UNIVERSITY **POLITEHNICA** OF BUCHAREST

Faculty of Applied Chemistry and Materials Science

DOCTORAL THESIS

Summary

Aplicațiile fizicii nucleare în datarea și caracterizarea materialelor

Applications of nuclear physics in dating and characterization of materials

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TABLE OF CONTENTS

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INTRODUCTION

Since the discovery of the atomic nucleus by Ernest Rutherford in 1911 so far, the huge progress made in the field of atomic and nuclear physics led to the development of techniques and methods of analysis that have stimulated multiple research in various fields.

An important area that uses these techniques is radiometric dating. Based on the change in time of the isotopic composition of certain elements in the analyzed materials, following their radioactive decay, their age can be determined.

Radiometric data is the only way of quantitatively measuring geological times and provide an absolute time scale for the evolution of the solar system and Earth.

The appearance of Accelerator Mass Spectrometry (AMS) in 1979, brought together the most advanced techniques and methodologies in the field of nuclear physics, and has revolutionized radiological dating. Its great advantage is that it uses low amounts of sample and shorter measurement times. Moreover, using this method it is possible to determine concentrations of isotopes with very long half-lives, which was impossible by the radioactive decay method.

In this context, many radiocarbon dating centers have appeared, and the artefact dating is done with very good accuracy so that objects up to 60,000 years old can be dated under certain conditions.

In 2012, a compact AMS system based on a 1 MV Tandetron, and purchased from High Voltage Engineering Europe (HVEE), the Netherlands, was installed at IFIN-HH and the activities specific to the development of the first radiocarbon dating laboratory from Romania (RoAMS) were started.

This doctoral thesis is structured in 5 chapters; it begins with a brief introduction and ends with general conclusions, original contributions and further development perspectives.

Chapter 1 summarisez archaeological empirical methods used by archaeologists to obtain the age of a artefact. Radiometric dating methods based on laboratory analyzes are then presented, the most commonly used being the radiocarbon method. Because this method is the one used in the studies presented in this thesis, this chapter includes useful information that were essential for obtaining reliable and high accuracy radiocarbon dates.

Chapter 2 describes the equipment, installations and methods used, emphasizing important aspects in their mode of operation so that the obtained results meet the requirements for which they were chosen. These aspects may include avoiding sample contamination in the chemical pre-treatment process and during the measurements, eliminating carbon isotopic fractionation as much as possible, and correcting it if it has occurred. Also, the choice of apparatus and methods must ensure the highest possible carbon extraction efficiency of the sample in order to obtain sufficiently high currents in the AMS source.

Chapter 3 presents the first sample preparation made in the chemistry laboratory, established at the RoAMS center. Based on these samples, the chemical treatment protocols have been developed, established on literature studies and on multiple attempts to adapt to the conditions of the new laboratory. The success of these studies, theoretical and experimental, was materialized by obtaining the first dating results, with high precision and confidence, for the most common materials: bone, wood and coal.

Chapter 4 presents two useful case studies in which the samples to be dated are poorly preserved, either because they did not contain enough collagen, or they showed contamination with ^{14}C from the enviroment. In both studies, HPLC separation was used. In the first study, four of the most common amino acid standards used in radiocarbon dating were characterized, in order to determine how the use of HPLC influences isotopic carbon fractionation, for the first time, a correlation between the age obtained by radiocarbon (both directly and using HPLC) and how each amino acid is produced was made. The second study consisted in dating some osteological materials, which presented difficulties in assigning the age, based on the dating of the constituent amino acids. A comparison was made between the data obtained on amino acids (mainly hydroxyproline, but others , to less studied) compared to raw collagen.

In Chapter 5, the study of the conservation degree of bone collagen using nuclear magnetic resonance (NMR) was described. The results showed that this nuclear technique is useful for this

purpose and the approach of future studies using directly solid samples (without a pretreatment) for NMR analysis will bring important information regarding not only the degree of conservation, but also the collagen degradation during pre-treatment procedures performed in the process of preparing bone samples for ^{14}C dating through AMS.

The originality and complexity of the research allowed that the obtained results to be presented at conferences and symposia. Thus, the results of the studies obtained in this thesis as well as the subsequent studies, were presented at 38 national and international conferences and symposia, 7 articles were in ISI-listed specialist journals and others were the subject of some chapters in 7 specialized books.

1. Radiocarbon dating in the context of dating with other nuclear techniques

Archaeological discoveries provide important information that can be used to understand how human relations worked and how human settlements developed in the prehistoric times or in regions or groups of people that were not mentioned in historical writings. Archeology analyzes the remains of ancient cultures which are most often found in the soil, but also on its surface or under water. Usually the consist of artifacts (whole objects or fragments) and in by-products (rubble, ash, coal), the latter coming from the destruction of structures built in ancient times.

Archaeologists have two main ways of determining the chronology of artifacts, namely the use of relative dating methods and absolute dating methods.

Relative dating methods evaluate the age of an artifact by reporting and comparing it to other objects found in its vicinity. They determine only the sequential order of past events, but do not determine the period time in which they occurred.

The stratigraphic method is the first and most important method of relative dating, which is applied in the archaeological excavation stage itself. When an archaeological site is excavated, the sides show the stratification of the settlements. The fossils and sedimentary rocks, are placed in a vertical order from the oldest to the newest.

The typological method is based on the fact that the style of an artifact changes over time, although its function remains the same. With the help of the typological method, a scheme is drawn up that establishes the chronological sequence of artifacts from simple to complex.

If the two methods have not yielded conclusive results, or for additional information, other relative methods such as the comparative, cross or cartographic methods may be applied.

A more accurate dating system in archeology is known as absolute dating, and it can assign a calendar interval to the studied object.

Among the absolute dating methods we can list: radiometric techniques (carbon radioactive method, uranium series dating, potassium-argon 40 method, thermoluminescence dating), dendrochronology, palynological method (pollen analysis), paramagnetic resonance method, etc. Absolute dating is largely dependent on laboratory analysis.

1.1. Radiocarbon dating

Following studies of the influence of cosmic radiation on particles in the atmosphere, Willard Frank Libby discovered that the neutrons for cosmic radiation interact with the nuclei of nitrogen atoms in the atmosphere transforming they into ^{14}C. The observation that all living things incorporate ^{14}C from carbon dioxide in the atmosphere through photosynthesis or through the food chain, has led to the development of the radiocarbon dating method.

With the end of nutrition processes (death of organisms) the exchange of ^{14}C with the external environment stops, allowing the determination of the abundance of the remaining ^{14}C (following disintegration) in the body determine the time elapsed since its death.

When an organism dies, it no longer assimilates ^{14}C and its concentration decreases in time the law of radioactive disintegration where: A_0 and A represent the number of atoms of ^{14}C at time t = 0, respectively, remaining after a certain time t, Λ is disintegration constant which is equal to $\ln 2 / T_{1/2}$, (Fig. 1.1).

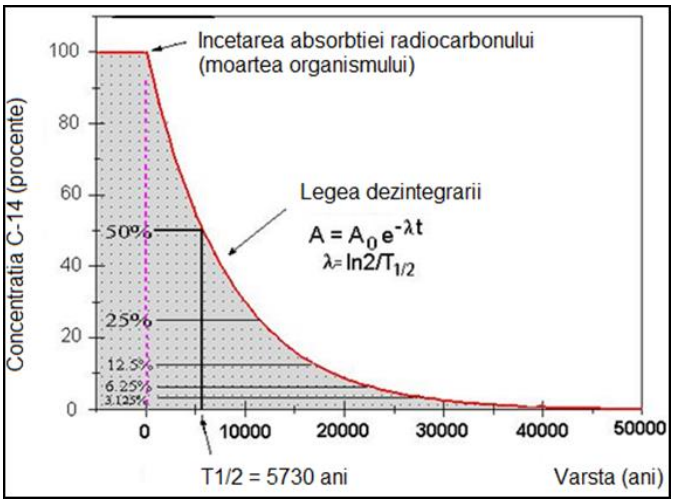

Fig. 1.1. ^{14}C dating principle

To date an archaeological sample, the amount of ^{14}C is determined and compared to the amount of ^{14}C contained in a standard material.

Measurement of the ^{14}C concentration in a sample is performed either using beta decay counting devices (the modified Geiger counter used by Libby itself, the proportional gas meters, with liquid scintillation, etc.), or the counting of atoms using the accelerated ions mass spectrometry method (AMS). The advantage of using AMS is that you can measure with very high accuracy only a few milligrams of sample in a very short time.

Samples ages ranging from several hundred years to 60,000 years can be measured. The materials that can be dated by radiocarbon are bone, wood, peat, organic sediments from lakes, remnants of plants and animals, coal, shells or corals.

The most famous applications of this method are the dating of the Turin Shroud, the manuscripts from the Dead Sea, and the determination of the period in which Ötzi the Man of the Glaciers lived [1-6].

2. The equipments and experimental methods used in radiocarbon dating

For samples taken from nature, chemical processing is required in order to be transformed into specific samples for AMS measurement.

Chapter 2 presents the equipment used in the steps of analyzing the concentration of carbon, nitrogen and the ratio C/N, in the process of graphitization and collagen separation by HPLC.

It summarizes both the description and operating principle of the devices and some issues that need to be taken into account during their operation to avoid contamination with foreign carbon and carbon isotopic fractionation.

Finally, the methods and experimental setups of NMR and AMS used for the characterization of bone collagen and for determining the ^{14}C content of the studied samples are briefly presented.

2.1. Elemental analyzer

The elemental analyzer used in the studies presented in this thesis is Vario MICRO Cube from the Elementar company (Fig. 2.1) in the CN working mode.

Fig. 2.1. Photo of the elemental analyzer type vario MICRO Cube

The analyzer is based on the catalytic combustion of the samples, the elimination of the components whose concentration do not interest, the separation of the desired components and the measurement of their concentration by thermal conductivity.

Because with this elemental analyzer the samples can be directly measured, i.e. as they are taken from nature, this system is very useful in determining the amounts of carbon in the sample before it is chemically pre-treated.

The diagram of the "vario MICRO" analyzer comprises four main units (noted in Fig. 2.2 with Roman numerals) [7]: mechanical input unit of the samples and the dosing system with O_2 (I); furnace area and reaction zone (II); component separation system of the analyzed gas mixture (III); detector (IV).

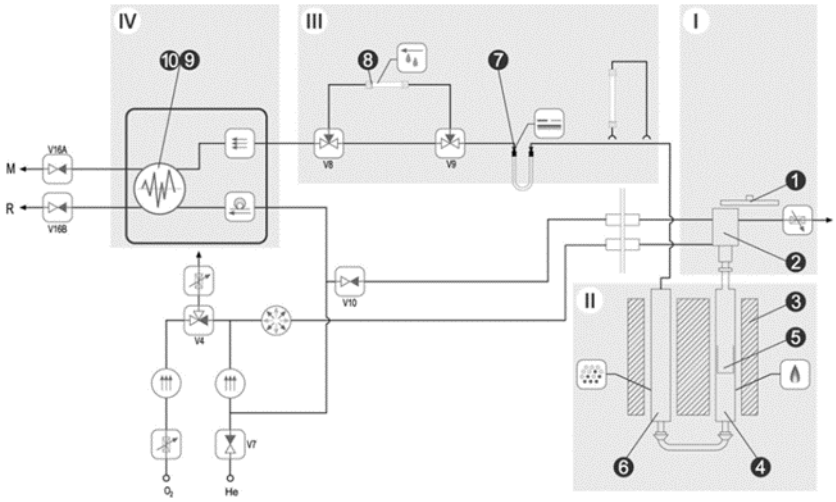

Fig. 2.2. The diagram of elemental analyzer and its functional units.

Unit I comprises the carousel (1) in which the samples from a series of analyzes are introduced and allows the transfer of the sample into the the ball valve (2). Unit II comprises the furnace (3), the combustion column (4), the ash crucible (5) and the reduction column (6). The component separation system of the gas mixture analyzed (unit III) comprises the adsorption column (7) and an additional adsorption tube (8). Unit IV is represented by the thermal conductivity detector (TCD) which consists of two enclosures: the measuring cell (10) through which the mixture of analytical gases passes and the reference cell (9) through which the helium passes.

2.2. Automated graphitization system

After combustion of the chemically pre-treated samples with the elemental analyzer, carbon dioxide must be converted into graphite in order to be measured by AMS. For this a fully automated graphitization system (Automated Graphitization Equipment 3) was used, built by L. Wacker et al. [8]. An photo of this graphitization system, used at RoAMS, is shown in Fig. 2.3.

Fig. 2.3. Photo of the automated graphitization system AGE 3

The diagram of the graphitization system AGE 3, coupled to the elemental analyzer and data processing system, is shown in Fig. 2.4. The graphitization procedure comprises four main stages: combustion of samples using the elemental analyzer (I), loading of the reactors (II), graphitization (III) and data processing (IV) [9].

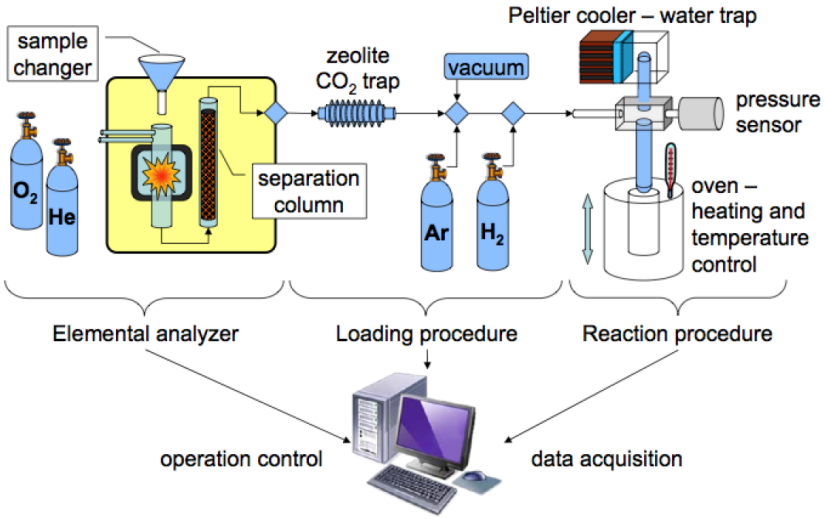

Fig. 2.4. Simplified operational scheme of the graphitization line [10].

2.3. The amino acid separation system by HPLC

For the separation of amino acids, the RoAMS Laboratory of IFIN-HH was equipped with a modular HPLC system from Shimadzu company. It contains an automatic module control system, autosampler, a solvent delivery unit, a chromatographic column, a UV/VIS photodiode detector, a fraction collector and a computer (for recording and data processing). The components of the HPLC system are enclosed separately in independent modules (Fig. 2.5).

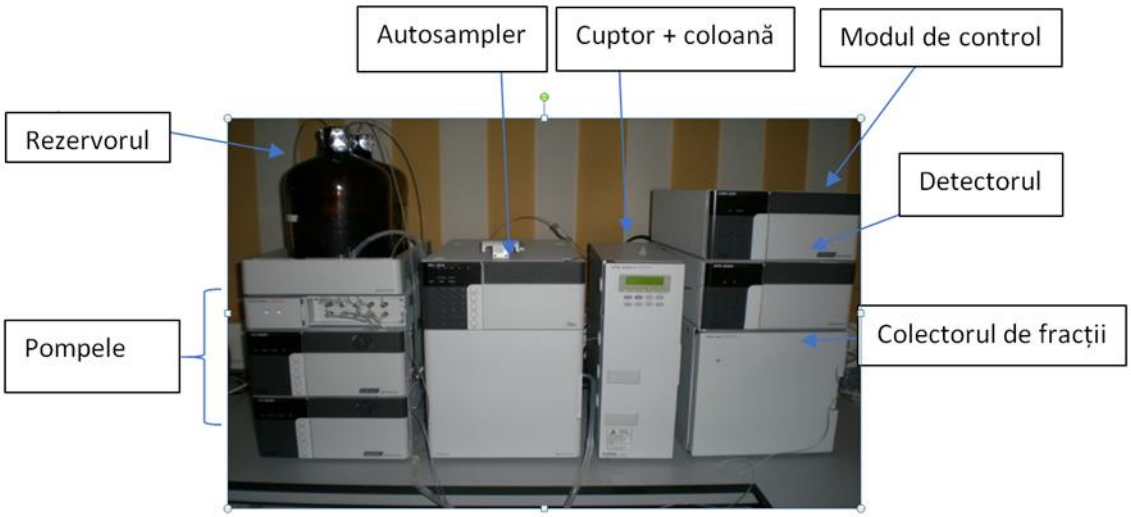

Fig. 2.5. Components of the HPLC system used to separate the amino acids.

The control system allows the control modules and provides information on their state of operation and maintenance. In order to increase the accuracy and a minimum waiting time, it was chosen that the solvent delivery unit to provide a circulating flow in high pressure gradient mode, using two pumps.

The detector in liquid chromatography must have a rapid response (in real time) and the measured value must be different for the analytes of interest eluting from the column and the components of the mobile phase.

2.4. NMR Spectrometry

To characterize the conservation degree of the bone collagen and the possibility of recovering broken collagen chains in the chemical process, the NMR facilities from the IFIN-HH and the Advanced Research Center for Innovative Materials, Products and Processes - CAMPUS, "Politehnica" University of Bucharest were used.

Nuclear magnetic resonance spectrometry (NMR) is a quantitative, non-destructive analytical technique that provides detailed information on the molecular structure, dynamic processes and allows the determination of the concentration of molecules and direct observation of chemical reactions. NMR spectrometry is used in studies on the structure and interaction between proteins, metabolic studies, to elucidate the many biological processes and their responses to external stimuli at molecular level [11].

NMR spectrometry was developed by analogy with spin electron resonance, based on the concept of quantum nuclear spin mechanics, which is an intrinsic form of the nuclei of atoms.

The main components of an NMR spectrometer are superconducting magnet, transmitter, receiver and the acquisition and control unit (Fig. 2.6.). The magnet is the most important part of the NMR spectrometer. This includes the probe into which the sample to be analyzed is introduced and a few other homogenization, stabilization and cooling systems with helium and liquid nitrogen. The magnetic field is fixed by a separate spectrometer in the NMR spectrometer, adjusted to the NMR resonance frequency of the deuterium. The deuterium signal comes from the deuterium solvent used for sample preparation and also provides the zero reference. The probe, inserted inside the coils of the magnet, is the interface between the sample and the instrument itself. It excites the nuclear spin (it acts as a transmitter) and detects the NMR signal (the role of a receptor) [12].

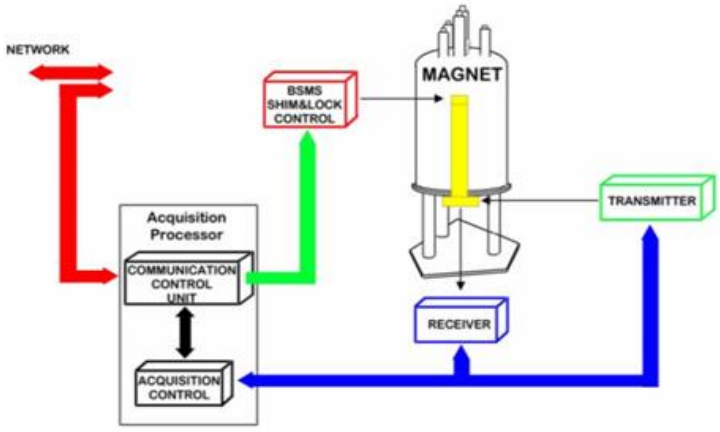

Fig.2.6. Diagrama of the NMR spectrometer from IFIN-HH [13]

The computer (the acquisition and control unit) controls all the spectrometer components. The RF components under the control of the computer are the RF frequency source and the pulse programmer.

Figure 2.7. shows the NMR spectrometer type Bruker Avance III 600MHz from Center for Advanced Research on New Materials, Products and Innovative Processes – CAMPUS, University Politehnica of Bucharest.

Fig.2.7. Photo of the NMR spectrometer Bruker Avance III 600MHz

2.5. AMS facility

Today Accelerator Mass Spectrometry (AMS) is the method of determining ^{14}C concentrations with the best measurement accuracy. It is a very powerful alternative for the ^{14}C radioactive decay method, which besides the enviable precision, also has the advantage of using very small sample quantities (about 1000 times smaller) and short measurement times.

In principle, mass spectrometry is based on the fact that ions with the same energy are deflected in the magnetic field according to their mass. This effect allows the ions of different masses to be separated and thus the isotopic composition of a material can be determined. To increase the sensitivity of the analysis, a particle accelerator and a multiple system of electric and magnetic filters were added to this mass spectrometer. This new, improved version has become the technique of accelerated ion mass spectrometry (AMS).

The general scheme of an AMS set up installation is shown in Fig. 2.8.

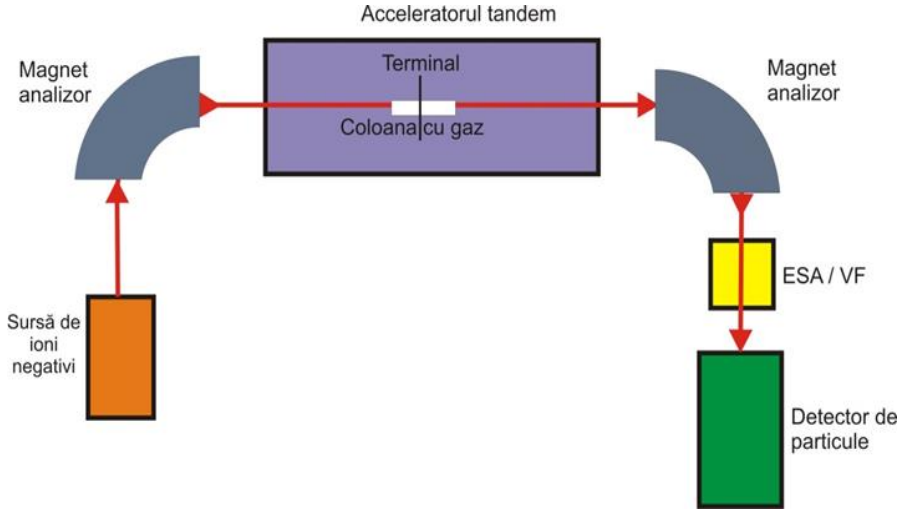

Fig. 2.8. General scheme of AMS installation

Applying the AMS method allows the measurement of the concentration of a rare isotope in the studied sample. Although the atoms are individually identified and numbered, the experimental conditions of AMS mean that in practice only isotopic ratios are measured. To determine the concentration of an isotope, the isotopic ratios are normed to calibration standards.

This system was built by High Voltage Engineering Europe (HVEE), and is based on a 1 MV Tandetron accelerator on which the high voltage is applied through a Cockcroft-Walton type source.

Figure 2.9. shows the technical drawing with all the components of the system: (1) two ion beam injectors, (2) electrostatic deflection system (electrostatic beam switching deflector), (3) multiple beam changer injector , (4) Faraday cup and Q-Snout device, (5) tandetron accelerator 1 MV with gas stripping channel, (6) magnet analyzer, (7) Farady cups for ^{12}C, ^{13}C and ^{14}C measurement, (8) electrostatic analyzer, (9) particle detector and (10) high voltage power supply Cockcroft-Walton type (Cockcroft-Walton high voltage generator) [^{14}].

The facility has two injectors equipped with a SNICS ion source type SO-110, a 50 samples carousel and ionic focusing lenses.

To increase the measurement accuracy through the analyzer magnet at 90°, the three carbon isotopes are injected almost simultaneously: ^{12}C, ^{13}C and ^{14}C. This is achieved by a bouncer system, so that different species of ions can be injected into the tandem accelerator depending on their current value.

After the magnet, in addition to the central measuring cup, two Faraday cups are placed (eccentric), in which the currents of stable isotopes are measured, one fixed for ^{12}C and one mobile for ^{13}C.

Through a 120° electrostatic filter (ESA = electrostatic analyzer), a new selection of the ion beam is made, allowing through it only the ions characterized by the same energy / charge state (E / q) ratio.

Finally, the ions are analyzed by the detection system having a detection efficiency of almost 100%. This is a gas (methane) ionization chamber with two anodes for measuring ΔE-Eres (energy loss and residual energy) and an inlet window of silicon nitride (Si_3N_4) with a thickness of 75 nm separating the gas from the detector vacuum.

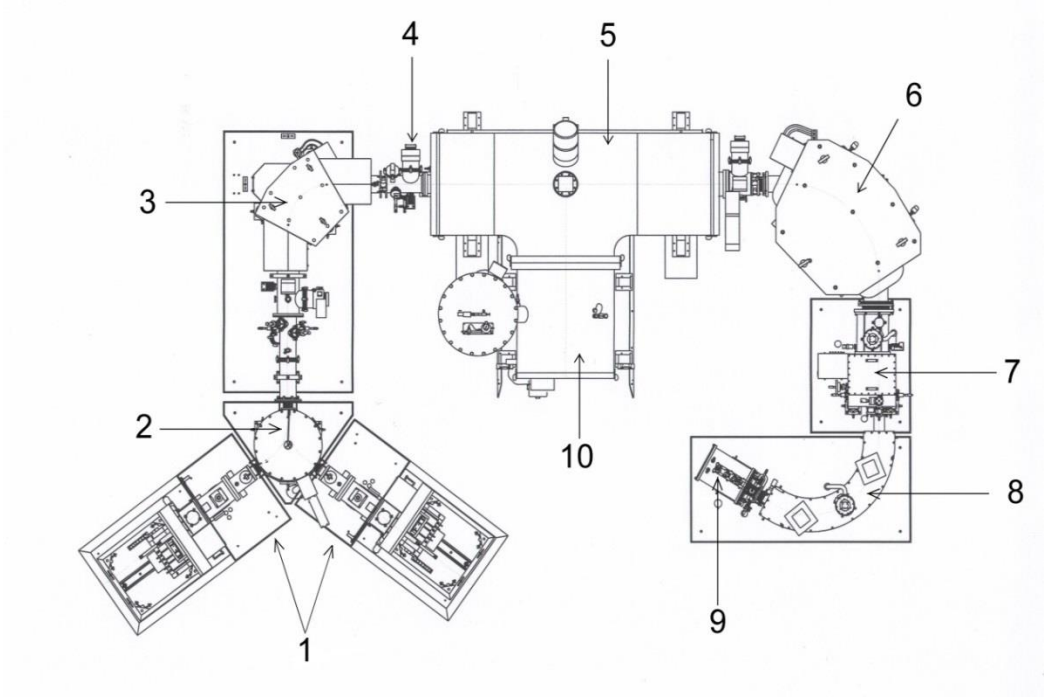

Fig. 2.9. 1 MV Tandetron AMS facility from IFIN-HH

Ion separation is based on the difference between their gas stopping powers. The electrodes for ΔE and E_{res} are 10 cm long and 20 cm long. Optimal separation is performed when the intersection of the stopping power curves occurs exactly at the separation limit between the two electrodes [15]. Using the signals from the two electrodes a biparametric spectrum is formed as shown in Fig. 2.10.

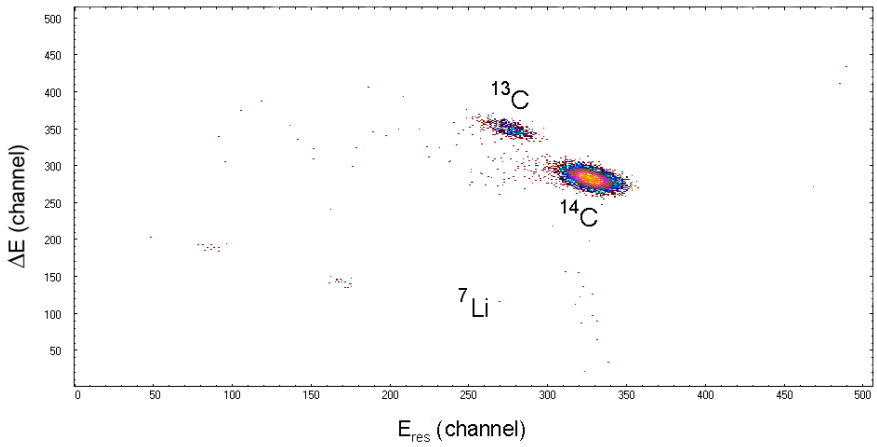

Fig. 2.10. Biparametric spectrum obtained for a graphite sample using the charge state +2

3. Dating of various materials by radiocarbon using the AMS facility from IFIN-HH

By purchasing a compact AMS system based on a 1 MV Tandetron [16, 17] from High Voltage Engineering Europe (HVEE), in 2015 the first radiocarbon dating laboratory in Romania was established, which through the inter-comparison exercise between radiocarbon centers (SIRI VI), received the RoAMS laboratory code [18].

This chapter presents the first sample preparation made in the newly established chemistry laboratory. So far there have been made many studies on the processing of various materials for their radiocarbon dating. Currently, organic materials such as bone collagen, coal, wood, plant residues, and inorganic materials such as water and carbonates are processed.

3.1. First radiocarbon dating in Romania using the AMS method

The installation of the 1 MV Tandetron AMS system from IFIN-HH represented the beginning of a program for developing the method of radiocarbon dating by AMS based on some research investment projects [19-21], that aims to support the Romanian historians and archeologists for dating thousands of archaeological artefacts.

After equipping the chemistry laboratory, the first processed sample was a fragment of a pig bone found during the rehabilitation and restoration works of the former „Oteteleşanu Mansion" in Măgurele - Ilfov. Part of the raw bone was processed and analyzed by another method, LSC (Liquid Scintillation Counting) method in other IFIN-HH laboratory.

In order to estimate the amount of collagen in the sample, FT-IR (Fourier Transform Infrared Spectroscopy) analyzes were performed, which showed that the organic matter in the bone is largely destroyed, but is still at the limit of ^{14}C dating. Based on these results, a few milligrams of the raw material were directly graphitized using the elemental analyzer and graphitization system presented in Chapter 2.

The first results obtained by the two methods, AMS and LSC, are in agreement, and converted to calendar ages from to Neolithic, which corresponds to 5000 - 4500 BC.

The calibration curves for the two methods of analysis AMS and LSC are shown in Fig. 3.1 [22].

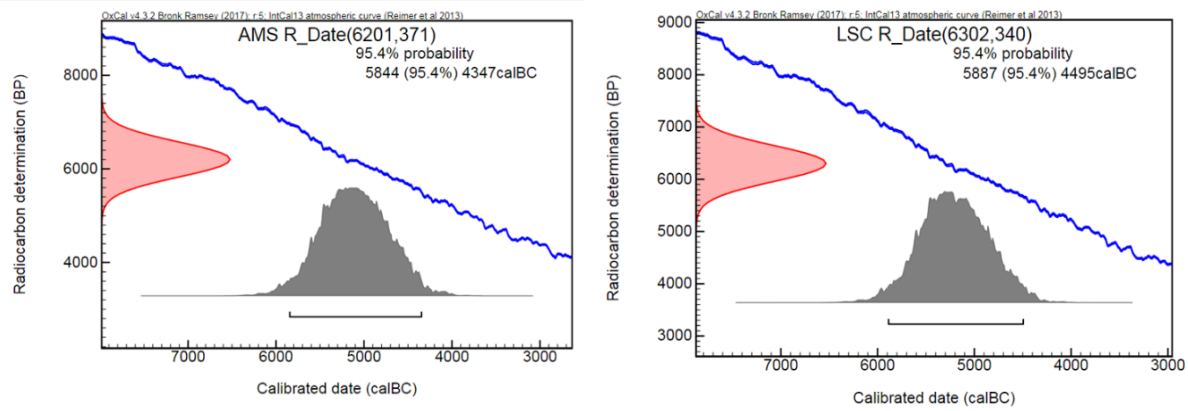

Fig. 3.1. ^{14}C data calibrated with the OxCal program, obtained by the AMS method (left) and by LSC method (right).

The results obtained from the calibration of the radiocarbon data are shown in Table 3.1.

Table 3.1. The experimental data obtained for the two methods of analysis

Method	Radiocarbon age (years BP)	Calibrated age (cal BC)	Probability for σ =2 (%)
AMS	6201 ± 371	5844 – 4347	95,4
LSC	6302 ± 340	5887 – 4495	95,4

Prior to the actions related to obtaining the international laboratory code, an inter-comparison of the results obtained in our laboratory with the data obtained on the same set of samples by a certified laboratory was performed [23].

The set consisted of 10 samples of osteological material (discovered in archaeological sites on the territory of Romania) previously dated at the AMS Ceter in Poznan, Poland. The material and the results obtained in Poznan were provided to us by archaeologists from the National Museum of History of Romania, Bucharest.

Before to the actual chemical pre-treatment, an elemental analysis of the bone was performed using the elemental analyzer (pre-screening). The protocol followed for these treatments was the one recommended by the AMS Laboratory in Zurich [24] that was adapted to our laboratory.

The graphitized samples were measured by the AMS method. The results for the standard samples show the quality of the measurements at the AMS facility of IFIN-HH, reflected by the reproducibility errors and a measurement of around 1%. The three higher values of inaccuracy,

considered acceptable for the studied historical periods, are a consequence of the low graphite content. The results of the AMS measurements are shown in Table 3.2.

Table 3.2. Values measured with AMS obtained at IFIN-HH and Poznan. The value δ13C presented in the table is obtained at the AMS installation from IFIN-HH.

Sample cod	Radiocarbon age (years BP)		$\delta^{13}C$ (‰)
	RoAMS	Poznan	
RoAMS 1	5528 ± 220	5355 ± 35	-20.70
RoAMS 2	6478 ± 200	6225 ± 35	-13.00
RoAMS 3	2230 ± 102	2090 ± 30	-16.01
RoAMS 4	6049 ± 80	6000 ± 40	-23.41
RoAMS 5	5022 ± 42	4830 ± 40	-19.41
RoAMS 6	5984 ± 30	6095 ± 35	-24.97
RoAMS 7	5587 ± 33	5440 ± 40	-19.18
RoAMS 8	6215 ± 50	6150 ± 40	-17.68
RoAMS 9	5385 ± 33	5445 ± 35	-21.42
RoAMS 10	N/A	4750 ± 40	N/A

Comparing the results obtained at IFIN-HH with those dated in the Poznan Center, it was concluded that they are within acceptable errors. The uncertaintley of the ages obtained by us decreased from hundreds of years (the first 3 results) to several decades due to the improvement of the chemical protocol for sample processing.

3.2. Dating osteological material

Collagen is the organic bone material that can be ^{14}C dated. Its content in bones is affected by variations in temperature, humidity, pH and microbial activities in the soil in which they were found.

In addition to these phenomena, old inorganic carbonates from soil and groundwater can be deposited on the surface of the bone, while humic substances can penetrate through the porous material of the bone, adding an undesirable influence on the degradation.

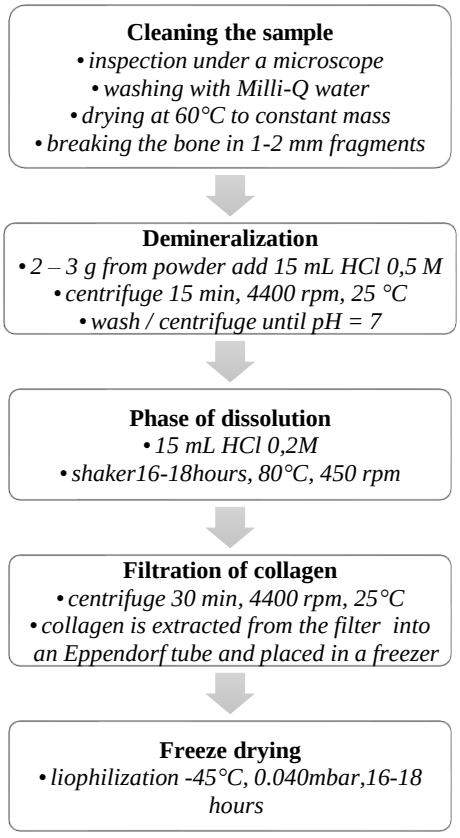

Fig. 3.2. The collagen extraction protocol from the bone.

Removing these contaminants until pure collagen is obtained involves a number of precautions and chemical treatments, influencing the accuracy of the data.

Depending on the state of conservation, various processing procedures are required. In this chapter, bone samples for which the standard protocol [25] was applied, were studied later on it was decided that thi is the protocol to be used in the RoAMS Laboratory (Fig.3.2.).

Various bone samples from three archaeological sites have been studied, whose age was either unknown or estimated based on other objects found in the vicinity (Tab. 3.3.).

Table 3.3. Description of bone samples dated at RoAMS

Sample cod	Description of sample	Provenance	Estimated age
TAR	6 animal bone fragments	Tărtăria site, County Alba	Neestimata
LUD	6 human and animal bone fragments	Luduș site County Mureș	Sec. XVII-XII î. Hr.
SOI	Mandible goat	Șoimuș site County Hunedoara	Unknown (>10.000 years)

For the six bone fragments from ***Tărtăria site*** (TAR 1 – TAR 6), the radiocarbon ages obtained from the AMS measurements grouped these samples as dating from two relatively distinct calendar periods. The AMS data obtained for TAR 1 – TAR 4 samples indicate that they come from the same stratigraphic context; for TAR 5 and TAR 6 samples the results showes that they are earlier than the other samples from the same site even though they were taken from a complex where stratigraphy indicates a later period.

The ^{14}C data obtained by AMS and calibrated for the six samples taken from ***Luduș site***, are quite large: from the beginning of the 18th century BC until the end of the 13th century. For the samples analyzed from Luduș radiocarbon data indicate two horizons of the settlements: one earlier represented by samples LUD 1, LUD 5 and LUD 6 and one later represented by samples LUD 2, LUD 3 and LUD 4.

For the bone fragment from ***Șoimuș site***, a calendar interval of 5732 – 5637 BC with a probability of 95.4% was obtained. This result represented the highest age obtained after dating a bone in the RoAMS Laboratory, namely 6796 ± 33 BP. The age obtained is very close to that estimated by the archaeologists, (6950 BP), which confirms that dating involving pre-treatment, collagen extraction, graphitization and AMS measurements has reached a level comparable to other radiocarbon laboratories in the world.

Radiocarbon ages and calibrated ages for all of the above samples are presented in Table 3.4.

Table 3.4. AMS results and ages for bone samples from Tărtăria, Luduș, Șoimuș

Archaeological site	Sample code	Radiocarbon age (BP years)	Calibrated age (cal BC)	Probability for σ =2 (%)
TĂRTĂRIA	TAR 1	6082 ± 33	5204 – 5171 5074 – 4897 4866 – 4852	4,4 89,6 1,4
	TAR 2	6063 ± 33	5055 – 4878 4871 – 4848	90,5 4,9
	TAR 3	6023 ± 32	5001 – 4834	95,4
	TAR 4	5996 ± 32	4977 – 4796	95,4
	TAR 5	6112 ± 33	5207 – 5146 5138 – 5093 5082 – 4945	18,7 6,8 70,0
	TAR 6	5966 ± 32	4943 – 4770	95,4
	LUD 1	3346 ± 73	1876 – 1841 1820 – 1797 1781 – 1492 1483 – 1452	3,0 1,6 88,6 2,3

LUDUȘ	LUD 2	3186 ± 73	1626 – 1277	95,4
	LUD 3	3147 ± 66	1607 – 1583 1559 – 1553 1546 – 1257 1250 – 1232	1,8 0,4 92,0 1,3
	LUD 4	3101 ± 66	1506 – 1195 1141 – 1134	95,0 0,4
	LUD 5	3422 ± 36	1876 – 1841 1821 – 1797 1782 – 1629	8,9 4,1 82,4
	LUD 6	3345 ± 78	1876 – 1841 1821 – 1796 1782 – 1451	3,3 1,9 90,1
ȘOIMUȘ	ȘOI	6796 ± 33	5732 – 5637	95,4

3.3. Dating wood

Dating wooden objects, papyruses, vegetable residues involves the extraction of cellulose ($(C_6H_{10}O_5)$ n) from the sample.

The cellulose extraction method is based on the procedure applied at the ETH Zurich center in Switzerland [[26]], (Fig. 3.3.)

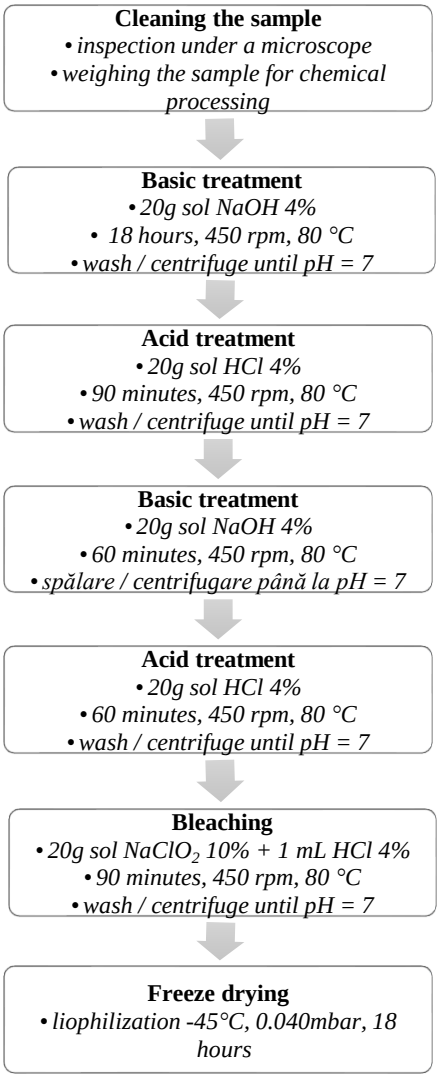

Fig. 3.3. The cellulose alpha extraction protocol.

Two wooden samples were studied, consisting of a trapeze (table) from the Monastery Corbii de Piatră, Argeș county, and a monoxile (boat carved from a single tree trunk) discovered in 2015 in the

town of Ostrov, Constanța county. The radiocarbon age obtained for the two samples falls within the calendar range estimated by archaeologists (Fig. 3.4.).

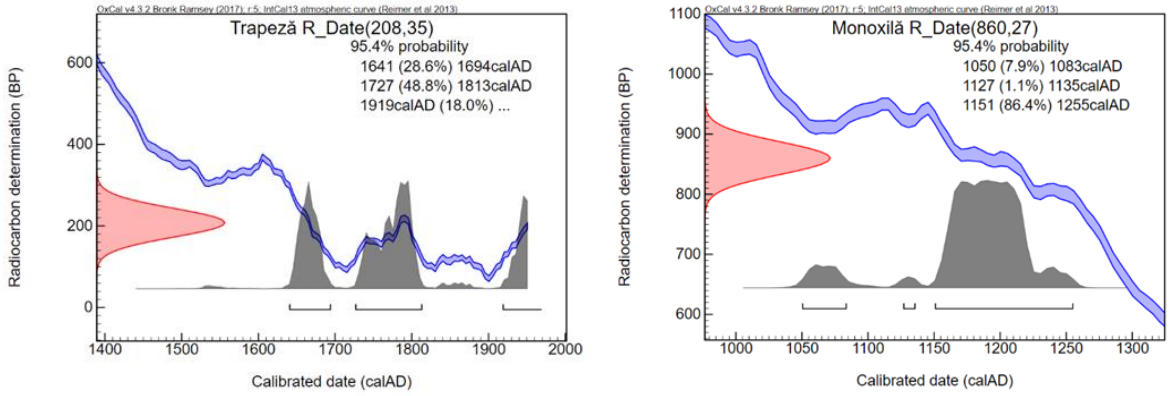

Fig. 3.4. ^{14}C data calibrated for the trapeze sample (left) and for the monoxile sample (right).

3.4. Dating charcoal

The chemical treatment of charcoal was performed using the Acid-Base-Acid (ABA) method, used at the radiocarbon center at the University of Arizona and whose protocol is described by A.J. Timothy Jull et al. [27]. This protocol is schematically shown in Fig. 3.5.

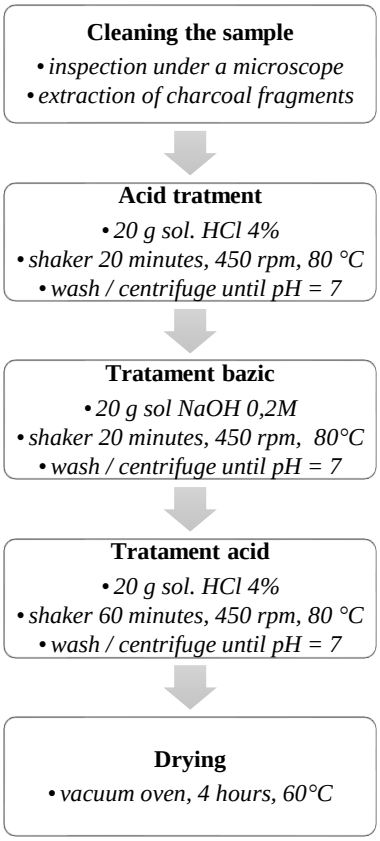

Fig. 3.5. Protocol for the chemical treatment of charcoal.

Two sets of charcoal samples were studied whose age was not known exactly, they are presented in table 3.5. The archaeological site Poiana Cireșului represents the oldest human settlement in Romania (> 10000 years).

The ages obtained for the three charcoal samples from Enisala – Palanca are grouped around the same calendar interval, suggesting that they belong to the same historical period, in line with what was estimated by the archaeologists.

For the charcoal sample from Poiana Cireșului a value it was confirmed that this site is one of the oldest archaeological sites in Europe. The obtained calendar range was 18580 - 18151 cal BC with a probability of 95.4%.

Table 3.5. Description of charcoal samples dated at RoAMS

Sample	Description of sample	Provenance	Estimated age
PAL	Carbonized fragments	Enisala – Palanca site, County Tulcea	14^{th} – 15^{th} c.
CIR	Charcoal sample	Poiana Cireșului site County Județul Neamț	Unknown (> 10000 years)

Obtaining such an old chronological interval with an error of only 60 years, confirms that the charcoal has been well processed and the AMS measurements have been made in excellent conditions.

The experimental data and the ages obtained for the charcoal samples from the two sites are presented in Table 3.6.

Table 3.6. Ages obtained by AMS and calibrated for coal samples

Archaeological site	Sample cod	Radiocarbon age (BP yeras)	Calibrated age (cal BC/AD)	Probability For σ =2 (%)
PALANCA	PAL 1	485 ± 31	1405 – 1 453	95,4
	PAL 2	397 ± 29	1435 – 1522 1575 – 1625	75,5 19,9
	PAL 3	410 ± 29	1431 – 1521 1592 – 1620	84,0 11,4
POIANA CIREȘULUI	CIR	16850± 63	18580 – 18151	95,4

The calibration curves for the two sets of samples are shown in Fig. 3.6.

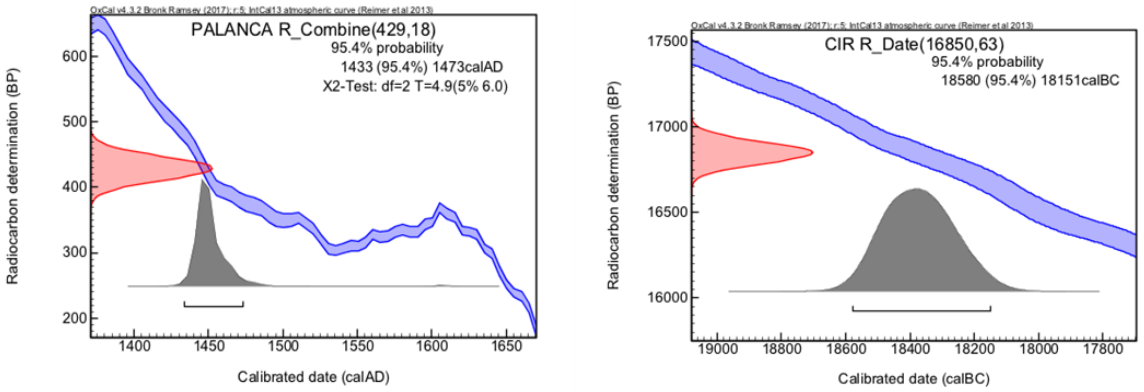

Fig. 3.6. Combination of calibrated ^{14}C data, for the samples from the Enisala - Palanca site (left) and for the sample from the Poiana Cireșului site (right).

4. High performance liquid chromatography (HPLC) used in radiocarbon dating

This chapter presents two studies in which HPLC was used. The first aimed at characterizing four of the most common amino acid standards used in radiocarbon dating. The characterization was performed both directly on standards and after passing through the HPLC system. The second study consisted of dating some osteological materials, which presented difficulties in assigning the age, based on the dating of the constituent amino acids. The most abundant amino acids in bone collagen were investigated: glycine (Gly), proline (Pro), hydroxyproline (Hyp), alanine (Ala) and glutamic acid (Glu). Since in some situations Gly could not be completely separated from Thr (threonine), their mixture was studied.

4.1. Characterization of the amino acid standards used in radiocarbon dating

Two sets of experiments were performed. The first included the graphitization of amino acid standards and their direct dating through AMS, and the second consisted of the separate passage of each amino acid standard by HPLC, then graphitization and dating.

Four types of amino acid standards provided by Sigma-Aldrich® were used in this study: Gly, Ala, Pro and Hyp. Solutions of 10 mg / mL were made from each standard.

Chromatographic analyzes were performed with the Shimadzu system, the percentage of C and N and the mass ratio C / N were determined with the elemental analyzer varioMICRO Cube and the analysis of stable carbon isotopes and radiocarbon dating were performed using the 1 MV tandetron accelerator from the RoAMS laboratory from IFIN-HH.

To verify ^{14}C contamination in the process of HPLC separation, all amino acid standards were graphitized in the same manner, both directly and after being passed through the HPLC column. When using HPLC the contamination with foreign carbon can come from two sources, namely: the *„^{14}C dead"* and the effect is an older radiocarbon age of the samples, and the *„modern carbon"* and the effect is an younger radiocarbon age of the sample [28].

The experimental values of the C/N ratio obtained for each amino acid (analyzed directly and passed through the HPLC column) are very close to the theoretical values and those specified by the manufacturer (table 4.1.).

Table 4.1. Comparison between experimental and theoretical data obtained for amino acid standards

No.	Amino acid	C/N (direct)	C/N (HPLC)	C/N (theoretical)
1	Gly	1,718±0,018	1,8112±0,009	1,715
2	Ala	2,625±0,030	2,651±0,026	2,572
3	Hyp	4,323±0,075	4,423±0,007	4,287
4	Pro	4,258±0,002	4,338±0,039	4,287

The fractionation of each amino acid standard passed by HPLC was determined to the $\delta^{13}C$ value of the non-HPLC processed amino acid standards. This fractionation is mentioned in the literature, but there is little or partial information on the fractionation introduced by HPLC [29-32]. The obtained data showed that the use of HPLC does not significant influence the age of the three amino acid standards. Only in the case of alanine a smaller ages was obtained a smaller age than that on the same type of crude amino acid (table 4.2.).

Table 4.2. Experimental ages and $\delta^{13}C$ values of amino acids measured at 1 MV by AMS

No.	Amino acid	Amino acid without HPLC			Amino acid after using HPLC		
		Radiocarbon age (BP years)	$\delta^{13}C$ (‰)	Percent of Modern Carbon (pCM, %)	Radiocarbon age (BP years)	$\delta^{13}C$ (‰)	Percent of Modern Carbon (pCM, %)
1	Gly	25,370 ± 347	-45,7±0,38	4,25	24,395 ± 292	-18,0±0,39	4,80
2	Ala	31,930 ± 139	-24,4±0,33	1,88	24,077 ± 77	-11,1±0,38	4,99
3	Pro	-505 ± 30	-29,0±0,39	106,49	-531 ± 29	-17,3±0,39	106,83
4	Hyp	-419 ± 44	-20,8±0,31	105,35	-357 ± 44	-12,4±0,30	104,54

Analyzing the obtained radiocarbon ages, it was possible to make a correlation between the age and the manufacturing process of he four amino acid standards.

Because very high ages were obtained for glycine and alanine, it can be said that these standards were produced by direct chemical synthesis using hydrocarbons which are derived from petroleum. On the other hand, the small (modern) ages obtained for proline and hydroxyproline confirm that they were produced by the fermentation method that uses contemporary (modern) atmospheric CO_2.

4.2. Using HPLC for dating osteological material that has difficulty in assigning an age

4.2.1. Experimental part

Four bone samples from two archaeological sites, in different conservation states were studied, see Table 4.3.

Table 4.3. Description of bone samples that presented difficulties in assigning an age

Archaeological site	Sample cod	Description of the sample	Estimated age
Capul Dealului, Constanța	CDE 1	Bone awl	$11^{th} – 12^{th}$ c.
	CDE 2	Astragalus	$11^{th} – 12^{th}$ c.
Curtea Domnească, Târgoviște	CDT 1	Human bone fragment	15^{th} c.
	CDT 2	Human bone fragment	15^{th} c.
	CDT 3	Human bone fragment conatminated in laboratory	15^{th} c.

The samples were processed according to the chemical procedure shown in Fig. 3.3. Prior to the separation of the amino acids by the HPLC system, it was necessary to break the protein chain from the collagenous bone in its constituent amino acids by the hydrolysis reaction.

Depending on the amount of amino acid, for measurements of ^{14}C and $\delta^{13}C$, either the AMS facility (for quantities greater than 1 mg of amino acid) of IFIN-HH or the Hertelendi Environmental Studies Laboratory (for samples smaller quantities), Hungary were used. The Hertelendi installation is an AMS system of type MICADAS (Mini Carbon Dating System). This system was developed to allow not only the measurement of graphite powder samples, but also the measurement of CO_2 gas directly from the combustion process.

From each sample, one part was used for direct dating of raw collagen, and the other part was hydrolyzed and injected into the HPLC column to separate the individual amino acids.

4.2.2. Results and discutions

For the bone awls sample (***sample CDE 1***), two fractions were collected by HPLC: Hyp and a mixture of Pro + Gly. For the astragal sample (***sample CDE 2***) the only amino acid obtained by HPLC and with a very good resolution and in a sufficient quantity was Thr. The sample amounts of collagen and amino acids used for AMS analysis are presented in Table 4.4.

Table 4.4. Experimental data and AMS analysis obtained for sample CDE 1, CDE 2

Sample cod	Sample name / Weight (mg)	Radiocarbon age (years BP)	Calibrated age (cal AD)	Probability for σ =2 (%)
CDE 1	Collagen (3,38)	1354 ± 39	612 – 720 741 – 767	85,1 10,3
	Hyp (10)	1398 ± 39	575 – 679	95,4
	Pro+Gly (7)	1523 ± 44	423 – 620	95,4
CDE 2	Collagen (3,20)	1378 ± 37	595 – 693 748 – 762	93,6 1,8
	Thr (1,20)	1467 ± 45	434 – 455 469 – 488 534 – 657	2,6 2,6 90,1

The global range obtained for the CDE 1 sample shows that this household object is of the same age as the few ceramic fragments estimated by archaeologists to be from the $4^{th} – 7^{th}$ c. AD.

Dating the Hyp fraction isolated from raw collagen can lead to a certain age, due to the purity and characteristic of Hyp of being found in large quantities only in mammalian bones [33]. In our case, the calendar range for Hyp overlaps well with that estimated by archaeologists for the ceramics found at the same place.

The calendar age obtained for the amino acid mixture does not seem to be a plausible value, because the time interval is much too large and earlier.

For the CDE 2 sample, by comparing the calibrated age with the ceramics dated in the $4t^h$ – 7^{th} c. AD from the same archaeological site, we can say that the age obtained for the raw collagen is plausible.

The AMS measurements for the amino acid Thr did not provide a reliable age of the astragal fragment, despite the very good amount and separation resolution obtained by the HPLC system. These ages are older than the age the raw collagen.

The explanation could be the external contamination with ^{14}C „dead carbon" that could be introduced due to defective leakage of the mobile phase through the HPLC column [34].

From the first bone fragment (***sample CDT 1***), four individual amino acids were separated with a good resolution. The experimental data and the quantities obtained are presented in Table 4.5. Collagen, Thr and Hyp were analyzed in the RoAMS laboratory, and the other two amino acids Pro and Ala, being in smaller quantities (1,87 mg and 0,47 mg) were measured at the Hertelendi Laboratory.

Table 4.5. Experimental data and AMS analysis obtained for sample CDT 1

Sample name	Weight (mg)	Radiocarbon age (years BP)	Calibrated age (cal AD)	Probability for σ =2 (%)
Collagen	3,60	614 ± 33	1293 – 1404	95,4
Pro*	1,87*	603 ± 18	1300 – 1368 1381 – 1404	74,7 20,7
Thr	3,32	713 ± 37	1225 – 1234 1242 – 1310 1360 – 1387	1,7 79,9 13,8
Ala*	0,47*	677 ± 18	1276 – 1306 1363 – 1385	66,3 29,1
Hyp	3,37	557 ± 38	1303 – 1366 1383 – 1434	49,4 46,0

**indicates that the samples were analyzed at the Hertelendi Laboratory*

The calendar intervals obtained for each amino acid are relatively close and fall within the global range 1225 – 1434 calAD, which is approximately the same interval obtained for collagen 1293 – 1404 calAD.

From the second bone fragment (***sample CDT 2***) Ala and Hyp were separated with good resolution; Pro could not be completely separated from Gly and therefore were collected together as a mixture of amino acids. The experimental data and the quantities obtained from each collected amino acid are presented in Table 4.6. Due to the very small quantity only 0,11 mg and 1,11 mg, Ala and Pro were measured at the Hertelendi Laboratory where, for Ala analysis, the gas source of the AMS facility was used.

Table 4.6. Experimental data and AMS analysis obtained for sample CDT 2

Sample name	Weight (mg)	Radiocarbon age (years BP)	Calibrated age (cal AD)	Probability for σ =2 (%)
Collagen	3,34	709 ± 31	1255 – 1308 1361 – 1387	83,2 12,2
Mix Gly+Pro	4,05	580 ± 39	1298 – 1421	95,4
Ala*^	0,11	888 ± 92	990 – 1279	95,4
Hyp*	1,11	713 ± 19	1264 – 1295	95,4

** indicates that the samples were analyzed at the Hertelendi Laboratory and the symbol ^ shows that the gas source was used*

From the raw collagen and Hyp, almost identical intervals were obtained in both laboratories, namely 1255 - 1308 calAD for collagen and 1264 - 1295 calAD for Hyp.

The tomb from which the CDT fragment 2 was collected is older (is deeper) than the one from which the CDT fragment 1 was collected and the bone remnants of the two tombs belong to two different time periods that differ by about 100 years.

The results obtained for these tests led to the conclusion that the two individuals were not contemporaries, but lived in periods separate for about 100 years.

For the human bone fragment contaminated in the laboratory (***sample CDT 3***) by HPLC, Pro, Ala and Hyp were separated. The experimental data and the quantities obtained for these amino acids are presented in Table 4.7.

Table 4.7. Experimental data and AMS analysis obtained for sample CDT 3

Sample name	Weight (mg)	Radiocarbon age (years BP)	Calibrated age (cal AD)	Probability for σ =2 (%)
Collagen	3,40	655 ± 31	1278 – 1325 1344 – 1394	45,5 49,9
Pro*	2,44	704 ± 18	1267 – 1299	95,4
Ala*^	0,55	569 ± 84	1268 – 1468	95,4
Hyp*^	0,15	748 ± 83	1049 – 1085 1124 – 1137 1150 – 1401	3,9 1,2 90,4
R_Combine (Pro*, Ala*^ Hyp*^)	N/A	700 ± 18	1268-1300 1370-1380	91.6 3.8

** indicates that the samples were analyzed at the Hertelendi Laboratory and the symbol ^ indicates that the gas source was used.*

The three amino acids (Hyp, Pro, Ala) were dated at the Hertelendi laboratory, using the MICADAS facility. Because very small quantities were obtained from the Hyp and Ala fractions, they were measured using the MICADAS gas ion source.

Calibrating the radiocarbon age using the IntCal calibration curve for collagen led to two calendar intervals: 1278 – 1325 calAD with a probability of 45.5% and 1344 – 1394 calAD with a probability of 49.9%.

Ala and Hyp dating led to longer calendar periods, due to the larger statistical errors, resulting as a consequence of the very small amount of sample.

The range obtained for Ala, 1268 – 1468 calAD (with a probability of 95.4%), which is more recent than the interval obtained for collagen, can be explained by a possible contamination in the direct transfer of CO_2 from the AGE reactor to source of the AMS facility.

In the case of Hyp dating, the global range, which is much extended and much older, can be explained by „dead" carbon contamination due to defective leakage of the mobile phase through the HPLC column [28].

However, using the R_Combine function of OxCal v.3.2, it was observed that the ^{14}C values for all the dated amino acids (Pro *, Ala * ^ and Hyp * ^) are convergent. These are presented in Table 4.7. (last line).

The comparison of the intervals obtained for the raw collagen and the constituent amino acids, showed that they are very close, which indicates that myrrh contamination was successfully eliminated by ultrafiltration of collagen and that direct dating of collagen extracted from CDT 1 and CDT 2 samples provided plausible information, even if they were treated with an old myrrh.

5. Characterization of bone collagen by nuclear magnetic resonance spectrometry (NMR)

The organic fraction of the bones is composed of proteins (mostly collagen) and lipids. Over time, depending on the conditions of conservation, the bones may incorporate external carbon from biogenetic sources leading to a younger age when radiocarbon dating is used.

Studies on the conservation of organic fraction using MRI spectrometry are very few or almost missing [35- 37].

This chapter presents a study on the characterization of the conservation degree of collagen extracted from the bone material studied and dated in Chapter 4. The study is based on the comparison of the NMR spectra obtained for the amino acids of the collagen extracted and filtered through the

Amicon Ultra Filter, which retains only the molecules with the molecular mass greater than 30kDa and those from fragments of collagen chains that passed through the filter and remained in the 0.2M HCl solution. The collagen chain may be destroyed due to external factors in which the osteological material was stored, but also due to its faulty chemical processing for ^{14}C dating.

5.1. Determination of the parameters of the NMR system using the analyzed amino acid standards

In order to identify the peaks of the amino acids in the NMR spectra, the individual ^{13}C NMR spectra were first developed for the amino acids that were analyzed and dated in Chapter 4, as well as for their mixing. Five types of amino acid standards provided by Sigma-Aldrich were used: glycine, alanine, proline, hydroxyproline and threonine. From each standard were made solutions with deuterated water D_2O / H_2O (1: 9), in a concentration of approximately 0.1 mmol / mL.

To choose which method will be used for collagen NMR spectrometry, two spectra were made: the proton spectrum (^1H) and the carbon spectrum (^{13}C). Analyzing the two types of spectra it turned out that the ^{13}C spectrum shows the peaks of the amino acids with better resolution and has a wider frequency range than the ^1H.

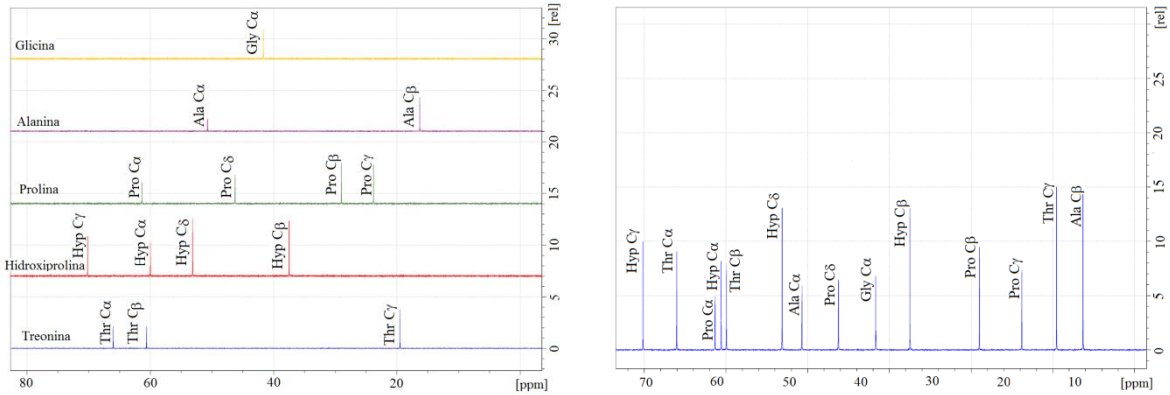

Fig. 5.1. ^{13}C NMR spectra of individual amino acid standards (left). ^{13}C NMR spectrum of the mixture amino acid standards (right).

In Figure 5.1. ^{13}C NMR spectra are presented for each amino acid standard analyzed and the mixture of the amino acid standards used.

The approximate positions of the peaks for each type of amino acid of interest are presented in Table 5.1. The position of the peaks obtained is in accordance with the literature data [38, 39].

Table 5.1. The position of the peaks in ^{13}C RMN spectrum for each standard of the amino acid used

No.	Amino acid standard	Cα	Cβ	Cγ	Cδ
1	Glycine	41,69	-	-	-
2	Alanine	50,74	16,35	-	-
3	Proline	61,37	29,02	23,81	46,27
4	Hydroxiproline	60,58	37,50	70,19	53,17
5	Theronine	66,06	59,96	19,57	-

5.2. NMR analysis for collagen extracted from osteological material

The four bone samples from two archaeological sites presented in Chapter 4 were studied.

The first stage of the study was the displacement of the amino acid peaks of interest for a real sample of unaltered (contemporary) collagen. For this, the peaks corresponding to the amino acids of interest in the ^{13}C NMR spectrum obtained for the collagen extracted from the contemporary bone were compared with those obtained for the mixture of their standards.

The ^{13}C NMR spectra obtained for the collagen extracted from the two samples ***(CDE 1 and CDE 2)*** taken from the Capul Dealului site, Constanța, are presented in Figure 5.2. To identify the

constituent amino acids, the spectrum of the mixed amino acid standards is presented above the collagen spectrum.

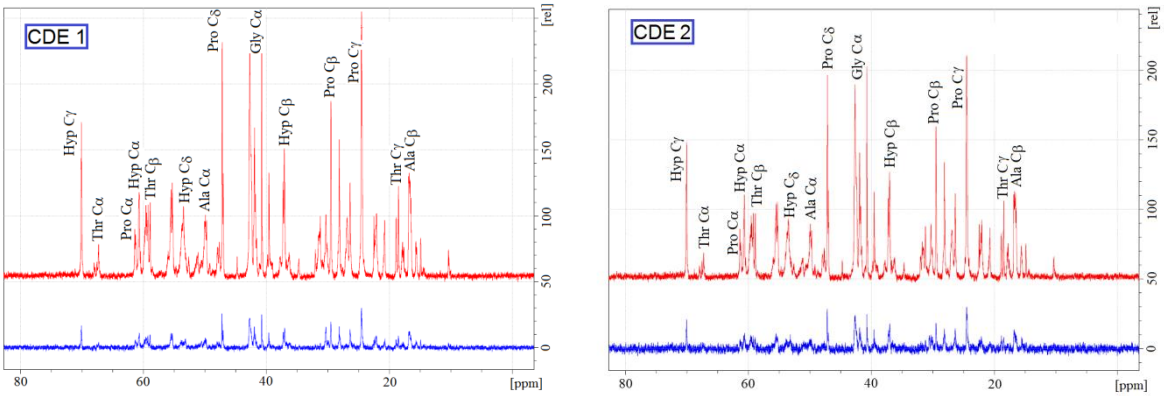

Fig.5.2. ^{13}C NMR spectrum obtained for collagen extracted from CDE 1 sample (left) and CDE 2 sample (right).

From the spectra obtained for the collagen extracted from the two samples, it is observed that all the peaks corresponding to these amino acid standards are present.

The deviation of the positions of the peaks obtained for the collagen of the two samples is below 0.89% which confirms that the amino acids have been correctly identified.

To evaluate the amino acid content of bone collagen extracted from these samples, the mass ratio of the peaks corresponding to the amino acids was also calculated. The mean value of the ratio of 1,00 ± 0,07 shows that the amino acid content of the two samples is almost identical.

In addition to the peaks of the amino acids of interest, ^{13}C NMR spectra also contain peaks of other amino acids, which were not indexed because they are not the subject of the study.

The ^{13}C NMR spectra obtained for the collagen extracted from the two samples (***CDT 1 and CDT 2)*** taken from the Royal Court site in Târgoviște, are presented in Figure 5.3. To identify the constituent amino acids, the spectrum of the mixed amino acid standards is presented above the collagen spectrum.

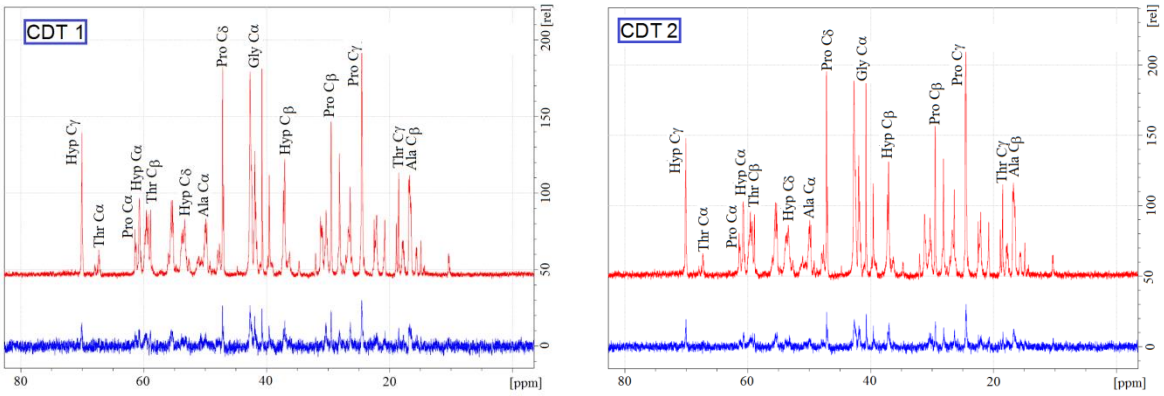

Fig.5.3. ^{13}C NMR spectrum obtained for collagen extracted from CDE 1 sample (left) and CDE 2 sample (right).

From the spectra obtained for the collagen extracted from the two samples, it is observed that all the peaks corresponding to these amino acid standards are present.

The deviation of the positions of the peaks obtained for the collagen of the two samples is at most 1% which confirms that the amino acids have been correctly identified.

To evaluate the amino acid content of bone collagen extracted from these samples, the mass ratio of the peaks corresponding to the amino acids was also calculated. The mean value of the ratio of 1.00 ± 0.04 shows that the amino acid content of the two samples is almost identical, but their weight is different.

In addition to the peaks of the amino acids of interest, ^{13}C NMR spectra also contain peaks of other amino acids, which were not indexed because they are not the subject of the study.

5.3. Establishing the degree of collagen conservation for osteological material dated by AMS

To determine the degree of collagen conservation, it was necessary to process the residues from each non-hydrolyzed collagen sample. After separation of the collagen and ultra-filtration through Amicon Ultra Filter 30kDa, the remaining residue was evaporated. For NMR analysis the dry residue was then reconstituted in 1 mL of D_2O / H_2O solution (1: 9).

To highlight the amount of collagen fractions that passed into solution after filtration, in Figure 5.4 were added the spectra obtained for collagen extracted from each sample. In this way a qualitative comparison can be made of the collagen fraction that was lost through the 30kDa filter.

It can be seen that all the peaks corresponding to the amino acid standards are present in the spectra obtained for the collagen fragments in the residue, except for the Cα peaks corresponding to alanine and proline for the CDE 2 sample and Cα corresponding to threonine for both samples. The deviation of the positions of the peaks obtained for the collagen of the two samples is at most 1.23% which confirms that the amino acids have been correctly identified.

The average value of the peak intensity ratios corresponding to the amino acids of interest for the collagen fragments is 1.78 ± 0.68 and shows that the content of amino acids that passed through the filter in the case of the CDE 1 sample is higher than for the CDE 2 sample.

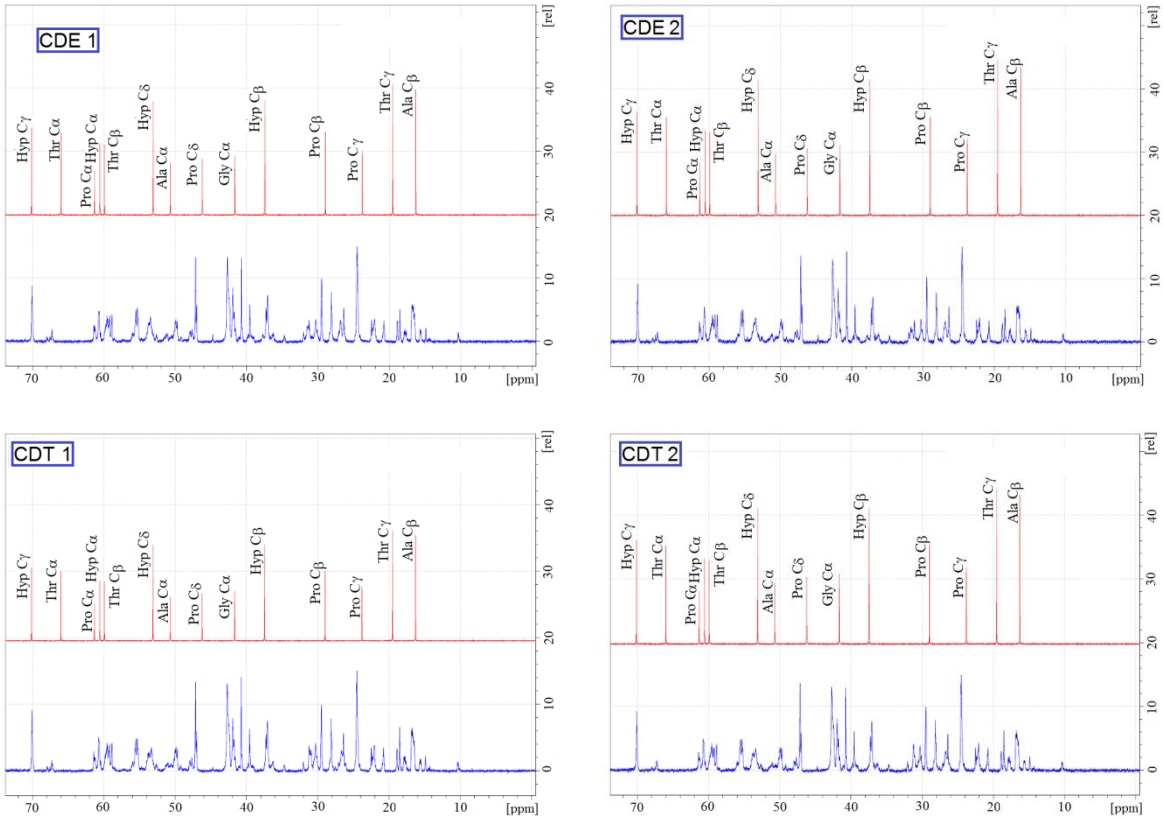

Fig. 5.4.13 C NMR spectra obtained for collagen fragments remaining in the acid residue after filtration.

From the spectra for CDT 1 and CDT 2 it is observed that in the collagen fragments from the residue are very absent many peaks (alanine and threonine completely missing). The deviation of the positions of the peaks obtained for the collagen of the two samples is at most 0.55% which confirms that the amino acids have been correctly identified.

The mean value of the ratio of 0.96 ± 0.23 shows that the collagen fragments present in the residue are approximately in the same concentration.

Although radiocarbon dating of collagen and individual amino acids showed that CDE 1 and CDE 2 samples belong to the same historical period [40], ^{13}C NMR analysis of collagen and collagen fragments indicated that collagen of sample CDE 1 is more degraded than of the sample CDE 2. One possible explanation is that the first sample, representing a household object made of a fragment of

animal bone, was subjected to aggression through its use, while the second sample is just a buried bone fragment.

Regarding the samples from the second archaeological site, the results showed that the collagen is less damaged, which was somehow expected, as they are much younger. CDT 1 collagen is shown to be the best preserved, supporting the assumption that these human remains have been treated with myrrh. Another explanation for the slightly different conservation degree of CDT 1 and CDT 2 samples may be that the bone fragments from which the samples were extracted were not of the same type (e.g. femur, tibia, skull, etc.) and the amount of collagen in them differs, which explains the difference between the proportions of each type of amino acid in the sample.

To verify that the quantities of individual amino acids that could be separated from the collagen fragments that passed through the filter can be dated by ^{14}C, the quantities of the most representative amino acids from the studied samples were calculated (Table 5.2.).

Table 5.2. Amounts of datable amino acids contained in the collagen and collagen fragments extracted from the studied samples

	Collagen (mg)				Collagen fragments (mg)			
	CDE1	CDE2	CDT1	CDT2	CDE1	CDE2	CDT1	CDT2
Gly	1.411	1.213	1.755	1.170	0.439	0.219	0.181	0.239
Ala	0.810	0.708	0.960	0.659	0.348	0.160	0.094	0.110
Pro	7.333	6.159	8.917	6.254	2.164	1.051	0.756	0.952
Hyp	8.192	6.925	10.514	7.236	2.196	1.241	0.825	1.092
Thr	4.173	3.422	5.096	3.552	1.154	0.564	0.309	0.469

For dating the separated amino acids from the collagen fragments that have passed through the filter, a quantity of more than 2 mg is required from each of them. From Table 6 it can be seen that only two amino acids can be separated from the CDE1 sample that can provide a sufficient amount of graphite to be dated by AMS [41].

6. Conclusions and perspectives

C1. GENERAL CONCLUSIONS

The radiocarbon dating establishment RoAMS Center, from "Horia Hulubei" Institute of Physics and Nuclear Engineering opened the horizon to new applications of Nuclear Physics, among which, radiocarbon dating is one of the most used.

In addition to the financial effort made to purchase the AMS installation based on a 1 MV Tandetron, a special effort was made to equip the chemistry laboratory dedicated to these measurements.

Chemical pre-treatment protocols were developed, specific for each material and state of degradation or contamination.

For a preliminary verification of the pre-treatment protocols of the samples, of performances of the AMS installation and of the calculations and corrections applied for the determination of the ages, a study was made of inter-comparison of the results obtained in the RoAMS center with those obtained in a certified AMS center Poznan, Poland. In this study, we were supported by historians from the National Museum of History of Romania, Bucharest, who offered us a set of 10 bones and their ages, determined by those from Poznan. Following this study, the graphitization process of the samples and the measurement technique by AMS of the ^{14}C content were improved, leading to very low statistical measurement and repeatability errors.

With the certification of these results, the final working protocols for bone pre-treatment were established and the other, simpler methods of pre-treatment of wood (cellulose in general) and coal were developed. To the same end, the collaboration with researchers from ETH Zurich provided the opportunity to develop and improve the pre-treatment protocols of the analyzed samples.

The AMS Center in Debrecen, Hungary supported us in the case of AMS measurements of very low carbon samples, they have an AMS installation equipped with an ion source in which CO_2 gas resulting from the combustion of samples in the elemental analyzer is directly introduced .

The elaborated chemical processes and the parameters established for the technical equipment used were validated by the successful dating of 13 bone fragments, 2 wooden objects and 4 samples in the form of coal. By dating, valuable information was obtained for the archaeologists and historians who provided it to us.

For bone samples that have difficulty in assigning age individual separation and dating of the amino acids from the constituent collagen was used. For this purpose, studies on the influence of the use of HPLC on carbon isotopic fractionation and dating using the ^{14}C concentration measured by AMS were required. Studies have shown that isotopic fractionation exists but does not significantly influence radiocarbon dating.

By using the separation of collagen amino acids, the degree of elimination of external ^{14}C contaminants was also verified, through the controlled contamination, in the laboratory, of a bone fragment. It was obtained that the use of the special Amicon Ultra filter, which retains only molecules with a molecular weight over 30kDa, was very efficient.

The obtained results using ^{13}C NMR to analyze the amino acid content of the residue after filtration with Amicon, showed that this nuclear technique is useful in estimating the amount of amino acids in altered collagen and is useful in deciding whether it is worth separating by HPLC and then each resulting individual amino acid is ^{14}C dated. The study was performed for the first time and is an important step in characterizing the degree of preservation of bone collagen using NMR, when the usual processing led to a small amount of dateable carbon.

C2. ORIGINAL CONTRIBUTIONS

This thesis is a scientific premiere for our country, being the first to address radiocarbon dating through AMS. Even if it is not a novelty worldwide, the development and use of this method makes important and original contributions to scientific knowledge in archeology and history in general.

In the studies I used the latest equipment and methods in order to obtain the necessary performance to more accurate dating, I had to consult the most recent publications in the literature.

I participated in all the studies presented in this thesis together with the team of the Tandem Accelerators Department (DAT).

I participated in the tests and pre-treatment tests of the samples performed for the final elaboration of the protocols that are currently used today. These tests included both laboratory chemical treatments and automated processing using the elemental analyzer, graphitization system and HPLC system.

The studies related to the separation of amino acids from collagen were carried out exclusively by me, but I was supported by the team in the stages regarding the determination of the carbon isotope content at the AMS installation. This study showed that the separation of individual amino acids from collagen makes an important contribution to bone dating, if the collagen in it is damaged or contaminated.

For the first time, at my initiative, a correlation between the age of standard amino acids and the procedure for their production: synthesis or fermentation, was made.

In addition to participating in the studies on the materials presented in this paper, but based on the experience and protocols gained from them, I participated in the team and other studies addressed, such as groundwater dating, hydraulic lime mortar, seeds, tree rings (useful in dendrochronology).

We participated, contributing to the chemical processing of samples, studies on the performance of the AMS plant in our center, not only on measurements of carbon concentrations, but also 26Al, 10Be and actinides (^{236}U, ^{239}Pu, ^{240}Pu, etc.).

The results of the studies obtained in this thesis, as well as subsequent studies, were presented at 38 national and international conferences and symposia (see Annex I).

I contributed to the chemical processing part of the samples that were the subject of the 7 articles were in ISI-listed specilaist journals and others were the subject of some chapters in 7 specialized books.

The articles on the separation of amino acids by HPLC and the characterization of bone collagen by NMR were developed in proportion of 80% by me, as a result of which I was the first author and corresponding author.

C3. PERSPECTIVES FOR FURTHER DEVELOPMENT

The experience of the first five years of operation of the laboratory has highlighted the complexity and practical uniqueness of each sample - and therefore the need for a varied approach to pre-treatment in order to decontaminate it, depending on the initial characteristics.

Osteological material of an animal or human nature often presents in varying degrees of degradation and / or contamination, becoming evidence that presents difficulties in assigning the age. The solution to these problems is to use the separation of individual amino acids from collagen and for me it is an important topic that I want to develop further.

Based on the first studies and the obtained results, I want to continue the study of using the process of isolating the amino acids that make up bone collagen, in order to obtain increased efficiency and better separation, so that dating can be done based on several amino acids.

This study involves the use of other mobile phases (other than the ultra-pure water that has been used so far) to avoid the smaller contaminations caused by "column leakage" or the introduction of an isotopic carbon fraction. I also want a study to determine the ^{14}C background, contemporary or old, introduced using HPLC separation. This will only be possible by meeting bone samples that are very old and for which there is very accurate information about their age. These tests are very rare and expensive, so we could not make these determinations in the studies presented in this doctoral thesis.

Studies that I have decided to address are important in that a significant number of samples, which cannot be dated using current chemical protocols, can be dated by dating the constituent amino acids. Those samples that have, for example, destroyed collagen chains, and that pass through a special 30 kDa filter, will be "saved" for dating. So, I will recover the solutions they go through, I will separate the amino acids that make up the collagen by HPLC, even if the chain is destroyed, and we will date each one individually. Dating of individual amino acids has a great future.

Those samples that have, for example, destroyed collagen chains, and that pass through a special 30 kDa filter, will be "saved" for dating. So, I will recover the solutions they go through, I will separate the amino acids that make up the collagen by HPLC, even if the chain is destroyed, and we will date each one individually. Dating of individual amino acids has a great future.

I will continue the study using ^{13}C NMR directly on solid osteological material to determine the amount of amino acids of interest in radiocarbon dating, focusing on hydroxyproline. This analysis will be useful for me to choose the bone fraction that ensures a sufficient amount of carbon for dating with high precision. Approaching future studies using direct solid samples (without a pre-treatment) for ^{13}C NMR analysis, will also bring me important information regarding the degradation of collagen in the pre-treatments performed in the process of preparing bone samples for dating with ^{14}C.

ANNEX I

AUTHORS PUBLICATIONS AND CONFERENCE PARTICIPATIONS

Scientific articles

1. Verifying the influence of the HPLC method on carbon isotopic fractionation of amino acids standards for radiocarbon dating, *Oana Gaza*, Tiberiu B. Sava, Catalin S. Tuta, Corina A. Simion, Doru Ghe. Pacesila, Dan G. Ghita, Horia Iovu, U.P.B. Sci. Bull., Series B, Vol. 80, Iss., 2, 2018.
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