EXPERIENCES OF PRODUCTION AND HOMOGENEITY ANALYSIS OF AN AMS ^{14}C SUCROSE STANDARD FOR HIGH-ACTIVITY MEASUREMENTS

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ABSTRACT. Accurate accelerator mass spectrometry (AMS) measurements rely on standards with well-known isotopic ratios. For radiocarbon measurements, a number of standards with different properties are commercially available, of which the IAEA-C6 sucrose standard with a ^{14}C value of 150.61 pMC is the most active. When analyzing biological samples resulting from studies using ^{14}C-labeled substances, the activity content can be up to 100 times this value. Thus, there is a need for a standard material with higher activity content than IAEA-C6 for making accurate AMS measurements on this type of sample. This paper describes the attempts of producing a standard with an activity content of about 10 times modern carbon. The material chosen has to be chemically inert, preferably non-toxic, commercially available in ^{14}C-labeled form, and the activity must be homogeneously distributed within the material. Two different standard materials were considered: urea and sucrose. Sucrose was chosen for the new standard, since it is non-toxic, inexpensive, and organic and on combustion, forms only carbon dioxide (CO_2) and water (H_2O). In this paper, we discuss our experience in the production and homogeneity analysis of this material, from the crystallization of the sucrose solution to the graphitization of the samples. When using an online combustion method and a septa-sealed vial reduction method, the AMS measurements indicated that the activity was not homogeneously distributed throughout the material. Contrary to this, measurements of the sucrose solution prior to recrystallization indicated that the activity was more homogeneously distributed before than after the recrystallization. In order to determine whether the inhomogeneity depended on the graphitization method (i.e. the combustion or the reduction method) or on the material itself, 3 different graphitization methods and 2 different methods of recrystallization were tested.

INTRODUCTION

Accelerator mass spectrometry (AMS) is an extremely sensitive tool for quantifying radiocarbon. The use of this technique in the biological sciences has increased widely over the past few years. The recent application of microdosing in pharmaceutical development has further extended the use of AMS as measurement method for analyzing biological samples resulting from studies using ^{14}C-labeled substances (Lappin et al. 2006). It is often the case that these samples have ^{14}C content orders of magnitude higher than modern levels, in contrast to archaeological samples, which are generally less active than modern levels. AMS measurements are made relative to a standard of known activity. In order to acquire as accurate AMS measurements as possible, it is appropriate that the activity of the standard used is in the same order of magnitude as the samples analyzed by the system. The use of standards with much lower isotopic ratio than the samples could potentially cause scaling problems (Ognibene and Buchholz 2005). IAEA has 8 different reference materials (IAEA-C1 to -C8), of which the IAEA-C6 sucrose standard is the most active standard with a ^{14}C content of 150.61 pMC. Thus, we perceived the need in the biological sciences for a standard material with higher activity content.

In this paper, we report on our attempts to produce a new high-activity standard with a ^{14}C content of about 10 times modern carbon. Material produced for use as a reference standard must meet certain conditions. The material must have a long shelf-life, i.e. it should not be contaminated by bacteria or change its chemical form when stored; this excludes the possibility of using a solution, since a solution is more susceptible to bacterial contamination than a solid material. Furthermore, it should be commercially available in ^{14}C-labeled form. It should preferably be non-toxic and the ^{14}C must be homogeneously distributed within the material. Another important consideration is the ease

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by which the material is converted into CO_2 and that the material does not produce combustion products that are solids at $-196\ °C$ (i.e. the freezing temperature of liquid nitrogen), which can introduce impurities in the CO_2 sample.

We consider and test 2 materials as candidates of a new ^{14}C standard: urea and sucrose, both organic, non-toxic, cheap, and commercially available in ^{14}C-labeled form. Two different crystallization methods are tested for producing a solid material from a solution containing a mixture of dissolved natural sucrose and ^{14}C-labeled sucrose. In the process of investigating the isotopic homogeneity of the solid sucrose produced, 3 different graphitization methods are used and evaluated.

MATERIALS AND METHODS

Urea

Urea consists of carbon, nitrogen, oxygen, and hydrogen ($(NH_2)_2CO$). This material is soluble in water (solubility 108 g/100 mL at 20 °C), so the ^{14}C concentration can be easily modified by dilution. Another advantage of this material is that it is present in some of the samples that are commonly analyzed (blood and urine samples). Commercially manufactured, unlabeled urea, produced from synthetic ammonia and carbon dioxide, was tested. Tests at room temperature in glass containers revealed that urea was only slightly soluble in ethanol but dissolved readily in water. The water-urea solution was left at room temperature (~20 °C) until it evaporated to dryness, leaving well-defined, large crystals.

A small amount (8–10 mg) of the urea crystals were combusted in order to form CO_2 from the sample using online combustion as reported in Sydoff and Stenström (2010). For urea, only these combustion tests were performed (see the section Results below).

Sucrose

Sucrose consists of 1 molecule of glucose and 1 molecule of fructose that is joined by an oxygen atom ($C_{12}H_{22}O_{11}$). Like urea, it is soluble in water (200 g/100 mL in 25 °C), and so the activity concentration can be easily modified by dilution. This material has the advantages of being non-toxic, inexpensive, and on combustion, only forms carbon dioxide (CO_2) and water (H_2O).

Four different sucrose batches were produced, aiming at an activity content of ~2300 Bq/kg carbon, i.e. ~1000 pMC. The basic process was to solve unlabeled sucrose and add an appropriate amount of it in ^{14}C-labeled form, mix the solution for several hours, and then evaporate it to dryness. Thus, for each batch commercially available ^{14}C sucrose solution (GE Healthcare; 10729993) with an activity of 7.4 MBq/mL was diluted with water (35 μL of the active solution was diluted to 1000 mL) resulting in a ^{14}C solution with an activity of about 259 Bq/mL. These solutions were added to sucrose solutions containing 2000 g of sucrose (VWR; 27480.294) dissolved in 2500 g of H_2O. To prevent contamination of the AMS sample preparation laboratory, the procedure was carried out in a laboratory devoted to work with radioactive materials. The solutions were then moved to our sample preparation laboratory devoted to ^{14}C-labeled AMS samples, where they were mixed using a magnetic stirrer for various mixing times for the different batches.

Two methods of crystallization were tested. The first method, referred to as the furnace method, was to simply evaporate the solution to dryness in a furnace at low temperature (60 °C) for several hours (12–48 hr) with an amount of solution between approximately 100 and 200 mL covering the bottom of an aluminium box (11 × 17 cm); the larger the surface of the solution, the shorter the evaporation

time. When the sugar solution had been evaporated, the solid sugar was ground to powder with a mortar in small sections and all the different sections were then thoroughly mixed.

A second method of evaporation was tested, referred to as the vacuum method. The idea was to mimic the method used for the industrial production of sucrose. In industrial production, the sugar solution is boiled under low pressure and, as the moisture is removed, crystallization begins. The reason for the low pressure in the boiling tank is that the boiling temperature can be held at a lower level in order to avoid burning of the sugar.

The apparatus for crystallizing sugar under low pressure consisted of a reaction flask with a volume of 1000 mL provided with 4 × NS29 ports. Three ports were closed with glass plugs and the fourth was connected to a vacuum pump, along with a valve to adjust the pumping rate and a vacuum gauge to monitor the pressure. A cold trap, consisting of a stainless steel pipe (length 100 cm, diameter 25 mm) immersed in LN_2, was placed between the reaction flask and the vacuum pump. This was needed to trap the water vapor produced during combustion was trapped and frozen in the pipe to prevent water from getting into the vacuum pump. Plastic tubing with a diameter of 15 mm connected the reaction flask and the cold trap. Approximately 300 g of an 80% sugar solution (2500 g of water and 2000 g of sugar) was boiled in the flask (placed in a water bath) until all moisture was evacuated and good vacuum was achieved.

The appearance of the crystallized sugar was white and porous. However, it was quite hygroscopic, readily attracting humidity from the air. One explanation for this could be that amorphous sugar with very small particles was produced by this process, giving a large surface area to absorb moisture from the air. This can be removed by placing the sugar in an oven at ~50 °C for approximately 2 hr. This apparatus worked well for small volumes of sugar solution (approximately 200–400 g of solution), but for larger volumes it became more complicated. It appeared that very short tubes between the reaction flask and the cold trap were required since the water vapor condensed in the tube before it reached the cold trap, filling the tubes with water, which prevented further evaporation from the sugar solution. Small adjustments of the system, including the use of a high-capacity vacuum pump, provided a good way of recrystallizing relatively small amounts of sucrose. However, it is a relatively complicated method and the fact that the solution has to be recrystallized in many separate batches may possibly lead to inhomogeneity in the samples. It is also problematic that the solutions have to be stored for different time periods while waiting to be recrystallized, which could cause bacterial contamination if not stored appropriately.

Graphitization and AMS Measurement

The sucrose samples were converted to graphite along with samples of Ox-I and IAEA-C6, which acted as primary and secondary standards in the AMS measurements. For background measurements, liquid paraffin was used since it is a ^{14}C-free material, which also can be used as a carbon carrier in samples of low carbon content (e.g. HPLC fractions) or to dilute active samples (Lappin et al. 2006).

Three separate methods were used to prepare graphite from the samples. The first method employed was online combustion (using ~2 g of wire-form copper oxide for samples containing ~2 mg of carbon) followed by reduction in septa-sealed vials (Sydoff and Stenström 2010). The use of online combustion has been shown in several studies to give high-precision results in AMS and is commonly used in the preparation of e.g. archaeology samples (Skog et al. 1992, 2010; Skog 2007). To evaluate the septa method, some samples were graphitized using a second method: online combustion (as reported by Sydoff and Stenström 2010) followed by conventional reduction method (Vogel

et al. 1984) in a graphitization system available at our sample preparation laboratory for ^{14}C-labeled samples. The third method, aiming to evaluate the online combustion, included combustion in sealed quartz tubes according to the principle reported by Getachew et al. (2006) (using 60–70 mg of wire form copper oxide for samples containing ~1.2 mg of carbon) followed by reduction using the conventional reduction method (Vogel et al. 1984). All samples were measured at the Single Stage AMS (SSAMS) system at Lund University, which has precision of ~0.5% for samples processed at the Radiocarbon Dating Laboratory in Lund (Skog 2007; Skog et al. 2010). All samples reported in the current study have been processed in our sample preparation laboratory devoted to ^{14}C-labeled AMS samples. Due to the handling of ^{14}C-labeled materials, the samples prepared at this laboratory are not expected to achieve as high precision as those from the Radiocarbon Dating Laboratory, where only modern or submodern samples are handled. Furthermore, the laboratory devoted to ^{14}C-labeled AMS samples is located in another building than the Radiocarbon Dating Laboratory, to exclude the risk of contaminating archaeological and geological samples.

RESULTS AND DISCUSSION

Urea

After combustion of the urea there was evidence of non-condensable gases (probably N_2). Other compounds such as nitrogen monoxide (NO) and nitrogen dioxide (NO_2), which are condensable in LN_2, may also have been formed during the combustion. The production of these nitrogenous gases can cause a high pressure in sealed combustion tubes, with possible explosion risk. For these reasons, we abandoned urea as a useful reference material and considered sucrose which produces only water and carbon dioxide on combustion.

Sucrose

The results from the AMS measurements for the 4 separate batches of sucrose solutions are depicted in Figure 1a–h. Fractionation correction was made for all AMS measurements (Skog et al. 2010). Somewhat different activity concentrations were achieved in the separate batches due to differences in the preparation of the solutions.

As described above, 2 different methods of evaporation and 3 different methods of graphitization were used, along with various mixing times for the solutions. The methods used for each batch are listed in Table 1, along with the relative standard deviations obtained from scattering of the pMC values of the different samples (see Figure 1).

For batch 1, the sucrose was evaporated and recrystallized by the furnace method, combusted via online combustion, and reduction was made with the septa method. The sucrose solution was mixed for 24 hr before evaporation. The scattering of pMC data from the AMS analysis of 10 samples resulted in a relative standard deviation of 11.7% (Figure 1a). The large scattering of these data could be due to the relatively short mixing time. Furthermore, the sugar was extremely compact and not easily ground to powder.

The mixing time for the sample solution of the second batch (samples 11–16) was extended to 48 hr. These samples were evaporated by the vacuum method, which yielded a dry sugar that was porous and more easily ground, unlike the dry sugar in the first batch. As for batch 1, the samples of batch 2 were graphitized via online combustion and reduction in septa-sealed vials. The scattering of the pMC values of the 6 samples analyzed resulted in a relative standard deviation of 4.12% (Figure 1b), which is less than for batch 1, suggesting that the material of batch 2 was more homogeneous than batch 1.
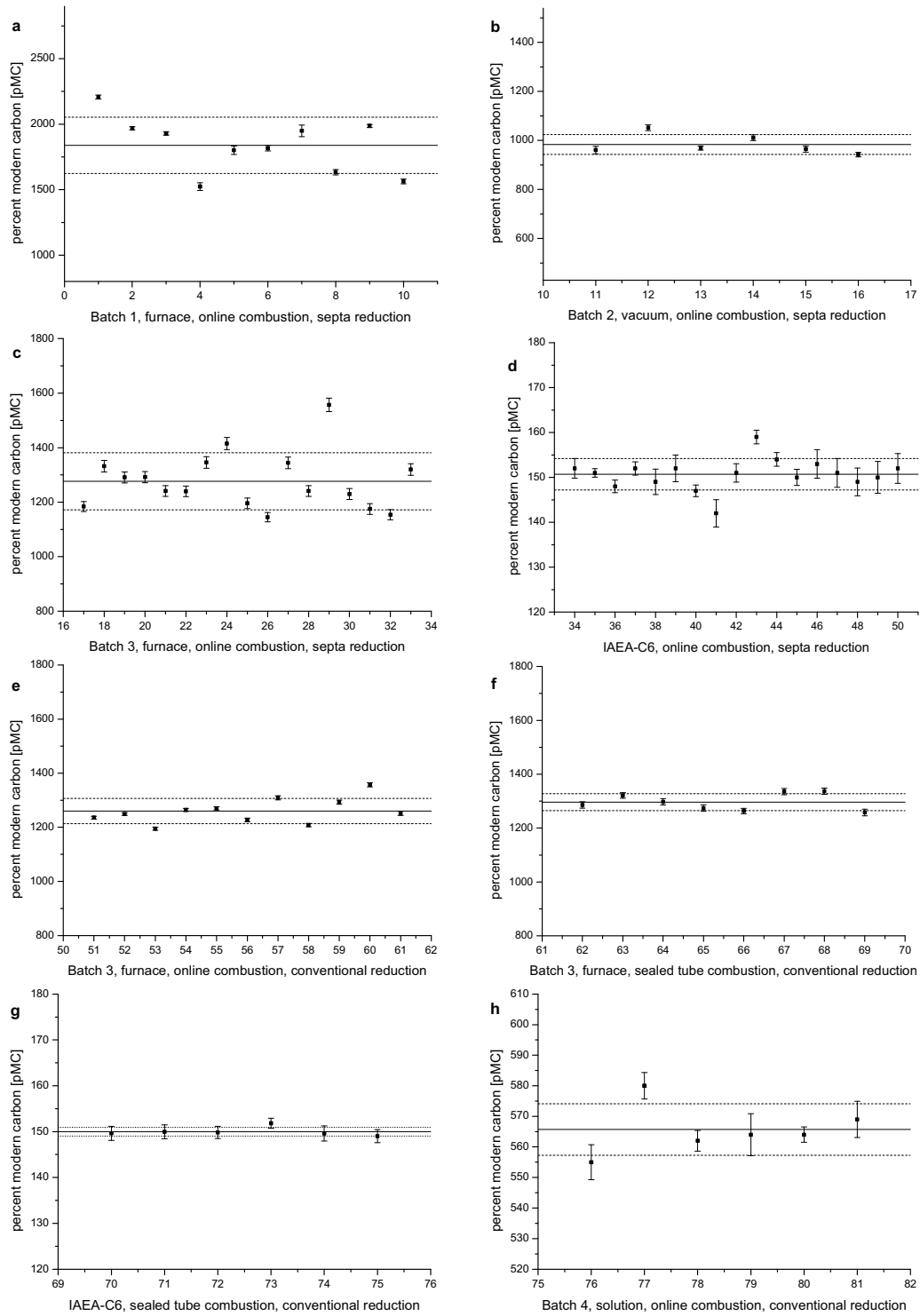
Figure 1 Diagrams a–h show the pMC values from the AMS measurements of the different batches of sucrose samples

Table 1 Description of parameters varied between different batches of sucrose standard. Evaporation method "solution" refers to samples graphitized after centrifugation of sucrose solution with no recrystallization. "Mixing time" is the time during which the active sucrose solution was mixed before evaporation. "Relative SD" refers to the relative standard deviation obtained from the scattering of the pMC values displayed in Figure 1.

Batch nr	Sample nr	Evaporation method	Combustion method	Reduction method	Mixing time (hr)	Relative SD (%)
1	1–10	Furnace	Online	Septa	24	11.7
2	11–16	Vacuum	Online	Septa	48	4.12
3	17–33	Furnace	Online	Septa	72	8.22
IAEA-C6	34–50	—	Online	Septa	—	2.32
3	51–61	Furnace	Online	Conventional	72	3.71
3	62–69	Furnace	Sealed-tube	Conventional	72	2.43
IAEA-C6	70–75	—	Sealed-tube	Conventional	—	0.64
4	76–81	Solution	Online	Conventional	48	1.48

In an attempt to further increase homogeneity, mixing of the solution was extended to 72 hr for batch 3. Evaporation was done by the furnace method. Seventeen samples (numbers 17–33) were graphitized using the same methods as for batches 2 and 3 (online combustion and septa reduction). The scattering of the pMC data of these samples showed a relative standard deviation of 8.22% (Figure 1c), which is higher than the results for batch 2 but somewhat lower than the results for batch 1. In order to determine whether the high standard deviation originated from inhomogeneity in the produced material or from the graphitization method (online combustion followed by reduction in septa-sealed vials), 17 samples of IAEA-C6 (numbers 34–50) were graphitized with the same method. The scattering in the IAEA-C6 data (Figure 1d) resulted in a relative standard deviation of 2.32%, i.e. significantly lower than for the homemade sucrose material, implying that homogeneity of this material had not been achieved.

Eleven additional samples (numbers 51–61) were taken from batch 3. These were combusted in the same way as samples 17–33 from the same batch, but graphitized by the conventional method. The scattering of the pMC data (Figure 1e) gave a relative standard deviation of 3.71%, demonstrating that the conventional reduction method was superior to the septa reduction method. However, still this value indicated that batch 3 was inhomogeneous.

Furthermore, another 8 samples from batch 3 (numbers 62–69) along with 6 samples of IAEA-C6 (numbers 70–75) were combusted in sealed quartz tubes and graphitized with the conventional method in order to exclude the possibility that the online combustion method could be the reason for the apparent inhomogeneity in the samples. The scattering of pMC data in the 2 data sets differed even if sealed-tube combustion was performed in both cases (Figure 1f and 1g). The relative standard deviation for the sucrose standard samples was 2.43%, while the corresponding value for the IAEA-C6 samples was only 0.64%. This indicated that the sucrose batch 3 indeed was inhomogeneous. The results of the measurements using the sealed-tube combustion and conventional reduction method also exclude the possibility that the apparent inhomogeneity was related to the online combustion procedure. Furthermore, internal controls on the AMS measurements at Lund did not indicate that any of the variability observed was due to variations in the SSAMS measurement itself.

An additional test was done in which a number of solution samples (76–81, batch 4) were graphitized directly, using online combustion and the conventional reduction method. This was a test of whether the inhomogeneity was due to recrystallization, irrespective of crystallization method used

(furnace or vacuum method). For this batch, the sucrose solution was mixed for 48 hr. Prior to graphitization, 6 samples of solution were dried in a vacuum centrifuge. The scattering of the pMC data (Figure 1h) resulted in a relative standard deviation of 1.48%, which is lower than the relative standard deviation for samples 51–61, graphitized by the same graphitization method (3.71%, Figure 1e). This might suggest that the recrystallization procedures increased the inhomogeneity of ^{14}C in the material.

CONCLUSION

The results of the AMS analyses showed that the activity in the sucrose produced was inhomogeneously distributed throughout the material despite the extremely thoroughly blending of the sugar solutions. It is indicated that the inhomogeneity was a result of the recrystallization and not of the choice of combustion or reduction method.

The tests of different graphitization methods, available at our preparation laboratory for ^{14}C-labeled AMS samples, showed that conventional reduction gave less scattering of the pMC data than septa reduction, when using online combustion. Furthermore, the sealed-tube combustion method gave slightly less scattering of the pMC data than the online combustion procedure, when using the system for conventional reduction. Repeated measurements of IAEA-C6 showed that the combination of sealed-tube combustion and conventional reduction was superior to the combination of online combustion and septa reduction.

As such, none of the prepared batches of sucrose were suitable for use as a high-activity reference standard. The recrystallization of the sucrose is a complicated process and measurements of the sucrose solution prior to crystallization indicated that the crystallization increases the inhomogeneity of ^{14}C in the material. Further developments of the crystallization method have to be investigated in order to be able to produce a homogeneous high-activity ^{14}C sucrose standard.

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