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Thermal kinetics analysis of bone tissue organic phase

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The rapid self-regeneration properties of deer antler tissue recommend this material as a potential bone regenerating biomaterial, because after high temperature treatments and gamma sterilization it represents a special source of hydoxyapatite. In order to investigate the decomposition of the organic phase existing in the bonelike antler tissue and the apatite type structural stability of the inorganic phase, thermal analyses were conducted at different heating rates, both on non-irradiated and gamma irradiated samples.

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1. Introduction

The research focussed on development of new biomaterials that promote bone tissue regeneration is receiving great interest by the biomedical scientific community due to the growing incidence of bone disfunctions and their major impact on human health. The efforts are focused on the designing of materials with nanostructure similar to that of the natural bone and having good biocompatibility with human tissue. Earlier X-ray diffraction studies on deer antler tissue [1] revealed that after high temperature thermal treatments a well defined crystalline structure is obtained, similar to that of the pure mineral hydroxyapatite. Bone regeneration is promoted by mechanisms of osteoconduction (bone growth from existing bone by stimulation of osteoblasts to form new bone), osteoinduction (stimulation of mesenchymal cells to differentiate into osteoblasts) and osteogenesis, i.e. bone formation in sites where bone did not exist previously [2].

The inorganic part of bone tissue mainly consists in a crystalline phase of hydoxyapatite with nanometric crystal sizes with a length of about 100 nm, 20-30 nm in width and thickness of 3-6 nm [3]. This inorganic phase is located into an organic matrix mainly consisting of type I collagen [4]. Hydroxyapatite is known to be bioactive, osteoconductive, non-toxic, non-inflammatory and non-immunogenic agent [5].

An ideal osteoinducing biomaterial of biological origin should be free of organic components in order to avoid antigenic and immunological contamination [6]. Therefore the investigated material – deer antler tissue – undergone thermal treatments for removal of the organic phase. Another process a biomaterial should be submitted is sterilization by gamma irradiation. Radiation offers several advantages as a sterilization method that makes it attractive in a growing number of situations over the conventional methods of sterilization [7]. This type of sterilization has proved to be very effective, but it can also have adverse effects on the biomaterials leading to their decomposition or changing their physicochemical or therapeutic properties [8].

Table 1. Values of the activation energy calculated from the linear plot of ln ($T_{peak-1}^2/ß$) vs. $1000/T_{peak-1}$ for thermal decomposition of the organic phase of deer antler tissue samples.

Sample	T_{peak-1} (ºC) Heating rate ß (ºC/min)			E_{a1} (kJ/mole)
	3	5	10	
3 non-irr	363.3	371.27	388.54	5.843 ± 0.7
3G-irr	361.35	372.42	400.22	3.587 ± 0.6
4G-irr	364.33	379.16	400	4.161 ± 0.7
6G-irr	364.96	370.02	397.23	4.112 ± 1.4

The antler tissue is the only mammalian tissue capable to fully regenerate annually at a tremendous growth rate. Regeneration of the antler is stem cells based process, almost a recapitulation of the initial antler formation [9]. Antler tissue from male red deer of different ages is investigated here as a potential biomaterial for bone regeneration applications. Earlier studies of scanning electron microscopy on this material [10], indicate that the surface morphology of the deer antler shows high porosity and fibrousity, which can be easily correlated with its properties of fast and complete regeneration.

Table 2. Values of the activation energy calculated from the linear plot of ln ($T_{peak-2}^2/ß$) vs. $1000/T_{peak-2}$ for the combustion of the organic phase of deer antler tissue samples.

Sample	T_{peak-2} (ºC) Heating rate ß (ºC/min)			E_{a2} (kJ/mole)
	3	5	10	
3 non-irr	443	456	474	7.243 ± 0.1
3G-irr	440	455.3	485.79	4.62 ± 0.4
4G-irr	451	462	484	6.911 ± 0.7
6G-irr	441	448	478	5.397 ± 1.6

Thermal analysis has been used to investigate the thermal behavior and kinetics of chemical reactions and phase changes of the solid-state amorphous materials. Many scientists describe methods for calculating the kinetic parameters like activation energy or reaction rate constant for crystallization, thermal decomposition reactions, devitrification mechanisms or bulk surface nucleation from DTA/DSC or TG techniques [11, 12].

2. Experimental

The samples were selected by the age of the male red deer (3, 4 and 6 years old respectively). Antler tissue was prepared in powder samples and degreased with acetone for 48 h and with ethylic ether for 30 min, then heated at 40ºC in the oven until the complete removal of the solvent. A part of the material was gamma irradiated (for 33 hours, average absorption dose 32,7 ± 1,9 kGy). The non-irradiated samples were labeled with the age of the deer (3, 4 or 6) and the irradiated samples with 3G, 4G and 6G. Thermal analyses were conducted on Shimadzu type derivatographs DTG-60H (differential thermal and thermo gravimetric analyses) and DSC-60 (differential scanning calorimeter). Heating rates of 3, 5 and 10ºC/min and a temperature range of 28-500ºC were chosen for kinetics DSC measurements, while DTA/TG analysis was performed with a heating rate of 10ºC/min for a temperature range of 28-1200ºC. For the DTA/TG analysis were used alumina open crucibles and for the DSC analyses were used aluminum open crucibles. The measurements were made in a dynamic nitrogen and air atmosphere at a flow rate of 70ml/min each.

3. Results and discussion

The DTA and TG runs for gamma-irradiated samples are represented in Fig. 1. The profile of the DTA curves presents first a small endothermic peak at ~70ºC accompanied by a decrease in mass of ~ 6-8 %. This event was evidenced in many biomaterials at temperatures bellow 100ºC [13] and can be related to the removal of the condensation water. At the temperatures of 354ºC for 3G sample and 357ºC for 4G and 6G samples respectively, one can observe a large and narrow exothermic peak, followed by a shoulder type event, both peaks accompanied by a massive loss of mass, of ~25 % and 11 % from the total mass of the sample. These processes can be related, in good agreement with other results on biogenic apatite [14], to the thermal degradation and the combustion of the organic phase of the antler tissue. The DSC scans of 3 years old non-irradiated, and 3, 4 and 6 years old irradiated samples (Figs. 2 – 5) were recorded in order to perform a kinetic analysis of the thermal decomposition processes affecting the organic phase (collagen) of the bone-like deer antler tissue. The more accurate DSC experiments revealed that the shoulder type event that follows the main exothermic peak in the DTA curves (Fig. 1) is also an exothermic event that could be related to the combustion of the organic phase of the antler tissue.

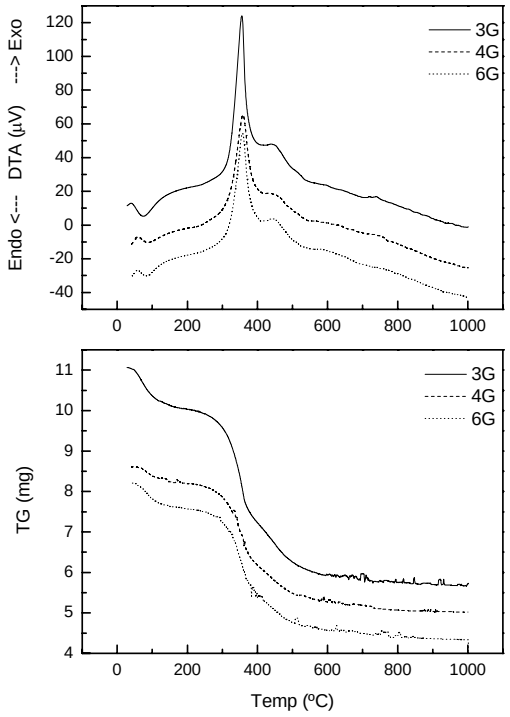

Fig. 1. TG and DTA curves of γ-irradiated deer antler of 3, 4 and 6 years.

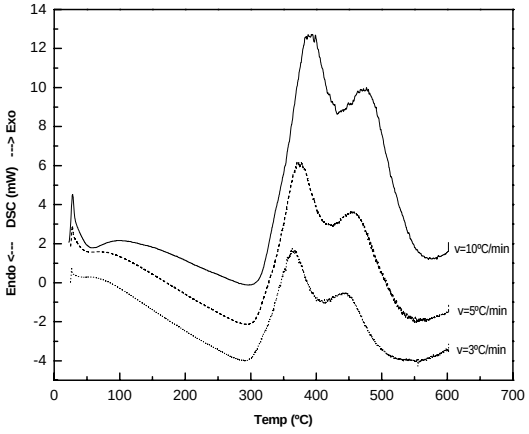

Fig. 2. DSC curves of non-irradiated 3 years deer antler tissue.

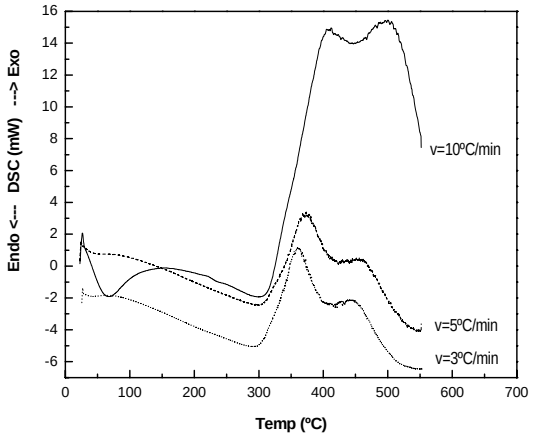

Fig. 3. DSC curves of γ-irradiated 3 years deer antler tissue.

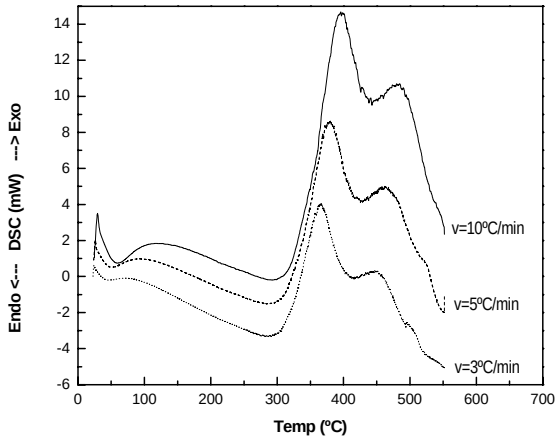

Fig. 4. DSC curves of γ-irradiated 4 years deer antler tissue.

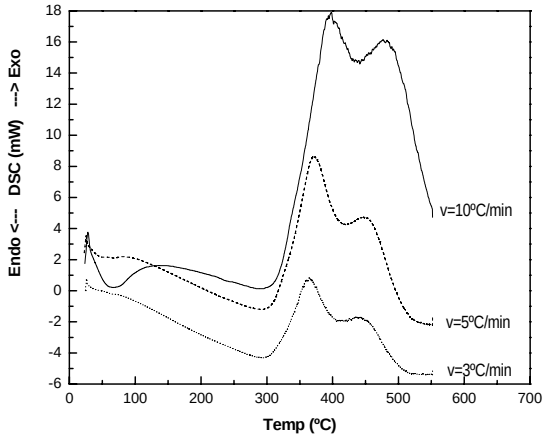

Fig. 5. DSC curves of γ-irradiated 6 years deer antler tissue.

Gamma irradiated samples present the same DSC thermogram characteristics, indicating that sterilization did not affect their structural stability. From DSC measurements it can be seen that the maximum of the peaks shifts towards higher temperatures when the heating rate is higher and this can be understood as a delay in occurring the decomposition process when the temperature is rising more rapidly due to increasing of the available energy in the sample, which creates evolving products. These products take some of the energy from the surroundings and maintain a vapor pressure atmosphere, and, therefore, the transformation rate of the decomposition reaction is delayed.

A simplifying assumption in modeling a thermally activated reaction is that the transformation rate during a reaction is the product of two functions, one depending only on the temperature (T) and the other one depending only on the fraction transformed, α:

$$\frac{d\alpha}{dt} = f(\alpha)k(T) \tag{1}$$

The temperature depending function is generally assumed to follow an Arrhenius type dependence:

$$k = k_0 \exp(-\frac{E}{RT}) \tag{2}$$

From equations (1) and (2) follows that for transformation studies by performing experiments at constant temperature, T_i, E can be obtained from the relation:

$$\ln(t_f) = \frac{E}{RT_i} + C_1 \tag{3}$$

where t_f is the time needed to reach a certain fraction transformed, while C_1 is a constant dependent on the reaction stage and kinetic model.

The ascertaining of the activation energy of a reaction, using non-isothermal experiments, requires the determination of the temperatures at which an equivalent stage of the reaction is obtained for various heating rates. In this approach, one of the most frequently used methods to determine the activation energy is the Kissinger method, based on the Arrhenius integral:

$$f(\alpha) = \frac{A}{\beta}\int_0^T \exp(\frac{E_a}{RT})dT \tag{4}$$

where A is the frequency factor, T is the temperature, R is the universal gas constant, β is the heating rate and E_a is the activation energy. This mathematical equation describes the thermogravimetric curve, where f(α) represents the reaction mechanism and the second term in the equation cannot be solved analytically, but only by approximation methods.

In order to determine the activation energy of decomposition of the organic phase (E_a) we considered the

Kissinger's formula, which is most commonly used in analyzing data in DSC. This formula that holds in very general cases has the form

$$\ln(\frac{T_{peak}^{\ 2}}{\beta}) = \frac{E_a}{RT_{peak}} + const. \quad (5)$$

where R is the universal gas constant and ß is the heating rate. The value of E_a is obtained from the slope of ln $(T_{peak}^2/ß)$ vs. $1000/T_{peak}$ plot. The values obtained for the first exothermic peak are plotted in Fig. 6, while the values for the second exothermic peak are represented in Fig. 7.

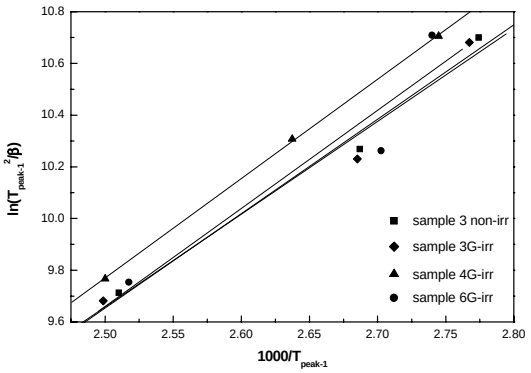

Fig. 6. Plot of ln (T_{peak-1}^2/ß) vs. $1000/T_{peak-1}$ of thermal denaturation of the organic phase of deer antler tissue samples.

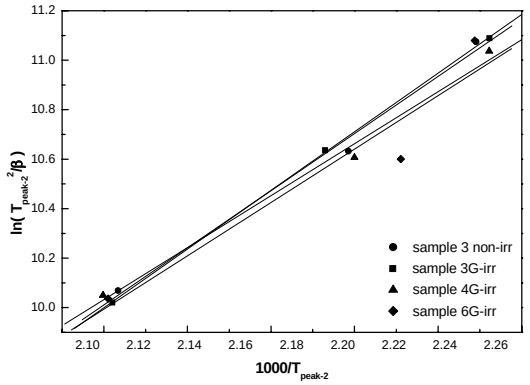

Fig. 7. Plot of ln (T_{peak-2}^2/ß) vs. $1000/T_{peak-2}$ of combustion of the organic phase of deer antler tissue samples.

The values of the activation energy (E_a) calculated from the linear fitting of Kissinger's equation, and peak temperatures are summarized in the tables 1 and 2. Analyzing these results it can be observed that activation energy has a greater value for the non-irradiated sample (5.843 kJ/mole for the first peak and 7.243 kJ/mole for the second peak) than for the irradiated samples (3.587÷4.161 and 4.62÷6.911 kJ/mole for first and second peak, respectively). This can be related to the fact that gamma irradiation predominantly affects the collagen matrix, having in view that in the inorganic phase induces only paramagnetic defects by trapping an electron at a nonbridging oxygen vacancy [10].

It is worth to mention that the highest activation energy for the gamma-irradiated samples (4.161 kJ/mole for peak 1 and 6.911 kJ/mole for peak 2) is determined for the 4 years old deer antler and this can be related to a better mechanical and strength characteristics of this bone matrix compared with that of the younger or older bone.

4. Conclusions

Samples of different ages (3, 4 and 6 years) deer antler tissue, prepared in powder form, non-irradiated and gamma-irradiated were studied as potential biomaterials for bone regeneration. Kinetic analysis performed on results obtained from thermal measurements indicates that gamma sterilization did not affect structural stability of the material and has a greater impact on the organic phase of the bone-like antler tissue. The diminishing of the activation energy after gamma irradiation can be related to the effects of gamma radiation on the organic matrix. For gamma-irradiated samples the activation energy of 4 years old deer antler sample is greater than that of 3 and 6 years old deer antler, and suggests better mechanical and strength characteristics for 4 years old deer antler.

Acknowledgments

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