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Developments towards improvement of the background for Radiocarbon Dating of ultra-small DNA samples

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Abstract

Radiocarbon (^{14}C) dating using Accelerator Mass Spectrometry (AMS) is a well established method. Standard methods of AMS typically require a sample size of about one milligram carbon, which, however, is not available in our recent efforts towards dating DNA from neurons from human brain samples using the ^{14}C bomb peak [1]. We are presently developing methods to reliably measure samples in the range of ~10 μg C. In decreasing the sample size, the main challenge is the control of the laboratory background which typically stays constant, and finally dominates the measurement result. In order to keep carbon contamination at the lowest possible level, an argon atmosphere – instead of air - was provided during sample handling, sample preparation, and ion source loading. Most of these tasks were performed in an argon glove box.

Since we couldn't find a significant difference in the carbon background for the ultra-small samples between sample preparation in laboratory air and argon atmosphere, we tested our argon-controlled set-up with mg-size samples of geological graphite (nominally zero ^{14}C content), and found a radiocarbon age of 77 000 years BP. This is the lowest ^{14}C background we have measured at VERA so far. It corresponds to a fraction modern carbon ($F^{14}C$) value of $(6.3 \pm 1.4)\times10^{-5}$. This is about one ^{14}C half life "older" than samples processed in laboratory atmosphere. However it was not possible to measure such a low value with ~10 μg graphitized carbon samples. Typically 65000 years BP were observed, because contamination from other sources seems to dominate the overall background.

In a different investigation, we studied the mass of carbon contamination incorporated during different steps of sample preparation using small samples highly enriched in ^{13}C. The amount of contamination with natural carbon (99% ^{12}C) present in CO_2 from sample combustion can be assessed by measuring $^{13}CO_2/^{12}CO_2$ ratios using an RGA (residual gas analyzer). Additional contamination from the graphitization process was determined by measuring $^{13}C/^{12}C$ ratios with AMS. An overall amount of 0.12 to 0.15 μg C contamination was found in this way [2].

Introduction

The main goal in our project is to develop a method to measure ultra small ^{14}C samples (<10 μg) with accelerator mass spectrometry while keeping uncertainties as small as possible. Therefore it's crucial to have total control over all the influences from sample preparation to AMS measurement. One of the bigger impacts on the measurement will be the contamination of the samples. While the sample size decrease the contamination will usually not and finally would dominate the result.

The major application for our method is the dating of human DNA from brain tissue using the bomb peak. It is currently unclear whether or not neurogenesis takes place in the adult human brain. (Neurogenesis means that new cells are formed after birth). Because the size of such samples is very small, the development of new methods to measure them is necessary.

In the current state we are able to measure sample masses down to 10 μg C with a precision of 1%. The amount of dead carbon contamination is determined by using ^{13}C enriched DNA material ($^{13}C/^{12}C$=99:1). The enriched material is treated as normal samples including freeze drying and combustion of the sample. The produced CO_2 is measured with an RGA connected via a capillary to the graphitisation line [2]. To check the RGA measurement and also to determine the contamination introduced during graphitisation the gas is graphitized and measured in our AMS facility VERA. The results of this measurements are shown in figure .

In our approach to reduce the carbon contamination we also set up an argon glove box system to determine the influence of ambient laboratory atmosphere. In this case possible contamination from atmospheric air may be avoided.

Background measurement of geological graphite using an argon glove box

To provide the best possible background measurement, one needs a material with an as low as possible ^{14}C content. In our usual procedure we use "dead" CO_2 from a bottle provided by "LindeGas AG". In this case we have no idea about the real amount of ^{14}C in it. The best previous measurements for this blank material resulted in a radiocarbon age of about 65000 years BP for samples of about 100 μg C. However Fig. 1 shows higher ^{14}C background, which was identified as coming from contaminated O-rings

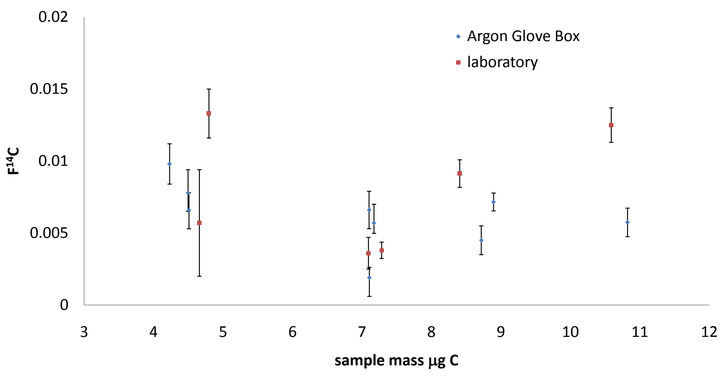

Figure 1: Measurement of dead carbon blanks from Linde CO_2

For our approach to determine the origin of contamination we needed a material that has almost no ^{14}C in it. Therefore we used geological graphite. We were able to get a piece of untreated graphite ore from the mining company "Kropfmühl Graphit AG" near Passau, Germany.

Figure 2: Geological graphite ore

For the measurement we prepared half of the samples in a laminar air flow box in laboratory air and half of the samples in our argon glove box system in order to find out if and how much of carbon from ambient atmosphere is brought into the sample preparation cycle.

To prevent contamination during sample cleaning steps we decided to use the graphite ore without any chemical pre-treatment.

<u>The following steps took place inside the laminar air flow box.</u>

In a first step a small piece of graphite ore was cut from the original piece. Then the small piece was cut into half to get a fresh and clean surface. From the surface we scratched some pieces with as little tool interaction as possible. Half of the pieces were filled directly into our standard AMS target holders made from aluminium and the other half was powdered and pressed into modified holders with copper inlets, such as the ones we use for our small-sample project. The produced AMS targets were stored inside polypropylene vials (PP) until the AMS measurement took place.

Figure 3: Argon Glove Box

The same sample preparation procedure took place inside the argon atmosphere where the samples also were stored until the AMS measurement. The box is especially equipped with an activated charcoal trap to remove organic solvents. We added a NaOH trap to remove CO_2.

During sample treatment the O_2 and CO_2 concentrations were kept bellow 5 ppm and 10 ppm, respectively.

Sample transfer to the AMS source

Samples prepared in laboratory air were mounted inside the laminar air flow box into our AMS sample wheel together with IAEA C-3 and C-6 standards, a high purity graphite sample and 5 used large samples for tuning operations. Then the sample wheel was transferred into the argon glove box. To avoid transport of trapped air from the sample wheel to the inside of the argon box, the wheel was transferred disassembled. Then samples stored under argon where mounted into the target wheel inside the box. To prevent contact with ambient atmosphere while transferring the sample wheel to the AMS source, we used a transportation box that could be sealed hermetically. The wheel was loaded into the source using a glove bag filled with argon. Enfolding the source, we can assure a completely air-free sample preparation cycle from obtaining samples until AMS measurement.

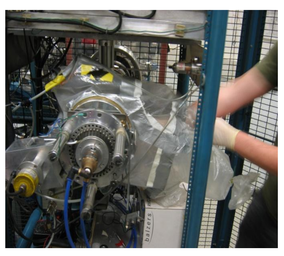

Figure 4: Mounting the sample wheel into the AMS source

AMS measurement

The measurement was performed with our standard radiocarbon setup of VERA.

Eight samples of graphite were prepared and measured. Five samples ran through preparation under argon and three samples were prepared inside the laminar air flow box. The weight of the samples was not determined to avoid possible contamination during the weighing process. But all of the samples can be assumed to have a mass of a few milligrams which would be a standard size of samples for AMS ^{14}C dating.

All samples were mounted into the source using an argon glove bag.

All samples gave negative ion currents in the range of a few tens of mA C^-, which is normal due to the size and material of the targets.

Results

It can be assumed that geological graphite with an age of millions to billions of years will represent a good machine blank of our AMS machine.

Nr.	sample name	$F^{14}C$	σ_{F14C}	comments
1	graphbox_1	1.01E-04	3.70E-05	Ar box
2	graphbox_2	9.00E-05	2.40E-05	Ar box
3	graphbox_3	8.80E-04	3.00E-04	Ar box,outlier not shown
4	graphbox_4	6.30E-05	1.40E-05	Ar box
5	graphbox_5	1.30E-04	3.70E-05	Ar box
6	graphlab_1	1.70E-04	2.70E-05	lab air
7	graphlab_2	1.33E-04	5.40E-05	lab air
8	graphlab_3	2.94E-04	5.20E-05	lab air

Table 1: results from geological graphite samples

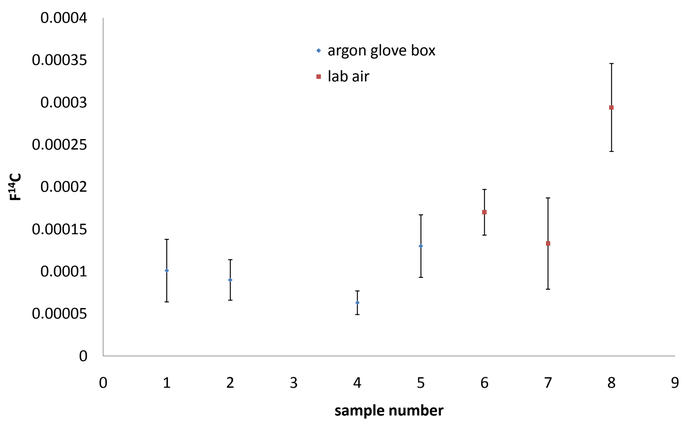

Figure 5: results from geological graphite samples

The use of ^{13}C enriched material to determine carbon contamination

We used ^{13}C enriched DNA material ($^{13}C/^{12}C$=99) to determine the contamination during the whole process. The isotopic ratio $^{13}C/^{12}C$ found in the final AMS sample is a direct measure for the total contamination of the sample, since the contamination is expected to have natural isotopic ratios ($^{13}C/^{12}C$=0.01). The material was stored in an aqueous solution in a refrigerator. The concentration of the sample material was 1.006 mg C ≡ 1 ml DNA solution. Sample pre-treatment was either performed in a laminar air flow box or in our argon glove box system, to investigate the influence of ambient atmosphere to the contamination of the samples. The sample treatment was carried out as identically as possible in both kinds of atmospheres. The only difference, besides the atmosphere, would be that in the argon glove box one has a very clean and controlled atmosphere of argon, but it isn't dust free in contrast to the laminar air flow box. But it can be assumed that if dust is introduced into a sample the effect would be quite large and noticeable as an outlier.

Typically a standard size of 0.5 ml water + DNA solution is used for further use. The purity of the used water plays an important role. For our purposes we used Ultra-pure DNase/RNase-free distilled water. The influence of the quality of the used water is shown in Table 2.

0.5 ml	CO_2 in graphitization reactor (μg C)
deionized bi-distilled laboratory water (no storage)	0.20 ± 0.10
ultra-pure water kept in not rinsed cleaned vials	0.25 ± 0.06
ultra-pure water kept in rinsed cleaned vials	0.08 ± 0.04
ultra-pure water kept in rinsed cleaned vials (pH 3)	0.07 ± 0.03

Table 2: carbon contamination from different types of water

For our work 2 μl of DNA solution (3μg DNA/μl) with 498 μl of ultra pure water is mixed in a cleaned PP vial and then filled into a cleaned sample vial. Vials were closed with the sample vial valves and were ready for sublimation and combustion.

Sublimation and combustion of DNA samples

The pre-treated sample solutions were transferred inside the closed sample vials to our graphitisation unit and connected to the combustion/graphitisation line. To get solid DNA sample material the solution was freeze dried inside the sample vials. This procedure takes about two hours to complete depending on the sample size. After that the samples were baked in vacuum at 220 °C for additional cleaning . After about 20 minutes the sample vials were filled with about 200 mbar of Oxygen from a gas bottle (Air Liquid Austria, ≥ 99.5% purity), which was fed through a liquid nitrogen cooled cold trap to remove carbonaceous contamination. The sample material was combusted for about 30 minutes at 900 °C to form CO_2.

Graphitization of DNA samples

After combustion the CO_2 was cryogenically purified and transferred into the graphitisation reactors using liquid nitrogen. 2.5 times the CO_2 sample pressure of H_2 was filled with an excess of 50 mbar into the reactors. The graphitisation reactions then will start as soon as the ovens are in place to heat the iron catalyst. The reaction took place at two different temperature steps. Depending on the sample size the reaction lasts between 2 and 15 hours.

AMS measurement of DNA samples

A total of nine ^{13}C enriched DNA samples were prepared and graphitized (Table 3). To determine the contamination resulting from ambient atmosphere six samples (out of the nine) were prepared under argon atmosphere inside the glove box. Samples from the argon glove box haven´t come in contact with ambient atmosphere since their arrival at the laboratory until the AMS measurement. Therefore we can guarantee an air-free preparation and measurement of the samples.

sample name	nominal m_{DNA} [μg C]	p_{CO2} [mbar]	m_{DNA} [μg C] from graph. Reactor	prepared in
19110_R1	2.17	4.94	2.19	Ar box
19110_R2	2.17	2.48	1.10	Ar box
19110_R3	2.17	5.71	2.53	lab air
240210_DNA1	2.17	4.21	1.86	Ar box
240210_DNA2	2.17	4.55	2.01	Ar box
240210_DNA3	2.17	16.15	7.15	lab air
250210_DNA4	2.17	4.56	2.02	Ar box
250210_DNA5	2.17	3.55	1.57	lab air
250210_DNA6	2.17	5.73	2.54	Ar box

Table 3: DNA sample data

Results

The measurement of the $^{13}C/^{12}C$ ratios were performed with the usual AMS setup used for radiocarbon measurements at VERA.

Nr.	sample name	$^{13}C/^{12}C$ AMS	abs. SD $^{13}C/^{12}C$	$m_{contam.}$ [μg C]
1	19110_R1	4.48	0.01	0.37
2	19110_R2	4.23	0.01	0.19
3	240210_DNA1	3.02	0.02	0.44
4	240210_DNA2	2.09	0.01	0.63
5	250210_DNA4	2.63	0.05	0.53
6	250210_DNA6	4.06	0.01	0.46
7	19110_R3	3.10	0.11	0.58
8	240210_DNA3	0.31	0.01	5.49
9	250210_DNA5	3.38	0.08	0.34

Table 4: DNA sample Results

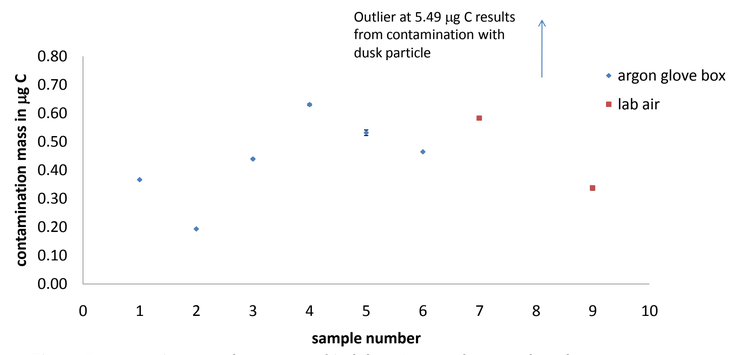

Figure 6: comparing samples prepared in laboratory and argon glove box

Conclusions

- For the untreated mg-size geological graphite samples we observed a reduced ^{14}C background when handling samples under argon.
- Previous measurements on μg-size samples established an overall carbon background of 0.12-0.15 μg C without Ar treatment[2].
- Measurements with μg-size samples enriched in ^{13}C did not show an effect when working under argon as compared to laboratory air.
- The remaining (small) carbon background [2] is therefore likely due to other sources.

References

[1]Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H., Frisen, J., "Retrospective birth dating of cells in humans", Cell **122**, 133-143 (2005).

[2] Liebl, J., Avalos Ortiz, R., Golser, R., Handle, F., Kutschera, W., Steier. P., Wild, E. M., "Studies on the preparation of small ^{14}C samples with an RGA and ^{13}C enriched material", Radiocarbon 52, 1394-1404 (2010).