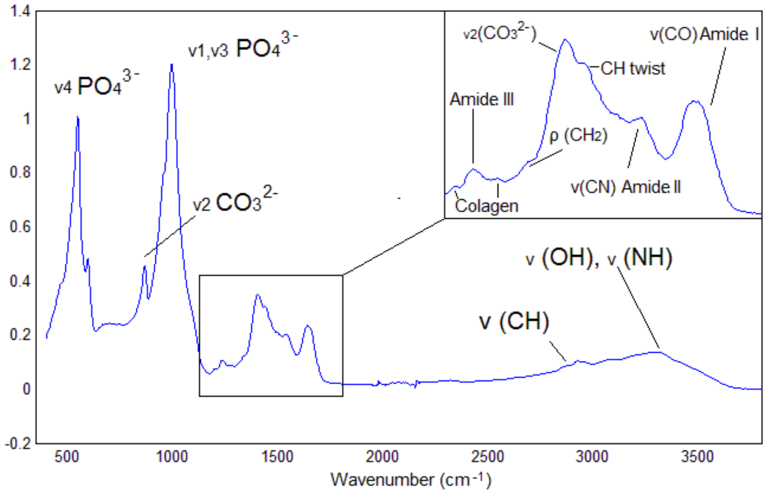


**Figure 2: Typical spectrum of bone tissue**

The Figure 3 shows the graphic of ratio between amide I sub-bands (1660 $cm^{-1}$ e 1690 $cm^{-1}$) of the six groups. To a better understand of the data comportment, a tendency line was applied. It is possible see that there is a gradual increase of the sub-bands proportion on the three first groups, than a suddenly increase on 1 KGy group, where the ratio reaches its maximum value, before this point the curve started to decline. The statistic test showed significant difference only in groups 1 kGy and 15 kGy in relation of the other groups.

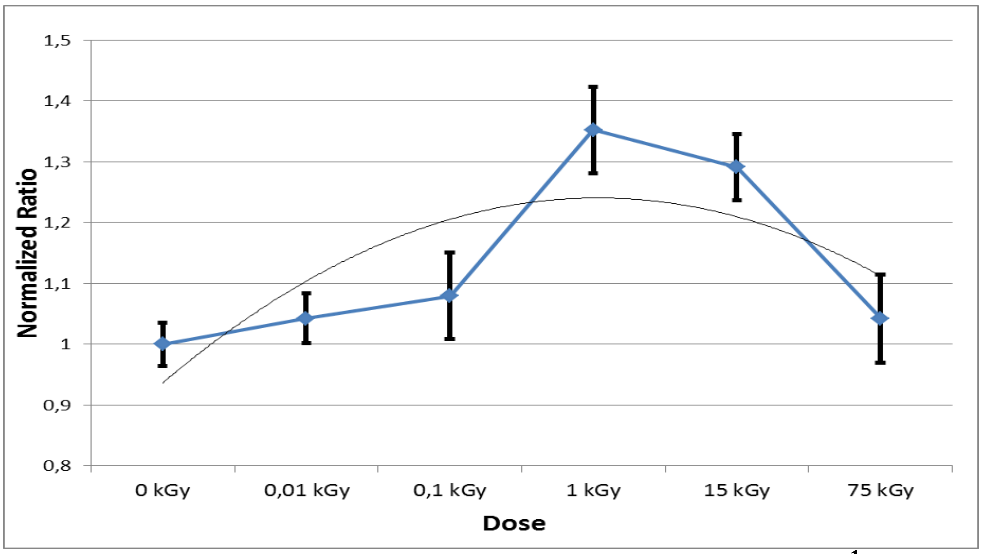


**Figure 3: Graphic of ratio between amide I sub-bands (1660 $cm^{-1}$:1690 $cm^{-1}$).**

The ratio of amide I sub bands are related with the organic matrix of the bone, representing the proportion between mature and immature collagen crosslink [4], [23]. How expected, the results showed that ionizing radiation was able to affect the organic components of the bones [4], since a statically difference was observed in the groups irradiated with 1 kGy and 15 kGy. However, it was not possible see the dose-effect relation, once none statistic difference was observed in the highest dose (75 kGy) when compared with the non-irradiated group and irradiated with smallest doses.

Considering that the 75 kGy dose is the highest used in this study, it was expected that if a lower dose promoted any changes in the organic components of the bone tissue, a similar change, with higher magnitude, would also be detected in the group irradiated with 75 kGy. However, the ATR-FTIR analyses show changes in bone organic matrix promoted by 1 kGy and 10 kGy doses, and no significant change on samples irradiated with 75 kGy was observed.

It is possible that water bands had affected the results. Despite the samples were dehydrated before the analyses, water of environment could have affected the amide I band, since the water molecules have vibration mode in this same range. More studies must be realized for a better comprehension about why no alteration was observed for the highest dose.

The Figure 4 shows the graphic of crystallinity index. It is possible to observe a gradual decrease of the index value from the group non-irradiated until the group irradiated with 0.1 kGy. From the dose of 1 kGy the index have a gradual increase, suffering a suddenly high on the group of 75 kGy. The statically analysis shows a significant difference between the group irradiated with 0.1 kGy and the control group and between the group irradiated with 75 kGy and the other groups irradiated.

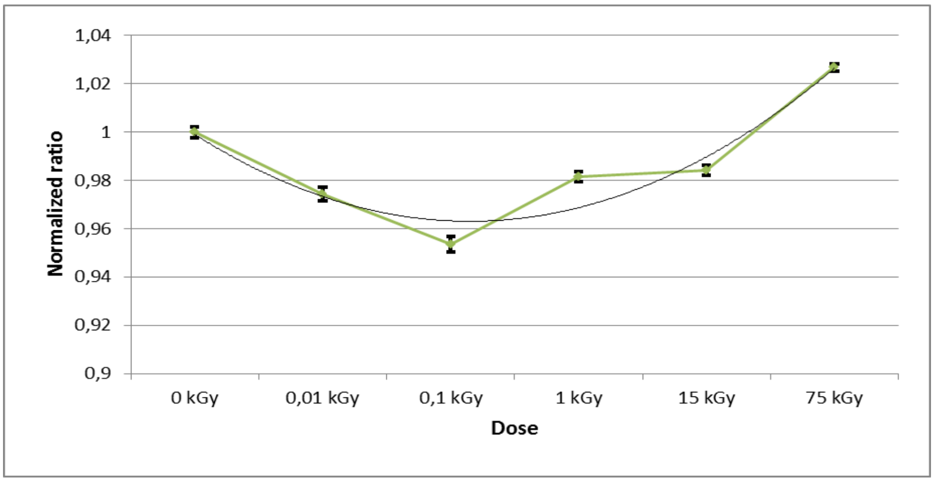


**Figure 4: Graphic of ratio crystallinity index.**

The cristallinity index is calculated using different vibration modes of phosphate and carbonate bands. It is related with the organization of the hidroxyapatite matrix as commented before. The results suggest that until the 0.1 kGy the doses affect the hydroxiapatite crystal in order to disorganize it. However, the crystal returns to be organized for highest doses values.

As observed in the organic analyses, the data acquired from crystallinity index showed a non-linear and non-exponential dose-effect relation. In both analyses the groups irradiated with intermediate doses were more affected than the group irradiated with 75 kGy. The water band have do not have any influence on the bands used in this analyze. In this way, the hypothesis about the levels of humidity in the environment influenced the results was discarded.

Data from both parameters analyzed by DMA, were normalized by the control group, emphasizing only the effects caused by the different doses. In the results of DMA there are no significant difference in the effects observed in the elastic modulus and delta tangent value related with the frequency used in the moment of measurement, showing that the radiation does not influence the tissue response to different frequencies of oscillating strains. In this way, it was used the mean value of all frequencies to analyze how both parameters were affected by each irradiation dose.

The Figure 5 shows the normalized elastic modulus and delta tangent mean values for different samples of from each dose group. It is possible see that both parameters presented similar behavior, however the damping values presented higher relative changes in relation to the control group than the elastic modulus, indicating that the chemical changes promoted by ionizing irradiation affects more the mechanical characteristics related with the organic components.

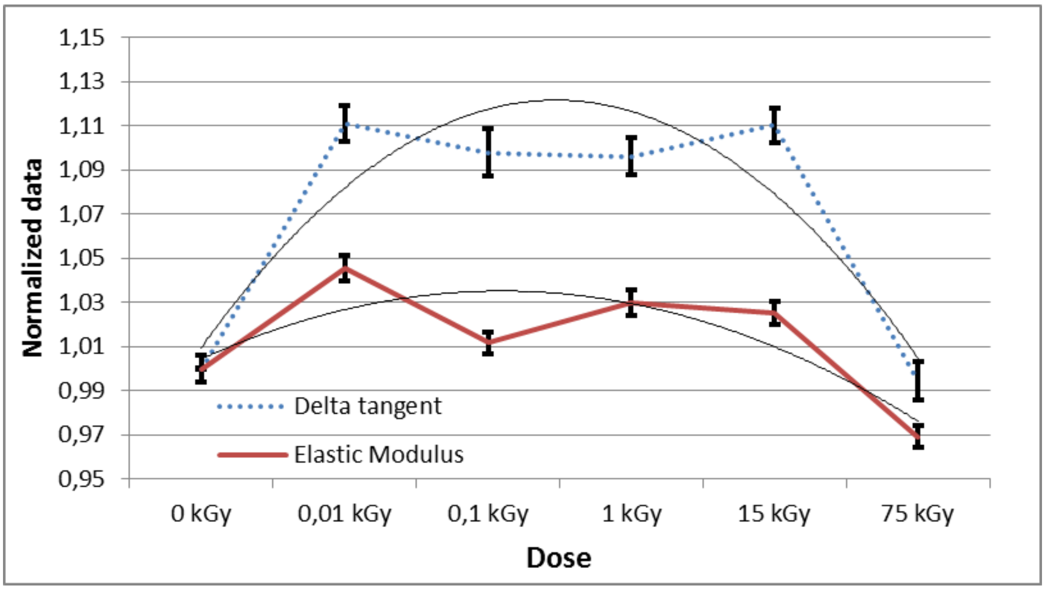


**Figure 5: Normalized mean of elastic modulus and damping values from each dose group.**

Despite that the group irradiated with 0.1 kGy has the highest relative elastic module value, it did not presented statistically difference in relation of the control group. Furthermore, it was not observed differences between the groups irradiated with 1 kGy and 15 kGy, either. Except for these two cases, all the other groups had significant differences when compared one with other.

Analyzing the damping value, only the group irradiated with 75 kGy did not presented significant difference with the control group. In fact, this group presented statistical difference with all other irradiated groups, suggesting that the mechanical changes caused by a small dose is not the same that the promoted by a higher dose.

As observed in the FTIR results, the DMA analyze did not show a relation between dose and effect as was expected. However, the fact that this happened in both techniques suggest that this comportment is not caused by experimental factors, like the sensibility of the equipments.

Table 1 shows the correlation between spectroscopic analysis and mechanical analysis. Values of coefficient of linear correlation (r) close to ± 1 present strong the correlation. For values of r close to 0, there is a weak or none correlation.

It was observed that the cristallinity index have a strong negative correlation with the elastic modulus. The amide I sub-band ratio showed a positive moderated correlation with the damping value and the elastic module. These results are consistent with the expected, what reinforce the quality of results of both techniques.

**Table 1: Coefficient of linear correlation (r)**

| | Cristallinity Index × Elastic Modulus | Amide I sub-band ration × Delta tangent | Amide I sub-band ration × Cristallinity index |
|---|---|---|---|
| r | -0.73 | 0.56 | 0,42 |

The main motivation of this study was to assess chemical and mechanical changes that different doses of ionizing irradiation causing in bone tissue. The knowledge about how different irradiation doses affect the bone is useful in situations where bone exposure of ionizing radiation is necessary, like in bone sterilization.

The FTIR spectroscopy already proved to be useful in the analysis of chemical changes in organic components in mineralized tissue [4], [17], [23], [27]. There are many analyses that can be made; it will depend of what is the desired information. In this work, in order to analyze the organic and inorganic matrix of bone tissue the bands of carbonate, phosphate and amide I were used.

In the other hand, the DMA is an established analyses technique to detected mechanical changes in mineralizes tissue [28] . In this study the elastic modulus and the damping value of bone were evaluated. They were chosen because of their relation with the organic and inorganic matrix of the bone. In this way, it was possible correlate the results of FTIR and DMA.

Both techniques showed that doses of ionizing irradiation affect the bone tissue in different way. However, the expected relation dose-effect was not observed. Since the techniques had a good correlation, insuring the data quality, more studies will be necessary to a better understanding how different ionizing irradiation doses affect the chemical and mechanical aspects of the bone tissue.

## 4. CONCLUSIONS

It was possible to evaluate the effects of different of ionizing radiation in bone tissue. It was found by ATR-FTIR spectroscopy analysis that there were changes in the organic components and crystal hidroxyapatite organization. The similar pattern was observed in the dynamic mechanical analysis. In both techniques, it was not possible to establish a linear or exponential dependence between dose and effect.

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