

Cover Page



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Title: Refining 14C dating of bone >30,000 BP : establishing an accurate chronology for the Middle to Upper Palaeolithic transition in France

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4 Establishing ^{14}C dating at MPI-EVA

In this thesis I focus on the pretreatment of bone to obtain pure collagen and convert it into graphite for AMS measurements to obtain reliable radiocarbon dates. The individual steps of pretreatment involve extraction of collagen from bone, cleaning all the equipment used in the procedures and the conversion of collagen to graphite (graphitization). I chose to use the extraction method (method C in chapter 6; paper (Talamo and Richards, 2011)) that best avoids lab contamination. In the field I collected good quality bone samples, which were selected due to their potential for high carbon yields.

All the bone samples presented in this thesis were subject to the following pretreatment procedures, usually in batches of up to 12 samples:

- Entry in database
- Pulverisation of bone
- Decalcification
- Removal of humics
- Gelatinization
- Cleaning of the filters and checking for the removal of contamination
- Ultrafiltration
- Freeze drying

These procedures are outlined in detail below.

4.1 Database entry

A S-EVA number is assigned to the sample and it is inserted in our database. Important fields of the sample record are S-EVA number, submitter name, sample code assigned by the submitter, name of the project or site, weight of the sample as received and a photo. All the subsequent pretreatment steps are entered into the database.

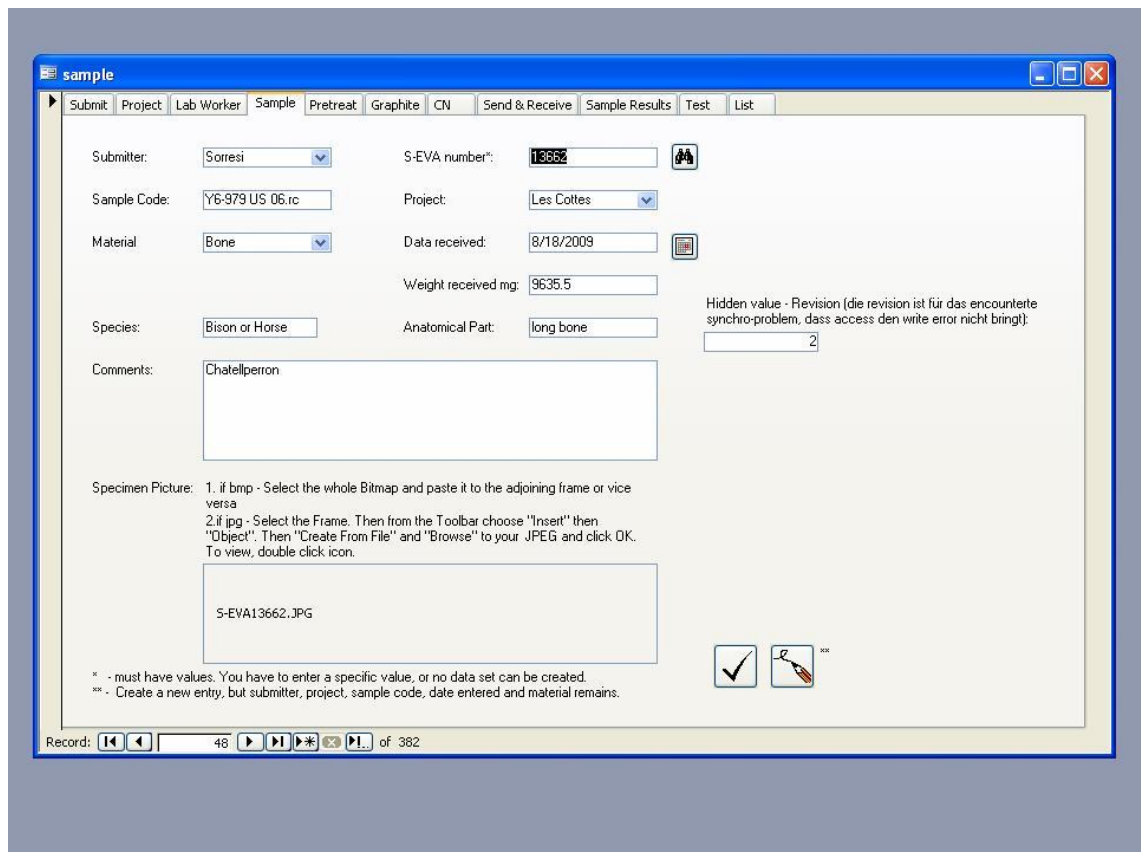


Figure 4.1 Entry page of the database at MPI

4.2 Pulverisation of bone

The bone is first cleaned by sand-blasting and then, using a dental drill, 500 mg of bone powder is taken. In the case of bone fragments a mortar is used to grind the bone. Bone powder is essential for a fast and efficient decalcification.

4.3 Decalcification

The sample is kept in 0.5M HCl at room temperature until no CO₂ effervescence is observed, which usually takes 4 hours. This interval is divided into two 2-hour segments after which the sample is rinsed in ultrapure water and centrifuged. Then it is kept overnight in 0.5 M HCl in a refrigerator.

4.4 Removal of humics

The following day 0.1M NaOH is added for 30 minutes to remove humics, which could have been introduced to the bone sample by ground water during the interval between burial and excavation. The NaOH step must be complemented by a final HCl step (15 minutes), to remove potential contamination from modern CO₂ taken up by the NaOH.

4.5 Gelatinization

The gelatinization step follows the method outlined in Longin (1971), at pH3 in a heater block at 75°C for 20h.

4.6 Cleaning of the filters and checking for the removal of contamination

The cleaning procedures for the ultrafilters are essential for a valid radiocarbon date (Higham, et al., 2006b). The ultrafilters are Sartorius “Vivaspin 15” of 30 KDalton size with 50ml plastic centrifuge tubes. The cleaning is designed to remove carbon-containing humectants. It is very important not to clean the filters more than 24 hours in advance as they may soften or dry out. The ultrafilters are rinsed 5 times in the centrifuge with ultrapure water for 15 to 20 minutes. Then they are bathed in ca 1 liter of ultrapure water in the ultrasonic bath for one hour, and after that rinsed 3 times.

Before the 4th centrifuge step, 1 ml of ultrapure water is added to one of the filters, and removed for analysis of remaining carbon. For this measurement the water sample is freeze-dried, another *ca.* 20 µl ultrapure water is added and inserted with *ca.* 8 mg chromosorb into a large tin capsule. The amount of carbon is determined by combustion in the EA (see step below). The burn yield must be below 5 to 10 µg C for this sample to indicate that the filter is not contaminated by carbon-containing humectants.

The Eeze-Filter™ (Elkay Laboratory Products (UK) Ltd.) is bathed for 20 minutes in *ca.* 1 liter of ultrapure water.

4.7 Ultrafiltration

The gelatine obtained in step 5 is filtered in the Eeze-filter to remove mineral particles. Then the liquid is transferred to the ultrafilter and centrifuged until the liquid in the filter is below 0.5 ml.

4.8 Freeze-drying

The filtered sample is frozen to a solid. The tube is sealed with parafilm and kept in a -28°C freezer. The tubes are kept in an inclined position so that the solution is thinly distributed along the tube, with no more than 10 mm at the thickest part. The samples stay in the freezer for at least 12 hours so that they are solidly frozen. Then the samples are transferred to the freeze-drier and lyophilized for 48 hours.

The specific lab protocol procedures for each sample (Figure 4.2) is entered into the database (Figure 4.3).

Collagen extraction protocol

S-EVA Notes _____

Date _____ Total Bone _____ mg

Sample taken _____ mg Rest _____ mg

Date _____ HCl for 2h HCl for 2h Fridge all night _____

Date _____ HCl for 2h Wash 3 times H2O
 NaOH 30min Wash 3 times H2O
 HCl 15min Wash 3 times H2O

Date Heater in with 10ml of Ph3 _____ Time in _____

Date Heater out _____ Time out _____ 75°C for 20h

Cleaning Eeze – Filter

Date _____ 20 minutes in Ultrasonic water

Cleaning Ultrafilter procedures

Date _____

15 min centrifuge with H2O pure

15 min centrifuge with H2O pure

1 Hour in Ultrasonic water

15 min centrifuge with H2O pure

15 min centrifuge with H2O pure

15 min centrifuge with H2O

Date Centrifuge and Samples in the fridge _____

Date Freeze – dryer in _____ Time in _____

Date Freeze – dryer out _____ Time out _____

Collagen mg _____

Circa 0.5mg of Collagen for C/N _____

Sent Collagen _____ mg **Remaining Collagen at MPI** _____ mg

Sent Collagen to _____ **Date** _____

Graphite **Sent graphite to** _____ **Date** _____

Radiocarbon Age _____ **Collagen back from the AMS Lab** _____ mg

Figure 4.2 Lab protocol with all the procedures made during the pretreatment

The screenshot shows a Microsoft Access window titled 'sample'. The menu bar includes File, Edit, Insert, Records, Window, and Help. The form contains the following data:

| | | | |
|----------------------|-----------|-----------------|-------------------------------------|
| Lab Worker: | Talamo | Sample Code: | Y6-979 US 06.rc |
| S-EVA number: | 13662 | Weight used mg: | 1609.1 |
| Date entered: | 8/27/2009 | Rest mg: | 7938.2 |
| HCL date: | 8/31/2009 | To be date: | <input checked="" type="checkbox"/> |
| Heater date: | 9/1/2009 | % Collagen: | 5851.272 |
| Centrifuge with ULF: | 9/2/2009 | Collagen mg: | 27.5 |
| Freeze dry date: | 9/7/2009 | | |

At the bottom of the form, there is a 'Record' field showing '61 of 418' and a 'Form View' indicator. A 'Submit' button with a checkmark icon is located at the bottom right of the form area.

Figure 4.3 Input of the lab protocol of the pretreatment and calculation of the % collagen

Important parameters are the date of the various steps, the weight used and the final weight of the collagen. At this point the collagen yield is available, which should be a minimum of 5 mg for 500 mg initial bone powder (1% yield limit). The minimum amount of collagen for graphitization is 3 mg.

4.1 Graphitization steps

All the collagen obtained after the pretreatment outlined above is graphitized according to the following procedures:

- Loading of collagen into tin caps
- Combustion in an Elemental Analyser (EA)
- Determination of carbon yield and C:N ratio
- Determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in a mass spectrometer
- Cleaning the CO_2 gas containers and conditioning of the iron catalyst
- Collection of CO_2 in the rigs
- Addition of hydrogen

- Conversion of CO₂ into graphite in the graphitizer
- Check of graphitization parameters
- Preparation of blank samples
- Preparation of shipment to an AMS facility and submission to the AMS laboratory for radiocarbon measurement.

4.1.1 Loading collagen into tin caps

The collagen is loaded into tin capsules, which are pre-cleaned in cyclohexane and acetone. An empty tin capsule is combusted to check that the blank contribution is < 2 µg C.

4.1.2 Combustion in Elemental Analyser (EA)

The collagen is combusted in the EA (CHN analyzer) system, in a sequence of up to 10 samples limited by the amount of available gas containers. Each sample combustion is preceded by the combustion of an empty tin capsule to purge the system. The sample is injected into the furnace together with a stream of helium and oxygen. The combustion furnace is at a temperature of 1000°C and with the addition of tin the combustion temperature reaches 1500°C; the subsequent reduction furnace is used to complete the combustion at 600 °C (Figure 4.4).



Figure 4.4 Elements of the graphitization: combustion in the EA (middle), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determination in the mass spectrometer (right) and the graphitizer (left).

The helium acts as carrier gas. The combustion products are sent through several gas chromatographic columns (GC) to purify and to separate the components of interest, nitrogen (N), CO₂ carbon (C), and hydrogen (H). This information is recorded by the EA software (Figure 4.5).

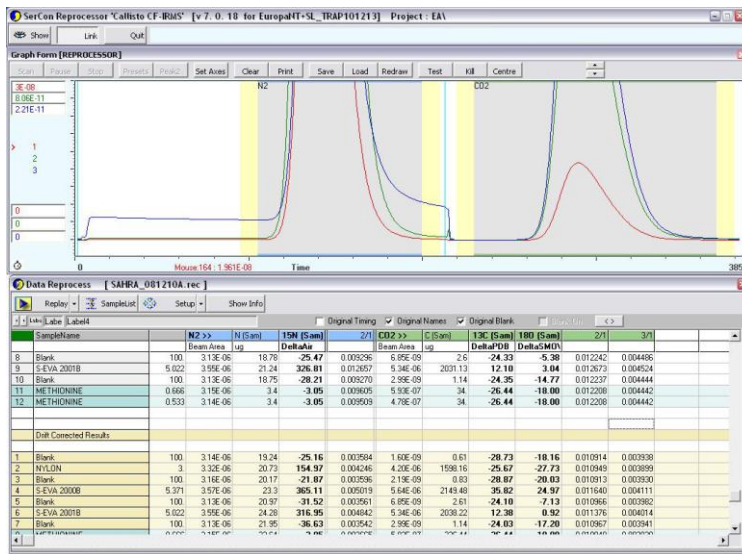


Figure 4.5 Protocol of the elemental analyser. The peaks represent the separation of C (CO₂) and N

4.1.3 Determination of carbon yield and C:N ratio

After the successful combustion of a sample the key parameters for quality control of bone collagen are available. These are the amount of carbon and nitrogen in the sample, which is used to determine the C:N ratio. These data and isotopic data from the next step are then entered into the database (Figure 4.6)

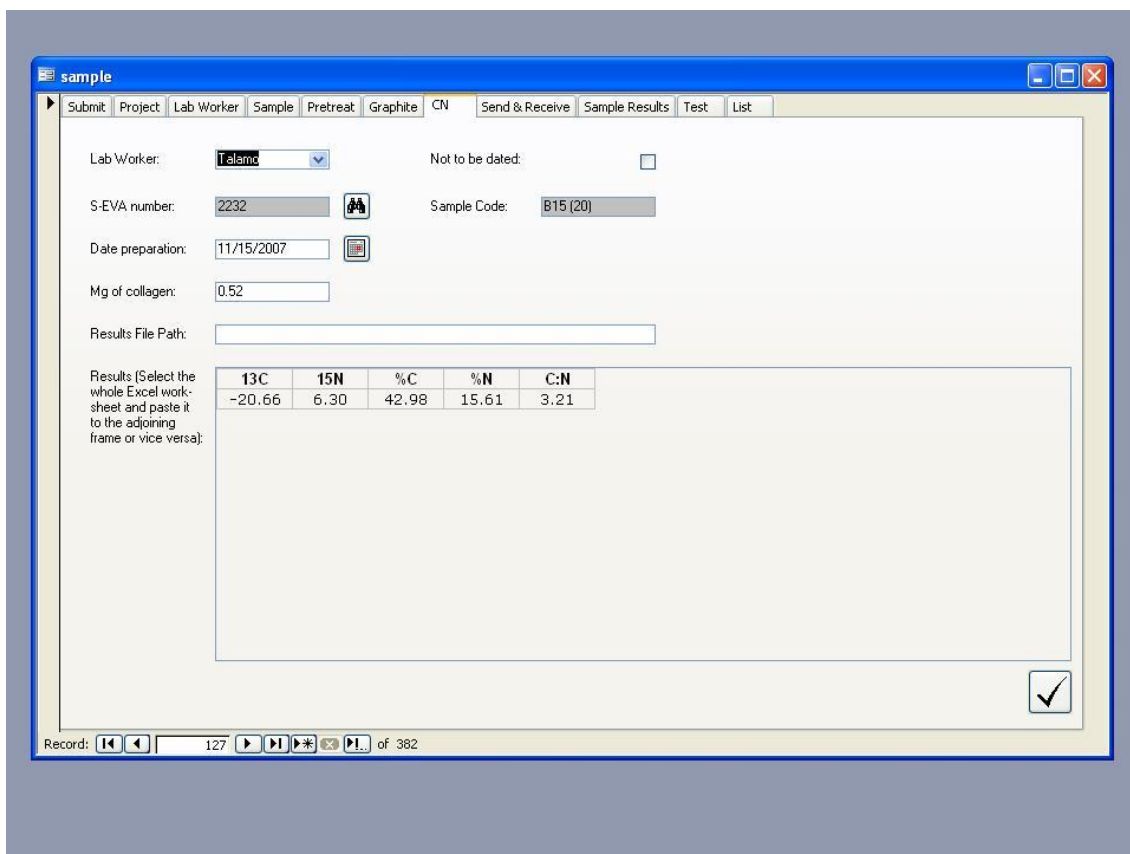


Figure 4.6 Input of isotope data into the database

4.1.4 Determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in a mass spectrometer

A small fraction, approximately 1%, of the purified gases are sent to the mass spectrometer (Figure 4.7), connected to the EA, to measure the stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (Table 4.I).

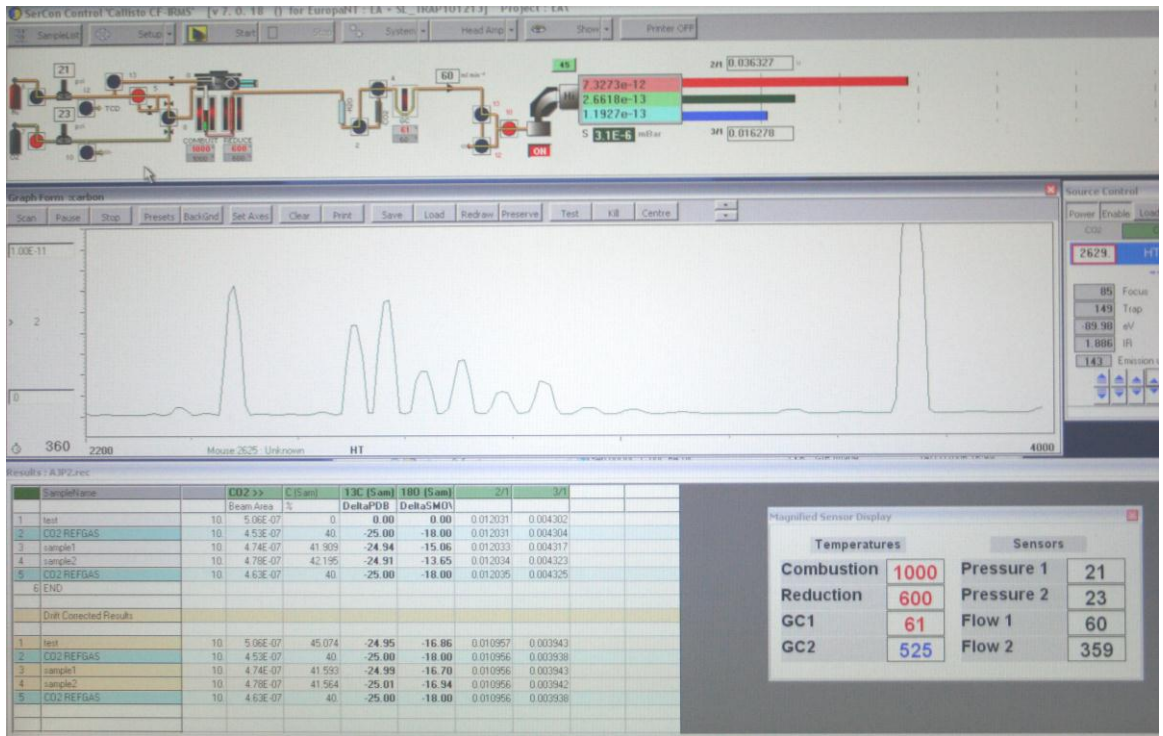


Figure 4.7 Output page of the mass spectrometer for $\delta^{13}\text{C}$.

Table 4.1 Example of a determination of stable isotope ^{13}C and ^{15}N for the reference material of Nylon 66

| Name | Weight/Vol | ^{13}C (Sam) | ^{15}N (Sam) | C | N | C:N |
|--------------------|----------------|-----------------------|-----------------------|-------|-------|------|
| | mg | DeltaPDB | DeltaAir | % | % | |
| EVA 0008 Nylon | 4.092 | -29.5 | 1.6 | 60.90 | 11.80 | 6.02 |
| EVA 0008 Nylon | 4.802 | -29.5 | 1.6 | 60.90 | 11.80 | 6.02 |
| Nylon 66 B2 Sample | 4.708 | -29.5 | 1.2 | 60.81 | 11.85 | 5.99 |
| Nylon 66 B3 Sample | 4.745 | -29.5 | 1.2 | 60.83 | 11.80 | 6.01 |
| | average | -29.54 | 1.52 | 60.86 | 11.83 | |
| | stdev | 0.04 | 0.24 | 0.05 | 0.03 | |

4.1.5 Cleaning the CO₂ gas containers and conditioning of the iron catalyst

The CO₂ gas containers are glass tubes closed by metal valves, called rigs (Figure 4.8).

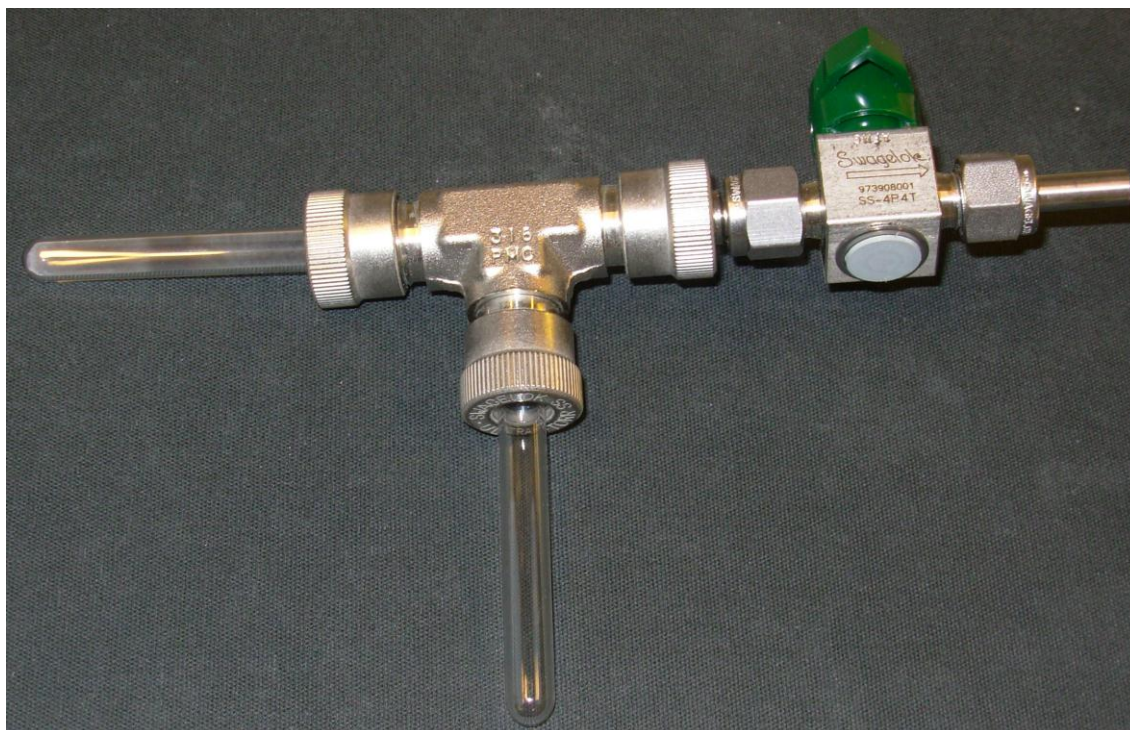


Figure 4.8 CO₂ gas container (rig) filled with iron catalyst.

The rigs are filled with 1.5 to 3 mg iron catalyst (Aldrich Chem. Co. <10micron 99.9%) and the optimal ratio of iron to carbon was determined to be 3 to 1 (Vogel, et al., 1984). To avoid contamination from absorbed CO₂ or particulates the iron and the glass surfaces are cleaned by adding H₂ (99.999%) at 500 mbar into the rigs and placing them in the oven at 450 °C for 1 hour.

4.1.6 Collection of CO₂ in the rigs

Most of the CO₂ is collected in a rig attached to the gas collection system (Figure 4.9) and is trapping using liquid N₂.

Hydrogen is added to the frozen CO₂ in a quantity sufficient to guarantee a complete reduction of CO₂. In our system an excess of H₂ is used with the ratio H₂:CO₂= 2.2 : 1



Figure 4.9 Graphitization system manufacture by the Oxford laboratory

4.1.7 Conversion of CO₂ into graphite in the graphitizer

The rig is placed in the oven at 560 °C for 6 hours, where CO₂ is reduced to carbon and water vapour. The latter is removed by cooling one finger of the rig (Figure 4.10).



Figure 4.10 Reduction of CO₂ to graphite using iron as catalyst in an oven (top section); water vapour is removed by immersing the vertical finger of the rig into a cooling bath (left and right section).

4.1.8 Check of the graphitization parameters

During the reduction of CO₂ to carbon hydrogen is consumed at the ratio 2:1 with respect to carbon. Therefore the pressure in the rigs after reduction will be low reflecting the excess amount of hydrogen. Typically we use 400 mbar of hydrogen which results in a residual pressure of *ca.* 80 mbar. This pressure is checked by reconnecting the rigs to the gas collection system. All these parameters are entered in the database (Figure 4.11).

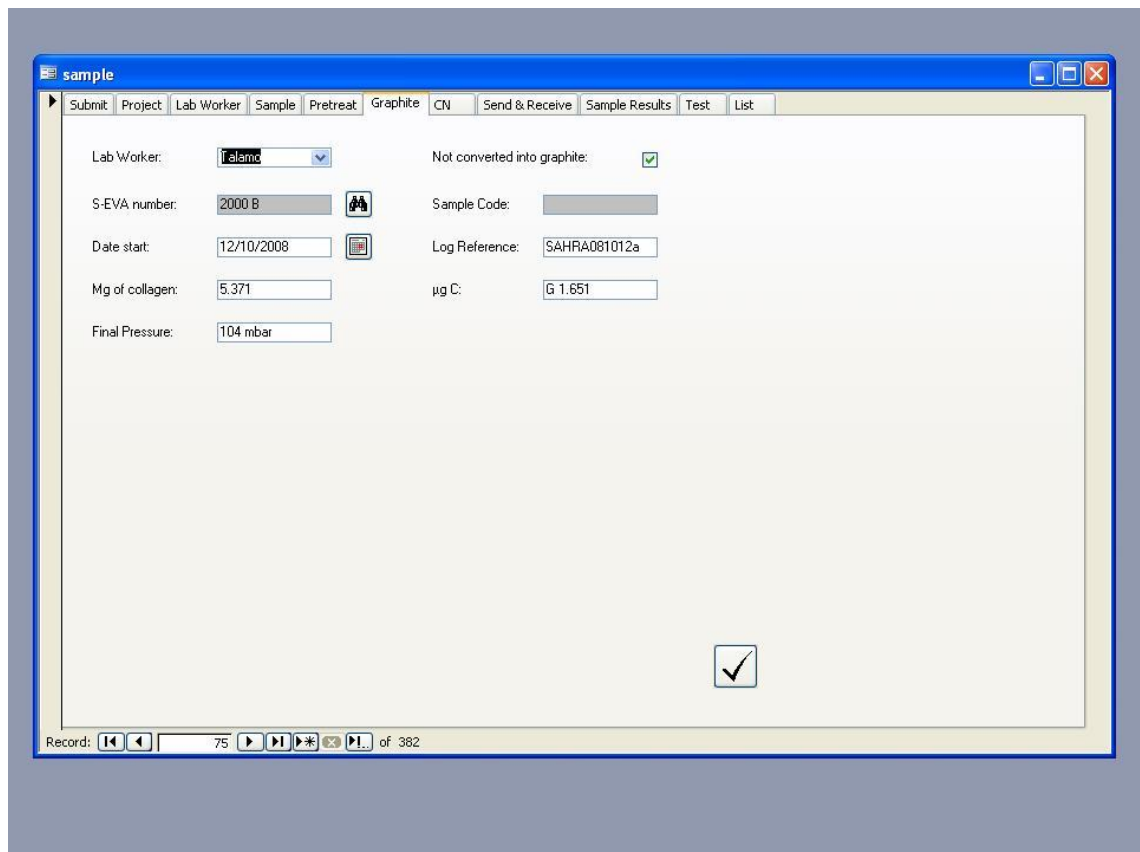


Figure 4.11 Input of graphitization parameters into the database

4.1.9 Preparation of blank samples

All steps of pretreatment and graphitization may contribute exogenous carbon (contamination). Therefore ^{14}C free material (old bone, Nylon 66, Pliocene wood) is pretreated and graphitized in the lab. These samples are called blank samples and they are prepared at the same time as the archaeological samples, and are also sent to the AMS facilities to establish the level of ^{14}C activity in the blanks.

4.1.10 Preparation of shipment to an AMS facility and submission

At this point the samples are ready to be sent to an AMS facility, where the graphite will be pressed into a target and measured in batches in the accelerator. A batch of target usually consists of a number of samples, standards and blanks. For this thesis the samples were submitted to the laboratories of Oxford, Kiel and Mannheim/Zurich. All dates of shipments and the dating results are recorded in the database providing the final list of samples and their ages (Figure 4.12 – 4.13 – 4.14)

sample

Submit | Project | Lab Worker | Sample | Pretreat | Graphite | CN | Send & Receive | Sample Results | Test | List

Lab Worker: Talamo Not to be sent:

S-EVA number: 2000 D Sent Collagen:

Date of shipment: 12/19/2008 Sent Graphite:

Date expected: 2/16/2009 Sample Code:

Sent to: Oxford

Collagen mg sent: 7.8

Collagen mg rest: 43

Record: 14 of 382

Figure 4.12 Table of shipment and dating results of a sample

sample

Submit | Project | Lab Worker | Sample | Pretreat | Graphite | CN | Send & Receive | Sample Results | Test | List

Lab Worker: Talamo

S-EVA number: 1601.1 Sample Code: SP1461

Sent to: Kiel AMS Lab No.: KIA 37396

C14 Age: 38260 Err+- 1d: 560 Cal BP: Results PDF (Important Note: The Result File must be saved in a subfolder of the database file - That means at present on humfsshared in "humfsshared\Research Projects\CT14\Subfolder") KIA Results\TalamoS090721.doc

Err+- 2d: d 4C: -12.81

Result Graph Path:

Comments:

Secure Results: Check here, after you entered C14 Age, Err+-, Cal BP and d 4C, so that the results are locked. They will be changeable again, if you deactivate this button.

Final EVA number:

Record: 14 of 382

Figure 4.13 Input of data as reported by the AMS facility

sample - Microsoft Access

Home Create External Data Database Tools

Views Clipboard Font Rich Text Records Filter Sort & Filter Window Find Simplified Traditional Translate with Options Chinese Translation

Submit Project Lab Worker Sample Pretreat Graphite CN Send & Receive Sample Results Test List

Radio group for query for:

- Site
- Submitter (Surname)
- C14 Age
- Pretreatment (Collagen mg exist, but no Age)
- Graphite (Final pressure exist, but not Age)
- Not yet pretreated (weight pretr. doesn't exist)

If query needs a value, please enter here:

les cottés

Execute Query Print Results

| S-Eva | Sample Code | Sent to | Lab Nr. | Sample Side | Weight Used | Collagen mg | % Collagen | C14 A... | Err+- 1d |
|---------|------------------|----------|---------------|-------------|-------------|-------------|------------|----------|----------|
| 9695 | Z4-1258 | Mannheim | MAMS_10803 | Les Cottés | 1466.8 | 49.7 | 3.39 | 38130 | 470 |
| 9696 | Y4-279 | Mannheim | MAMS-10804 | Les Cottés | 7492 | 22.9 | 3.06 | 43980 | 650 |
| 13677 M | US08.0 Z3-356 | Mannheim | MAMS_10830 | Les Cottés | | | 0 | 39460 | 540 |
| 13678 | US08.rc Z3-289 | Mannheim | MAMS_10831 | Les Cottés | 793.8 | 30.1 | 3.79 | 37940 | 460 |
| 13679 | US08.rc Y4-311 | Mannheim | MAMS_10832 | Les Cottés | 618.8 | 20 | 0.32 | 38310 | 500 |
| 13671 | US04.4 Y5-1083 | Mannheim | MAMS_10826 | Les Cottés | 1011.5 | 16.1 | 1.59 | 33170 | 250 |
| 13672 | US04.9 Y6-1681 | Mannheim | MAMS_10827 | Les Cottés | 1013.9 | 13.4 | 1.32 | 33570 | 270 |
| 13675 M | US08.0 Z3-362 | Mannheim | MAMS_10828 | Les Cottés | | | 0 | 39550 | 560 |
| 13676 M | US08.0 Y5-1654 | Mannheim | MAMS_10829 | Les Cottés | | | 0 | 40430 | 610 |
| 13663 | Y5-1225 US 06... | Mannheim | MAMS_10814 | Les Cottés | 1933.5 | 13.7 | 0.71 | 32550 | 250 |
| 13665 M | S6-557 US 06... | Mannheim | MAMS_10816 | Les Cottés | | | 0 | 34590 | 300 |
| 13667 | Z4-3286 US 06.1 | Mannheim | UnK 41 | Les Cottés | 826.6 | 15.8 | 1.91 | 35754 | 318 |
| 13668 M | Z4-3368 US 06.1 | Mannheim | MAMS_10824 | Les Cottés | | | 0 | 37330 | 430 |
| 13669 | US04.5bj R5-785 | Oxford | UnK 43 | Les Cottés | 701.5 | 18.9 | 2.69 | 33535 | 224 |
| 13668 | Z4-3368 US 06.1 | Oxford | UnK 42 | Les Cottés | 642.1 | 21.2 | 3.3 | 37016 | 290 |
| 13666 | X6-205 US 06.07 | Oxford | UnK 40 | Les Cottés | 542.9 | 11.6 | 2.14 | 35273 | 244 |
| 13664 | Y5-2785US 06... | Oxford | UnK 37 | Les Cottés | 1887.9 | 17.7 | 0.94 | 40560 | 400 |
| 13665 | S6-557 US 06... | Oxford | OxA-V-2381-52 | Les Cottés | 1205.5 | 26.2 | 2.17 | 33670 | 400 |
| 13677 | US08.0 Z3-356 | Oxford | UnK 56 | Les Cottés | 670.8 | 45.7 | 6.81 | 37441 | 257 |

Record: 838 of 838 No Filter Search

Form View Num Lock

Figure 4.14 Example of a summary sheet of an archaeological site (Les Cottés)