ACTA PHYSICA DEBRECINA XLVII, 95 (2013)

PRE-TREATMENT OF ARCHAEOLOGICAL BONE SAMPLES FOR ^{14}C MEASUREMENT IN THE HERTELENDI LABORATORY

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Abstract

Preparation of bone material for radiocarbon dating is still a subject of several investigations. Development of preparative methods requires sufficient amounts of bone material, proper sample pre-treatment methods as well as the possibility of verification of the gained ages. Here we document a chemical procedure and some results on international and inner comparison samples at the Hertelendi Laboratory of Environmental Studies (HEKAL), Debrecen. We also show results using the new MICADAS system and compare these results to internationally-recognized standards and internal blank materials. The results of ^{14}C measurements of comparison samples are not only suggestive for the reliability of the sample preparation system at HEKAL Lab but also good performance of the Environ MICADAS ^{14}C system.

I. Introduction

Bone, teeth and ivory are very complex sample materials which are routinely used in radiocarbon dating. During the last 60 yr, numerous bones of humans and animals have been dated and artefacts made of ivory and turtle shell also provide collagen-like material suitable for dating. Nevertheless, ^{14}C dating of bone collagen is challenging because its preservation is influenced by several environmental factors, including temperature, moisture, pH, and microbial activity [1]. Although bone mineral is partly made of carbonate-apatite containing carbon suitable for dating [2], separation of the organic fraction is the prevailing method for dating bone material and ivory at the moment. Bone organic matter could be altered physically and chemically through dissolved organic carbon and post-depositional incorporation of humic substances into the bone material due to either humification processes (Maillard reaction) occurring in the bone or an interaction with the burial environment. Humic acids migrating with groundwater might attach to the porous bone structure and build cross-links within the collagen [3]. These contaminants could cause ^{14}C dates of bone collagen to be either too young or too old depending on the variations of local environment and soil chemistry. Therefore, the main focus of the treatment methods for ^{14}C dating is on the removal of such contamination.

Dried, defatted fresh bones contain about 20% collagen. The organic fraction is often described as "collagen" or protein remnants, i.e. the remainder after the dissolution of the mineral part. The most common protocol to extract collagen is the method described by Longin [4]. In this procedure collagen is defined as a gelatin-like, acid insoluble remnant that remains after the carbonates precipitated at the surface of the bone are removed in the acid step of dissolving the mineral fraction. Theoretically, this treatment produces total bone organic matter that includes untwisted triple-helical gelatin-like collagen molecules. However, humic substances absorbed by bone cannot be completely removed through weak acid treatment. An improved protocol adds a weak base solution (0.1–0.125M NaOH) to remove humic acids, and then uses near-boiling (95°C) weak acid (pH 3) to solubilise bone collagen [5]. Although this protocol provides collagen that generally yields more accurate ^{14}C dates, NaOH decreases collagen yields, and does not completely remove humic contaminants [6-8]. Refined molecular compound-specific methods of

purification of collagen have been developed, including ninhydrin derivatisation [9], ion-exchange techniques [10], the isolation of single amino acids (usually hydroxyproline) [11-13], collagenase digestion [14], and isolation of tri-peptides [15]. Other methods involving the removal of the light humic fraction (<30 kD) using ultrafiltration or cleaning protein remnants in ion-exchange columns [15-16] showed that a separation of collagen-specific peptides obtained from collagenase cleavage yields organic material that can be separated using chromatography (HPLC) and then AMS ^{14}C dated. The most effective method of pre-treatment for accelerator mass spectrometry (AMS) dating has been a subject of continual research over more than 20 yr since the method was first used.

Due to the installation of the new Environ MICADAS AMS facility at Hertelendi Laboratory of Environmental Studies (HEKAL) many, new sample preparation lines have been constructed and tested since the year 2011. In this paper we present the preparation system and method of our laboratory adapted to AMS preparation of archaeological bone samples and we discuss the first ^{14}C results of some comparative measurements.

II. Experimental methods

II.1 Physical and chemical pre-treatment of the samples

In the Hertelendi Laboratory of Environmental Studies, we have experience of several years in the preparation and ^{14}C measurement of bone samples. Initially, we prepared samples for gas-proportional counting (GPC), but due to the low concentration of organic material in bone, the GPC measurement required at least 50g of initial sample amounts and the measuring time was also relatively long (7 days).

In the case of our AMS bone preparation technique first, the sample is placed in an appropriate size beaker cleaned prior with gas nitrogen blowing. Afterwards the beaker is filled with distilled water and placed into an ultrasonic machine pan. The bone samples contaminated with dirt can easily pollute the soaking water so decanting steps are repeated until water is no longer cloudy or dirty. When the sonicating steps are done the remaining water is decanted off the sample and the baker is placed in a drying oven at 50°C for an overnight.

Next day, after the bone has dried out its entire exterior is removed using a Dremel® drill device. Meanwhile a clean mortar, pestle and a clean set of small sieves (0 – 1mm sieves) are prepared for grounding of the sample. When drilling has been done, crushing of the larger bone shards with mortar and pestle begins. This is a repetitive process, crushing all the bone at once is forbidden since particles with powder size can clog the Teflon filters used in the next step, but the extraction of the collagen at high temperature is more difficult from large pieces. The 0,5 – 1,0 mm fraction is used for the bone cleaning process and generally 500-1000 mg, but in extreme situations 300 mg of these grains are weighted into glass columns.

In respect to the chemical cleaning of the samples the well known ABA (acid-base-acid) process has been applied. A continuous flow bone sample preparation line (Fig. 1.) has been developed which is similar to that one used at Oxford Radiocarbon Accelerator Unit, Oxford, England (ORAU). In our unit Omnifit ™ glass columns are used as flow cells to automate the ABA cleaning system [8]. From 3 types of reagents, each one is injected via a 4 way valve and inert plastic tubing to an Ismatech™ IPC 12 channel peristaltic pump ensuring a constant flow rate. Reagents are selectively pumped into the reaction cells containing small grains of bone samples, with a sequence of 0.5 M HCl and 0.1 M NaOH solution, interspersed with flushing by distilled water. At the end of the process, the reagents together with the dissolved contaminants are accumulated using a collection bottle for each cell. During the seventeen-hour-long process, reagents follow a well-defined sequence that is controlled by a computer program and a special electronic driver device.

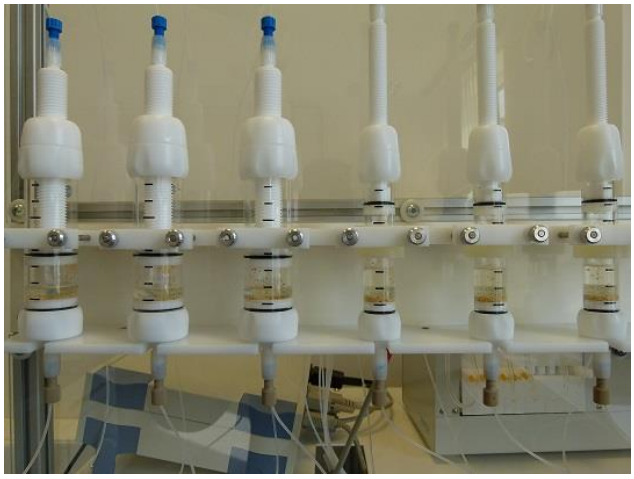

Fig. 1. Automated bone ABA cleaning system made by ATOMKI

After setting of the pH, the cleaned sample is transfused into a clean test tube containing 5 ml aqueous solution, and it is placed into a heating block at 75°C for 24 hours. Dissolved collagen/gelatine is filtered via a syringeless, 0.45 μm glass fibre filter (Whatman™ AUTOVIAL 5) into a pre-weighted clean vial in order to remove the insoluble residue. After a 5 hour-long freezing step in a commercial freezer, the gelatine sample is freeze-dried at the vacuum of 10^{-1} mbar which takes about 1-2 days.

II.2. Combustion and graphitisation

For the combustion of the samples, a system similar to the one used at the University of Arizona has been applied [17]. Collagen can be combusted either in the presence of CuO or O_2 gas. In the case of CuO combustion the temperature of 1100°C necessary for the oxidation is produced by a gas torch. A Watlow® tube oven with a precision temperature controller is used for heating during the stepped combustions. To check the combustion process, a vacuum-tight stainless steel Swagelok® manometer is used. The gas is passed through a tube section kept at 1000°C and filled with quartz pearls (Fig. 2/2) ensuring the complete conversion of CO to CO_2.

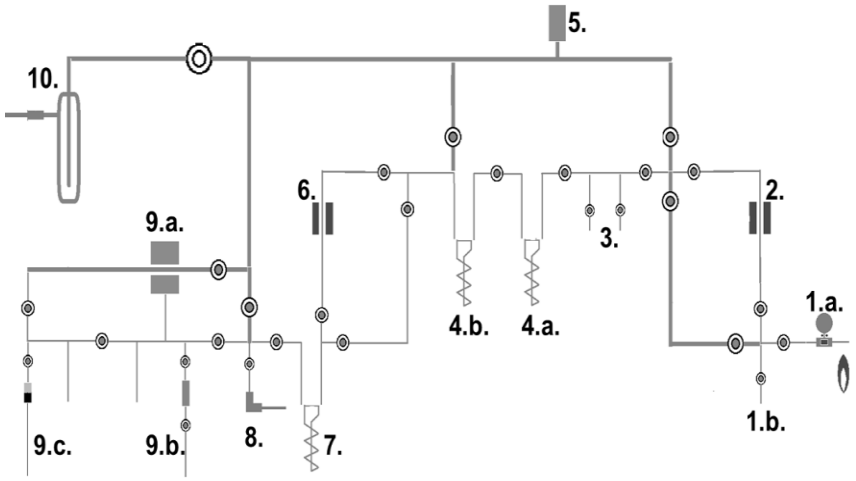

Fig. 2. Scheme and main parts of the on-line combustion and CO_2 purification line, adopted from UA.

(1.a/b: combustion cell, with manometer and oxygen gas inlet; 2: afterburning furnace; 3: off-line produced CO_2 sample inlet valves; 4. a/b cold/freezing traps; 5: WRG Edwards pressure gauge; 6: cleaning furnace with Cu/Ag; 7: cold trap; 8: pre-cleaned CO_2 sample inlet (like OxII gas or blank CO_2 gas); 9.a: known volume with pressure sensor; 9.b/c: cleaned CO_2 sample storage vessel/tube; 10: high vacuum pumping with lN_2 trap. All the round symbols are Kotes glass valves)

The quartz oven is followed by two spiral cold traps with a functional length of ~500 mm. The first trap (Fig. 2/4.a.) is cooled by a mixture of isopropyl-alcohol and dry ice to -78°C, in order to remove the water vapour and nitrogen dioxide generated in the combustion process. The next cold trap (Fig. 2/4.b.) is cooled by liquid nitrogen to -196°C and is used to freeze out the revealed CO_2 and to pump away the waste gases. Gases frozen out at -196°C pass through a catalyst oven (Fig. 2/6) operating at 500°C and filled with elemental copper and silver to eliminate sulphur, nitrogen oxides and halogens. After the oven, there is a second -78°C trap to remove the water vapour generated during the reduction process. The determination of the quantity of

the final CO_2 gas is performed in a calibrated volume using an MKS Baratron pressure gauge (Fig. 2/9a). The calibrated volume can be split into two equal parts. One half of the sample gets graphitised (Fig. 2/9.b.), while the other half gets reserved in a sealed glass tube (Fig. 2/9.c.).

All the graphite targets were prepared by a sealed tube graphitisation method in HEKAL [18]. Amounts of the reagents and catalyst used were kept constant, independent of sample size, using 10 mg titanium-hydride (Alfa Aesar, #012857), 60 mg zinc (Aldrich, #324930), and 4.5 mg iron powder (Aldrich, #20,930-9). During the pre-treatment process, the reagents and the iron catalyst are weighed into the reaction tubes which are then kept at 300°C for 1 hour. After the transfer of the CO_2 gas and sealing of the reaction tubes, the graphitisation process consists of two steps: (1) 3 hours annealing at 500°C to release the hydrogen and reduce the iron powder, (2) 5 hours regular graphitisation process at 550°C.

All of the ^{14}C measurements reported below were performed by our Environ MICADAS AMS at the Hertelendi Laboratory [19]. The time and conditions of the measurements were set to collect at least 200,000 net counts for every single target in case of a modern sample. The overall measurement uncertainty for a modern sample is <3‰, including normalisation, background substraction and sample preparation counting statistics.

III. Results of the measurements

To assess bone- and collagen-extraction procedures at HEKAL, we studied known ^{14}C-age bone samples that had been previously analysed earlier at the NSF ^{14}C AMS Facility, Tucson, AZ, USA [20] using different sample preparation techniques.

Estimating the background of the bone preparation method fossil CO_2 gas standards were measured in parallel with real bone background samples. These background fossil mammoth and bison bones in good condition were found in a stone mine close to Budapest. The collagen yield ranged from 13% to 15%, which is very good regarding bones with such old ages. In order to present a more representative and accurate picture, the results are given in pMC value

instead of BP ages. It can be seen that the results of the bone blanks are in the same pMC range with the values of fossil CO_2 blanks (Fig. 3.).

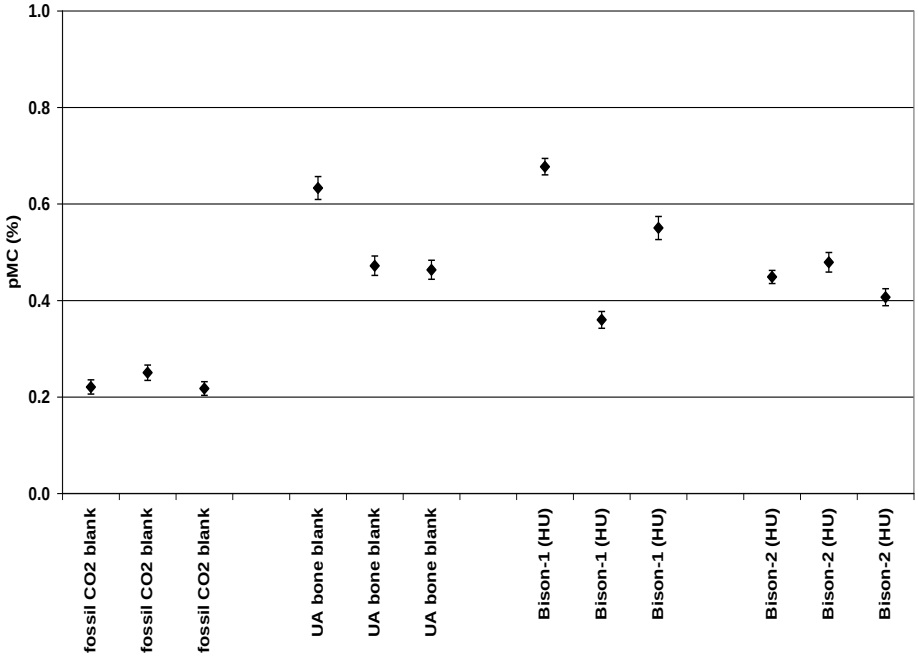

Fig. 3. Comparison of the results of fossil CO_2 gas and real bone blank samples.

In order to measure the ^{14}C contamination contribution to the results originating from the AMS preparation and analyses, an old (10 kyrs BP) *Dent Mammoth* bone sample [20] was prepared and analysed several times. The results (DeA-codes in Fig. 4.) are in excellent agreement with the earlier published NSF results (AA- code in Fig. 4.). This confirms that the ^{14}C contamination level at our laboratory is insignificant for bone samples of ~0.5 g. In this case the results are given in BP ages.

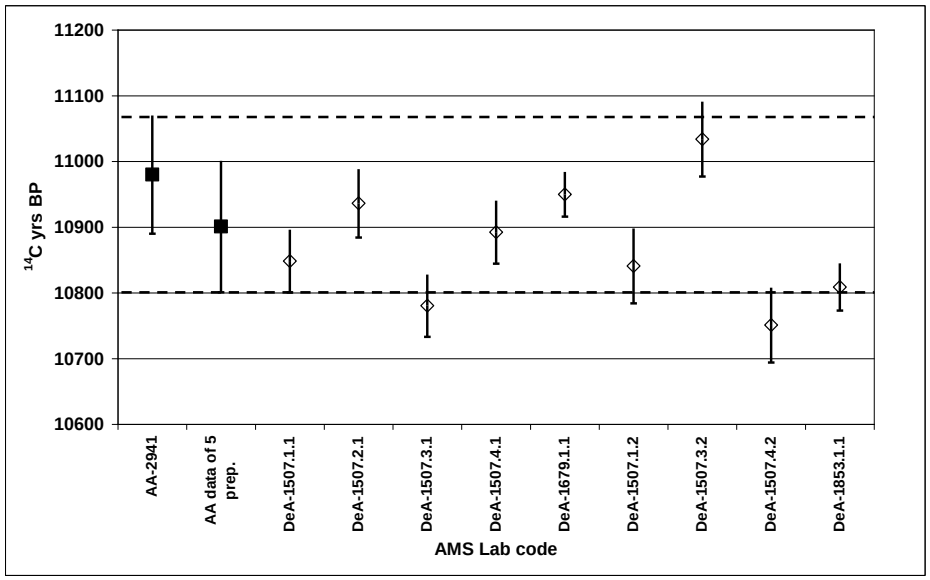

Fig. 4. Comparison of NSF (AA-) and HEKAL (DeA-) results for the same old mammoth bone (Dent Mammoth Bone).

To investigate the sample-preparation reproducibility a known-age bone sample, previously dated by GPC at HEKAL, was prepared several times for AMS ^{14}C analyses (0.5 g bone for each preparation). The obtained results (Fig. 5.) show very good reproducibility and excellent agreement within the given errors.

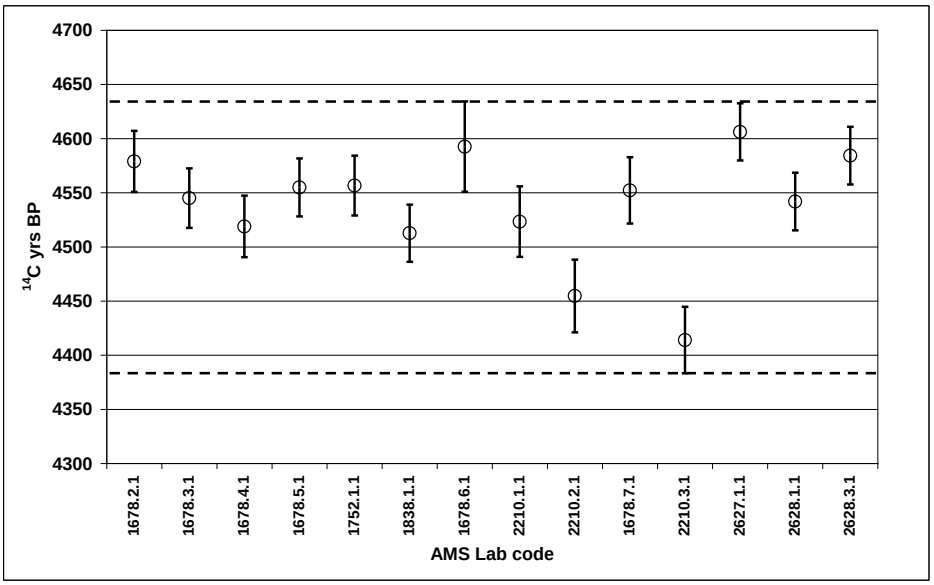

Fig. 5. Reproducibility test using an AMS dated bone sample of HEKAL.

IV. Conclusions

In this paper we presented a preparation system and method of our laboratory adapted to AMS preparation of archaeological bone samples. We have prepared and measured numerous international and inner laboratory standard and blank bone samples. With respect to the results of the standards excellent agreement was obtained with the earlier published NSF results. This confirms that the ^{14}C contamination level at our laboratory is not significant for bone samples of ~0.5 g or more. The inner standard results show very good reproducibility and excellent agreement within the given errors. Future plans include the development of this method with the elaboration of pre-treatment of inorganic bone fraction (bioapatite) for ^{14}C measurements.

V. Acknowledgments

These infrastructure developments were financed by Isotoptech Ltd. (Debrecen, Hungary) and the Hungarian Academy of Sciences and were performed in part under the New Hungary Development Plan Project No. GOP-2.1.1-09/A-2009-2008 and GOP-1.3.1-09/A-2009-0032. The research was also supported by the Hungarian NSF and National Development Agency (ref code: OTKA MB08-A 81515). The work/publication is supported by the TÁMOP-4.2.2/B-10/1-2010-0024 project. This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.

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