THERMAL STABILITY OF COLLAGEN FROM COW FEMUR BONES

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In the study of natural materials, the denaturation temperature of collagen is important for revealing the processes of maturation and of molecular defects linked with connective tissue diseases. Thermal stability tests usually measure the temperature at which the collagen macromolecule denatures from its hydrogen-bonded triple helical structure to a random coil [1]. In the present work, the evolution of the thermal denaturation of collagen from cow femur bones is studied through coupled studies of scanning electron microscopy (SEM) and dynamic mechanical analysis (DMA). It is shown that SEM studies are mandatory for analyzing the results from any kind of collagen thermal stability mechanical tests. A FEI Quanta 200 scanning electron microscope operated at 15 kV, under vacuum, was employed to characterize the bone samples. Coating for samples was not used in SEM studies. DMA measurements were performed at frequencies close to 1 Hz, as a function of temperature, in torsion, under pure Ar atmosphere at standard pressure. The heating and cooling rates were of 1K/min. The maximum shear strain of the sample was 2×10^{-4}. Parallelepiped samples were cut with a jeweller saw longitudinally to the femur bone. Results from SEM and DMA exhibit differences between different samples, however, the results shown in this work represent the general trends of the whole set (seven) of studied samples. Figure 1 shows the tan(ϕ) spectra and the behaviour of the square frequencies (proportional to the elastic shear modulus) for a sample during two consecutive warming runs. During the first warming up to 420 K, the following peaks in tan (ϕ) and their peak-temperature were measured, which are in agreement with previous works [2]: The melting of iced water (273 K), the denaturation of albumin and haemoglobin proteins and also the melting of fats (320 K), the loss of water (350 K) and the loss of crystallization water (390 K). However, it should be stressed, that the cortical part of the femur also exhibits the peak related to the loss of water at around 350 K, which was absent in the cortical part from cow ribs bones [2]. In addition, the elastic modulus, exhibits a decrease up to around 320K followed by an increase which is promoted by the shrinkage of the mesostructure due to the loss of water [2]. During the second warming, the tan (ϕ) exhibits still some relaxation process at temperatures around 320 K, related to the partial restoration of collagen [2]. In addition, an intense peak in tan (ϕ) develops at around 500 K. The modulus reveals also a step down related to the relaxation process. The temperature of appearance of the tan(ϕ) peak at around 500K can be related to the thermally activated irreversible process of denaturation of the collagen triple helix, which involves the uncoupling of the chains leading to the helix $\rightarrow$ random coil (THRC) transition [1, 3]. Infrared spectroscopy (IR) studies performed in the same bone samples (thermally treated) also reveal changes in the intensity of the amide groups of collagen, between around 1700 cm^{-1} and 1200 cm^{-1}, which are in agreement with the mechanism above proposed for the tan (ϕ) peak at 500 K. In addition, IR confirms that changes in the apatite did not appear for warming the sample up to 640 K. Nevertheless, the THCR transition is not the unique-thermodynamic effect which must be considered from the mechanical point of view when bone samples are warmed. Indeed, the change in the morphology of the bone as a function of temperature is a crucial point often not considered in collagen thermal stability mechanical tests. Figure 2 shows a SEM micrograph for a cortical femur sample in the fresh-state, i.e. prior to start the thermal treatments. SEM micrographs reveal the loss of water as the temperature increases up to

420 K due to the compactness of the mesostructure, Figure 3; which results in agreement with previous works in cow ribs bones [2]. In addition, a further increase in temperature up to 640K, leads both to a further compactness of the mesostructure and to the appearance of large cracks at mesoscopic level. Consequently, it should be pointed out that the relaxation phenomenon at around 500 K is promoted by both the transition of the collagen helical structure towards a random coil and the appearance of large cracks due to the shrinkage of collagen.

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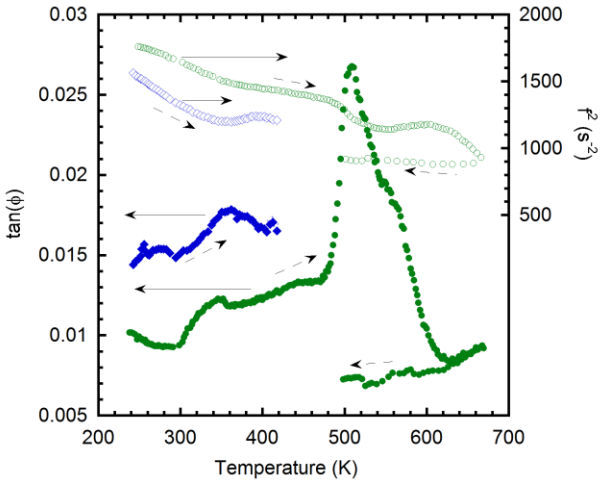

Figure 1: tan(ϕ) and square frequency (f^2) for the cortical part of cow femur sample. Rhombuses: warming up to 420 K. Circles: warming up to 640 K.

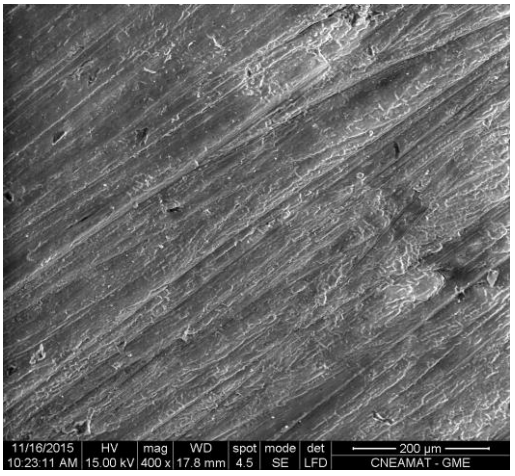

Figure 3: SEM micrograph for a cortical femur sample after warming up to 420 K.

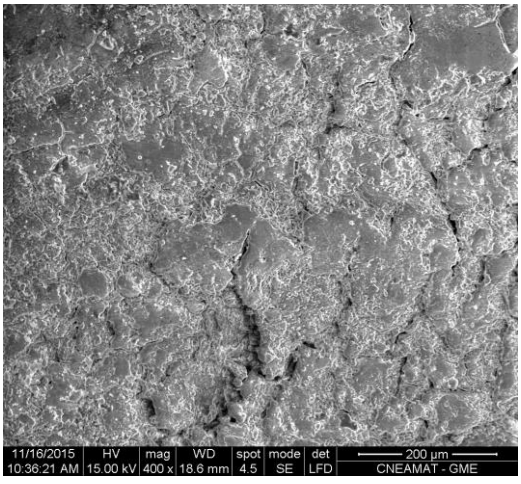

Figure 2: SEM micrograph for a fresh cortical femur sample (prior to warming).

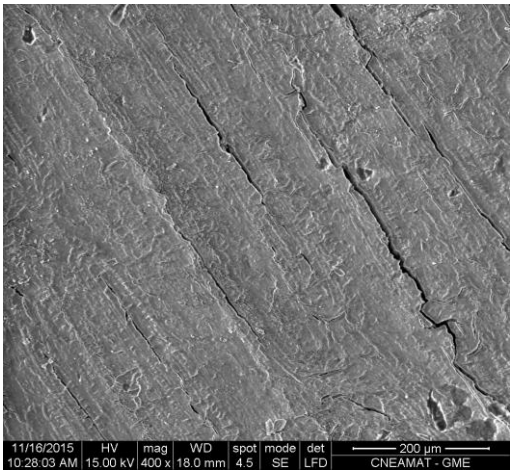

Figure 4: SEM micrograph for a cortical femur sample after warming up to 640K.