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DIPLOMARBEIT

Titel der Diplomarbeit

„Reduction of the background in the handling of ultra-small ^{14}C samples:

A clean atmosphere glove box and other approaches“

Verfasser

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[…] „Daß ich erkenne, was die Welt im Innersten zusammenhält“, […]

Johann Wolfgang Goethe

Faust, Der Tragödie erster Teil

Verse 382, 383

Content

1 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 many interesting applications like in our recent efforts towards dating DNA from small human brain tissue samples using the ^{14}C bomb peak. We are presently developing methods to reliably measure samples in the range of ~10 μg C. The goal of this thesis was to contribute to the development of the measurement procedures, and to apply them to a problem in the field of nuclear physics.

In decreasing the sample size, the main challenge is the control of the laboratory background which typically is expected to stay constant in mass, and finally can dominate the measurement result. Among other efforts to keep carbon contamination at the lowest possible level, an argon atmosphere – instead of air - was provided for sample handling, sample preparation, and ion source loading. Most of these tasks were performed in an argon glove box. This system can provide an atmosphere with less than 2.5 ppm O_2 and a CO_2 content of about 10 ppm. A slight improvement in background is observed if ^{14}C free CO_2 is treated without atmospheric contact of the catalyst used for graphitization. We tested our argon-controlled set-up also with mg-size samples of geological graphite (nominally zero ^{14}C content), and found a fraction modern carbon ($F^{14}C$) value of (6.3 ± 1.4) x 10^{-5}. This is the lowest $^{14}C/^{12}C$ ratio we have measured at VERA so far and corresponds to a radiocarbon age of 77 000 years BP. The same samples processed in laboratory atmosphere appear about one ^{14}C half life "younger", which corresponds to a contamination mass of several 100 ng of modern carbon. This amount would significantly affect measurements on samples with ~10 μg carbon. However, 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. Contamination from other sources seems to dominate the overall background, while the contamination from the atmosphere seems to be smaller than 100 ng modern C for these samples.

The search for other factors contributing to background revealed that one critical point is the correct pre-treatment of the iron catalyst used for the reduction of CO_2 to graphite. We have observed a difference between oxidizing Fe to FeO in laboratory air or under pure oxygen and argon, and we observed a difference when using different temperatures during the reduction of FeO back to Fe.

One application of our small graphitization unit in the field of physics is to detect traces of ^{14}C emanating from solid nitrogenous materials which have been exposed to a flux of neutrons. The task was to determine if ^{14}C can escape due to recoil, a question, which to our knowledge was not yet addressed so far. Such losses would complicate the interpretation of a previous measurement series of the cross section $^{14}N(n,p)^{14}C$ and of other neutron capture cross sections of astrophysical relevance. We have shown that such emanation at the level of several percent can occur in organic materials, which has to be taken into account in the design and evaluation of such experiments.

2 Zusammenfassung

Altersbestimmungen durch die Radiokohlenstoff Methode mittels Beschleuniger Massenspektrometrie (AMS) ist heutzutage eine gut etablierte Methode. Standard AMS Messverfahren benötigen Probengrößen von etwa 1 Milligramm. In vielen interessanten Anwendungen stehen aber solche "großen" Probenmengen nicht zur Verfügung. Ein Beispiel wäre die Datierung von DNA aus dem menschlichen Gehirngewebe mithilfe des sog. ^{14}C bomb peak. Zurzeit entwickeln wir Methoden um Proben mit Massen im Bereich von 10 μg C reproduzierbar zu messen. Das Hauptproblem beim Verwenden von kleinen Proben besteht darin, dass während die Probengröße kleiner wird, der Labor Hintergrund konstant bleibt und letztendlich das Ergebnis dominieren würde. Um die Kohlenstoff Kontamination auf einen möglichst geringen Level zu halten, wurde, um direkten Kontakt mit Laborluft zu vermeiden, eine „Argon Glove Box" installiert. Probenpräparation, Bestückung des Probenrades bis hin zum Einbau des Probenrades in die AMS Quelle wurde in der Argon Glove Box oder unter Argon Atmosphäre durchgeführt. Die Atmosphäre der Argon Glove Box weißt einen Sauerstoff Anteil von weniger als 2.5 ppm und einen CO_2 Anteil von weniger als 10 ppm auf.

Weil wir keinen signifikanten Unterschied zwischen Proben die unter Argon präpariert worden sind und Proben die unter Labor Bedingungen hergestellt worden sind, finden konnten, haben wir unser Argon Glove Box System mit Proben aus geologischem Graphit getestet. Dies sind Proben im mg Bereich die nominell keine ^{14}C Atome mehr enthalten sollten. Mithilfe der Argon Glove Box konnten wir so ein ^{14}C Alter von 77000 yBP für diese Proben bestimmen, was einem $F^{14}C$ (fraction modern carbon) Wert von $(6.3 \pm 1.4) \times 10^{-5}$ entspricht. Dies sind die ältesten jemals bei VERA gemessenen Kohlenstoff Proben. Das bedeutet, dass Proben aus der Glove Box um etwa eine Halbwertszeit von ^{14}C älter gemessen worden konnten als Proben die unter Labor Atmosphäre präpariert worden sind. Dieser Unterschied entspricht einer Kontamination der Proben mit „modernem" Kohlenstoff von einigen hundert Nanogramm. Wir waren wir nicht in der Lage einen solch niedrigen Wert für kleine Proben (~10 μg) zu messen, was bedeutet das der Hauptanteil des Kohlenstoff Hintergrundes aus anderen Quellen stammt, während der Anteil der Laboratmosphäre zum Hintergrund wenig als 100 ng moderner Kohlenstoff zu sein scheint.

Ein weiter Punkt zur Verringerung des Hintergrundes ist die korrekte Aufbereitung des verwendeten Eisen Katalysators, welcher für die Reaktion von CO_2 zu Graphit benötigt wird. Wir konnten einen Unterschied zwischen an der Laboratmosphäre oxidiertem und in reinem Sauerstoff und Argon oxidiertem Eisen finden und es scheint einen Einfluss der Temperatur während der Reduktion von FeO zu Fe zu geben.

Eine andere Anwendung für unsere kleine Graphitisierungsanlage im Bereich der Physik stellt die Messung von Spuren von ^{14}C in, mit Neutronen bestrahlten, Stickstoffhaltigen, Materialen dar. Die Aufgabe bestand darin, herauszufinden ob es den ^{14}C Atomen möglich ist, mittels der gewonnenen Rückstoßenergie, den Festkörper zu verlassen. Würde es einen signifikanten Anteil derer geben, so würde das die Interpretation einer früheren Messserie, die sich mit Wirkungsquerschnitten von Neutronen Einfang Reaktion, im Rahmen der Astrophysik,

beschäftigt, verkomplizieren. Wir konnten zeigen, dass der Verlust von ^{14}C in organischen Proben einige Prozent betragen kann, was bei der Durchführung und Auswertung solcher Experimente berücksichtigt werden sollte.

3 Goals

The main goal in our project is to develop a method to measure ^{14}C in ultra small carbon samples (<10 μg) with accelerator mass spectrometry with an accuracy as high as possible. Therefore it is crucial to have total control over all influences from sample preparation to AMS measurement. One of the bigger impacts on the measurement is the contamination of the samples, because while the sample size decreases the contamination does usually not and finally would dominate the result.

One major application of our method is currently be the dating of human DNA from brain tissue using the bomb peak. *Neurogenesis* means that new neuronal cells are formed in the brain. It is very unclear whether or not neurogenesis takes place in adult humans (Bédard, et al., 2004), (Bhardwaj, et al., 2006), (Spalding, et al., 2005). Because the size of the resultant carbon samples is very small, the development of new methods for their measurement is necessary.

In the current state, achieved also with the help of the work presented in this thesis, 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 like normal samples, including freeze drying and combustion of the sample. The produced CO_2 is measured for $^{13}C/^{12}C$ with an RGA (Residual Gas Analyzer, a small quadrupole mass spectrometer) connected via a capillary to the sample preparation line. To verify the RGA measurement and also to determine the contamination introduced during graphitisation the CO_2 gas is graphitised and measured in our AMS machine.

In our approach to reduce the carbon contamination we have set up an argon glove box system to determine the influence of ambient laboratory atmosphere. By these means, we can avoid contamination of a sample from atmosphere from the arrival at our laboratory until the measurement in the accelerator.

Our ability to measure such ultra-small radiocarbon samples opened the opportunity to address a yet unanswered question on the field of experimental nuclear physics. Many nuclear cross section measurements are based on the detection of the reaction product in the matrix of the irradiated sample. However, the recoil from the reaction is usually large enough to displace the product from the lattice, to cause significantly lattice damage along its track, and to leave it in a chemically reactive form. Such lattice damage is known to increase the mobility of radiogenic atoms (as an example, radiogenic ^{234}U is more readily leached from minerals then primordial ^{238}U) (Dawood, 2008). We think that especially products which can form gaseous compounds are at risk to get lost from the sample matrix as emanation. Such losses would lead to an underestimation of the cross section under investigation.

The energy-dependent neutron capture cross section $^{14}N(n,p)^{14}C$ is presently being studied at our Laboratory. Chemical compounds containing both nitrogen and carbon in a stochiometric ratio (e.g. Uracil $C_4H_4N_2O_2$) have been irradiated already, and the $^{14}C/^{12}C$ ratio was measured using standard radiocarbon procedures. Possible losses of ^{14}C during irradiation have not been

considered. To address this question, we have flame-sealed small amounts of material in evacuated quartz vials, irradiated them, and have measured the ^{14}C content of the gases released during irradiation. To allow dependable preparation of graphite targets, ^{14}C free CO_2 gas was added. The expected small number of ^{14}C atoms in the sample recommended to minimize the amount of the carrier gas, to obtain a sufficiently high isotopic ratio for the AMS measurement. Additionally, a low ^{14}C blank is required. Both requirements are well met by our micro-graphitization line.

Theory

4 Radiocarbon Dating

4.1 Why ^{14}C

Because of its special electron configuration (i.e. half filled L-orbit) carbon is able to form highly complex molecules. Organic carbon has to two stable isotopes, ^{12}C and ^{13}C and the long-lived radioisotope ^{14}C with a half-life of 5700 ± 30 years (National Nuclear Data Center, Brookhaven National Laboratory, USA).

	abundance	half life	decay	decay energy	decay product
^{12}C	0.99	stable	-		
^{13}C	0.01	stable	-		
^{14}C	1.2×10^{-12}	5730	β^-	0.156 MeV	^{14}N

Table 4-1 Carbon isotopes

Carbon is a very good "nuclear clock" for dating biological materials, which comes from the fact that ^{14}C in living organisms is in a steady exchange with its environment and because ^{14}C has a half life which is suitable to measure ages in the range of ten thousands of years. Since its natural abundance is very low, AMS is a suitable method.

4.2 ^{14}C cycle

^{14}C is formed through neutron capture in the upper atmosphere by the reaction

$$^{14}_{7}N + ^{1}_{0}n \rightarrow ^{14}_{6}C^{*} + ^{1}_{1}p$$

When a neutron from cosmic radiation hits a ^{14}N nucleus, ^{14}C is formed through an (n,p) reaction. Finally, ^{14}C decays back into ^{14}N. This leads to a relatively stable equilibrium, which is almost constant over a larger time scale, besides some effects which are described later. The new-built ^{14}C reacts with the atmospheric O_2 to form CO_2. The next step in the cycle is that plants inhale the CO_2 through photosynthesis and therefore the ^{14}C enters the biosphere. This cycle leads to a continuous exchange of the carbon isotopes between atmosphere and living organisms. If an organism dies, the cycle is broken and the "clock starts to tick": The ^{14}C content starts to decrease with its half life. Thus, if one can measure the amount of ^{14}C in an organic sample one can determine the time passed since its death.

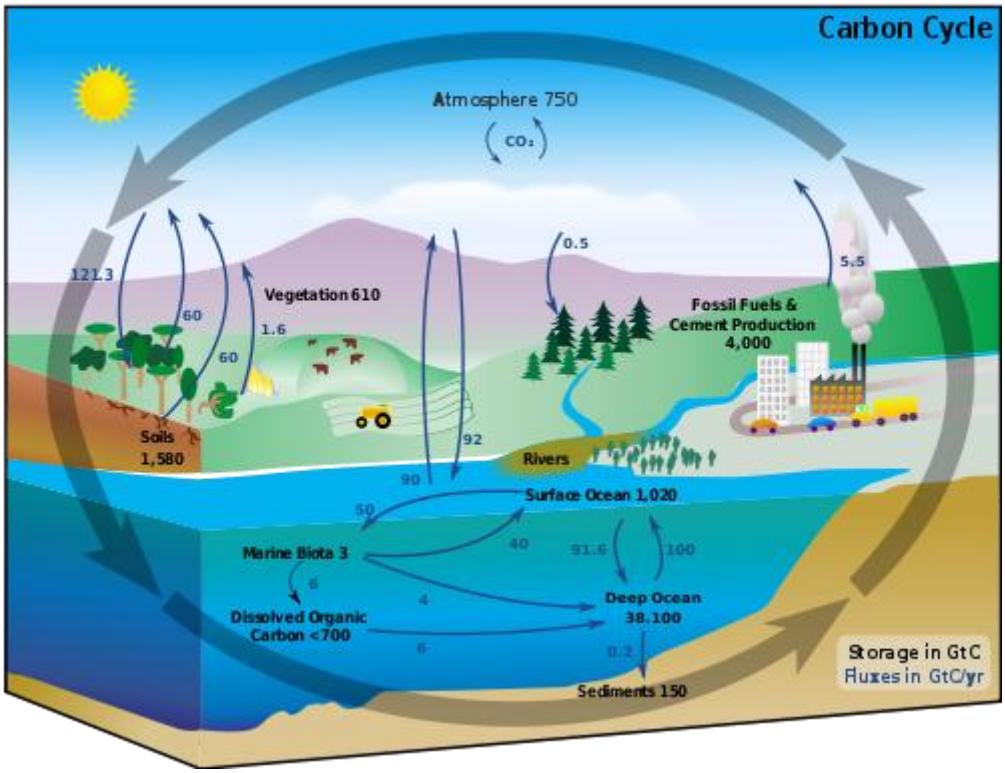

Figure 4-1 This diagram shows the storage and the annual exchange of different carbon reservoirs in gigatons of carbon. Human activities, mainly burning fossil fuels, add 5.5 gigatons of carbon per year (Illustration courtesy NASA Earth Science Enterprise).

4.3 Fluctuation in time of $^{14}C/^{12}C$ in atmospheric CO_2

The equilibrium level of the carbon isotopes is modified by several effects, which raise the need for corrections.

4.3.1 Natural effects

There are several effects that are causing variations in the natural equilibrium of ^{14}C in the atmosphere. Over the last 5000 years this effects led to a variation of about 5% in the ^{14}C content in the atmosphere. (Levin, et al., 2000).

- The modulation of the cosmic radiation through sun activity (DeVries-Effect) causes short term variations of the production rate of ^{14}C (Lingenfelter, et al., 1963).
- The variation of the geomagnetic dipole field results in a change of the production rate of approximately a factor 3 (Levin I., 2004).
- The exchange between terrestric carbon reservoirs leads to variation in the $^{14}C/^{12}C$ (Levin I., 1980).

4.3.2 Suess-Effect

When industrialisation started some 150 years ago, mankind began to use fossil oil and coal, which consist of 100% "dead" carbon (i.e. carbon without any ^{14}C). Therefore a lot of ^{14}C-free carbon was blown into the atmosphere, leading to a decrease of $^{14}C/^{12}C$. This corresponds to apparently "older" sample ages, if decay-dating would be carried out based on the assumption of constant atmospheric $^{14}C/^{12}C$. This effect is taken into account by the calibration curve used for radiocarbon dating, but nevertheless prevents precise dating after 1650 AD.

4.3.3 Nuclear weapon effect

When the atmospheric nuclear tests reached their point of culmination in 1963, the amount of ^{14}C in the atmosphere was almost doubled. After the Nuclear Test Ban Treaty in 1963 the concentration of radiocarbon started to decrease but has not yet arrived at its original concentration. An important observation was the fast homogenisation of ^{14}C throughout the atmosphere which takes place in a few years (Levin I., 1980), (Levin I., 2000), (Levin I., 2008). This effect had a major impact on the whole method of radiocarbon dating. With the help of the bomb peak it is possible to measure recent samples with an accuracy of a few years.

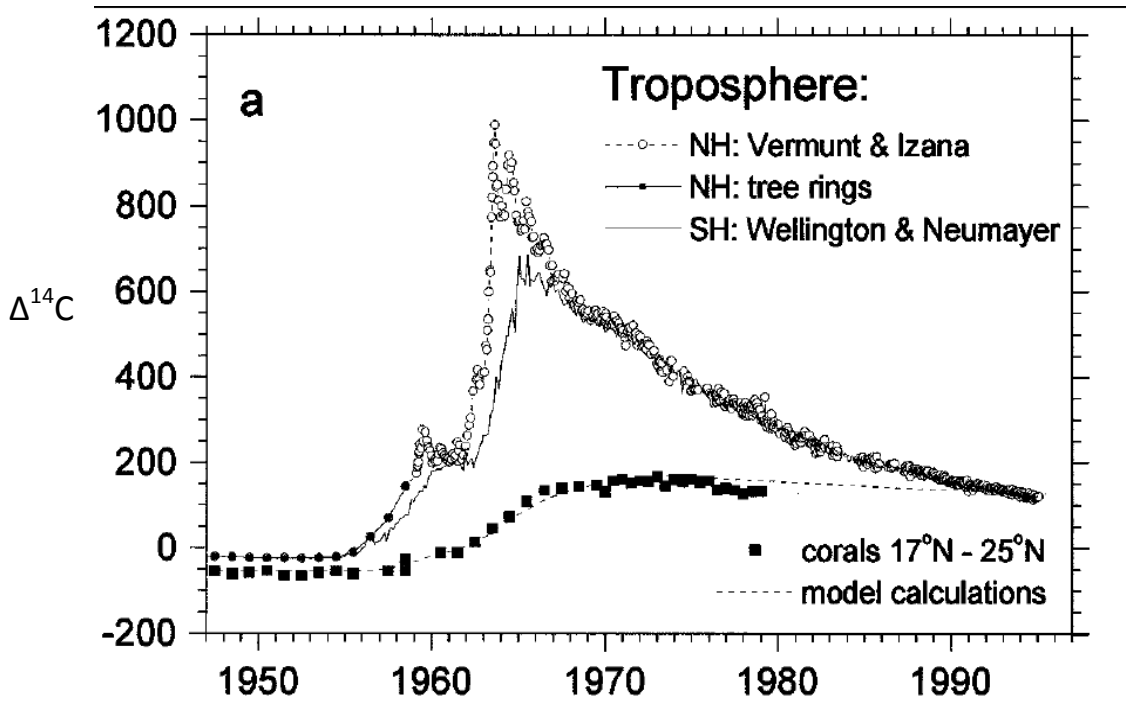

Figure 4-2 Long-term observations of ^{14}C in atmospheric CO_2 in the northern and in the southern hemisphere (Levin, et al., 2000), $\Delta^{14}C$ denotes the decay-corrected excess of ^{14}C above the absolute international standard activity

4.4 Aspects to be considered in a ^{14}C measurement

4.4.1 Fractionation

Because the isotopes ^{12}C, ^{13}C and ^{14}C have different masses they do not behave exactly the same in chemical reactions or transport phenomena, resulting in a slight fractionation. For example during photosynthesis the uptake of the different carbon isotopes is different (Smith, 1972). Fortunately, for all processes investigated so far, the effect for ^{13}C is exactly half of that observed for ^{14}C (Stuiver, et al., 1977). This allows to measure the fractionation factor of ^{13}C and to apply a correction for ^{14}C.

4.4.2 Reservoir effects

In some cases sample materials get in contact with large reservoirs of dead carbon. For example the oceans aren't in equilibrium with the atmosphere and therefore the water on the surface of the oceans appears typically a few hundred years old. Organisms from this habitat did not incorporate the same starting concentration of carbon isotopes as was present in the atmosphere. Another example would be the dating of groundwater. For that purpose the dissolved inorganic carbonate is used as sample material. But because also carbonates leached from the rock may contribute to the carbonate budget, the measurement without a correction would give a quite too old age (Hard-Water-Effect) (Broecker, et al., 1959), (Rea, et al., 1995).

4.4.3 Contamination

If a sample got into contact with a material with different radiocarbon age and the contamination isn't cleaned properly one also gets a wrong age determination. If the amount and the ^{14}C content of the contamination are known, a correction of the result is possible, however usually at the cost of dating precision.

4.4.4 Calibration

A raw radiocarbon date cannot be used as a calendar date, because the $^{14}C/^{12}C$ of the CO_2 in the atmosphere was not constant over time.

To get a calendar date from a radiocarbon date one has to compare it to the so called calibration curve. This curve is created from samples that can be dated with alternative methods also, to get an independent age measurement. Therefore one can establish a connection between calendar age and radiocarbon age. For an unknown sample, the inverse

transformation yields the calendar age; nowadays, probabilistic mathematical methods are used for this transformation (see Figure 4-3).

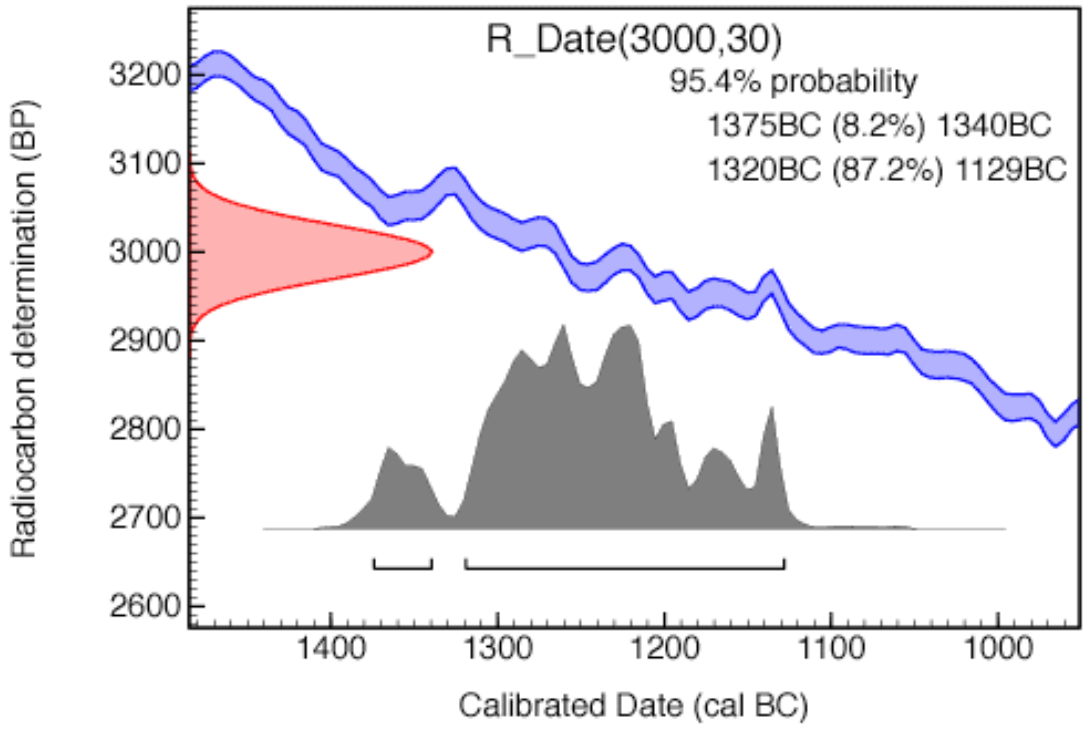

Figure 4-3 Example for a radiocarbon calibration (c14.arch.ox.ak.uk). On the y-axis the raw radiocarbon date (resulting from a measurement) is assigned as Gaussian distribution. On the x-axis the calibrated calendar date is assigned. The blue indicated line is the calibration curve for this time interval. The intersection of the raw radiocarbon date with the calibration curve now gives one the possible calendar date.

4.5 Different types of radiocarbon measurement techniques

4.5.1 Proportional counting method from Libby

Radiocarbon dating was first invented by Willard Frank Libby (1908–1980). He used a gas proportional counter to directly measure the decay of ^{14}C. Because of the long half-life and the low abundance of ^{14}C, this method typically needs a large amount of sample material (grams) and long measurement times (weeks) to get a precise result.

4.5.2 Liquid scintillation spectrometry

This method also concentrates on the decay of ^{14}C but with a different type of detection. The sample carbon is transformed into CO_2, then reacted with molten lithium to form Li_2C_2 and finally catalytically trimerised to benzene, which is used as liquid scintillator; decays are counted with a photomultiplier. Because of the possibility to use even more carbon than in the gas counter, one can achieve a higher precision within a shorter time.

4.5.3 Accelerator mass spectrometry (AMS)

The nowadays most common method to determine radiocarbon ages is AMS. This method does not use the decay of ^{14}C, but counts the ^{14}C atoms directly. This leads to an enormous reduction of sample size required, which allows measuring mg-sized samples with a precision well below 1% for $^{14}C/^{12}C$ ratios. While the classic decay based methods need a sample mass of several g carbon, AMS dating typically uses sample masses of about 1 mg. With the optimized methods investigated in this thesis, we are able to measure sample masses of less than 10 μg carbon. Thus, compared to decay counting, the carbon sample size could be decreased by a factor one million.

5 DNA

5.1 What is DNA

DNA is the short form for Deoxyribonucleic acid which is the substance used to encode the building plan of nearly all living beings (the exception from this are RNA (ribonucleic acid) viruses, which are however not counted as life forms nowadays).

Chemically DNA is a polymer made from repeating units called nucleotides. The DNA chain is 2.2 to 2.6 nm wide, and one nucleotide unit is 0.33 nm long. One nucleotide consists of:

- a nucleobase (adenine, guanine, cytosine, or thymine)
- A five-carbon sugar
- One to three phosphate groups

The cell nuclei contain most of the genetic material, mainly the DNA molecule and a large variety of proteins which finally form chromosomes.

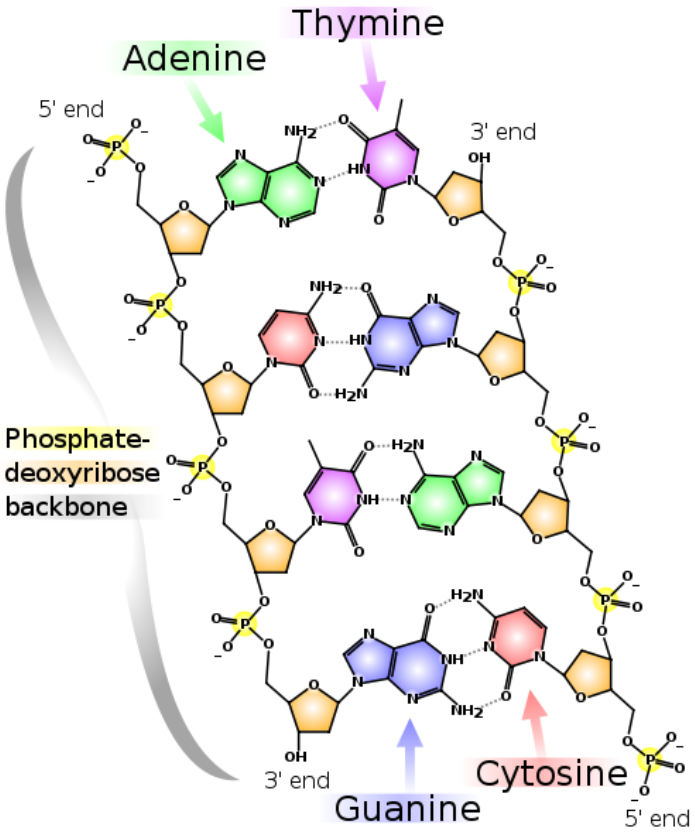

Figure 5-1 Chemical structure of DNA (wikipedia.com)

5.2 The reason for dating DNA

The reason for dating DNA is to find a dependable way to verify if neurogenesis takes place after a human being is born (Bhardwaj R. D., 2006), (Eriksson, 1998), (Spalding, et al., 2005). Until now it is very unclear whether neuronal cells are formed after the early childhood. Therefore a method to determine the age of a cell population is of great interest since it can directly determine whether a cell turnover takes place or not.

Currently our measurements concentrate on two sections of the adult human brain: The cerebellum, which serves as the motor control center in the human brain; and the olfactory bulb which is responsible for the sense of smell.

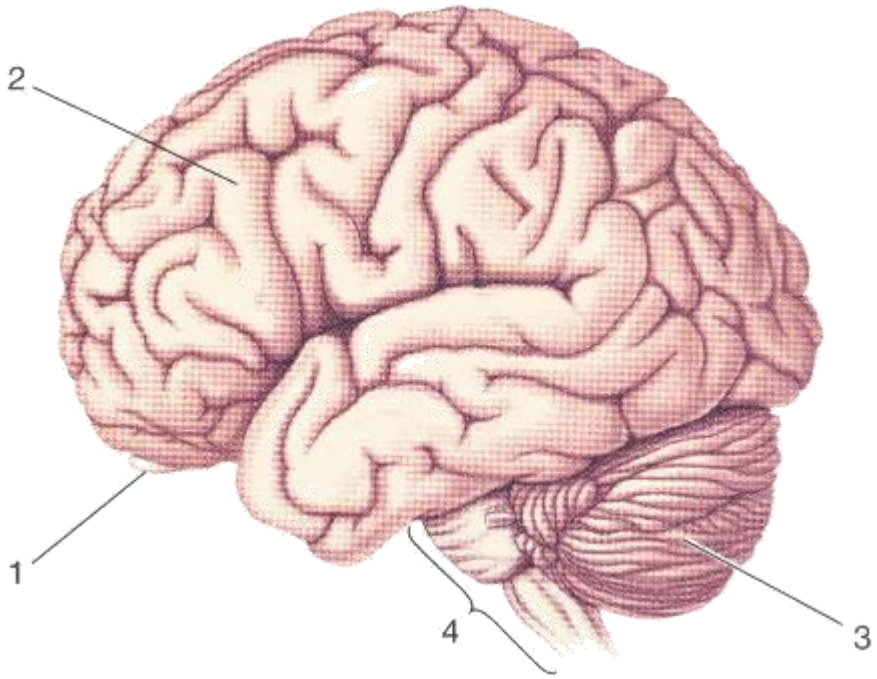

Figure 5-2 Lateral view of the human brain (mindsci-clinic.com). (1) Olfactory bulp, (2) Cerebrum, (3) Cerebellum, (4) Brain stem.

One human cell contains about 1.2×10^{11} C Atoms from DNA and so one needs roughly 15 million cells to be able to perform an AMS measurement of a 30 μg C sample. Assuming a detection efficiency of 2% (higher values were actually reached, see below) one would detect 30000 ^{14}C atoms which correspond to a precision of ~0.5%.

5.3 Problems when dating DNA

One of the main challenges when dating DNA is the time consuming extraction of sample material, which is performed by our collaborators at the Karolinska Institute, Stockholm Sweden. One sample takes about one week to produce and the success rate for producing a DNA sample of measurable size from a human olfactory bulb can be as low as 1 in 10 (Personal communication Olaf Bergmann[1]).

Another task results from the small size of the extracted samples. While in conventional radiocarbon dating sample sizes of about 1 mg are common, in our case of dating DNA we have to cope with sample masses below 10 μg, which leads to a necessity of extremely low contamination levels. Therefore our group developed a graphitisation unit that is able to process sample sizes down to a few μg of carbon with a reasonable error. Together with adapted pre-treatment procedures this allows us to measure ultra small DNA samples with an overall accuracy of about 1%.

5.4 Sample production from DNA material

5.4.1 Cell Nuclei extraction and purification

Extraction of cell nuclei is performed while keeping reagents and samples on crushed ice. Samples of interest from tissue are cut into small pieces and mixed homogenously in a lysis buffer. Through centrifugation the cell nuclei are separated, which is possible due to the consequent disruption of the cell membranes. The lysate is suspended in a sucrose solution and then carefully layered on top of a sucrose cushion solution in an ultracentrifuge tube. After centrifuging of the samples (2-20 hours at 26000 g at 4 °C) the supernatant is removed while the cell nuclei are collected at the bottom of the centrifuge (Spalding, et al., 2005).

5.4.2 Flow cytometry

Since the extracted and purified fraction contains cell nuclei from different kind of cells of the original sample a further separation process is needed. This is done by labelling the cell nuclei of interest with specific antibodies. For our case the epitope NeuN, which is located in a neuron nucleus (Mullen et al., 1992), is used for labelling (Spalding, et al., 2005). Once the cell nuclei are labelled they can be sorted by flow cytometry (fluorescence-activated cell sorting, FACS®). Fluorochromes are attached to the cell specific antibodies. Since the fluorochromes can be excited by light of specific wavelengths the cell nuclei can be separated according to cell

[1] Dept. of Cell and Molecular Biology, Karolinska Institute, SE-171777 Stockholm, Sweden

species (Hawley, et al., 2004). A final centrifugation of the solution allows collecting the cell nuclei in a concentrated form (some hundred µl volume).

5.4.3 DNA extraction

The cell nuclei solution is diluted with a lysis buffer in the next step. To destroy proteins, proteinase K is added, which breaks up the peptide bonds in the proteins amino acid (Ebeling, et al., 1974), (Hilz, et al., 1975). To degrade RNA, a ribonuclease cocktail is added after the sample is incubated overnight. After another short incubation period sodium chloride solution is added to the sample. To separate the DNA out of the sample, it is centrifuged at 13000 g. The supernatant which contains the DNA is mixed with ethanol to precipitate it. The now visible small DNA pellets are washed with ethanol four times and then transferred into sample vials (Spalding, et al., 2005). Before the samples are shipped to our laboratory the ethanol is evaporated and the residue dissolved in 500 µl ultra-pure water. To avoid contamination of the samples the production procedures are performed in a special clean room and the tools that are used for preparation are cleaned very carefully.

5.4.4 DNA quantification and purity analysis

DNA quantification

Because the four bases of DNA are absorbing light in the UV section an absorption spectrum can be used to determine the sample DNA mass. The absorption spectra of the four bases sum up to a maximum absorbance for DNA material in solution at a wavelength of 260 nm (Perkampus, 1992), (Mahon, et al., 1999). To quantify DNA mass in aqueous solution it is generally accepted to assume that an aqueous solution of doublestranded DNA with an optical density of 1.0 at 260nm in a 10mm pathlength cuvette has a DNA mass concentration of 50 ng/µl (Sambrook, et al., 2001).

DNA purity analysis

The purity of DNA sample solutions can be determined by the attenuation ratios of UV light at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}). Since unwanted fractions like proteins result in different UV absorption and therefore altering the absorbance ratios the impurities can be quantified. To guarantee a purity of 90%, absorbance ratios of A_{260}/A_{230}=2.0 to 2.2 and A_{260}/A_{280}=1.8 to 1.9 are required (Cantor, et al., 1980). In addition the sample purity was checked by using high performance liquid chromatography (HPLC). The HPLC analysis showed a typical protein mass contamination of 0.1 to 5%.

5.5 Sample preparation from DNA material

For my work I used ^{13}C enriched DNA material. $^{13}C^{15}N$ labelled Ralstonia eutropha H16 DNA with an enrichment of $^{13}C/^{12}C$=98, produced by Silantes GmbH, Gollierstr. 70 c, 80339 München, Germany was used to determine the contamination during the dating process (Liebl J., 2010). The material was stored in an aqueous solution in a refrigerator. The concentration of the sample material was 1.006 µg C per µl DNA solution. Sample pre-treatment was either performed in a laminar air flow box or in an argon glove box system, to investigate the contribution from ambient atmosphere to the contamination of the samples. The sample treatment was carried out as identical as possible in both atmospheres. One difference is that the argon glove box provides a very clean and controlled atmosphere, but isn't dust free in contrast to the laminar air flow box. However, it can be assumed that if dust is introduced into a sample the effect would be quite large and noticeable as an outlier.

An important role during sample preparation plays the cleaning and pre-treatment of the used tools.
Before working in the laminar air flow box, the whole inside of the box is cleaned with dilute NH_3. Every tool that is used is also cleaned with NH_3 and blown off with clean nitrogen to remove dust particles. The laminar air flow box is turned on at least 15 min before use to ensure proper function.

The quartz vials (200 mm length, 4 mm inner diameter) which are used for freeze-drying and combustion of the samples are cleaned as follows: the vials are rinsed three times with bi-distilled water and then filled with 10% HCl and put into an ultrasonic bath for 15 min. After that the vials are put into an oven and heated for 4 hours at 900 °C.

Typically a volume of 0.5 ml aqueous DNA solution is used. The purity of the used water plays an important role. We obtained best results with ultra-pure DNase/RNase-free distilled water (Gibco®). The influence of the quality of the used water is shown in Table 5-1.

0.5 ml	CO_2 in graphitisation 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 kepted in rinsed cleaned vials (pH3)	0.07 ± 0.03

Table 5-1 Contamination mass of different qualities of water (Liebl, et al., 2010)

For my work 2 µl of sample solution with 498 µl of ultra pure water is mixed together in a cleaned polyethylene vial (RATIOLAB, Reaktionsgefäss 1.5 ml, PP farblos) and then transferred into a cleaned sample vial. Vials are closed with modified Swagelok (Swagelok SS-4P4T) valves.

5.6 Sublimation and combustion of DNA samples

The closed vials containing the sample solution were taken out of the argon box and connected to the 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 water volume. Afterwards the samples are baked at 220 °C in vacuum as additional cleaning step. After about 20 minutes the sample vials are filled with about 200 mbar of Oxygen from a gas bottle (Air Liquid Austria, ≥ 99.5% purity), which was guided through a liquid nitrogen cooled cold trap to remove carbonaceous contamination. The vials were closed and the sample material was combusted for about 30 minutes at 900 °C to form CO_2.

5.7 Graphitisation of DNA samples

After combustion the CO_2 is cryogenically purified and frozen with liquid nitrogen into the graphitization reactors. 2.5 times the sample pressure of H_2 plus an additional 50 mbar is filled into the reactors. The graphitization reactions will start as soon as the ovens heating the iron catalyst inside the reactors are applied. The reaction takes place at two different temperature steps. Depending on the sample size the reaction lasts between 2 hours and 15 hours.

A detailed report of the graphitization process is described in section 8.3.3.

6 Accelerator mass spectrometry (AMS)

A disadvantage of conventional mass spectrometry is that it is not capable of measuring extremely low isotopic abundances. This is where the big advantage of AMS comes into play. If the half life is long AMS is chosen over decay counting for the reasons explained for ^{14}C in 4.5.3, despite decay counting is much cheaper than AMS. If the half life is short (up to a few years) decay counting will still produce the most precise results.

6.1 History

The first usage of an accelerator for the purpose of mass spectrometry (MS) was performed by L. W. Alvarez and Robert Cornog. They used a cyclotron to demonstrate that ^{3}He is stable (Cornog, et al., 1939). In 1977 Richard A. Muller from the Lawrence Berkeley Laboratory claimed that this method could be used for radioactive atoms, because operating particles at these high energies allows one to separate the isotope of interest from the background with high selectivity (Muller, 1977). At about the same time two groups at tandem accelerators realized the advantage of using negative ions for ^{14}C detection because the interfering ^{14}N does not form negative ions (Bennet, et al., 1977), (Nelson, et al., 1977), (Purser, et al., 1977). Inspired from these early works a lot of groups around the world started to use accelerators for mass spectrometry. Nowadays AMS has a lot of fields of application. For our purpose of dating human neuronal DNA with radiocarbon AMS is presently the only feasible method.

6.2 Principle

The first suppression of unwanted isobars takes place in the ion source. When measuring ^{14}C the disturbing stable isobar would be ^{14}N. But fortunately nitrogen does not form negative ions. The negative ions are then preaccelerated to pass a first mass spectrometer, which consist in the most cases of an electrostatic analyser (ESA), which is sensitive to energy, and a magnetic field which is sensitive to the momentum and thus able to separate masses. At this point the first “cleaning” of the beam from unwanted masses takes place. In the case of carbon $^{12}C^-$ and $^{13}C^-$ are separated from $^{14}C^-$. All negative ions with a mass of 14 amu enter a Tandem accelerator. Here the ions are accelerated in two steps. In the first stage they are accelerated towards the so-called accelerator terminal by applying a high, positive voltage (3 MV in the case of VERA). The terminal contains a canal filled with dilute gas, or a thin foil which strips off the outer electrons of the particles. They reach high positive charge states. In the case of carbon, the 3+ state is produced with the highest yield at 3 MV. When the now positive ions leave the terminal, they are accelerated again towards ground potential, but this time they gain a few times the energy as in the first acceleration, because of their multiple charge state. The ions pass another mass spectrometer also based on a magnetic field and an ESA. Additional components like Wien-filters or magnets can be used to enhance the suppression

rates of the high-energy mass spectrometer. Finally the beam reaches the detection area, where the particles can be detected with different kinds of detectors, depending on which isotope is measured and what information one wants to get.

6.3 Vienna Environmental Research Accelerator (VERA)

VERA is an accelerator system dedicated measurements with AMS. VERA was ordered 1994 with the financial help of the Austrian Federal Ministry of Science and Research, delivered in the fall of 1995 and started operation in 1996 (Kutschera, 1997). VERA is equipped with a 3 MV Pelletron Tandem accelerator (Model 9SDH-2) built by National Electrostatics Corporation (NEC). It is designed to theoretically measure radio nuclides or stable nuclei from the entire nuclear chart. Undergoing many new developments VERA is a system in a state of steady change. For example the last expansion of the facility was the installation of a second negative-ion source.

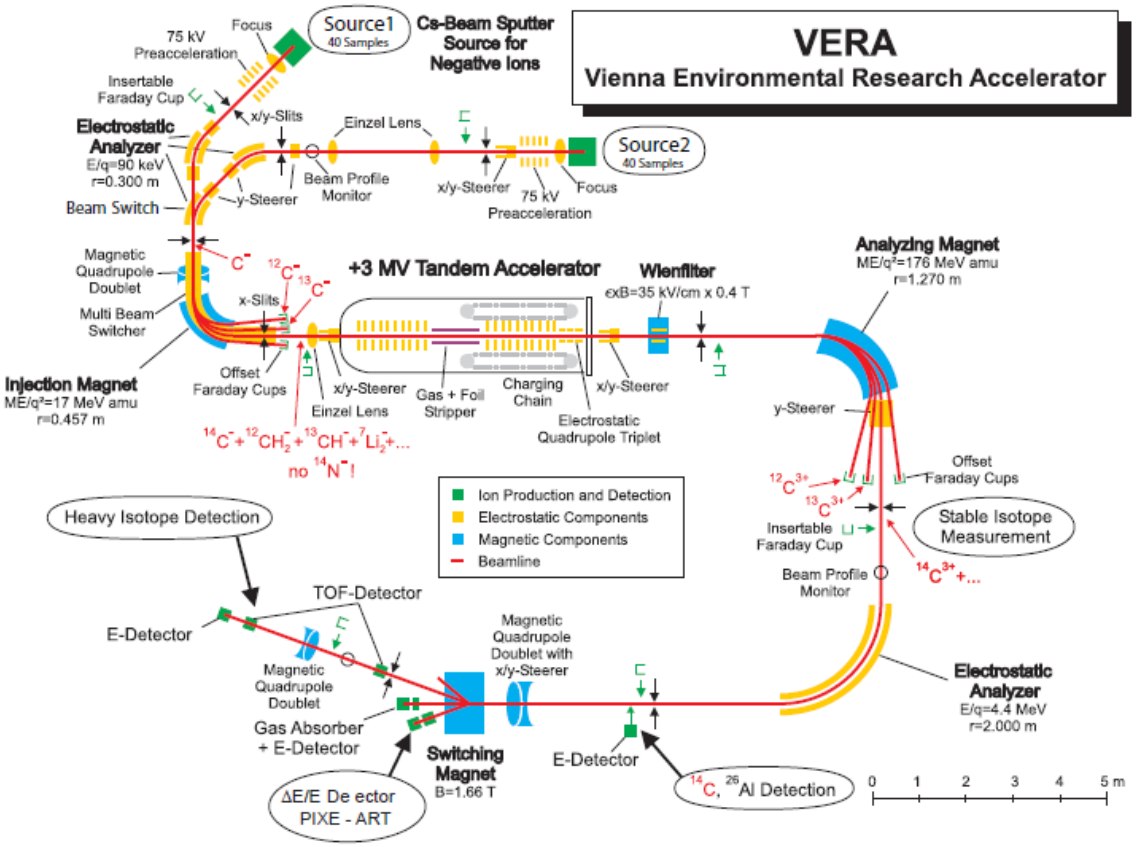

Figure 6-1 Schematic layout of VERA

6.3.1 Components of VERA

Ion source

The source that is used at VERA is a Cs-beam sputter source for negative ions. It is loaded with target wheels which hold 40 samples. Cs is heated inside an oven and the vapour is transported via a capillary to a hot tantalum surface (the ionizer), where positively charged Cs ions are formed. Because the sample wheel is at negative high voltage, the Cs ions are accelerated and focussed (also with the help of a so called Cs–focus electrode) onto the sample, which is therefore called "target" in the nomenclature of AMS. The targets are sputtered and form negatively charged ions. Through the same voltage that has accelerated the Cs^+ ions onto the target the negative target ions are accelerated through the central hole of the ionizer. Several stages of further acceleration result in an energy of 75 keV.

Low energy electrostatic analyser (LE ESA)

In the LE ESA a first stage of separation takes place. It is a 45° spherical electrostatic analyser with a maximum energy-to-charge ratio E/q = 90 keV. Because all regular ions coming from the source have an energy of 75 keV, the ESA serves as a first energy filter which removes mainly so called "sputter tails" and ions from molecular break up.

Injection magnet

The injector magnet is carried out as a 90° sector magnet with a maximum ME/q^2=17 MeV amu and a radius of r =0.457 m. Since the energy is fixed by the ESA, it serves as mass filter. When using carbon as target material, ^{12}C and ^{13}C are separated from ^{14}C and deflected into offset Faraday cups.

Because for a successful measurement of carbon one needs the ^{14}C count rate and also the ion currents of ^{12}C and ^{13}C, one has to be able to switch between these isotopes quite fast during the measurement. Since magnetic fields can be changed only slowly because of various reasons, the magnetic field of the injector magnet is held constant, while the electrically isolated magnet chamber is put on a variable high voltage. This is the so called multi beam switcher (MBS). The particles are accelerated when they enter the magnet chamber, are deflected in the magnetic field depending on their mass-energy product, before they are again decelerated at the end of the MBS. This design allows one to switch between different isotopes by changing the voltage of the MBS without changing the magnetic field of the injector magnet.

Tandem accelerator

After the injection magnet, the negatively charged ions reach the accelerator with an energy of about 75 keV. The accelerator used at VERA is a tandem accelerator designed for a terminal voltage of 3 MV; the high voltage potential applied to the so called terminal at the center of the accelerator is produced using the Pelletron principle.

Pelletron principle:

The charging of the high voltage terminal is performed by two charging chains, which consist of conducting cylinders (pellets) that are connected through isolators. On the earth potential side the pellets receive a positive charge by electrical influence, because they form a capacitor with an electrode put on high voltage via a power supply. On the high potential side the chain links are losing their charge to the outside of the high voltage terminal which forms a Faraday cage. The control of the terminal voltage is performed through variation of the charging voltage. The fine regulation is accomplished via a controlled corona discharge from earth potential to the terminal, emitted by so called corona needles.

For electrical insulation of the high voltage terminal the whole accelerator is mounted inside a pressure vessel which is filled with SF_6 gas at 6 bar. Evacuated acceleration tubes lead to and from the terminal. The stripper installed at the terminal is responsible for changing the charge of the ions, and consists of an about 1 m long channel filled with dilute gas. The ions collide with the gas molecules, loose some of their outer electrons, and reach high positive charge states. In the case of carbon at an energy of 3 MV the most likely charge state is 3+. The stripper also serves as a molecular dissociator. Most of the molecular isobars (e.g. $^{13}CH^-$, $^{12}CH^-_2$) break up into individual atoms. Since triply charged positive ions of small molecules do not exist, the $^{14}C^{3+}$ ions are now the only ion species remaining in the mass/charge ratio of 14/3. Despite the break-up products follow the $^{14}C^{3+}$ on their track from the terminal out of the accelerator, they can be easily separated later. Since the ^{14}C ions now are triply charged, they gain 3×3 MeV from the stripper to the exit of the second accelerator tube.

Figure 6-2 Tandem accelerator tank

Analyser magnet

The analyser magnet is the first part of the high energy mass spectrometer. It has a maximum bending power of MeV/q²=176 MeV amu and a radius of 1.27 m. After this magnet the stable isotope measurement takes place. Since the field of this magnet is not changed when the low-energy mass spectrometer is switched to the stable isotopes (in the case of carbon, ^{12}C and ^{13}C), they leave the field at different angles than the trace ion. They are thus measured in Faraday cups which are mounted offset from the nominal exit direction along the focal plane of the magnet.

High energy electrostatic analyser (HE ESA)

The last device before the detection is the HE ESA with an E/q=4.4 MeV and a radius of 2 m. It serves as a final filter for the energy distribution of the beam.

Detection

Finally the beam reaches the detection area. Here we can obtain additional information about the particles (energy, count rate, flight time, etc.). In the case of carbon measurements we use a Si detector (Ametek, Model Nr.: BU.016.150.100) to measure the count rate and the energy of ^{14}C.

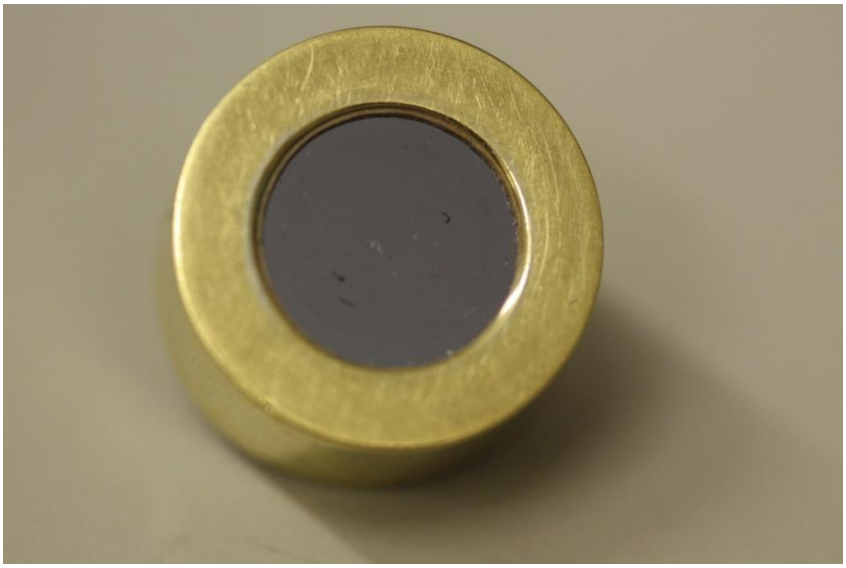

Figure 6-3 Si detector (active detection area of 150 mm²)

6.3.2 Tuning of the ion beam

After the target wheel is loaded into the ion source the tuning of the ion beam is performed automatically by the measurement control system. In short terms the measurement is performed by the following steps:

- Before the measurement one has to create a so called control.log file. In this file different parameters like the number of the tuning targets and their position, number of tuning runs, etc., is provided for the automatic tuning and measurement script.
- The script loads an already existing (and hopefully good) start setup for ^{14}C measurements (after this step usually one can already see a ^{14}C count rate in the detector).
- Optimisation of the injector setup by maximizing the $^{12}C^{3+}$ beam in the high energy offset Faraday cup with the automax program (Steier, 1998).
- With the quadrupole triplet after the accelerator the $^{12}C^{3+}$ beam is focused through a small aperture at the object point of the analyzer mass spectrometer, formed by narrow (horizontal and vertical) slits. The design of the beam line makes sure that the beam is also focussed at the further slits up to the detector.
- Maximisation of the $^{13}C^{3+}$ beam in the corresponding high energy offset Faraday cup, by changing only the magnet chamber potential and a pair of steerers in front of the accelerator tank, which can be changed simultaneous with the MBS by fast sequencing also. Similarly, maximisation of the $^{13}C^{3+}$ beam from $^{13}CH^{-}$ in a high energy offset cup, as a pilot beam for mass 14 in the injector.
- The ^{14}C beam is centred in the high energy beam line with the automax program by varying steerer elements to maximize the detector count rate.

Experimental

7 Using a glove box providing a carbon free working atmosphere

To create an air free environment we installed an Argon Glove Box system (Schmidt, et al., 1987). With its help it is possible to work in an atmosphere with controlled parameters like O_2 content, moisture content or CO_2 content.

Figure 7-1 Argon Glove box

The system contains two major parts. The first is the box itself where the sample preparation takes place and the second is the gas regeneration system in which the atmosphere is cleaned. The minor parts of the system are the vacuum pump, the moisture detector, the O_2 sensor, the antechamber and different tools for the running operation of the box.

7.1 Gas regeneration unit

Figure 7-2 Gas regeneration system

For our glove box we used argon. To guarantee a permanent good quality of the argon atmosphere we installed a gas regeneration unit from it-systems[2]. Gas is drawn from the glove box by a fan. As a first step of cleaning and also as protection for the regeneration columns the gas passes a HEPA (High Efficiency Particulate Air filter) filter and then an activated charcoal filter which reduces the content of organic molecules. After that the pre-cleaned gas runs through one of two regeneration columns. These are filled with a molecular sieve to remove moisture and activated copper to remove oxygen. Finally the gas is brought back to the glove box through a second HEPA filter.

A slight overpressure in the glove box is maintained by this unit. Pressure can be increased with argon from a constantly connected bottle (50 litre Volume, 200 bar pressure). Reducing the pressure inside the box is provided by an oil-free scroll pump, which is also responsible for creating the antechamber vacuum. The pressure can be controlled with two foot pedals, which allow comfortable operation for the user.

[2] Innovative Technology Inc., 2 New Pasture Road, Newburyport, MA 01950

7.2 Glove box

The glove box has a nearly prismatic shape with a volume of about 650 litres. Its body is made of stainless steel, while the transparent front window is made of Plexiglas. The front window is held by 40 M8 screws and is sealed with vacuum grease (Dow Corning High Vacuum Grease) prepared rubber border. The front window has two openings where gloves of different material can be attached. We used gloves made of a mixture of butyl and neoprene, which has the advantage of good gas integrity and a high resistance against chemicals (Piercan Model: 22750cb84398 4C 6/10) (Figure 7-5). The box was inherited from a previous experiment. Initially designed only to handle dangerous materials in a sealed cabinet and without controlled atmosphere, it was significantly modified in our workshop for our needs. The box was completely renewed, connections for the gas regeneration system were fitted and a new front window was mounted. At the left top of the box a burst foil was installed (Figure 7-4), which will break at approximately 30-40 mbar and thus protects the box and the operator from overpressure conditions. Next to the over pressure release a simple self-built moisture detector was installed (Figure 7-3). It is a polished aluminium tube extending about 20 cm into the chamber which can be filled with a dry ice/isopropanol mixture from the outside. By adjusting the temperature of the mixture, the dew point of the box atmosphere can be determined by the onset of condensation. For a faster measurement, a small ventilator is installed inside the box.

Figure 7-3 Self built device for moisture measurement

Figure 7-4 Overpressure valve

Figure 7-5 Gloves

On the bottom of the box, the inlet opening and the outlet opening with the HEPA filters are attached, from where the box is connected to the gas regeneration system. On the right side of the box the antechamber is attached, with which tools, samples, etc. are transferred into the box, without contaminating the atmosphere. The antechamber is cylindrical made of stainless steel with a volume of about 58 litres. It is closable from both sides with two vacuum tight doors. On top of the chamber a small manometer is installed to gauge the pressure inside. For pressure adaptation a small bypass line connects the antechamber and the inside of

the box. Antechamber operations - like transferring things in and out - are performed using the following procedure:

Transfer into the box:

Make sure that the inner door and the bypass is fully closed

Open the outer door and place objects inside the chamber. Close the door fully

Evacuate the antechamber to -290 mmHg

Fill box gas into the chamber using the bypass to a pressure of -150 mmHg

Repeat 3 Times

Fill the antechamber to ambient box pressure

Take out the objects inside the box and close the door fully

Figure 7-6 Antechamber

7.3 Controlling the atmosphere

To check the purity of the box atmosphere we used three different kinds of sensors. The O_2 and moisture sensors are built as live sensors which are immediately readable and the CO_2 measurement is carried out offline in our graphitisation unit. With these sensors it is possible to guarantee a working atmosphere that suits our purposes.

7.3.1 O_2 sensor

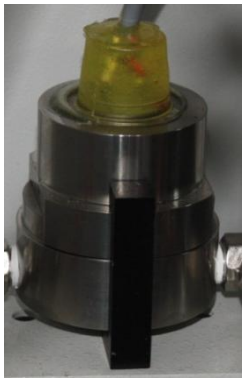

Figure 7-7 0_2 sensor

With a built-in O_2 sensor a steady record of the oxygen content is provided. When the sensor is exposed to gases containing oxygen, oxygen diffuses through a Teflon® barrier and then a thin layer of electrolyte between this barrier and the oxygen sensing cathode. The sample gas then reaches the catalyst surface where it is immediately reduced (IT dual column gas purification system, Operation manual, p. 34-35).

$$O_2 + 2\,H_2O + 4e^- \rightarrow 4\,OH^-$$

During the reduction process 4 electrons are taken up by each oxygen molecule. These electrons are furnished by the simultaneous oxidation of an anode such as lead, cadmium, iron, etc.

$$2\,Pb + 4\,OH^- \rightarrow PbO + 2\,H_2O + 4\,e^-$$

The flow of electrons from the anode to the sensing cathode via an external circuit results in a current proportional to the amount of oxygen in the sample gas.

The rate of diffusion of oxygen through the limiting barrier, Teflon®, varies with temperature, which results in a change in the current flowing between the anode and the cathode. The current increases at a rate of approximately 2.5 % per degree C increase in temperature. The current output of the sensor is compensated by using a negative coefficient thermistor combined with an appropriate array of electronics.

7.3.2 Moisture sensor

With the already described aluminium tube (Figure 7-3) we are able to determine the dew point of water which is left inside the box. Therefore we cool the tube with a mixture of dry ice and isopropanol and measure the temperature when the water inside the box condensates on the tube. This is an indicator for the dew point which allows determining the amount of water left inside the box.

7.3.3 CO_2 measurement

For the measurement of CO_2, a glass tube with a volume of 250 ml and a Swagelok valve (Type SS-6P6T) is transferred into the box and left there open for a couple of days. After that it can be assumed that the atmosphere inside the tube is the same as in the box. Then the tube is closed and brought to the laboratory where it is connected to the graphitisation line. Liquid nitrogen is placed under the tube to freeze out the argon and CO_2 that's in the tube. Since argon has a vapour pressure of about 300 mbar at liquid nitrogen temperature, it can simply be sublimated away while CO_2 and a little content of water stay frozen. After the argon is gone the CO_2 is cryogenically purified and transferred into the graphitisation reactor where the pressure ($p_{reactor)}$ is measured. With the knowledge of the volumes of reactor ($V_{reactor}$) and tube V_{tube}) it is possible to determine the CO_2 content ($CO_{2,\ arbox}$) inside the glove box.

$$p_{tube,CO_2} = \frac{p_{reactor} \times V_{reactor}}{V_{tube}}$$

$$CO_{2,arbox} = \frac{p_{tube,CO_2}}{p_{tube}}$$

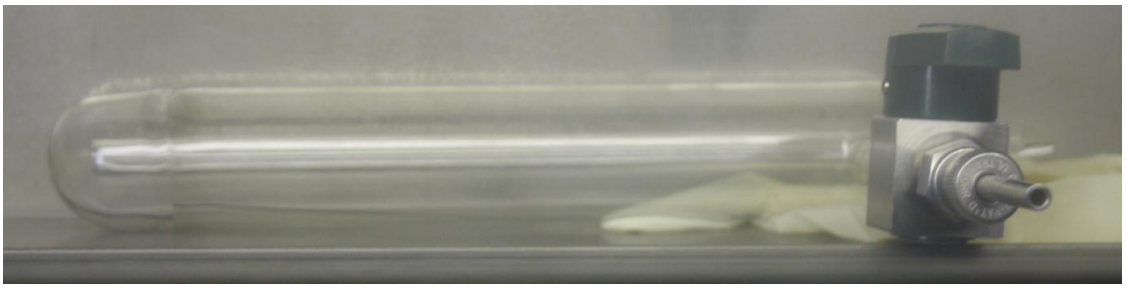

Figure 7-8 Tube for CO_2 measurement

7.4 Start-Up of the glove box

For the detection of any leaks in the box we used a SF_6 leak tester (DILO Armaturen, Babenhausen, ArtNr: 3-033-R001). The box was filled with air and a small amount of SF_6 and then possible spots of leakage are controlled by "sniffing" with the instrument.

Referring to the instructions provided with the gas regeneration system, it is recommended to purge the whole box with a minimum of three 50 litre bottles of argon before use. To speed up this process we used a plastic bag that can fill out the larger part of the inside of the glove box and can be connected to the argon gas supply. By inflating the bag the residual volume of the atmosphere in the box is decreased and the purge process takes place much faster.

The purging of the box was carried out until an O_2 content of less than 25 ppm was reached. Then the regeneration system is turned on and the O_2 content decreases to less than 5 ppm, which represents our working conditions.

8 Sample preparation

An important aspect when preparing ultra small ^{14}C samples is the clean handling of the samples and the therefore needed accessories. Since the smallest dust particle would cause a substantial increase of the background it is crucial to have an excellent (in terms of avoiding contamination from the environment) chemical pre-treatment.

8.1 Sample types

Depending on the type of material there are several different forms of chemical processes that are used. It depends strongly on whether or not the sample material is already CO_2, or if the material is solid.

Solid samples

The goal of sample pre-treatment is the removal of non-indigenous carbon by physical and chemical means. For example, for radiocarbon dating of archaeological samples like charcoal, textiles, peat, etc., the so called ABA (acid-base-acid) method is commonly applied (Wild, et al., 2008). This method was, however, not applied for the present work, since our solid samples had not been exposed to usual environmental contamination (see below).

Liquid samples

Sample materials in solution were loaded into quartz vials which had been baked in laboratory atmosphere at 900°C for 4h beforehand. The vials were closed by a PTFE plug valve and connected to our small sample graphitization setup directly. Gentle drying was carried out by freeze-drying. Vacuum during sublimation was provided by an oil-free scroll pump connected via a dry ice cold trap. Cooling for the frozen samples was provided by a compressor. Thermal contact to the sample vials was established by adjustable telescopic cold fingers of copper and aluminium. This allowed controlling the temperature of the frozen samples and thus the evaporation rate. As a measure for this rate we used the system pressure determined by a thermocouple gauge. A pressure of about 1 mbar allowed freeze-drying of 0.5 ml aqueous solution typically within 3 hours. Higher system pressures and thus more rapid drying resulted in significant sample material loss. We attribute this to entrainment of small sample particles by the vapour flow. For particulate solid samples we observed even scavenging of the whole

sample at higher evaporation rates. Once sublimation was completed a high vacuum from a turbo molecular pump (< 3.0 x 10^{-3} mbar) was applied while the vials containing the dried samples were baked in a tube oven at 200°C for 20 minutes.

8.2 Tools

All steps of treatment were performed inside a laminar air flow box or under argon atmosphere. Sample handling with chemicals was performed inside the laminar air flow box only, to avoid possible defects of the gas regeneration system of the argon glove box. All tools that were used, like micro spoons, tweezers, etc., were cleaned with 5% NH_3 before usage. All material was additionally blown off with a stream of N_2.

Quartz reactors and sample vials were cleaned as follows:

1. Rinsing with bidistilled water 3 times
2. Filled with 1mol/L HCl and put into ultrasonic bath for 10 min
3. Rinsing with bidistilled water 3 times
4. Rinsed with isopropanol
5. Baked at 900°C for 4 h at laboratory atmosphere

PFA (Perfluoroalkoxy polymer resin) gaskets of reactors and sample vials were renewed before every use.

8.3 Reactions

8.3.1 Conditioning of the Fe catalyst

To enable the graphitisation reaction a catalyst is needed (Vogel, et al., 1984). We are using Fe powder (Merck 3819, particle size 10 µm) for our purposes. To clean the powder from any carbonaceous contamination it is oxidised to FeO and then reduced to Fe again with H_2. Fe oxidation was done in two different ways.

First the Fe powder was baked at 815° C for 4 h in a muffle furnace under laboratory atmosphere. About 0.5 mg of iron oxide was pressed (0.3 GPa) into Cu holders (1 mm inner diameter, 1 mm depth, Figure 8-2). Cu holders were then put into graphitisation reactors and baked 30 min at 815°C in vacuum. After that about 800 mbar of H_2 (Linde Gas Austria, ≥ 99.9999% purity) was filled into the reactors and the catalyst was baked at 815°C for 2 hours. H_2 was renewed every 15 min. After about 1 hour of baking, a pressure decrease during H_2 renewal intervals could no longer be observed.

The high temperature of 815°C for reduction was used due to carbon contamination issues, even though SEM (scanning electron microscope) images of the catalyst showed an increased surface when applying a lower reduction temperature of 615°C (Figure 8-1).

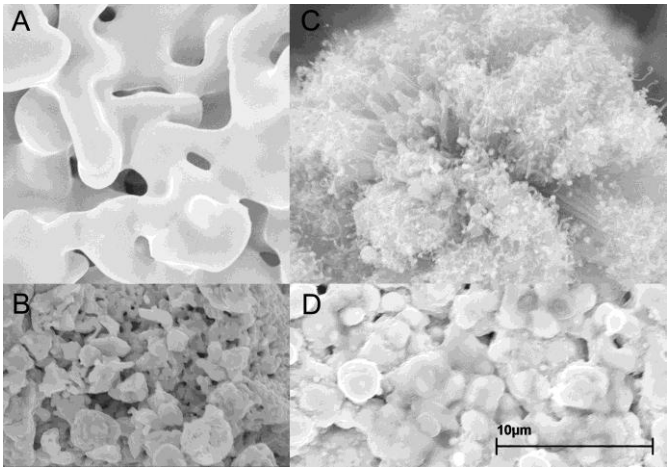

Figure 8-1 SEM images of vacuum and H_2 baked iron catalyst without graphite (A, B, D) and with graphite (C). Vacuum baking was carried out at 815°C for 30 min; H_2 baking was done at different temperatures for 2 hours for all samples. (D) shows pressed iron powder which was H_2 baked at 300°C, (A) and (B) show pressed iron oxide powder reduced to iron at 600°C (B) and 915°C (A). H_2 baking at higher temperatures significantly reduces the catalyst surface. Graphite produced on an iron catalyst prepared at a H_2 baking temperature of 815°C is seen in (C). Graphitization was carried out at 915°C during the first hour and then continued at 615°C (Liebl, et al., 2010).

Figure 8-2 Cu holder

Figure 8-3 Oxidised Fe powder

8.3.2 Combustion

The combustion of sample material usually is carried out by adding CuO for oxygen availability and silver for removal of sulfur and halogen compounds. Since this is another possible source of carbon contamination we decided to use pure oxygen instead. Oxygen from a gas bottle (Air Liquid Austria, ≥ 99.5% purity) was led through a liquid nitrogen cold trap to remove carbonaceous impurities. A pressure of about 220 mbar O_2 was filled into the quartz vials holding the sample materials. The vials were sealed with the PTFE plug valves. Combustion took place in tube ovens at 800°C for 30 min.

When using CuO as source of oxygen we observed a carbon background of (0.36 ± 0.14) µg C. By using pure oxygen for the reaction we achieved a background of (0.13 ± 0.06) µg C. Since the combustion procedure was integrated in our graphitisation unit, we were able to further reduce the background below 0.05 µg C (Liebl, 2010).

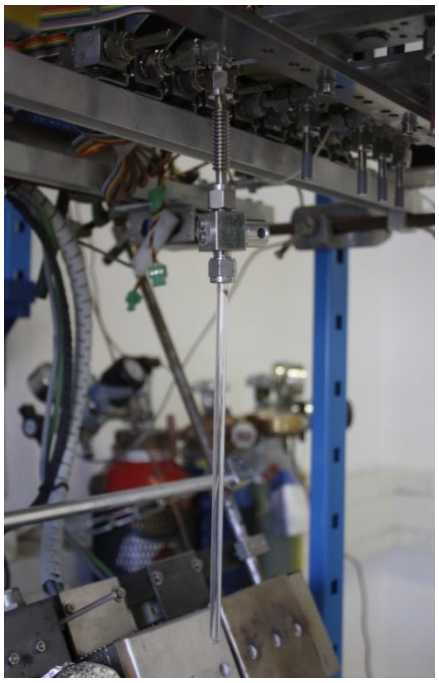

Figure 8-4 Combustion vial connected to the graphitisation unit

8.3.3 Graphitisation

Sample CO_2 was reduced to graphite by means of an iron catalyst (Vogel, et al., 1984).

Graphitization reactors were constructed which achieve a very small total volume of 0.5 to 0.8 cm^3 (depending on the dead volume of the pressure sensor used) by avoiding all unnecessary connecting pieces. They consist of a stainless steel body machined from one piece with two fittings only: one for the quartz vial for the Cu holder with the iron catalyst and one for the pressure sensor. The reactor valve consists of a commercial valve plug (Swagelok SS-4P4T) mounted directly into the reactor steel body. Also the cold trap to freeze out H_2O produced during the graphitization process is machined out of the same steel body (Figure 8-5). Since the volume of the quartz vials used can vary, the graphitization reactor volumes are determined before each graphitization by expanding air, trapped at atmospheric pressure inside the graphitization reactors, to a calibrated volume. The reactor leakage rate was checked with a He leak tester before every use.

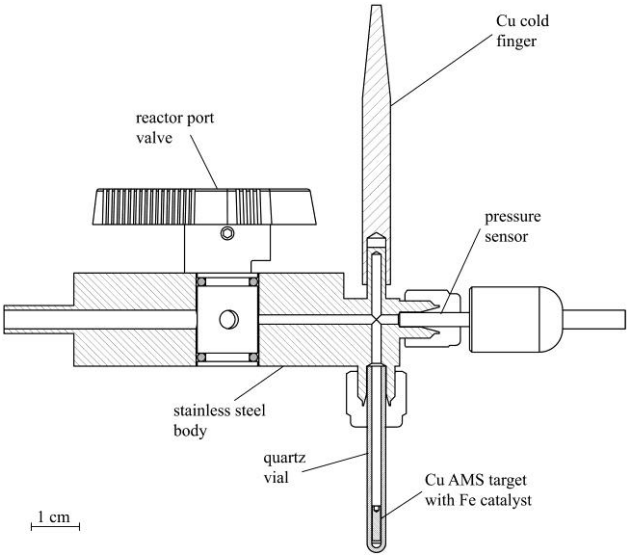

Figure 8-5 Graphitisation reactor cross section. Total reactor volume is 0.5 to 0.8 cm^3 depending on the dead volume of the pressure sensor attached. The Cu cold finger is cooled by a Cu cold box filled with dry ice. The Cu AMS target, holding the Fe catalyst for graphitization can be heated up to 915°C with a tube furnace.

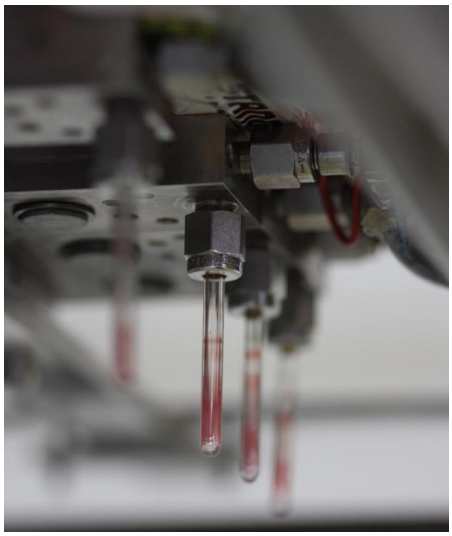

Figure 8-6 Picture of a mounted graphitisation reactor

Sample CO_2 is cryogenically purified before being filled into the reactor in which a readily prepared iron catalyst is present. While sample CO_2 is frozen by means of liquid nitrogen in the sample vial where also combustion took place (section 5.6), non-condensable gases are pumped off. Warming up the sample vial to dry ice temperature allows transferring CO_2 while H_2O remains frozen. Sample CO_2 is collected inside the graphitization reactor by cooling the reactor vial with liquid nitrogen. The CO_2 mass is measured manometrically. CO_2 is frozen again while H_2 needed for graphitization is added. The added H_2 pressure was controlled to be 2.5 times the pressure of sample CO_2 plus a constant offset of 50 mbar. Graphitization reaction steps for small samples are strongly dependent on the partial pressure of H_2O (Liebl, et al., 2010), (Nemec M., 2010). To remove water that is produced during the graphitisation reaction, copper cold fingers are attached to the reactors, which are connectable to a dry ice cooled cold box. The cold fingers itself are electrically heated, so that we are able to control the cold finger temperature.

The graphitisation reaction starts as soon as the ovens are lifted. In the first step, the reaction is running at 915°C and usually settles at a constant pressure within 45 minutes. The cold finger temperature is controlled to -28°C by cooling with dry ice and parallel electrical heating. After 1 h the oven temperature is lowered to 615°C and the cold finger heating is turned off to start the second reaction step (Figure 8- 7).

The completeness of the graphitization reaction was judged by the monitored pressure progression. Once the reactions were considered to be complete, graphitization ovens were removed quickly and the reactors were fan-cooled to laboratory temperature.

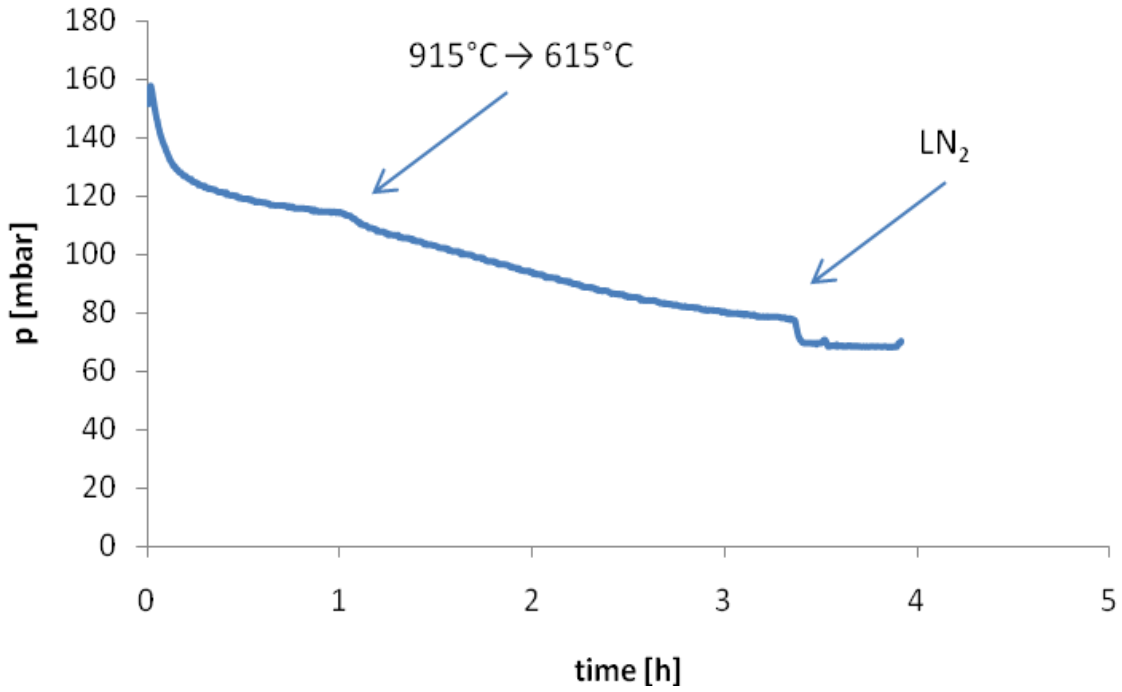

Figure 8-7 Graphitisation reaction of a 27 mbar sample (~ 10 µg C), where LN_2 was used to finish the reaction

If the reaction was not running until completion (i.e. the observed pressure drop inside the graphitization reactor was below three times the sample CO_2 pressure) liquid nitrogen was applied to the cold trap instead of dry ice (Liebl, et al., 2010). This was necessary for a few very small samples of less than 2 µg C in CO_2 only, and enabled the graphitization reaction to continue until completion.

9 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 commercial, "dead" CO_2 from a bottle provided by "LindeGas AG[3]", supposedly collected from a mineral water spring (Rom, et al., 2000). In this case we have no certain knowledge about the real amount of ^{14}C. The best measurements for this blank material so far resulted in a radiocarbon age of about 65000 yBP for samples smaller than 100 µg.

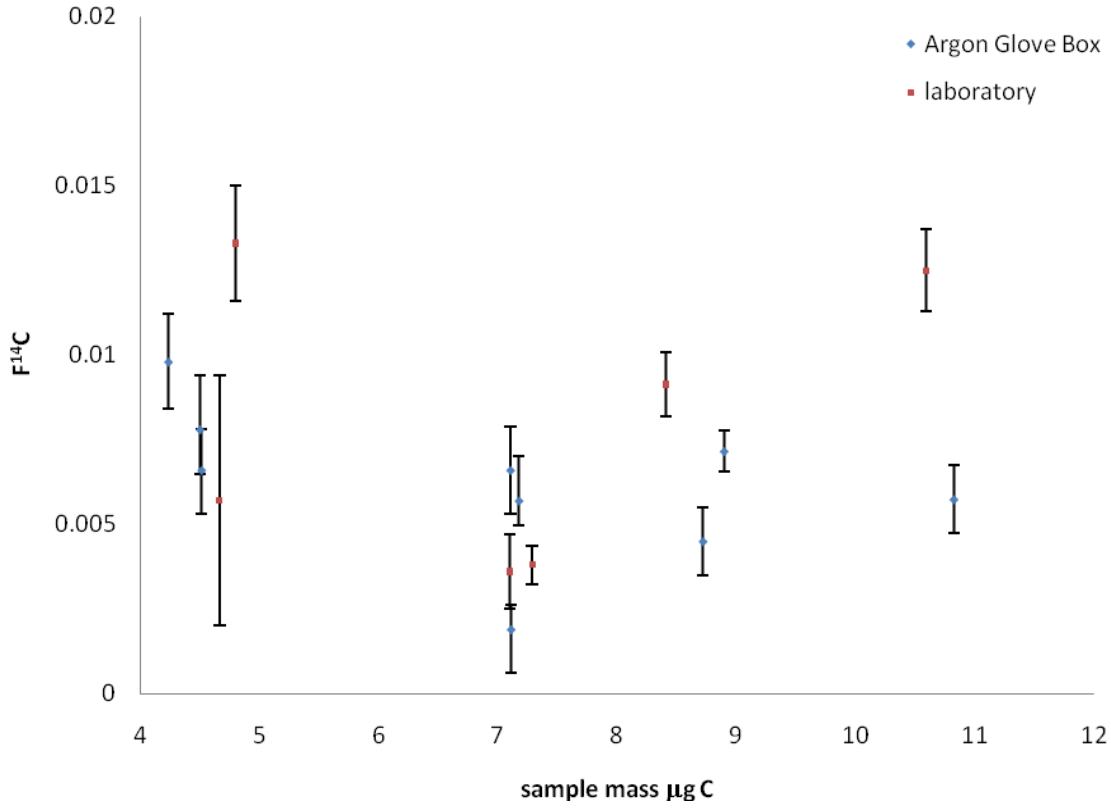

Figure 9-1 Measurement of dead carbon blanks from Linde CO_2

[3] LINDE GAS GmbH, A-2492 Eggendorf, Carl von Linde-Gasse 1

For our approach to determine the origin of contamination we needed a material that has practically no ^{14}C in it, and which can be used in the ion source directly, with a minimum of preparation steps. 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 9-2 Geological graphite ore

9.1 Sample preparation

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 to measure 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. Tools used and sample treatment was the same between the two atmospheres. Tool preparation is described in the pre-treatment section.

The following steps took place inside the laminar air flow box.

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 little pieces with as little tool contact as possible. Half of the pieces were filled directly into standard aluminium target holders for the McSNICS source (Ferry, 1993), and the other half was powdered and pressed into the copper pins described in section 8.3.1. These were then pressed into McSNICS target holders. The produced AMS targets were stored inside PP vials (RatioLab, 1.5 ml, #5615000) until the AMS measurement took place.

The same sample preparation procedure took place inside the argon atmosphere where the samples also were stored till the AMS measurement. During sample preparation the box parameters where as follows:

box overpressure	5-10 mbar
O_2 content	<5 ppm
CO_2 content	<10 ppm

Table 9-1 Argon glove box parameters

9.2 Sample transfer to the AMS source

Samples prepared in laboratory air were mounted inside the laminar air flow box into our AMS target wheel together with IAEA C-3 and C-6 standards, a high purity graphite sample and 5 other used, large carbon samples for tuning purposes. Then the sample wheel was transferred into the argon glove box. To avoid possible air transport from the sample wheel to the inside of the argon box, the wheel was transferred disassembled. Then the 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 small transportation box that could be sealed hermetically. The wheel was loaded into the source using a glove bag filled with argon. Evidence that the glove bag avoids air contact is provided by the fact that the ion source vacuum recovers almost immediately if the glove bag is used, and by the successful mounting of highly hydroscopic CaH_2 into our source with the same procedure (Wallner, et al., 2004). Therefore we think we have achieved a complete air free sample preparation cycle from sampling until AMS measurement.

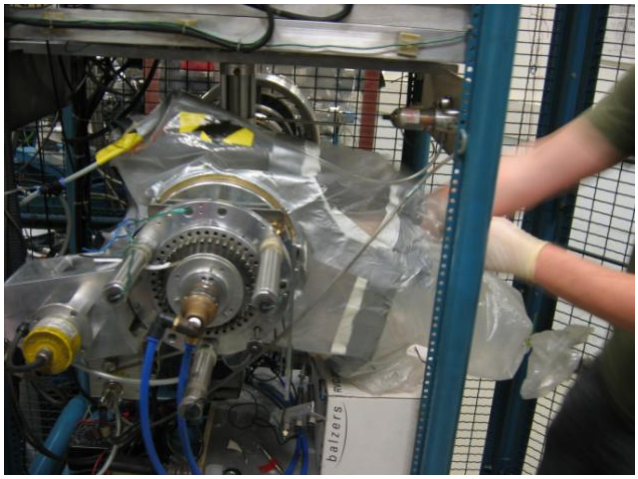

Figure 9-3 Using a argon filled glove bag to mount the sample wheel into the source

Figure 9-4 Close up of mounted Al cathodes in the sample target wheel

Figure 9-5 Sample target wheel

Results

10 Background from Fe catalyst preparation

We filled several mg of Fe powder into standard sample vials and connected them to the graphitisation line. The vial then was evacuated. To prevent loss of Fe powder due to the vacuum flow, 2 pieces of quartz filter are placed about 3 cm above the Fe powder. After the evacuation, O_2 (Air Liquid Austria, ≥ 99.5% purity) is cryogenically transferred into the sample vials. Then the Fe powder is baked for 2h at 900°C using the tube oven. Afterwards the sample vial is evacuated again and transferred into the argon glove box. Treating the Fe this way enables us to avoid any possible additional carbon background originating from ambient atmosphere

In Figure 10-1 the two different treatments are shown. As sample material 4-7 µg C from ^{14}C-free CO_2 was used.

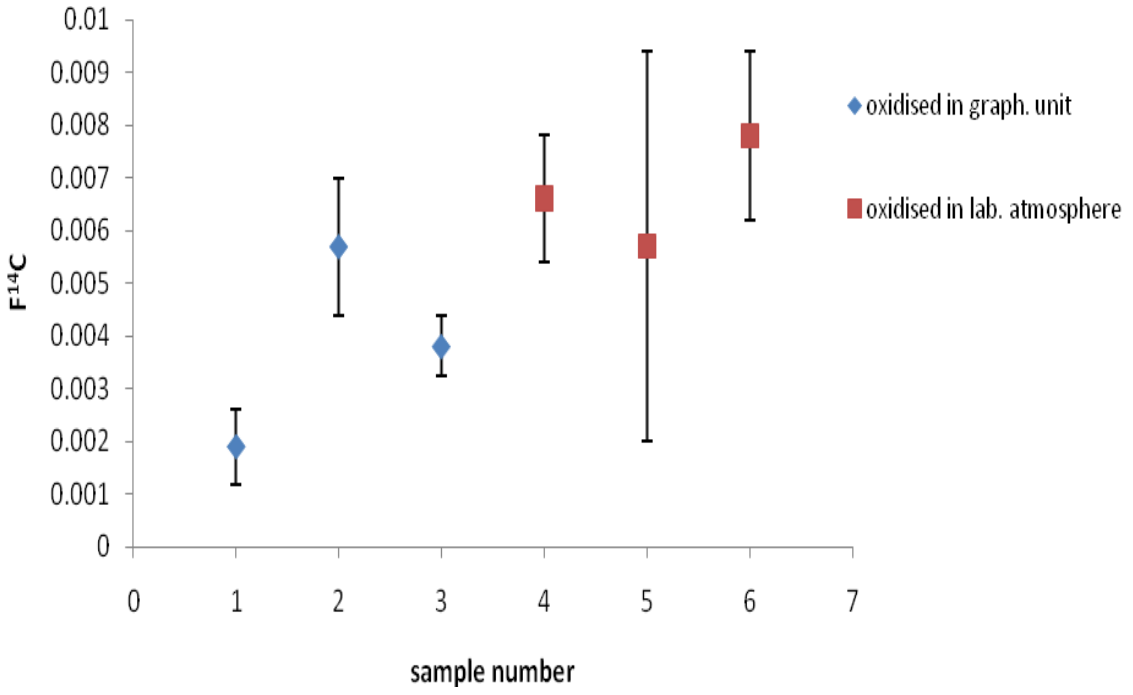

Figure 10-1 Comparing ^{14}C-free samples that are prepared with Fe oxidised in laboratory air or in argon atmosphere

As one can see in Figure 10-1 there is very likely a trend towards better blanks when iron oxide is not prepared under laboratory air, although the uncertainties are quite large. The average $F^{14}C$ measured for the catalyst oxidized in laboratory air is 0.0067 ± 0.0019, while that of the catalyst prepared without air contact is 0.0038 ± 0.0010. Unfortunately, this was found out relatively late in the run of this work, so all other samples prepared for this work were prepared with catalyst oxidized in air. However, oxidation inside the sample reactor should be explored for further improvements.

11 AMS measurement of geological graphite

The measurement was performed with our standard AMS setup, which is described in the AMS section of this paper.

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 mass 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 is a standard size for AMS dating.

sample name	atmosphere	comments
graphbox1	argon glove box	small pieces pressed into sample holder
graphbox2	argon glove box	small pieces pressed into sample holder
graphbox3	argon glove box	small pieces pressed into sample holder
graphbox4	argon glove box	small pieces pressed into sample holder
graphbox5	argon glove box	small pieces pressed into sample holder
graphlab1	laboratory	pulverized and filled into standard copper pin
graphlab2	laboratory	small pieces pressed into sample holder
graphlab3	laboratory	small pieces pressed into sample holder

Table 11-1 Geological graphite samples

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

11.1 Results

All samples were evaluated without blank correction. 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. One sample (graphbox_3) yielded only very small ion currents (150-250 nA instead of tens of µA), and cannot be expected to give a result useful for this investigation. All other samples gave a current in the range of a few tens of µA, which is normal for the size and material of the targets.

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
	graphbox_3	8.80E-04	3.00E-04	Ar box,outlier not shown
3	graphbox_4	6.30E-05	1.40E-05	Ar box
4	graphbox_5	1.30E-04	3.70E-05	Ar box
5	graphlab_1	1.70E-04	2.70E-05	lab air
6	graphlab_2	1.33E-04	5.40E-05	lab air
7	graphlab_3	2.94E-04	5.20E-05	lab air

Table 11-2 Geological graphite measurement results

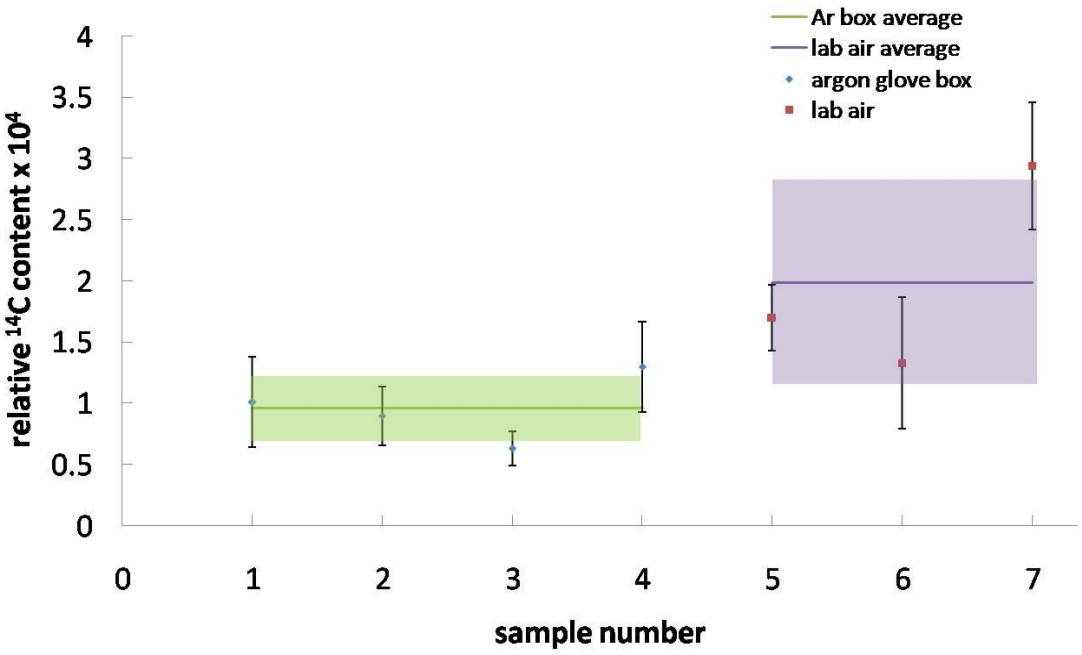

Figure 11-1 Geological graphite results

As we can see in the diagram there is a significant difference between samples that were prepared inside the argon glove box and samples which were prepared in laboratory air. Samples from the argon box appear about one half life of ^{14}C older than the other samples. Therefore we can conclude that there is an impact of ambient atmosphere to sample preparation, at least for mg-sized samples of graphite. The observed increase of the measured $F^{14}C$ corresponds to a background of several 100 ng of carbon. If the same amount is introduced into µg-size samples, it would lead to a significant deterioration of the results. However, it is unclear how this background scales with the sample mass.

12 AMS measurement of DNA samples

12.1 Sample characteristics

A total of nine ^{13}C enriched DNA samples were prepared and graphitised. To determine the contamination resulting from ambient atmosphere 6 samples 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 production and measurement of the samples.

The iron catalyst that is used for the graphitisation reaction (3819, particle size 10 µm) usually is pre-treated at laboratory atmosphere, which means it is oxidized at 900°C for 4 hours in a laboratory oven. In addition to complete the air free sample production we oxidized the iron catalyst in our graphitization unit under cryogenically cleaned oxygen. This batch of iron was also stored in an argon box until use to prevent air entering the preparation process. Also there was a possibility to bake the iron catalyst under argon atmosphere.

Therefore all critical steps of sample preparation can be performed under argon or in vacuum.

In Table 12-1 the differently treated samples are listed. The sample with the name 240210_DNA3 can be classified as an outlier, because the strongly increased CO_2 points to dust that was introduced during sample preparation.

sample name	nominal m_{DNA} [µg C]	p_{CO2} [mbar]	m_{DNA} [µg C] from graph. Reactor	storage	oxidation of Fe catalyst
19110_R1	2.17	4.94	2.19	Arbox	lab
19110_R2	2.17	2.48	1.10	Arbox	lab
19110_R3	2.17	5.71	2.53	Lab	lab
240210_DNA1	2.17	4.21	1.86	Arbox	graphitisation unit
240210_DNA2	2.17	4.55	2.01	Arbox	graphitisation unit
240210_DNA3	2.17	16.15	7.15	Lab	graphitisation unit
250210_DNA4	2.17	4.56	2.02	Arbox	lab and graphitisation unit
250210_DNA5	2.17	3.55	1.57	Lab	lab and graphitisation unit
250210_DNA6	2.17	5.73	2.54	Arbox	lab and graphitisation unit

Table 12-1 DNA sample data

12.2 Results

The measurement of the $^{13}C/^{12}C$ ratios were performed under parameters from an AMS setup which is commonly used for our radiocarbon measurements.

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

Table 12-2 AMS results for DNA samples

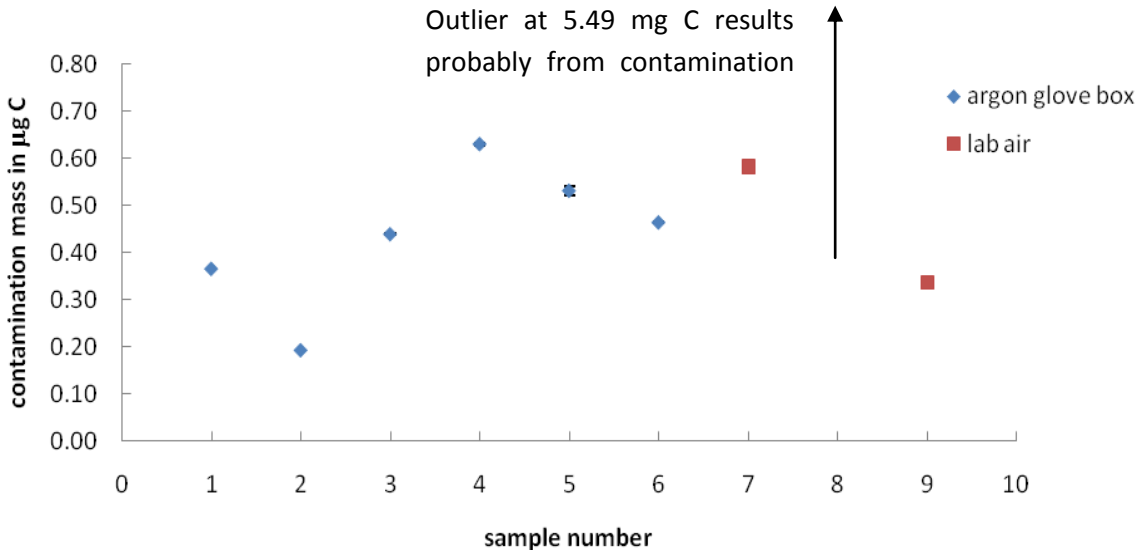

Figure 12-1 Contamination mass in abundance of different atmospheres

In Figure 12-1 the contamination mass is shown for the different preparation atmospheres. There is no evidence that different treatment of samples plays a role at this level of contamination.

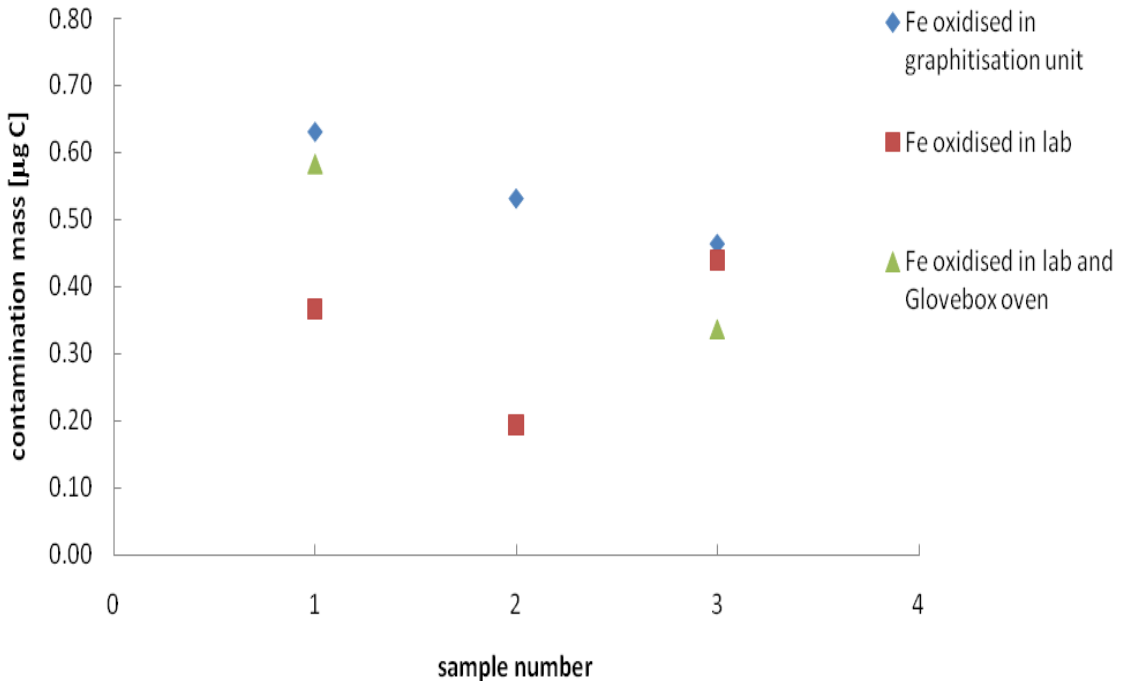

Figure 12-2 Contamination mass in abundance of different Fe catalyst oxidations

Also in Figure 12-2, where the different treatments of the Fe catalyst is shown, there is no evidence for a reduced background when oxidising the catalyst in high purity oxygen. This is in contrast to our findings presented in Figure 10-1, where ^{14}C-free CO^2 was used as carbon sample.

We conclude that the background reduction achieved by oxidation of the iron catalyst in pure oxygen without any atmospheric contact is obscured by the contamination introduced in the treatment of real DNA samples. The use of an air free environment did not result in a significant decrease of the contamination mass for such samples. This suggests that the dominating factor of background has to be found somewhere else in our sample preparation cycle.

13 Measurement of ^{14}C emanating from solid sample material

In nuclear astrophysics and fusion physics the production of long lived radionuclides is an important field. During the hydrogen burning phase of the CNO-cycle (carbon-nitrogen-oxygen cycle) of red giant stars, ^{14}N is a common product. Due to the large cross section of the reaction $^{14}N(n,p)^{14}C$ this acts as neutron poison, which means that neutrons needed for further reactions are eliminated from the cycle. This is also interesting for upcoming fusion reactors on earth, since the cross section of neutron energies > 7 MeV is not well known yet. Especially the production of nuclear waste from the shielding and surrounding material of the fusion reactors is under current investigation.

The energy-dependent neutron capture cross section $^{14}N(n,p)^{14}C$ is presently being studied at our Laboratory. Chemical compounds containing both nitrogen and carbon in a stochiometric ratio (e.g. Uracil $C_4H_4N_2O_2$) have been irradiated already, and the $^{14}C/^{12}C$ ratio was measured using standard radiocarbon procedures.

AMS measurements showed a lower ^{14}C concentration than expected from the cross section values found in the literature. An explanation for that would be that not all of the produced ^{14}C was still present in the solid material. The recoil from the reaction is usually large enough to displace the product from the lattice, to cause significantly lattice damage along its track, and to leave it in a chemically reactive form (Yankwich, 1956). Such lattice damage is known to increase the mobility of radiogenic atoms (as an example, radiogenic ^{234}U is more readily leached from minerals then primordial ^{238}U (Dawood, 2008)). We think that especially products which can form gaseous compounds are at risk to get lost from the sample matrix as emanation. Such losses would lead to an underestimation of the cross section under investigation.

The recoil energy of the produced ^{14}C atom can be determined through the conservation of the energy and the impulse of the system.

$$E_n + E_{^{14}N} = E_{^{14}C} + E_p + Q$$

$$p_n + p_{^{14}N} = p_{^{14}C} + p_p$$

$$E_n = 14\ MeV \ldots\ldots\ldots neutron\ energy$$

$$E_{^{14}N} \ldots\ldots\ldots energy\ of\ the\ target\ ^{14}N\ Atom$$

$$E_{^{14}C} \ldots\ldots\ldots energy\ of\ the\ produced\ ^{14}C\ Atom$$

$$E_p \ldots\ldots\ldots energy\ of\ the\ escaping\ proton$$

$$p_n \ldots\ldots\ldots neutron\ impulse$$

$$p_{^{14}N} \ldots\ldots\ldots impulse\ of\ the\ target\ ^{14}N\ Atom$$

$$p_{^{14}C} \ldots\ldots\ldots impulse\ of\ the\ produced\ ^{14}C\ Atom$$

$$p_p \ldots\ldots\ldots impulse\ of\ the\ escaping\ proton$$

$$Q = 7.17\ MeV \ldots\ldots\ldots Q - value$$

$E_{^{14}C}$ was computed to be ~2.2 MeV. To determine the range of the ^{14}C atoms in the sample material the SRIM[4] (stopping and range of ions in matter) program was used. With its help the average range of ^{14}C atoms in Uracil was computed to be about 4.6 µm.

Possible losses of ^{14}C during irradiation had been discussed after the disagreement with already published cross sections was observed during the measurement series. However, a first attempt to address this question, partly similar to our new approach presented below, but with a more provisional set-up, did not yield conclusive results. Though above-modern ^{14}C concentrations were positively detected, the chemical yield determined from a "dead" CO_2 tracer was very low and significant amounts of non-LN_2-condensable gases were found in the sample vial.

To resolve this question, we repeated this measurement with refined procedures. Sample material was filled into quartz glass vials (baked at 850°C), evacuated and flame sealed. Then the samples were irradiated with 14 MeV neutrons at the TU Dresden/Rosendorf. The intend is that if ^{14}C escapes the sample, it is trapped inside the wielded sample vial and can be detected. The samples were cracked in a hermetically closed, evacuated sample cracker (see Figure 13-3 next chapter). To allow dependable preparation of graphite targets, ^{14}C free CO_2 gas was added as carrier. The expected small number of ^{14}C atoms in the sample recommended to minimize the amount of the carrier gas, to obtain a sufficiently high isotopic ratio for the AMS measurement. Additionally, a low ^{14}C blank is required. Both requirements are well met by our small-sample unit.

Probe	material	mass [mg]	irradiated
A	Polyimide Resin	6.81	yes
B	Uracil	6.3	yes
C	Uracil	5.01	no - blank
D	Uracil	89	yes
E	Polyimide Resin	23.28	yes
F	empty	--	yes
G	Uracil	~ 5	no - test

Table 13-1 Samples from the $^{14}N(n,p)^{14}C$ experiment

[4] SRIM, James Ziegler, www.srim.org

As listed in Table 13-1 Uracil and Polyimide Resin were used as sample material.

Uracil

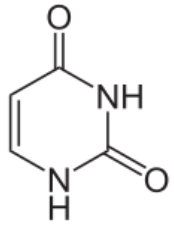

Figure 13-1 Structure formula of Uracil (www.wikipedia.com)

Uracil is a pyrimidine derivative and is found in the ribonucleic acid (RNA). It sum formula is $C_4H_4N_2O_2$. There it base-pairs with adenine and during the DNA transcription it replaces thymine. Additionally, Uracil protects the DNA and improves the efficiency of DNA replication, turning into thymine. The sampled uracil was in a crystalline powder form (grain size ~ 6µm, measured with an optical microscope) and should therefore most likely be in a gas sealed state. The carbon content of uracil is 40% (evaluated from the sum formula).

Polyimide

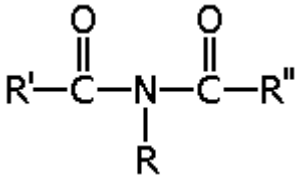

Figure 13-2 Structure formula of Polyimide (www.wikipedia.com)

Polyimide is a polymer of imide monomers. It s produced through the reaction of dianhydride and a diamine or a diisocyanate. Polyimide resin can also be described as thermosetting polyimide. These specific polyimide's have very good thermal properties. Normal operating temperatures can range between cryogenic and about 250 °C.

The main applications of polyimide's are fields were resistancy against temperature and chemicals, is needed while flexibility is also granted. Polyimide resin in specific is often used as an insulating and passivation layer in the production of digital semiconductor chips.

Because Alfa Aesar[5] was not able to provide an exact sum formula of the polyimide resin we have obtained it has to be noticed that in our measurement the amount of carbon present in the sample was determined through the yield of the combustion and has been about 57%. For the polyimide Kapton® ($C_{22}H_{10}N_2O_5$) the carbon content is 69%. This introduces some additional uncertainty into the interpretation of the measurement.

[5] Alfa Aesar, A Johnson Matthey Company Benzstraße 3, 76185 Karlsruhe, Germany

It was also intended to use adenine (which is a nucleobase with different applications in biochemistry) samples for the cross section experiments. However this is not possible due to the "modern" carbon content we found for our adenine material. In addition a number of blank (i.e. not irradiated) samples for these materials were produced and measured.

sample name	material	mass [mg]
100705_ade	Adenin	1.50
100719_ade2	Adenin	1.21
100705_poly1	Polyimide Resin	0.66
100705_poly2	Polyimide Resin	2.00
100719_poly3	Polyimide Resin	1.47
100719_Ura	Uracil	2.25
G_dust	Uracil	5.00
C_dust	Uracil	5.01

Table 13-2 Blank materials

The first trial to measure the ^{14}C emanation in sealed vials had encountered problems concerning large amounts of non-LN_2-condensable gases (NCG´s) of unexplained origin in the irradiated vials. Therefore the design of the new approach included the option to analyze them. The amount of the NCG´s was measured manometrically via the pressure sensors in the graphitisation reactors. To determine the composition of the gas, an RGA measurement was performed.

13.1 Sample preparation

The pre conditioning of the iron catalyst is performed as in our other applications (30 min vacuum baking at 800°, 2h H_2 baking at 800°C).

Figure 13-3 Detached tube cracker assembly with combustion vial (right) and flame-sealed sample vial (in front).

To allow the measurement of the gas inside the quartz vial, a stainless steel bellows was used as a tube cracker. By valves, the assembly was connected to the graphitization line and to a capillary leading to the RGA. At the bottom of the tube cracker a quartz vial is connected filled with a few grams of copper oxide for the combustion of the sample gas, to make sure that the ^{14}C emanation is in the form of CO_2. The tube cracker is evacuated. To remove any carbonaceous fraction from the copper oxide, it is baked for 30 min at 900°C and then evacuated again. Since the expected mass of the emanating gas is too small for reliable preparation, about 200 mbar (~80 µg C) of "dead" CO_2 (LindeGas AG) is filled into the graphitisation reactor and then cryogenically transferred into the tube cracker vial. After that the vial is cracked. To check if there is a larger amount of NCG´s the gas inside the tube cracker is expanded to the graphitisation reactor to measure the amount manometrically. For the analysis of the composition of the gas an RGA measurement is performed at this step. After that the sample gas is combusted inside the quartz vial below the tube cracker for 1 h at 900°C, to ensure that the ^{14}C-emanation is oxidized to CO_2. After that the carrier CO_2 together with the sample was transferred back into the graphitization reactor. The following steps of graphitisation were performed by our usual protocols.

The remaining solid sample material was removed carefully from the tube cracker and was filled into clean PP vials. To provide a homogenous distribution of sample material the PP vial was shaken a few times. From each type of sample material two aliquots with a mass of 600-2000 µg were prepared. The sample material was filled into our standard combustion vials. To prevent loosing material during evacuation 2 quartz filters were put into the middle of the vials. The combustion and graphitisation of the solid samples is performed with our usual protocols described in section 8.3.2.

13.2 Results

In contrast to the earlier attempt, the pressure measurement did not show noticeable NCG´s inside any of the sample vials. Also the RGA measurement showed no unexpected gases. We conclude that in the previous attempt (at which the author of the present work was not participating) a leakage of atmosphere into the sample must have occurred, which was not the case with the present assembly.

sample name	$m_{nominell}$	m_{sample}[µg C]	$^{14}C/^{12}C$	±	$m_{C\text{-}14}$ [µg]	±
B_dust	6.30E+03	2.6460E+03	6.61E-14	4.35E-15	1.66E-10	1.21E-11
B_gas		113.77	9.33E-14	6.60E-16	8.55E-12	1.82E-13
D_dust	8.90E+04	3.7380E+04	4.15E-14	6.50E-16	1.43E-09	3.32E-11
D_gas		127.70	2.48E-13	1.00E-15	2.93E-11	1.28E-13
A_dust	6.30E+03	6.66E+02	1.09E-14	3.80E-16	3.10E-11	2.08E-12
A_gas		9.90E+01	4.64E-14	6.30E-16	2.80E-12	2.70E-13
E_dust	2.33E+04	2.46E+03	2.75E-14	8.15E-16	3.34E-10	1.35E-11
E_gas		9.94E+01	2.20E-13	1.10E-15	2.01E-11	3.18E-13

Table 13-3 Results from the AMS measurement

sample name	material	$^{14}C_{gas}/^{14}C_{dust}$	±
B	Uracil	0.05	0.005
D	Uracil	0.02	0.001
A	Polyimide Resin	0.09	0.015
E	Polyimide Resin	0.06	0.003

Table 13-4 of the solid and gaseous content of the samples

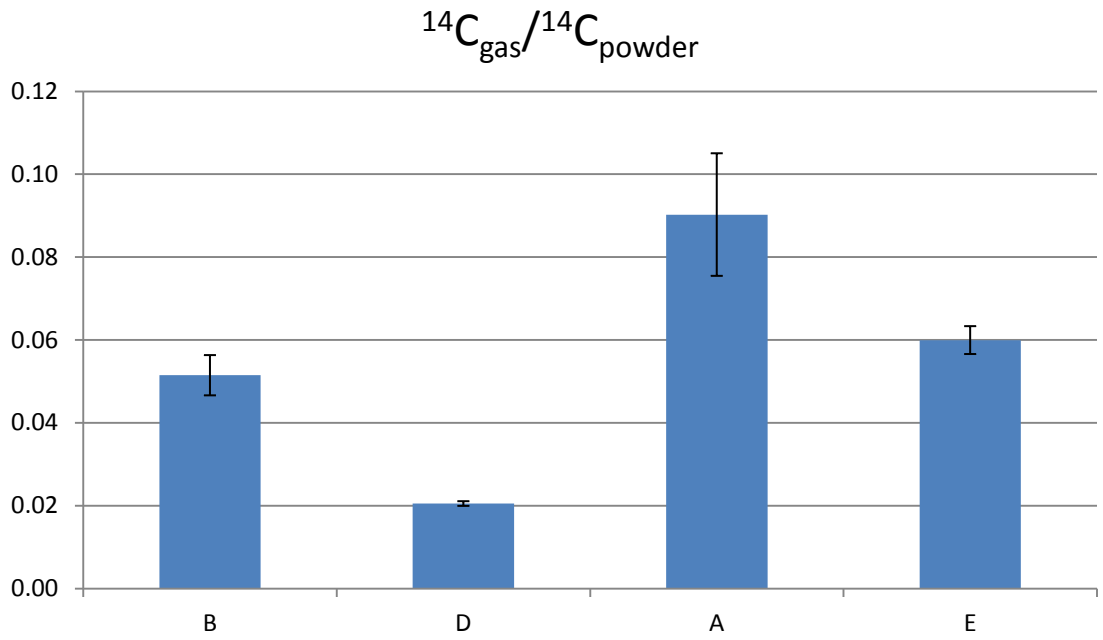

Figure 13-4 Graphical display of the $^{14}C_{gas}/^{14}C_{powder}$ results

In the case of Uracil as sample material the amount of ^{14}C that emanates from the solid material (labelled "^{14}C powder" in the figures) was 2 and 5 percent. For Polyimide Resin the loss was higher. In the worst case ~9% of the produced ^{14}C has entered the gaseous part of the sample.

We conclude that a significant fraction of radiogenic ^{14}C can emanate out of an irradiated, solid sample. The loss seems to depend on the type of sample material.
Since the range of the recoiled ^{14}C atoms (~4.6 µm) is only slightly lower than the grain size of Uracil (~6µm) one can suggest that a much higher percentage of sample loss should take place. But the recoiled atoms will most likely implant themselves into adjacent grains (Haas, et al., 1982). The conclusion is that maybe a relative high percentage of ^{14}C atoms can be emitted from the single grains but the overall loss of ^{14}C will be significantly lower due to the fact that the inner surface of the crystalline uracil powder is much bigger than the outer surface of the sample.

These facts should be taken into account when choosing materials for irradiation. Our data will be used to provide a correction for the already measured samples of our neutron cross section project.

14 Conclusion

The ability to perform preparation and measurement of ultra small samples (<10 μg) offers a wide range of applications in different fields of science. The method of dating ultra small samples allows to perform measurements on objects where only a small amount of sample mass is available. For this thesis the major applications were the radiocarbon dating of human DNA, to determine the cell turnover over different regions in the adult human brain, and the detection of ^{14}C emanating from solid materials which are exposed to a flux of neutrons. For all applications the minimization of the carbon background during sample preparation is presently the crucial factor. The main methodical task of this thesis was the investigation of the impact of using a clean argon atmosphere.

It was shown that for the untreated mg-size geological graphite samples a reduction of the carbon background can be achieved when they are prepared in an argon glove box. We were able to perform the "oldest" radiocarbon measurement at VERA so far. We have successfully prepared samples with an age of about 75000 yBP. This is about one half-life older than samples from laboratory atmosphere, i.e. corresponds to a reduction of the background by a factor of 2.

A similar effect is observed when the iron catalyst used for the graphitization is prepared completely without contact to laboratory air. For small dead CO_2 samples (4-7 μg C) also this resulted in a reduction of the background by a factor of approximately 2, from 0.007 to 0.003 $F^{14}C$.

However we were not able to observe this effect when preparing μg-sized DNA samples highly enriched in ^{13}C under argon atmosphere. This leads to the assumption that the major part of carbon background for ultra small DNA samples is not introduced by atmospheric air, but most likely due to other sources. This suggests that presently the cumbersome preparation inside the glove box will not lead to significantly lower overall background. However, once we are able to reach the same carbon background for ultra small DNA samples as for dead CO_2 or geological graphite, the clean argon atmosphere will provide a significant improvement. As an improvement of the present setup, it would be advantageous to install a laminar flow box inside of the argon glove box to prevent that dust particles can enter the preparation cycle.

The measurement of samples that were exposed to a flux of neutrons confirms that radiogenic ^{14}C is lost from samples during irradiation. For two uracil samples we observed losses of 2% and 5%, respectively. In the case of two polyimide resin samples a higher amount of emanated ^{14}C (6% and 9%) was observed. The assumption that the diffusion of ^{14}C is independent from the type of sample material couldn't be verified. Further experiments in this field should be performed. Despite we can only measure ^{14}C with our setup; our result is relevant for all irradiations where the product can form gaseous compounds.

Since dating of ultra small samples has a lot of applications in various fields of science it is strongly recommended to improve the overall performance of our protocols even more.

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Arriving at the last pages of my work, it is time to say thanks to a few people, which had a major influence on this work.

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