


LAC-UFF STATUS REPORT: CURRENT PROTOCOLS AND RECENT DEVELOPMENTS

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ABSTRACT. The Radiocarbon Laboratory of the Fluminense Federal University was installed in 2009, and its NEC Single Stage Accelerator Mass Spectrometry system has been operational since 2012. As the first ¹⁴C-AMS facility in Latin America, the LAC-UFF became a reference center for radiocarbon (¹⁴C) dating in Brazil. Over the years we have implemented pretreatment protocols for several kinds of materials, such as cellulose, soil, bone, and biofuels. In the present paper we describe our current protocols for the preparation of these types of samples. Moreover, after 10 years of operation, with the aim of expanding the range of materials we are able to analyze, we report the results of several tests to improve accuracy, precision and background levels. For example, here we discuss how isotopic fractionation during the graphitization and measurement steps has been controlled. Concerning results interpretation, our research group has been using OxCal chronological models to analyze different contexts such as stromatolite growth, tree rings, soil deposition and marine reservoir effect (MRE) determination.

KEYWORDS: ¹⁴C-AMS, chronological models, sample preparation, zinc reduction.

INTRODUCTION

Sample preparation is one of the most critical steps in the radiocarbon accelerator mass spectrometry (¹⁴C AMS) technique. The aim of this procedure is to isolate a pristine fraction of a given material, i.e., a fraction whose carbon isotopic signal reflects that of the specific time and context when the system stopped exchanging carbon with the surroundings. Methods for sample preparation strongly depend on the sample matrix and can vary among different laboratories.

The ¹⁴C AMS technique was established in the 1970s and since then many facilities have been established around the world, especially in Europe and the United States. However, the first sample preparation laboratory for ¹⁴C-AMS analysis in Latin America was only installed in 2009, at the Fluminense Federal University in the city of Niterói, Rio de Janeiro, Brazil. The Radiocarbon Laboratory of the Fluminense Federal University (LAC-UFF) became a complete facility in 2012 with the installation of a 250 kV Single Stage Accelerator (SSAMS) system built by the National Electrostatics Corporation (NEC). Since then,

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thousands of samples have been both prepared and measured in the LAC-UFF, mostly through collaborative scientific research.

Our main research areas are Brazilian archaeology (e.g., Lopes et al. 2016; Macario et al. 2009, 2014; Milheira et al. 2017; Oliveira et al. 2017), studies on the ^{14}C marine reservoir effect (e.g., Alves et al. 2015a, 2015b; Carvalho et al. 2015; Diaz et al. 2017; Macario et al. 2015b, 2016a) and palaeovegetation and climate changes (e.g., Coe et al. 2014; Oliveira et al. 2014; Aguilera et al. 2016; Barros et al. 2016; Macario et al. 2019; Jou et al. 2020). We have also worked with biopolymers (Jou et al. 2015; Santos et al. 2019), anthropological contamination (Oliveira et al. 2019) and historical objects (Oliveira et al. 2015). At the same time, we have improved our graphitization (Macario et al. 2015b; 2016b, 2017a, 2017b), measurement (Linares et al. 2015) and data handling methods (Castro et al. 2015). In this paper, we describe the current sample preparation methods used at the LAC-UFF.

SAMPLE PREPARATION

By physically and chemically treating samples, our aim is to remove exogenous carbon that may have been incorporated after the organism's death. Those exogenous carbon atoms change the isotopic ratio of the sample and lead to incorrect ^{14}C ages. Therefore, such treatments are a key step in order to obtain accurate results. The concept of original carbon, coeval to the organism's live or corresponding context, can be subjective and inevitably has different meanings for different sample matrices.

Physical Pretreatment

Physical pretreatment is necessary to remove possible contaminants that are visible to the naked eye or under a binocular microscope. For sediments, these contaminants include root and plant fragments that must be discarded by flotation, sieving, or hand-picking. Charcoal fragments, on the other hand, are observed under the microscope and sediment/rootlet detritus and other remains from the depositional environment are removed with the help of disposable stainless-steel blades. For bones, teeth and ivory, it is possible to physically clean samples by aluminum oxide jet abrasion. Additionally, for teeth samples, the manual separation of dentine and enamel before both collagen extraction and enamel treatment can be performed (Alkass et al. 2013; Kobayashi-Kinoshita et al. 2016). Shells are generally scraped with the aid of disposable stainless-steel blades, and sonicated with MilliQ water, if necessary.

The preparation of foraminifera and other microfossils follows standard procedures consisting of wet sieving through a 63- μm mesh, drying under temperatures $<60^\circ\text{C}$, weighting and then dry sieving through a second mesh which limits the size of the desired microfossil (Shirayama and Horikoshi 1989). Samples are observed in a microscope with a magnification of 20–80 \times and the specific individuals of interest are isolated (Andree 1984).

Structures such as stromatolites, shells and vermetids may present more than one organism, e.g., barnacles' incrustations and other remains that need to be removed with the help of a magnifying glass and clip. It is desirable to focus on specific individuals with known composition and feeding habits.

Chemical Pretreatment

Once the physical pretreatment is done, the chemical pretreatment is performed with the use of reagents for the further removal of contamination (Hedges et al. 1989). For calcium carbonate (CaCO_3) samples, the standard chemical pretreatment consists of etching with hydrochloric acid (HCl) to remove the outer part of the sample. For CaCO_3 samples, such as shells, corals and other macrofossils, 50% of the outer layer, which is more susceptible to the effects of recrystallization (Douka et al. 2010), is removed. Vermetids may require specific care, such as density separation (Moreira et al. 2020). For foraminifera and microfossils, only 25% is removed, as greater care is required not to lose the sample completely. The chemical pretreatment is performed at 90°C and consists of a wash with HCl with molarities of 0.1M or 0.5M and varying volume as described in Table 1, depending on the initial sample mass. Following the HCl wash, the sample is rinsed with MilliQ water and left in the oven at 90°C to dry.

The typical treatment used for organic samples is the acid-base-acid (ABA) protocol. For this, the selected sample fragment is placed inside a 13-mm-long Pyrex[®] test tube. Then, 1.0M HCl is added for reaction cycles of 2 hr at 90°C with the aim of hydrolyzing the inorganic fraction. The process is repeated until the supernatant is transparent and gas is no longer produced. Sodium hydroxide (NaOH) with molarity of 1.0M is then added in baths of up to 1 hr at 90°C until the supernatant is clear (light-straw colored). These washes remove humic/fulvic acids from decomposition processes that occur in the burial environment. A further acidification step eliminates possible modern atmospheric contamination introduced during the NaOH step (Goh et al. 1972; Hedges et al. 1989). Between different reagents, rinses with MilliQ water are performed at least three times to neutralize the pH of the solution. Following the last acidification, the sample is rinsed five times with MilliQ water and left in a furnace at 90°C until it completely dries (see Table 1).

Specific Care with Organic Samples

For wood samples, when the dating of specific growth rings is required, a bleaching step is performed after the ABA pretreatment. This additional step aims to extract the holocellulose, which is the most preserved compound of the wood growth ring (Leavitt and Danzer 1993; Hajdas et al. 2017). The initial mass used in wood chemical pretreatment is 20–30 mg. The holocellulose is extracted using a mixture of 3 mL of 1.0M sodium chlorite (NaClO_2) and 3 mL of 1.0M HCl at 70°C in the heating block/furnace for 3–4 hr, repeating the procedure until the sample's color becomes white (Santos et al. 2001; Southon and Magana 2010; Santos and Ormsby 2013; Hammerschlag et al. 2019). After bleaching, the samples are washed several times with MilliQ water until the pH becomes neutral. The samples are dried at 60°C within a heating block before combustion.

Seeds and leaves can be very delicate samples, requiring a mild ABA pretreatment. Sample amounts vary from 5 to 20 mg. The base treatment is performed only once with 0.75M NaOH, then with 0.5M NaOH until the supernatant becomes light-colored or transparent. All treatments are performed at 90°C and followed by rinsing with MilliQ water at least three times.

For materials such as parchment, which are usually preserved with chemical substances considered contaminants (e.g., glue and oil), solvent washes are performed to remove such preservatives before ABA or any other chemical pretreatment is applied. It is important to

Table 1 Summary of the LAC-UFF protocols.

Material	Initial mass (mg)	Chemical pretreatment			
		Name	Reagents	Temp.	Duration
Wood	20–30	ABA	1M HCl	90°C	2 hr
		—	1M NaOH	90°C	1 hr
		—	1M HCl	90°C	2 hr
Charcoal	20–30	Bleach	1M NaClO ₂ + 1M HCl	70°C	3–4 hr
		ABA	1M HCl	90°C	2 hr
		—	1M NaOH	90°C	1 hr
Seed	5–20	—	1M HCl	90°C	2 hr
		ABA	1.0M HCl	90°C	2 hr
		—	0.75M NaOH	90°C	1 x 1 hr
Collagen	400	—	0.5M NaOH	90°C	1 hr
		—	1.0M HCl	90°C	2 hr
		Decalcification	0.5M HCl	4°C	36 hr
		Decontamination	0.1M NaOH	RT	15 min
		—	0.5M HCl	RT	30 min
		Gelatinization	pH 3 solution	65°C	≈20 hr
Apatite	600	Ultrafiltration	Centrifugation (3000 RPM)	RT	10 min
		Organic removal	1.5% NaClO	RT	48 hr
		Decontamination	0.1M HCl	RT	12 hr
Parchment	3–10	Solvent extraction	Hexane/acetone/CH ₃ OH:CHCl ₃	45°C/45°C/RT	1 hr/1 hr/2 × 1 hr
		ABA	0.5M HCl	RT	30 min
		—	0.1M NaOH	RT	20 min
Shell and coral	15–40	—	0.5M HCl	RT	1 h
		Etching	0.1M HCl or 0.5M HCl	90°C	Overnight
		Stromatolite	Organics removal	H ₂ O ₂	40°C
Vermetid	1000	Etching	0.1M HCl or 0.5M HCl	90°C	Overnight
		Organics removal	H ₂ O ₂	60°C	3 × 3 hr/3D
		Density separation	SPT 2.8 g/cm ³	RT	2 D
Sediment	1000	Acid wash	1.0M HCl	90°C	2 hr

verify the origin of the contaminant in order to choose the right solvent. For instance, glue is usually obtained from petroleum, constituting a “¹⁴C-free” contamination, while oil, generally used for writing, can introduce modern contamination. Solvents such as acetone, hexane, and methanol remove these kinds of contaminants and at LAC-UFF we follow the work of Brock et al. (2013) for parchment pretreatment. The physical pretreatment performed in 3–10 mg of sample is followed by solvent extraction using hexane (45°C, 1 hr); acetone (45°C, minimum of 1 hr); 1:1 ratio of methanol:chloroform (CH₃OH:CHCl₃) at room temperature, twice for 1 hr (Oliveira et al. 2015). After that, the samples are left to air-dry overnight to ensure the complete removal of the solvents. Given that parchment does not resist high temperatures, the samples are sequentially treated with a mild ABA at room temperature. The mild ABA consists of treatments with 0.5M HCl for 30 min, 0.1M NaOH for 20 min, and 0.5M HCl for 1 hr. After each treatment, the samples are washed with MilliQ water. Before combustion, samples are freeze-dried.

Our standard chemical pretreatment for sediment involves a simple acid treatment performed several times. Samples are treated with 1.0M HCl at 90°C for 2 hr until the supernatant is clear. If the whole treatment cannot be completed in one day, the samples are left overnight in 0.1M HCl at room temperature. At the end of the chemical pretreatment, the sediment is washed at least five times with MilliQ water and left to dry in a furnace at 80°C. It is important to be careful with the number of times that HCl is used during the chemical pretreatment. The number of HCl cycles varies depending on the type of soil, with the acid removing the modern carbon from the soil sample. If too many acidic washes are carried out, the carbon of interest may eventually be removed.

For biopolymer samples, according to method B of the ASTM D6866-16 (ASTM International 2016), there is no need to perform any chemical pretreatment, but the solid samples may be pre-cleaned with ethanol on the external surface (Quarta et al. 2013).

Specific Care with Carbonate Samples

For samples such as corals, stromatolites and vermetids, residues of organic matter must be removed. Samples are conditioned in a clean glass beaker, where hydrogen peroxide (H₂O₂) 30% is added. The sample remains submerged at 40°C for 5 hr or until the reaction stops. After that, the standard chemical pretreatment for carbonate samples is performed.

Crystallographic analysis (for instance, x-ray diffraction—XRD) may be performed prior to the ¹⁴C-AMS measurements in order to check for recrystallization (Lisker et al. 2009; Burr et al. 2015), a type of rearrangement in crystal structure.

If recrystallization is confirmed, extra treatments are required (Douka et al. 2010; Moreira et al. 2020). The carbonate sample must be treated with a solution of 2.8 g/cm³ density using sodium polytungstate (SPT), which will separate the recrystallized lighter fraction (usually calcite) from the original fraction of the sample (usually aragonite). After that, the aragonite fraction is ready to proceed as a regular CaCO₃ sample.

Conversion to Carbon Dioxide

Organic samples (e.g., wood, charcoal, seed, leaves, sediment, parchment, collagen) are taken to the vacuum line to be pumped out in quartz tubes, while carbonate samples (e.g., shells, otolith, vermetid, hydroxyapatite) are connected to the line via septum-sealed vials. The carbonate

samples are converted to CO₂ by acid hydrolysis at room temperature using 1 mL of 85% H₃PO₄ injected with the aid of a needle into the evacuated tubes. All organic materials are converted to CO₂ by combustion. Sample combustion takes place in sealed quartz tubes containing prebaked cupric oxide (Fisher Scientific, carbon compounds 0.0004%) and silver wire (Aldrich ≥ 99.99% 0.5 mm diameter), at 900°C for 3 hr. Six mm tubes are used for regular sized samples and 9-mm tubes for soils, sediments and other large volume samples.

Liquid samples such as fuel need to be handled carefully because their volatile components can evaporate. Fuel samples are frozen inside prebaked quartz tubes (6 mm diameter and 7 cm length) in a dry ice/ethanol or liquid nitrogen trap, depending on the solidification temperature of the liquid, and then inserted into the combustion prebaked quartz tube (9 mm diameter and 15 cm length) containing Ag wire and CuO. Then they are pumped out in a liquid nitrogen trap (Jou et al. 2015). Combustion tubes are torch sealed and baked at 900°C for 3 hr.

Conversion to Graphite

Graphitization is performed in a muffle oven in batches of independently torch-sealed tubes. Since the installation of the sample preparation laboratory, the Zn reduction method for the conversion of carbon dioxide into graphite has been used. At the beginning, we followed Slota et al. (1987) and used quartz tubes with prebaked Zn and Fe (Anjos et al. 2013). After Xu et al. (2007), we changed to borosilicate tubes using TiH₂. Issues concerning isotopic fractionation led us to test different amounts of reagents and graphitization temperatures (Macario et al. 2015, 2016b, 2017a, 2017b).

Graphitization tubes consist of borosilicate glass tubes with 9 mm OD and 15 cm length, containing TiH₂ (Alfa Aesar 99%) and Zn (Aldrich 99.995% powder <150 μm), plus a 6-mm OD Durham tube, which sits inside the larger reaction tube, containing approximately 5 mg of Fe powder (Alfa Aesar –325 mesh, reduced, 98%). Graphite is pressed into NEC aluminum cathodes and samples are placed in a 40 samples wheel to be measured.

MEASUREMENT

At the LAC-UFF, measurement is performed in a SSAMS system built by NEC and especially designed for ¹⁴C measurements. The SSAMS is a single stage system, meaning that no acceleration takes place after the stripping process. With a high voltage deck at 250kV, a sequential beam injection line, tuning magnets, two off-axis Faraday cups, and a solid-state detector, the system is capable of measuring both ¹⁴C/¹²C and ¹⁴C/¹³C ratios. The machine was installed in 2012 and was immediately put into operation, with performance improvements over the years (Linares et al. 2015).

The ion source of the SSAMS is the Cs sputtering MC-SNICS type that allows automatic analysis of up to 40 samples in a batch. Typical parameters for the MC-SNICS operation are listed in Linares et al. (2015). In a normal run, 20 unknown samples, 6 OXII as primary standard, 5 control samples, 5 blank samples, and 4 background samples, are measured. Measurements of each cathode are performed in 15–20 runs of 140 s acquisition, with usually 60 s of warm-up. The acceleration tube operates at 240.2 kV and the stripper gas used was Argon 99.999% at 0.95 sccm (Linares et al. 2015). In 2019 the Argon was changed by Helium in the stripper and the results will be presented in (Carvalho et al. in prep.).

Occasionally, when the system is under maintenance, we have our graphite sent to the Center of Applied Isotopes Studies (CAIS) at the University of Georgia, in the United States (Ravi Prasad et al. 2015) and to the Australian National University (ANU) Radiocarbon Dating Centre (Fallon et al. 2010).

DATA HANDLING AND CALIBRATION

The data analysis at the LAC-UFF is performed using the software LACAMS (Castro et al. 2015). The fraction modern F of unknown samples is calculated individually for each measurement using the following equation (Castro et al. 2015):

$$F_i = F_{std} \frac{{}^{14}\text{C}/{}^{13}\text{C}^{(unk)}}{{}^{13}\text{C}/{}^{12}\text{C}^{(unk)}} \text{MSL}(t_i) \quad (1)$$

where the MSL

$$\text{MSL}(t_i) = f\left(\left(\frac{{}^{13}\text{C}/{}^{12}\text{C}}{({}^{13}\text{C}/{}^{12}\text{C})_i^{(std)}}\right), t_i\right) \quad (2)$$

is currently the mean value calculated from the isotopic ratios of the set of standard samples in the wheel, using the nearest measurements in time to the unknown sample. Both rare and stable isotope ratios are measured in the accelerator.

The blank correction for unknown samples is made in the isotopic ratios following the identical-size approach, equation 6 in Castro et al. (2015) and, as we use the secondary standard NBS-OXII, no blank correction is applied for standard ratios. The reported modern fraction of samples is the weighted mean of individual measurements and the corresponding error is the standard deviation of the mean.

We report the results in ${}^{14}\text{C}$ age, percent modern carbon or biogenic fraction (ASTM International 2016). At the LAC-UFF the software OxCal 4.3 is used to calibrate ${}^{14}\text{C}$ ages (Bronk Ramsey 2009). ${}^{14}\text{C}$ ages are calibrated and expressed in cal BP or BC/AD. The result after calibration is a probability distribution different from a Gaussian distribution due to the irregular profile of the calibration curves and the use of subjective information related to the context of the samples, justified by the Bayesian approach in chronological models to improve the calibrated results.

Data sets presenting chronological ties, e.g., ${}^{14}\text{C}$ ages from stromatolites, tree rings and sediment cores, can be used in chronological models. The OxCal 4.3 software allows the selection of a model that most accurately represents the data. Indeed, OxCal provides tools to verify the suitability of a model and, depending on the degree of agreement between the data and the model, it is possible to remove outliers or to change the type of model. For instance, natural systems can be represented by simple, Poisson, or uniform sequences, depending on the available prior information about their behavior (Ramsey 2008).

RESULTS AND DISCUSSION

Figure 1 shows the results for oxalic acid (OXII) around the value of pMC (134.06 ± 0.18). Samples that yielded low beam currents ($< 5 \times 10^{-6}\text{A}$) were not considered. Figure 1 also shows that we did not measure many samples in 2016 and 2017 due to maintenance on our

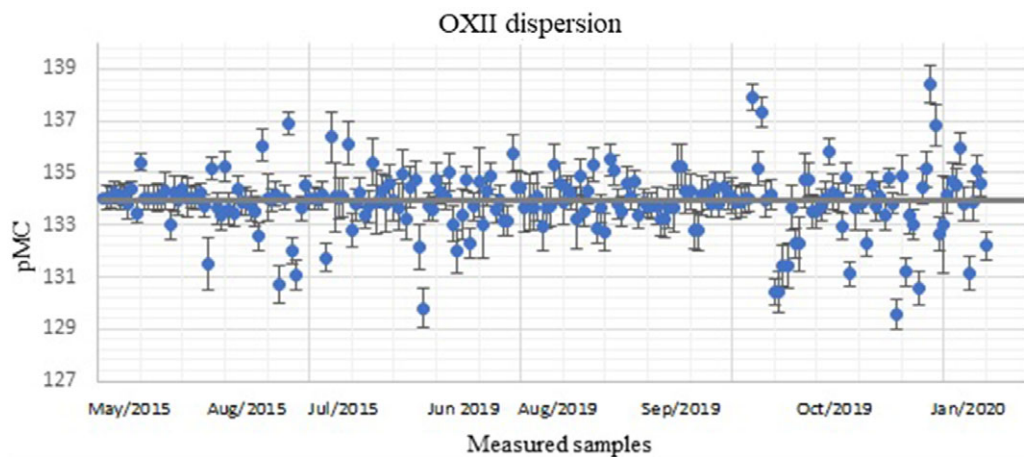


Figure 1 Results for Oxalic acid II (OXII) from 2015 to early 2020. The gray line indicates the consensus value for the OXII standard.

SSAMS system. During these years we have our graphite sent to the CAIS (Ravi Prasad et al. 2015) and to the ANU (Fallon et al. 2010).

In the same batch of organic and wood samples we analyzed reactor graphite (RG), an organic background sample processed together with unknown samples during chemical pretreatment (Figure 2A), IAEA-C3, a cellulose sample with $pMC = 129.41 \pm 0.06$ (Figure 2B), IAEA-C5, a wood sample with $pMC = 23.05 \pm 0.02$ (Figure 2C), and IAEA-C9 with pMC ranging from 0.12 to 0.21 (Figure 2D). For RG, IAEA-C5 and IAEA-C9 we performed only the ABA protocol; while for IAEA-C3 we further employed the bleach step to extract the holocellulose. Despite the variability shown in Figure 3, except for the background samples, the results are consistent with the consensus value.

As carbonate samples we analyzed IAEA-C2, a travertine with $pMC = 41.14 \pm 0.03$, and optical calcite (CA), a background sample (Figure 3A and 3B, respectively). The results are reported with ($\pm 1\sigma$) error. For the CA samples the dispersion is high and the values are higher than expected, even considering a $2\text{-}\sigma$ error.

The same happens with GR, whose values are plotted in Figure 2A. The background value for processed blank samples should be near zero for ^{14}C activity pMC , but the results show that the background of the LAC-UFF is a little higher than expected.

The preliminary results for bone samples were presented at CLARA (1st Latin American Radiocarbon Conference) and are also reported in Oliveira et al. 2020 (this issue). For collagen analysis, the samples extracted in the LAC-UFF come from the Sixth International Radiocarbon Intercomparison (SIRI) and our measured values were: SIRI-B ($35,083 \pm 256$ ^{14}C yr BP) and SIRI-C ($40,995 \pm 485$ ^{14}C yr BP). The SIRI-B (a mammal sample) is a bone from the North Sea with approximately 40,000 years BP. SIRI-C (a mammoth) is a background (~ 50 ka BP) sample from Latton Quarry, Wiltshire/Gloucestershire.

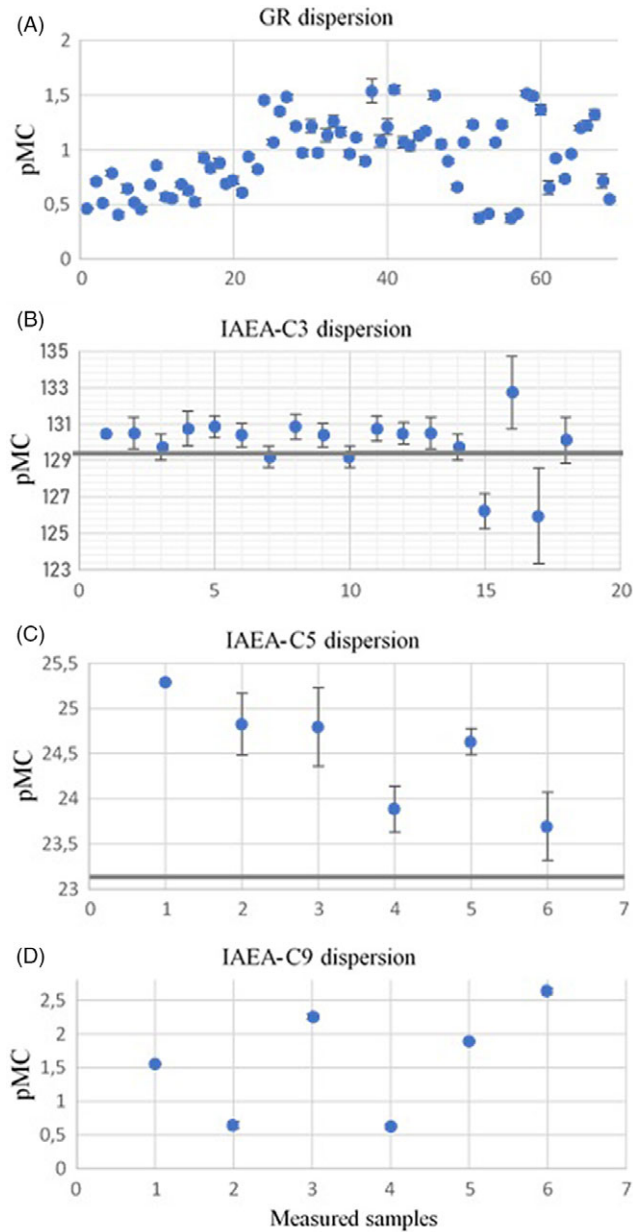


Figure 2 Results for process blanks and standards used with organic samples. (A) reactor graphite (GR); (B) IAEA-C3 cellulose; (C) IAEA-C5 wood; (D) IAEA-C9 wood.

Despite the background values the LAC-UFF were slightly high in the beginning, improvements were made and, in general, the values are in agreement with the consensus values and present small variability.

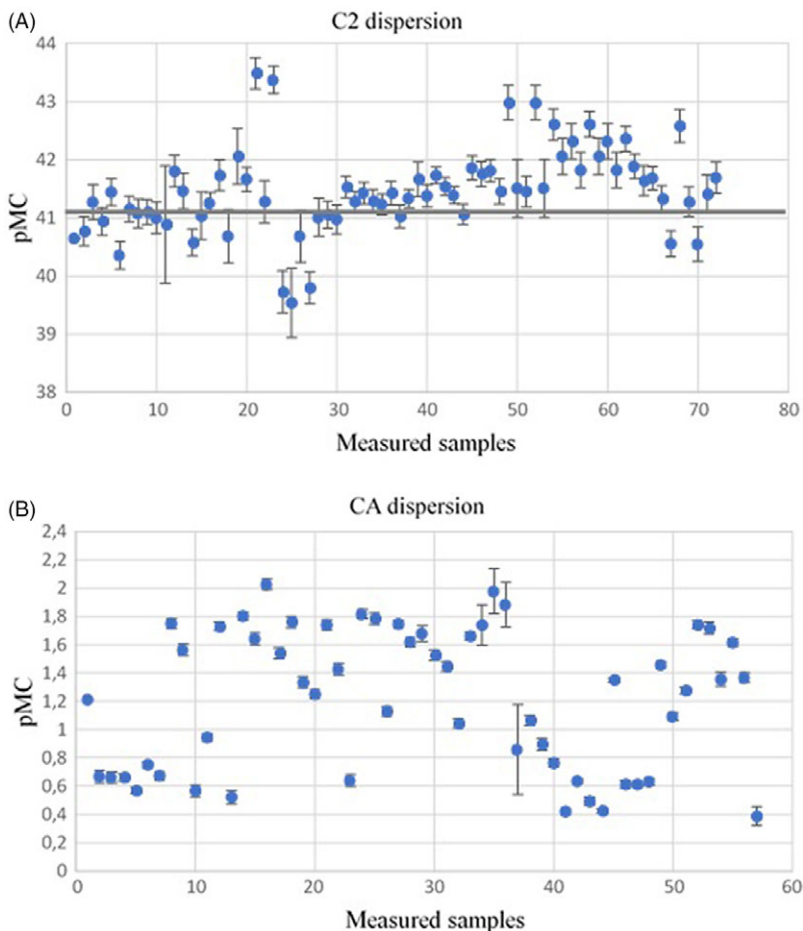


Figure 3 Results for standards (A) IAEA-C2 and (B) optical calcite (CA) used for chemical pretreatment of carbonate. The results are shown with ($\pm 1 \sigma$) error.

SUMMARY

This work shows the results for background and references samples since the first measurements made with the SSAMS installed at the LAC-UFF in 2012. Initially, in 2009, when the sample preparation laboratory was installed, samples were prepared in the LAC-UFF and the graphite produced was measured in laboratories abroad. The present paper demonstrates our ability to produce high quality graphite, shows that the results of the samples measured are consistent with consensus values, and describes the protocols performed in the LAC-UFF during its 10 years of operation.

CURRENT AND FUTURE PERSPECTIVES

The data handling will be improved with some new options for the LACAMS software. New blank corrections options will be added in the future, including mass dependent corrections that are mandatory when small sample are measured.

We are currently working on the refinement of our protocols for the preparation of materials such as water, different soil fractions and bone. All necessary equipment was recently acquired, and tests are currently being performed. In the case of bone and water, our first findings are reported in Oliveira et al. (2020) and Bragança et al. (2021) in this issue, respectively.

There is also work being developed at the LAC-UFF to investigate the degree of contamination in small carbonate samples. Future perspectives include the construction of new graphitization lines for water and gas samples. In addition, a study is being carried out to analyze soil fractions in order to obtain the most stable fraction in different soil types.

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