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TG-MS CHARACTERISATION OF PIG BONE IN AN INERT ATMOSPHERE

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A challenge for forensic examiners is the ageing and characterisation of bone fragments or decomposed skeletal remains. Due to the sensitivity of thermal methods to morphological states, thermal analysis has been selected as a technique which could overcome the difficulties. In this preliminary study, TG-MS was applied to the characterisation of bone fragments derived from the compact bone of pig rib specimens. TG-MS curves were collected by heating bone samples to 1000°C in an argon atmosphere. Under these conditions, both the organic and inorganic phases decomposed, producing a variety of organic fragments and carbon dioxide. Pyrolysis of the organic phase, which is composed predominantly of collagen, occurred resulting in the observation of ion fragments up to 110 amu. Selected fragments were monitored and their observation is discussed in terms of the decomposition of both the collagen phase and the inorganic carbonated hydroxyapatite phase.

Keywords: *compact pig bone, TG-MS*

Introduction

Bone is a complex composite material consisting of approximately 10% water, 30% organic phase (mainly collagen fibrils) and 60% inorganic material (predominantly carbonated hydroxyapatite). The chemistry of bones can provide a record of their history and have been well studied for archaeological samples. However, the structures of lesser aged bones, such as those that are encountered in a forensic context, have not been as widely studied. The dependence of bone properties on environmental factors has made the problem of estimating time since death more complex. A range of experimental techniques have been employed in studies to examine the ageing of bones, including electron microscopy [1, 2], X-ray diffraction [3] and infrared spectroscopy [1, 4], as well as thermal analysis [5–7].

Thermal analysis can provide valuable information regarding the organic and mineral content of bones. Thermal methods have been used for the characterisation of bone in several studies, having been applied to the characterisation of bone from various species [8, 9], the dating of archaeological [5, 6] and forensic remains [7] and to the characterisation of both the organic and inorganic phases for the development of synthetic analogues [10]. The current investigation focuses on the application of thermal analysis to the characterisation of pig bone specimens of known history. Thermogravimetric analysis (TG) coupled with mass spectroscopy (MS) for evolved gas analysis (EGA) was employed for this study. In order to use TG-MS as a characterisation tool for the decomposition of aged specimens, it is first necessary to identify suitable conditions for the thermal analysis. This paper reports the TG-MS analysis of the decomposition of a single specimen of compact pig rib bone with the intention of characterising a series of bone specimens on known age for later publication.

Experimental

Samples of pig rib bone were provided by the Centre for Forensic Science at the University of Western Australia from the remains of an ongoing research project on the environmental effects of tissue decomposition. The specimen of rib bone used in the current study was recovered from a 45 kg female pig slaughtered and conditioned as 'surface deposited' by placing the carcass directly onto the soil surface in open air for five years. The carcass was protected from animal scavengers by fencing. Compact bone samples were prepared by cutting the shaft of the rib bones in transverse segments. The compact bone specimens were cleaned only by scaping of the surface with a scalpel to remove fatty bone marrow from the interior and any residues from the exterior of the specimens.

The bone specimens were then examined by TG using a Setaram Setsys 16/18 thermobalance coupled with a Balzers ThermoStar mass spectrometer for EGA. Experiments were carried out by placing ap-

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proximately 15–20 mg of the bone sample into a platinum crucible and heating at a rate of 10°C min^{-1} from ambient temperature to 1100°C under flowing (20 mL min^{-1}) high purity argon gas. Temperature calibration was carried out using indium, tin, aluminium, gold and silver. Baseline curves measured under the same experimental conditions were acquired to account for buoyancy effects on the balance.

Initially, mass spectra were acquired over the full range of the mass spectrometer up to 300 amu. As each full scan covered approximately 3 min (or 30°C), a limited number of mass units were selected for analysis in this paper. The peaks in the spectra chosen for analysis were at 18, 28, 30, 32, 44, 54, 64, 67, 70, 72, 78, 81 and 91 amu. A second acquisition time for each mass unit was set, thus requiring 0.13 min (or 1.3°C) for each cycle.

Results and discussion

Typical mass loss data for the thermal decomposition of bone in an inert argon atmosphere is shown in Fig. 1. The mass loss occurs in three distinct steps. Step 1 is observed from ambient temperature to approximately 230°C with a peak at 100°C and a mass loss up to 230°C of 8.8%. Step 2 occurs in the range 230 to 560°C. This step appears to be more complex with a major peak observed at 345°C. A shoulder on this peak is observed at approximately 420°C. The mass loss between 230 and 650°C is 21.9% of the original mass. The third step occurs between 760 to 1050°C with the peak at approximately 900°C. The mass loss in this latter temperature range was measured to be 7.3% based on the original mass, giving a total mass loss of 38.0%. The thermal oxidation of this bone specimen in an air atmosphere produced a total mass loss of 41.2 with 27.4% removed in the step 2 region and only 2.5% removed in the step 3 region.

In an oxidising atmosphere the organic phase is expected to be removed up to 600°C. The mass loss of step 3 for the air atmosphere can therefore be attributed to decomposition of the inorganic phase, notably the removal of carbon dioxide from the carbonated apatite, as has been observed elsewhere [9, 11, 12]. Given the latter assumption, an estimate of the water content (9 to 11%) corresponds to the mass loss in step 1, organic phase corresponds to the mass loss in step 2 of the decomposition carried out in an air atmosphere (≈27%) and the inorganic phase content corresponding to mass loss up to the end of step 2 (≈61%). The carbonate content (CO_3^{2-}) can be taken as 1.36 times the mass loss in step 3 (assuming the dehydration of the inorganic phase is complete by this time) and corresponds to 3.4%. Similar values have been observed for cortical bone in Sprague–Dawley rats (trabecular bone 2.3% CO_3^{2-} and 62.0% inorganic phase and cortical bone 3.8% CO_3^{2-} and 66.4% inorganic phase) [9] and for cod clytherum, deer antler and whale periotic fin bone 4.2, 4.2 and 3.6% CO_3^{2-} and 65.3, 66.0 and 64.7% inorganic phase, respectively [8], although in the latter case a number of high mass% inorganic component bones were also investigated. In the inert atmosphere, the organic phase pyrolyses resulting in less mass loss in step 2 and greater mass loss in step 3 associated with the decomposition and evolution of the pyrolysis products.

EGA of the volatile products was carried out using mass spectrometry for the thermal decomposition in an inert atmosphere. Initially, the selection of the appropriate mass to charge (*m*/*e*) ratios for analysis was necessary and was achieved by scanning from *m*/*e*=2 to 300 amu as a typical TG run was being carried out. Figure 2 is a three dimensional plot of the spectrometer response during such a heating experiment to 1100°C. The data in Fig. 2 is limited to 120 amu as no measurable response was observed above 120 amu. A selection of scans from this data corresponding to each mass loss step is shown in

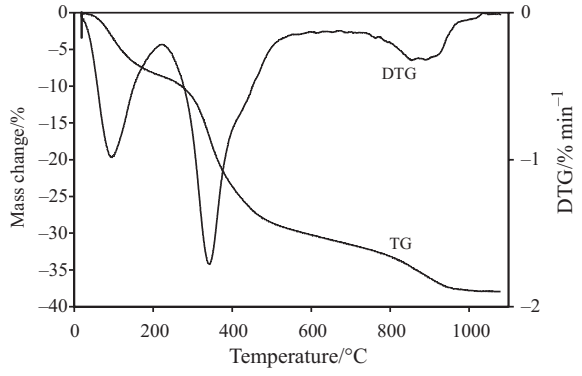

Fig. 1 TG and DTG curves for the decomposition of compact pig rib bone in an argon atmosphere

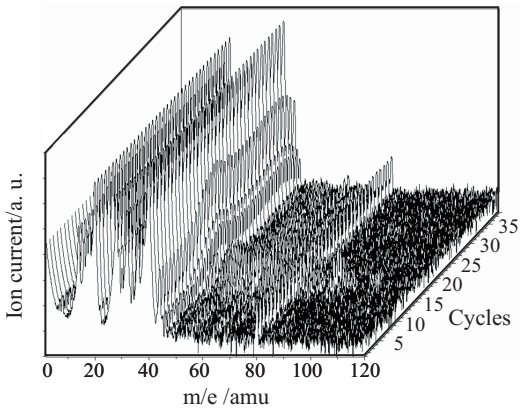

Fig. 2 Ion current, as a function of the *m*/*e* ratio in amu cycled through from 2 to 300 amu over the full temperature range (RT to 1100°C). Only *m*/*e* ratios up to 120 are shown as no observable ion current was measured above this value

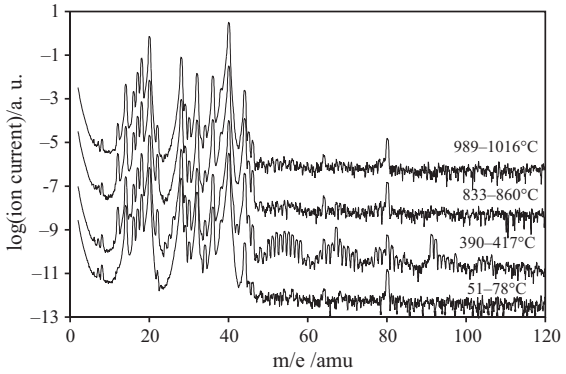

Fig. 3 Selected scans taken from Fig. 2 showing the *m*/*e* ratios in the range 2 to 120 amu coinciding with the three mass loss steps. The bottom curve corresponds to the second cycle and is representative of the background ion currents

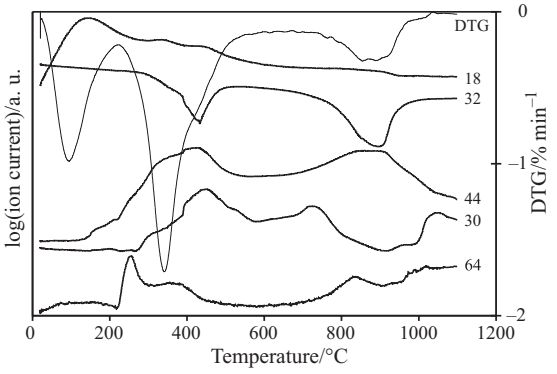

Fig. 4 Temperature dependence of the ion currents for ion fragments containing hetero atoms. Possible fragment species are listed in Table 1

Fig. 3. Based on this data a number of *m*/*e* peaks were chosen as representative of the decomposition products observed in each step. Peaks chosen for analysis were at 18, 30, 32, 44, 54, 64, 67, 70, 72, 78, 81 and 91 amu. Plots of ion current for these species are shown in Figs 4 and 5. In each figure the DTG curve is included for comparison. Figure 4 contains the evolved species associated with hetero atoms or small molecules. Figure 5 contains data which is predominantly associated with organic volatile products. Table 1 lists possible molecular identities of these evolved species; assignments were based on the molecular mass and base peaks reported in the literature for the pyrolysis of a variety of organic matter [13–17] and are, therefore, tentative.

The first mass loss (step 1) in the decomposition curves of bone in an inert atmosphere occurs around 100°C and is associated, based on the 18 amu peak in

Table 1 Evolved gas analysis

Fragment mass/amu	Molecular formula	Possible molecule	References
18	H_2O	water	
28	N_2	nitrogen	
30	NO	nitrous oxide	
	CH_2O	formaldehyde	
	C_2H_6	ethane	
32	O_2	oxygen	
44	CO_2	carbon dioxide	
54	C_4H_6	butadiene, cyclobutene, butyne	
	HCCCHO	propynal	
	C_3H_5N	propane nitrile	[17]
64	SO_2	sulphur dioxide	
67	C_4H_5N	crotonitrile, allyl cyanide, methacrylonitrile, cyclopropyl cyanide, pyrrole	[13–15]
	C_6H_{10}	4-methyl cyclopentane	[14]
70	C_5H_{10}	pentene, cyclopentane	
	C_4H_6O	butenal	
72	C_5H_{12}	pentane	
	C_3H_4O	acrylic acid	
	C_4H_8O	butanal, butene-ol	
78	C_6H_6	benzene	
81	C_5H_7N	methyl pyrrole (2- and 3-)	[13]
	C_7H_{12}	methyl cyclohexane	[14]
91	$C_2H_5NO_3$	2-nitroethanol, ethyl nitrate	
	$C_6H_5CH_3$	toluene	
	$C_6H_4(CH_3)_2$	1,4-, 1,3- and 1,2-dimethyl benzene	[14–16]
	$C_6H_5–C_2H_6$	ethylbenzene	

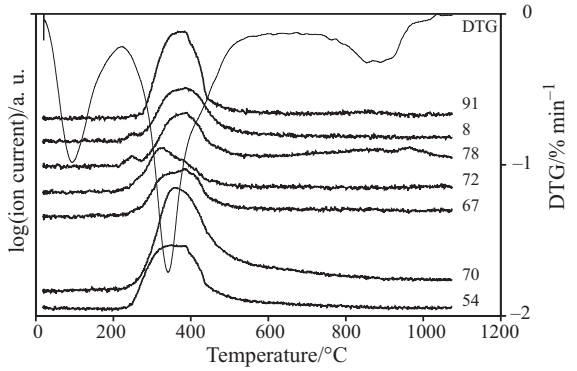

Fig. 5 Temperature dependence of the ion currents for ions corresponding to organic fragments. Possible fragment species are listed in Table 1

Fig. 4, with the loss of water, notably molecular adsorbed water. It is noteworthy that the 18 amu peak is at 150°C and, therefore, at slightly higher temperature than the step 1 DTG peak. This may be due to a delay in transfer through the capillary to the MS, although higher temperature peaks correlate well with the DTG curve. The shift may also be due to the onset of decomposition of the bone as the step 2 peak in the 44 amu curve is observed to begin around 130°C. Further heating results in the thermal decomposition of the bone in step 2 through a two sub-step process, observed in the DTG as a peak at 345°C with a shoulder at approximately 430°C. Two peaks associated with this step are observed in the 18 amu curve suggesting that water is a decomposition product. The 44 amu curve assigned to predominantly carbon dioxide, the 30 amu curve assigned to nitrous oxide, but may also be associated with organic fragments, the 64 amu curve associated with sulphur dioxide and the series of organic product fragment curves are also observed to have a peak associated with this decomposition step 2. Between the end of step 2 and the beginning of step 3 further heating results in a continued mass loss and is observed in the DTG curve as a minimum (the DTG curve does not return to zero in the 550 to 760°C region). In this minimum, 18, 30 and 44 amu fragments are continually evolved. Above 760°C step 3 occurs and coincides with EGA peaks at 44 amu (CO_2) and 64 (SO_2). Of the organic fragment curves, only the 78 amu curve (assigned to benzene) shows a distinct broad peak in this region.

Although the data of Figs 4 and 5 were recorded in an inert atmosphere, the purge gas still contains a small amount of oxygen (<10 ppm). This small contamination in the purge gas can be used to aid the identification of the origin of CO_2 evolution in step 3 as being due to the combustion of the organic pyrolysis products. Where peaks are observed in the 44 amu curve, corresponding negative peaks are observed in the 32 amu curve, corresponding to oxygen, suggesting that a significant portion of the steps 2 and 3 mass loss steps are associated with the removal of the organic phase. Although water loss is observed at a high temperature, it cannot be attributed in the same manner to the decomposition of the organic phase.

The 30 amu curve (Fig. 4) has been tentatively assigned to both the production of nitric oxide (NO) and formaldehyde. Both are possible products of the pyrolysis of proteins and amides. If these assignments are correct, then it is likely that the aldehyde would predominate at a lower temperature and NO at a higher temperature. Ethylene also has a *m*/*e* of 30 amu and could, therefore, also contribute to this evolution. The 30 amu curve has a number of peaks around 450, 730 and 1050°C with a series of shoulders associated with each peak. The 30 amu curve follows step 2 in the DTG, but with the magnitudes of the peak and shoulder reversed. It is also interesting to note the continued evolution of 30 amu species between steps 2 and 3 and the presence of the peak at 730°C which does not correspond to a peak in the mass loss data. This may be due to the occurrence of gas phase reactions, although it is notable that the DTG curve in this region does not return to 0% min^{-1}, but a significant and finite minimum indicating that material is continually lost throughout this region. Without further analysis, however, identification of the species responsible for this peak cannot be made with certainty.

Conclusions

In this preliminary study, TG-MS was applied to the characterisation of bone fragments derived from the compact bone of pig rib specimens. The aim of this initial study was to characterise the decomposition products of the bone fragments and interpret the TG-MS data. TG-MS curves were collected by heating bone samples to 1000°C in an argon atmosphere and, under these conditions, both the organic and inorganic phases decomposed producing a variety of organic fragments and carbon dioxide. Pyrolysis of the organic phase, which is composed predominantly of collagen, occurred resulting in the observation of ion fragments up to 110 amu. Selected fragments were monitored and could be associated with the decomposition of both the collagen phase and the inorganic carbonated hydroxyapatite phase.

By targeting changes in particular decomposition fragments, it may be possible to determine post-mortem age of a bone. The method shows promise as an approach to the study of bone ageing in a forensic context. Further TG-MS studies of bone of different ages are being undertaken. The bone samples will also be studied in different atmospheres in order to gain a more detailed understanding of the decomposition processes of forensic bones.

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