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Small sample Accelerator Mass Spectrometry for biomedical applications

M. Salehpour *, K. Håkansson, G. Possnert

Department of Physics and Astronomy, Applied Nuclear Physics Division, Ion Physics, PO Box 516, SE-751 20 Uppsala, Sweden

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ABSTRACT

The Accelerator Mass Spectrometry activities at Uppsala University include a group dedicated to the biomedical applications, involving natural level samples, as well as ^{14}C-labeled substances requiring separate handling and preparation. For most applications sufficient sample amounts are available but many applications are limited to samples sizes in the μg-range. We have developed a preparation procedure for small samples biomedical applications, where a few μg C can be analyzed, albeit with compromised precision. The latest results for the small sample AMS method are shown and some of the biomedical activities at our laboratory are presented.

1. Introduction

Over two decades ago, biological Accelerator Mass Spectrometry (AMS) was introduced by Turteltaub et al. [1]. AMS's exceptional selectivity and sensitivity for radiocarbon is highly relevant in organic environments, where minute amounts of ^{14}C-labeled compounds can be routinely measured in the zeptomole (10^{-21} mol) range [2]. One of the applications of biological AMS is within drug development research. After toxicity tests with animals, ^{14}C-labeled drug candidates are administered directly to humans, albeit at doses lower than one-hundredth of the pharmacological doses, never exceeding 100 μg. The methodology is referred to as microdosing [3] and can provide valuable data on new drug candidates at an early stage of drug development. Human pharmacokinetic data (which is how a drug is absorbed, distributed, metabolized and excreted), bioavailability results (various methods of delivering drugs) and metabolic profiling (break up process of drug molecules) have been interesting enough to start a number of commercial entities around the world to address the need for the pharmacological companies.

Other examples of biological applications that have been reported during the last decade have involved bomb-peak dating of various biological samples, including dating of composite samples such as human plaques [4], teeth [5], fat [6] or highly purified DNA samples of human brain or heart for measuring the regeneration rates of specific cells [7–9].

The compiled atmospheric $^{14}CO_2$ data from the 1950's to the present day is a key factor to facilitate dating the measured samples [10].

AMS of ultra-small samples (USS) in the microgram range has the obvious advantage of addressing a wider variety of samples which might not be available in mg-amounts, which is normally required in standard AMS. We identified a particular need for microgram samples when performing bomb-peak dating of human DNA samples. In these applications, purified DNA from post-mortem human subjects are bomb-peak dated to find an average age of the DNA using the atmospheric $^{14}CO_2$ data [10]. Many sample cell types from specific parts of the brain or the heart provide only about 5–20 μg C from an adult human. As an example, the human hippocampus, a special region in the brain which is partially responsible for memory and spatial orientation gives rise to only about 15 μg C per human. We have been involved with USS AMS for the last 5 years and have developed a sample preparation method to address our specific needs [11,12]. Our method is based on combining the sample preparation methods of Santos et al. [13] and Khosh et al. [14] as well as using the methodology of Hua et al. [15]. The application of this method has already resulted in new novel findings in medicine regarding regeneration in specific parts in the human brain such as the hippocampus [16], striatum [17] and oligodendrocytes which are special cells for axon myelination [18], as well as the effect of stroke on regeneration of new neurons [19]. Here, an update is given on the results for the USS method and also a few other recent biomedical related activities at our laboratory are outlined.

* Corresponding author.
E-mail address: mehran.salehpour@physics.uu.se (M. Salehpour).

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2. Experimental setup

The USS sample preparation method has been reported earlier [11,12] and is therefore only briefly outlined here. The small samples size sample preparation method differs from the standard mg-AMS in a number of ways, some of which are mentioned here. Introduction of minute amounts of background carbon (<1 μg) into a sample may not be detectable in a mg sample but in a μg-sized sample it has a significant impact. The background carbon must consequently be minimized experimentally, which influences more-or-less all the laboratory routines including handling, drying, combustion and graphitization. The laboratory environment must also be controlled, including all the chemicals, glassware, etc. Due to the size of the sample, graphitization will not initiate in standard reactors due to too low CO_2 partial pressure and need to be modified. Our reactors are approximately 0.6 mL in volume. Furthermore, corrections for the introduced carbon background need to be made. Below, some points are noted to differentiate the USS method compared to standard AMS samples preparation.

2.1. Lyophilization

Samples in water solution need to be lyophilized prior to combustion, as drying in air will introduce background carbon.

2.2. Combustion

Samples are introduced into prebaked quartz tubes including pre-baked CuO and are flame-sealed using a high temperature gas torch. The quartz tubes are subsequently placed in a muffle furnace and are baked at 950 °C.

2.3. Gas transfer

Any water in the residual gas is discarded by using a cold trap at −80 °C utilizing a mixture of ethanol and liquid nitrogen. Any residual, non-condensable gases are also pumped away while the CO_2 gas is frozen in a liquid nitrogen trap.

A portion of the gas can be sampled for the stable isotope ratio spectrometer for δ ^{13}C determination. The sample amount is measured using a calibrated volume and a sensitive gauge to measure the CO_2 gas pressure. The graphitization tube is flame sealed while the gas is frozen at liquid nitrogen temperature.

2.4. Graphitization

The graphitization reactor is approximately 0.6 mL in volume and uses zinc reduction chemistry. Unlike Khosh et al. [14] we have reported that TiH_2 is not necessary for the graphitization process [11,12]. The sealed graphitization tube is inserted in a heater block and the graphitization takes place at 550 °C for 6 h.

2.5. Handling and pre-baking

- All glassware (borosilicate) including the graphitization and all quartz tubes used for combustion including the CuO powder must be pre baked under oxygen flow.
- The graphitization reactor, with the zinc and the iron powder must be pre baked as well as all other surfaces e.g., aluminum foils, etc.
- All glassware and chemicals are stored in a CO_2-free environment.

We have been using the Uppsala University 5 MV Pelletron tandem accelerator (NEC Inc. Middleton, WI, USA) installed in 2001 with a choice of two ion sources depending on the amount of radiocarbon in the samples; one is used for natural/archeological samples and the other for labeled samples. Off-line δ ^{13}C-analysis is performed on an IRMS spectrometer on site.

3. Results

Up until the AMS-13 meeting in Aix de Provence in July 2014, we had analyzed 6672 samples which were prepared using the USS method. One important measurable parameter for the sample preparation is the secondary ion current from the sample which determines the precision of the measurements. We have been monitoring this and the latest data is shown in Fig. 1 where the secondary ion current dependence is shown for the low mass region below 200 μg C. It should be noted that no data points have been excluded in the figure even though some samples, for one reason or another may have been deemed as failed samples, e.g. too low current. The dependence of the current on the sample size is apparent, reaching a plateau at around 200 μg C. The data points for the lowest masses around 1 μg C are blank biological samples which are prepared from water solutions in order to determine the amount of background carbon. The mass dependence of the current limits the precision for samples with low masses. For examples, a 10 μg C sample is expected to produce approximately ten times lower current compared to a mg sample and consequently over 3 times lower precision in the range of 1–1.5 Modern. It is noteworthy that outliers, i.e. samples with too low current constitute a very small portion. As a whole the USS method is reliable and the percentage of samples that may not graphitize does not differ from the standard mg-size samples preparation (<<1%).

To determine the isotopic ratio for small samples, ^{14}C/^{12}C denoted as F ^{14}C, background subtraction is important. In the methodology of Santos et al. [13] and Hua et al. [15], two sets of standard samples are loaded for every run, one set being ^{14}C-free samples (e.g. old graphite) and the other set with a higher isotope ratio around 1 Modern. The two methods give similar results. We have been using the Hua method as the background can be

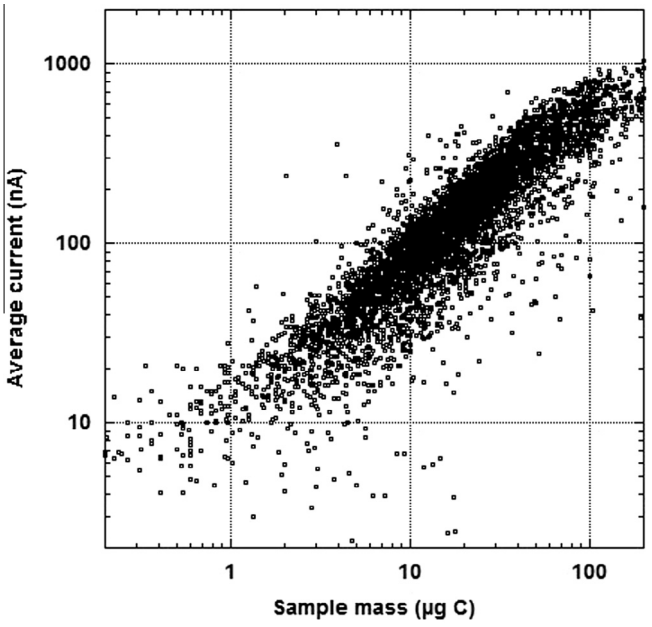

Fig. 1. Measured current of 12 MeV $^{13}C^{3+}$ ions after the high energy magnet in nA as a function of the mass of the samples in μg of carbon for 6672 samples. The plot is shown on a log–log scale.

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