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DEVELOPMENT OF AN AUTOMATED SYSTEM FOR PREPARATION OF ORGANIC SAMPLES

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ABSTRACT. We constructed an automated system to transform organic samples to CO_2, which included several options such as: combustion in 2 steps with collection of the 2 fractions, volatile fraction combustion, and ^{13}C sampling. The process includes organic matter combustion, CO_2 drying, quantification of the mass of carbon, CO_2 collection in a glass vial, and eventually ^{13}C sampling. The system is computer-controlled and -monitored. The apparent background age of the automated system reaches 0.191 ± 0.011 pMC (2 σ), equivalent to a ^{14}C age of about 51,700 yr BP, and requires only 30 min of handling, instead of the several days needed when using a manual procedure.

INTRODUCTION

The radiocarbon group of the Laboratoire des Sciences du Climat et de l'Environnement (LSCE) previously developed a semi-automated system to transform carbonate samples into CO_2 (Tisnérat-Laborde et al. 2001) in order to reduce the time and effort of sample processing. A further step of the automation consists of the elaboration of a system allowing transformation of all types of organic samples into CO_2. The system must be able to handle both simple samples (e.g. charcoals and vegetal macrorests) and complex samples (e.g. peat and sediment) requiring step combustion and/or including volatile fractions. An option permitting aliquot collection for further ^{13}C analysis is also necessary.

METHODS

Organic Sample Pretreatment

Sample preparation depends on sample type, ranging from classical Acid-Alkali-Acid treatment for routine charcoals to ABOx, for N-rich woods and Fe^{2+}-rich paleosols (Hatté et al. 2001), and ninhydrin extraction for bones (Tisnérat-Laborde et al., this issue).

To validate manual and automated lines and to measure backgrounds, we use a 0-pMC charcoal as an internal standard. This standard, known as "Afrique du Sud," is from inside the Border cave (South Africa) in a Paleolithic level (Middle Stone Age) dated to more than 70 kya. It is routinely prepared by using an Acid (HCl 1N 60 °C)–Alkali (NaOH 0.1N 60 °C)–Acid (HCl 1N 60 °C) treatment.

"Manual" Procedure to Transform Organic Samples into CO_2

Organic samples are sealed in quartz tubes under a vacuum with an excess of CuO and silver wire. Tubes are introduced into a furnace at 835 °C for 5 hr to transform the organic matter into CO_2. The quartz tubes are then broken under a vacuum to release, dry, measure, and collect CO_2. This procedure requires complex handling (installation of tubes, sealing of the tubes, vacuum setting, etc.) and is time consuming.

Graphite Target Preparation

The graphite target is obtained by direct catalytic reduction of the CO_2, using Fe powder as the catalyst with a ratio of 1:5. The reduction reaction occurs at 600 °C with excess H_2 ($H_2/CO_2 = 3$) and is

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complete after 5–7 hr. The iron-carbon powder is pressed into a flat pellet (1-mm diameter) and stored under pure argon in a sealed tube.

To reduce contamination from modern carbon or memory effects, all quartz and glass dishes are burned at 450 °C for at least 12 hr. A turbo-molecular pump reaching 10^{-6} mbar is used to evacuate the vacuum lines.

Results are presented as ^{14}C activity in pMC (i.e., normalized to a $\delta^{13}C$ of –25‰).

AUTOMATED SYSTEM DESCRIPTION AND FUNCTIONALITY

Technical Choices

We developed the automated system based on the manual procedure, rather than derivation from an elementary analyzer (EA) (Aerts-Bijma et al. 1997, 2001; Hedges et al. 1992). This latter technique is susceptible to carbon contamination from Sn capsules and memory effects caused by the gas chromatographic column. The Gröningen team reports a blank value of 0.56 ± 0.17 pMC and a memory effect of a factor 2 with the initial EA system (Aerts-Bijma et al. 1997). Modifications of the protocol (combustion of an empty Sn capsule between each sample) and of the line (elimination of the GC separation) significantly reduced the memory effect and the blank to 0.24 ± 0.05 pMC (Aerts-Bijma et al. 2001). The Oxford team reports a mass-dependent contamination, essentially due to the capsules combustion, of about 0.15 pMC on a 1 mg sample, which one must add to the background of the physical measurement of 0.25 ± 0.1 pMC (Hedges et al. 1992).

The automated system (hereafter, "BMOA") allows: i) combustion of 2–5 samples in the presence of 500 mg of CuO and Ag wire; ii) CO_2 drying; iii) measurement of the carbon amount; and iv) CO_2 storage in glass vials.

The new automated system called for flexible handling to accommodate both routine samples (i.e. charcoals) and decomposable samples or samples showing a volatile fraction (some peats and wood). To allow for different sample types, we motorized the furnace for vertical shifts. This solves the problem of a "cold wall" that exists with a static furnace. Indeed, with a furnace heating only the lower part of the quartz tubes, the vaporized volatile fraction rises and condenses on the quartz tube above the furnace. This fraction is not burned. On the contrary, if the furnace is raised above the sample before the heating phase and lowered only after the combustion temperature is reached, the volatile fraction will first encounter a high temperature zone, where it will be combusted to CO_2. Thus, volatile fractions never encounter a "cold wall." The obtained CO_2 is then representative of the whole sample.

With the idea of using this automated system for all types of samples, and because it is already shown that it can be advantageous to burn peat in 2 steps (Bird et al. 1999), a 2-step combustion option was added to our system. In addition, we have included a user-definable option to remove an aliquot of obtained CO_2 for ^{13}C analysis. Our line allows a partition of 10% for ^{13}C and 90% for ^{14}C.

Finally, the calibrated finger is shaped to optimize CO_2 freezing. Indeed, organic matter combustion evolves not only CO_2, but also nitrogen and sulphur oxides that interfere with CO_2 during the freezing. The finger shape combines a small volume (permitting good precision of CO_2 measurement) with a high surface area.

Description

The line and storage vials are made of glass. Samples introduced into quartz tubes are connected by a Cajon Ultra-Torr fitting with a Viton o-ring. Glass vials are connected via "Rotulex" unions with Viton o-rings.

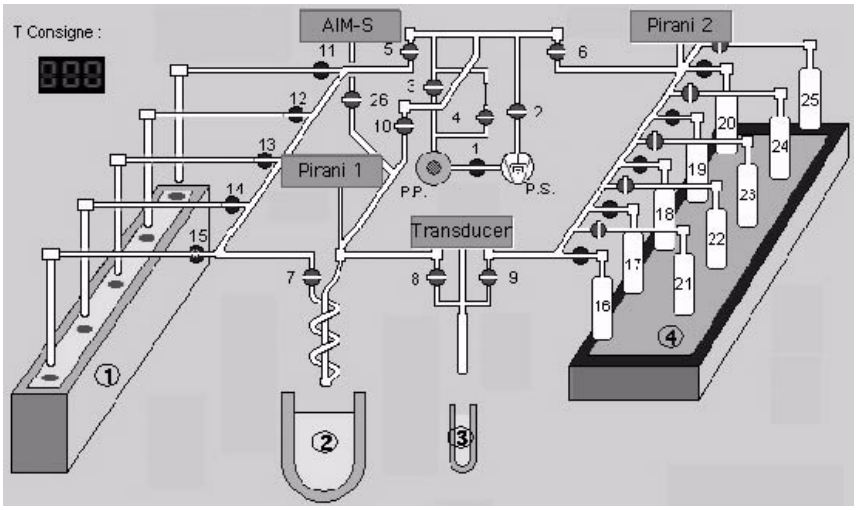

Figure 1 Schematic overview of the automated system. ①: movable furnace; ②: cold (–78 °C) or warm (70 °C) water trap; ③: cold (–173 °C) or warm (40 °C) calibrated finger trap; ④: cold (–173 °C) storage vials trap; P.P.: rotary pump; P.S.: turbo-molecular pump.

The vacuum is assured by a rotary and a turbo-molecular pump, and is controlled through 2 Pirani gauges and 1 inverted magnetron gauge (AIM-S). The amount of obtained carbon is calculated from the pressure gauge measurement in a calibrated finger.

All valves are either electro-pneumatic (vacuum system) or vacuum-actuated and connected to a vacuum reservoir emptied by a membrane pump.

The furnace (① on Figure 1) may be operated by a jack system operating as a step-to-step motor.

Five warm and cold traps are operated by a pneumatic-jack system:

- A cold trap (–78 °C, dry ice and ethanol) and a warm trap (70 °C water) to remove H_2O between 2 consecutive samples for the water trap (② on Figure 1);
- A cold trap (–173 °C, liquid nitrogen) and a warm trap (40 °C water) to expand CO_2 for the calibrated finger (③ on Figure 1);
- A cold trap (–173 °C, liquid nitrogen) for storage vials (④ on Figure 1).

Electro-pneumatic valves control the transfer of liquid nitrogen from an adjacent liquid nitrogen storage reservoir, which is secured by the filling level.

The entire system is monitored by a PC computer and controlled by software written in DELPHI (Turbo Pascal Objet). The computer collects external parameters by an IEEE interface card through a digital multimeter. The electro-pneumatic valves, the vacuum-actuated valves, and the jack systems are connected via actuators to a 24-channel relay Output Board PC, then to a Data Acquisition Board (Digital I/0).

Functionality

The software tests the vacuum and leaks on different parts of the line, monitors organic sample combustion and CO_2 transfers until final storage in a vial, and calculates the amount of produced and stored carbon.

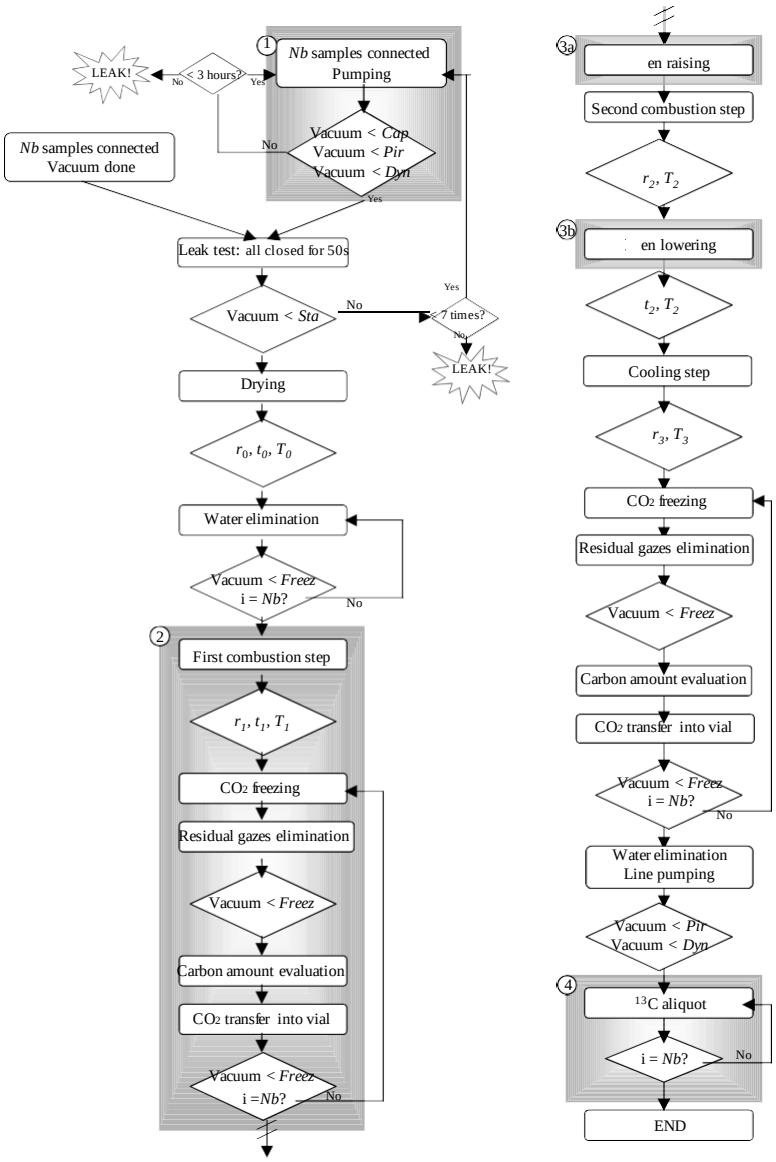

Figure 2 Synoptic of the automated system, including optional ways and user-definable user parameters. In *italics*, user-definable parameters. *Nb:* number of samples (2<Nb<5); Gauge thresholds (*Cap*: capillary to primary pumping, *Pir*: primary to turbo-molecular pumping, *Dyn*: limit vacuum under pumping, *Sta*: limit without pumping, *Freez*: limit pumping on freezing CO_2); furnace parameters: r_i, t_i, T_i: heating time, hold time, and temperature for i = drying (# 100 °C), first combustion step (# 450 °C), the second or single combustion step (# 900 °C) and cooling step (# 150 °C). The grey squares correspond to the following options: ① preliminary line pumping included in automated run; ②: combustion in 2 steps; ③: moving furnace; ④: ^{13}C aliquot sampling. The diamond boxes correspond to tests that have to be successful in allowing a passage to the following step. If the condition is not satisfied, either the operation continues until satisfaction (pumping, temperature rise, etc.), or a series of steps is carried out again for the following sample (until i = *Nb*), or a message preventing of a leak is sent if the time to obtain satisfaction is exceeded (<3 hr, <7 times).

Different options can be added to the basic run procedures:

- Preliminary line pumping before the combustion step, included in the automated system;
- Combustion in 2 steps with storage of the 2 fractions of obtained CO_2;
- Combustion with a furnace vertical shift (from top to bottom), allowing total combustion of decomposable or volatile fraction containing samples;
- Aliquot sampling of obtained CO_2 for further ^{13}C analysis.

Several parameters are user-definable:

- Number of samples (from 2 to 5);
- Timing of the different steps;
- Furnace parameters: heating rate, temperature hold time and temperature for drying, the eventual 2 combustion steps, and the cooling phase;
- The following gauge thresholds allowing continuation of the run:
  "Cap" threshold on Pirani 1 allowing a shift from primary pumping through a capillary to a direct primary pumping (necessary for fine sediments);
  "Pir1/Pir2" threshold on Pirani 1/2, allowing a shift from primary pumping to a turbo-molecular pumping;
  "Dyn" threshold on AIM-S, indicating that the limit vacuum is reached through the whole line;
  "Sta" threshold on AIM-S, controlling pressure increase on AIM-S in 50 seconds. It allows run continuation *id est* drying and combustions;
  "Freez" threshold on AIM-S, controlling the residual gas pumping on freezing CO_2.

Several parameters are logged during the automated protocol and are easily available during and after the run:

- Sample names;
- Results of combustion in terms of the amount of residual gases (measured in mbar on Pirani 1) and obtained carbon (in mg of carbon);
- Records of AIM-S pressure during leak testing, including results from Pirani 1 during CO_2 transfer from the quartz tube to the calibrated finger, pressure gauge during the CO_2 expanding in the calibrated finger, and Pirani 2 during the CO_2 transfer from the calibrated finger to the glass vial;
- Record of action messages showing progress and timing of the run.

RESULTS

Background Level of Manual Procedure

Figure 3 presents ^{14}C activity versus mass or inverse mass of carbon obtained for different amounts of the Afrique du Sud standard through a manual procedure. As expressed by the very low correlation coefficient, there is no relationship between carbon mass and activity. Considering a 2 σ error margin, all data are consistent (χ^2 test with 56 observations) with a mean of 0.138 ± 0.006 pMC (2 σ), equivalent to a ^{14}C age of about 54,400 yr BP.

For the common size range (between 0.8 and 2 mg) a mean apparent background age of 0.134 ± 0.006 pMC (about 54,700 yr BP) is measured.

Background Level of BMOA

The Afrique du Sud standard was used extensively to test different options and to cover a large range of mass. Results ranging between 430 and 1640 µg of carbon are presented in Figure 4.

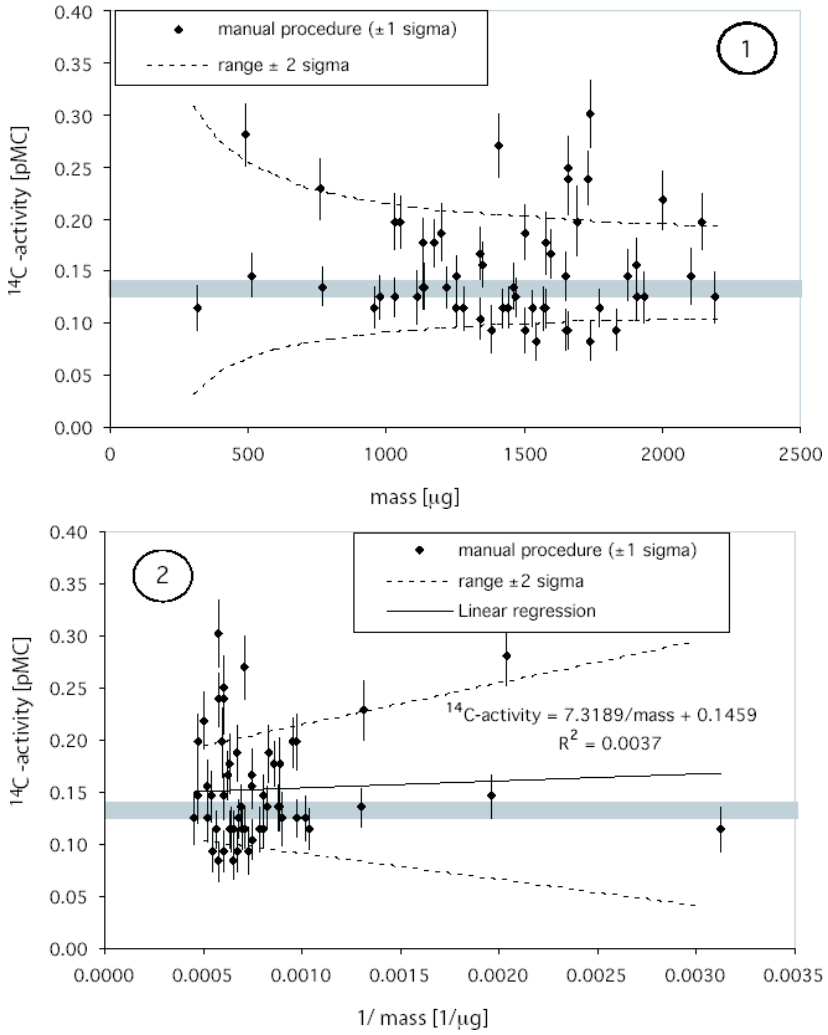

Figure 3 Background level of manual procedure. ①, above: ^{14}C activity (pMC) as a function of carbon sample mass (μg); ②, below: ^{14}C activity (pMC) as a function of inverse carbon sample mass (1/μg). The error bars are shown ± 1 σ (68% of overall confidence). Full lines represent regressions (equations and coefficients are noted above). Dotted lines are the range of variation within 2 σ. The grey bar represents the mean value (0.138 ± 0.006 pMC).

The results show that the relationship between mass and ^{14}C activities may be represented by:

$$^{14}\text{C activity} = (194 \pm 28) \times (1/\text{mass}) + (0.022 \pm 0.040) \quad (1)$$

with the mass in μg of carbon ($r^2 = 0.67$). However, this empirical relationship is not valid for samples containing more than 800 μg of C, as exhibited by the very low regression coefficient ($r^2 = 0.0015$) between ^{14}C activities and 1/mass. Considering the 2 σ error margin, all data are consistent (χ^2 test with 11 observations) with a mean of 0.191 ± 0.011 pMC (2 σ), equivalent to a ^{14}C age of about 51,700 yr BP.

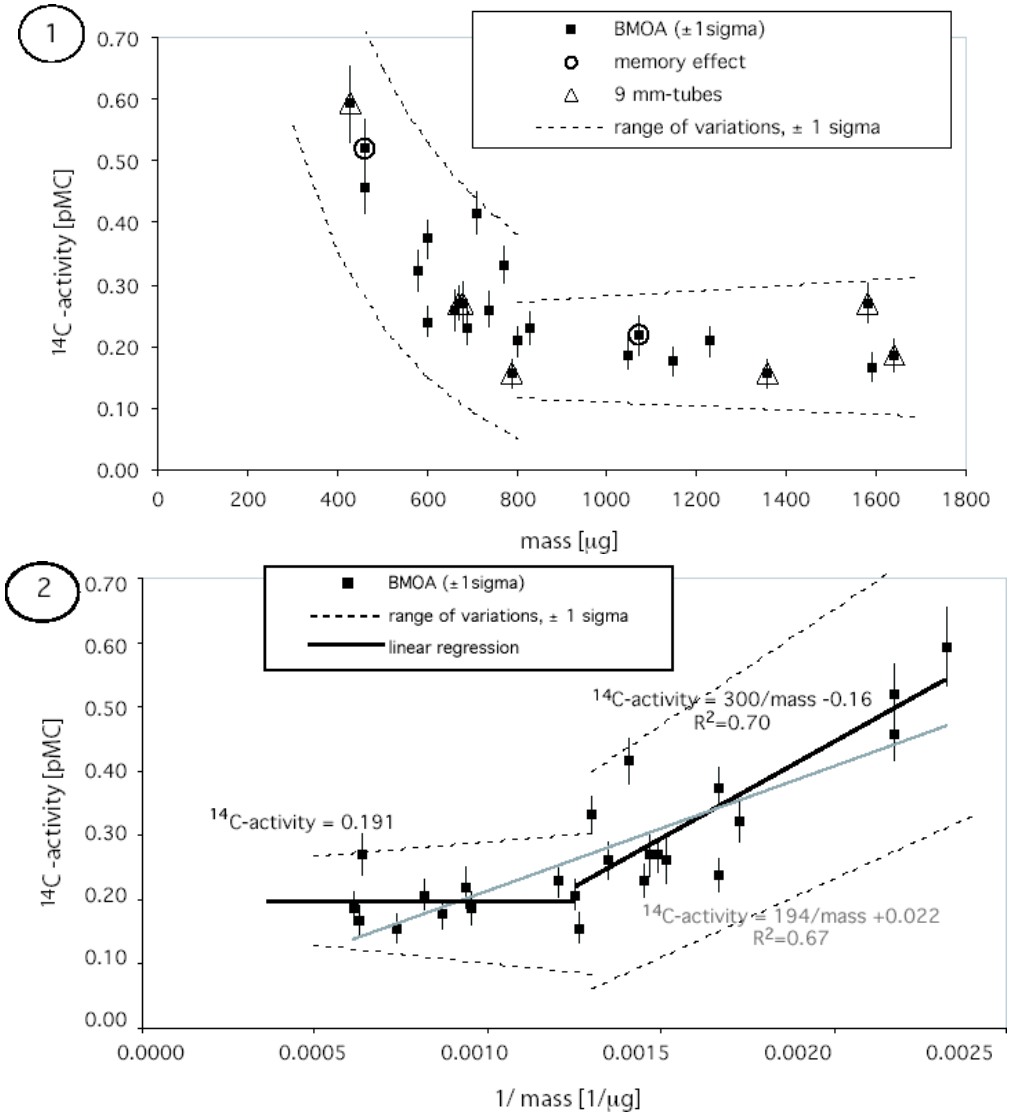

Figure 4 Background level of BMOA. ①, above: ^{14}C activity (pMC) as a function of carbon sample mass (µg). Open circles represent memory effect tests, whereas open triangles are for 9-mm tubes (see text). ②, below: ^{14}C activity (pMC) as a function of inverse carbon sample mass (1/µg). The grey line represents the linear regression tying ^{14}C activity and inverse carbon mass for all tests (equation and coefficient are noted below). The error bars are shown ± 1 σ (68% of overall confidence). Full black lines represent the couple of equations tying ^{14}C-activity and inverse mass (see text). Equations and coefficient are noted above. Dotted black lines are the range of variation within 1 σ.

Consequently, it is more appropriate to replace Equation 1 by the following 2 equations:

$$\text{mass} \leq 800\ \mu g\ ^{14}C\ \text{activity} = (300 \pm 55) \times (1/\text{mass}) + (-0.16 \pm 0.09) \quad (2)$$

$$\text{mass} \geq 800\ \mu g\ ^{14}C\ \text{activity} = 0.191 \pm 0.035 \quad (3)$$

Mass dependencies can be appreciated as a constant addition of 1.8 ± 0.4 µg of modern carbon (112 pMC as 2002 atmospheric CO_2) into a 0-pMC charcoal.

Memory Effects

In Figure 4, open circles symbolize the "memory effect" tests. These were performed using a small amount of the Afrique du Sud, which was run just after a large sample of "Sucrose" (IAEA-C6, 150.6 pMC). Values obtained are consistent with mean standard values obtained during normal runs, indicating that the procedure timing is well calibrated to induce no memory effect.

Quartz Tube Evaluation

Due to differences in dimensions between the international metric and imperial systems, tests were conducted to define the type of quartz tubes to be used. Cajon Ultra-Torr fittings correspond to a tube with an external diameter of 3/8", but common quartz tubes are manufactured in international metric units with an external diameter of 9 mm (i.e., 0.54 mm smaller than the Cajon fitting). Our tests showed that values obtained using the metric tubes (9 mm, open triangles in Figure 4) are consistent with values obtained using the 3/8" tubes. This indicates that no extensive leak exists with the association of Cajon Ultra-Torr fittings and the 9-mm tubes. The use of metric tubes allows for substantial lab-cost savings.

DISCUSSION

Ranges of background variation of the BMOA and the manual procedure are consistent for samples containing more than 800 µg of carbon (Figure 5). Nevertheless, the mean level of BMOA is definitely higher than that of the manual process, 0.191 ± 0.011 pMC versus 0.138 ± 0.006 pMC (2 σ). The small number of manual measurements for the lowest masses (5 lower than 800 µg of carbon) do not allow us to confirm an apparent background activity as low as that of the heavier samples. Nevertheless, it seems that the difference in background between the manual process and the BMOA is even larger for the samples lower than 800 µg than it is for the largest samples. Nevertheless, the BMOA background is lower than the blank value of the EA-based systems, even modified, which show a value of 0.24 ± 0.05 pMC. (Aerts-Bijma et al. 2001).

The particular nature of the BMOA procedure may explain its apparent greater background age compared to the manual procedure for 2 reasons:

1. The major difference between the manual procedure and the BMOA is that for the former, combustion occurs in a sealed tube, while in the latter, it occurs under a valve and an Ultra-Torr Cajon. Undoubtedly, flame-sealed tubes are more airtight than a "closed valve–Ultra-Torr Cajon" assembly. In particular, an increase of Viton o-ring porosity can occur consecutively with an increase of temperature (around 50 °C) during the combustion step and can allow small contamination by o-ring degassing. Perhaps a preliminary heating of the Viton o-rings would be sufficient to decrease the background level.
2. A leak due to differential thermal expansion between quartz, Viton and Stainless of Cajon is not very probable. Indeed, a more significant leak would be expected to occur in the case of the 9-mm tubes, for which the pressure on the o-ring is weaker than for the 3/8" tubes. Such leaking is not observed. Consequently, at this stage of the development, it does not seem necessary to implement a room with double walls around the Cajon connection, as proposed by Bird et al. (1999).

Nevertheless, the constancy and relatively low background obtained for samples containing more than 800 µg on BMOA allows for the treatment of the majority of organic samples. Moreover, this configuration requires minimal handling to obtain, dry, measure, and collect CO_2 from 5 samples; thus, only 30 min of human handling is necessary. Furthermore, an automated run of only 10 hr is

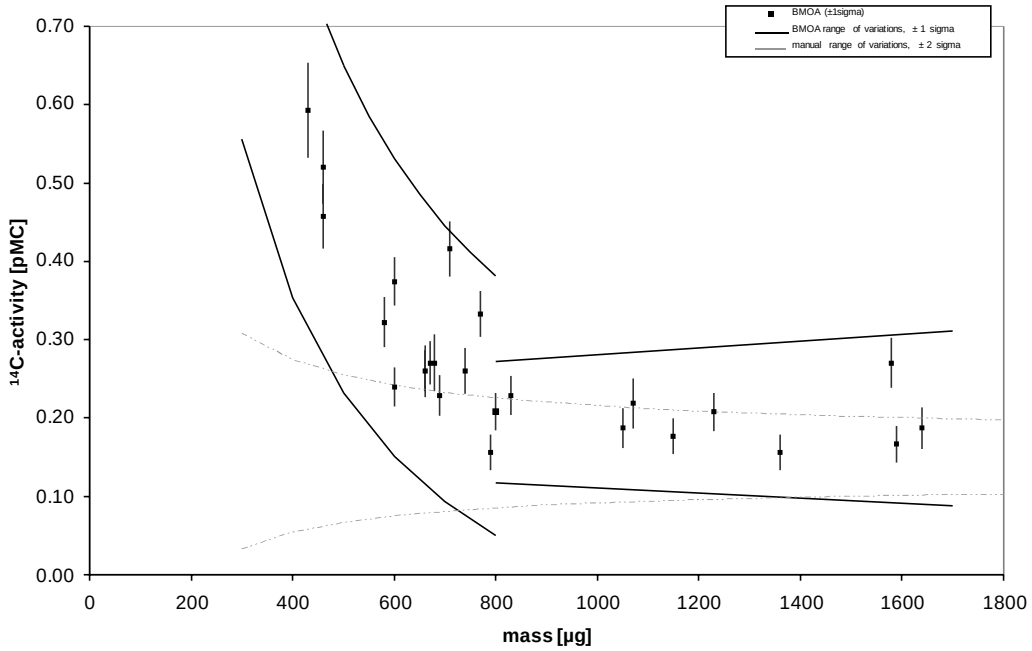

Figure 5 Background level of manual procedure and of BMOA with the ^{14}C activity (pMC) as a function of the carbon sample mass (µg). The error bars are shown ± 1 σ (68% of overall confidence). Full lines are the range of variation within 1 σ for the BMOA. Dotted lines are the range of variation within 2 σ for manual procedure.

necessary to obtain the collected CO_2, compared with the more than 8 hr distributed over 4 days necessary to arrive at the same result "manually."

CONCLUSION

We developed an automated system to obtain, dry, measure, and collect CO_2 from organic samples. This new procedure considerably reduces the human handling time, while preserving a background level sufficiently low to treat samples containing more than 800 µg of carbon, with a limiting age of approximately 51,700 yr. This system includes many options, such as allowing aliquot extraction for ^{13}C measurement and step combustion of specific samples.

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