

## Simultaneous AMS determination of $^{14}\text{C}$ content and total carbon mass in biological samples

U. Zoppi\*, A. Arjomand

Accium BioSciences, 550 17th Avenue, Suite 550, Seattle, WA 98122, USA

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### ABSTRACT

Accelerator Mass Spectrometry (AMS) is now recognized as one of the most powerful techniques available for conducting ultrasensitive clinical studies. However, since for biological applications the relevant quantity is the total  $^{14}\text{C}$  activity (i.e. dpm/mL sample), AMS  $^{14}\text{C}$  measurements must be combined with total carbon concentrations measured on a separate instrument using a different sample aliquot. This procedure is inherently a source of large inaccuracies, especially in non-homogeneous samples such as urine and fecal blends. To overcome this limitation we developed a new measurement technique whereby a small amount of  $^{13}\text{C}$ -enriched carbon carrier is added to each sample. Accurate measurement of the  $^{13}\text{C}/^{12}\text{C}$  and  $^{14}\text{C}/^{13}\text{C}$  ratios of the mix can be used to simultaneously calculate total carbon mass and  $^{14}\text{C}$  concentration of the original sample. In this paper we present our first test runs including a detailed error analysis demonstrating that sample mass and  $^{14}\text{C}$  concentration of the original sample can be determined with a precision and accuracy of better than 3%, thus significantly reducing the final uncertainty due to sample in-homogeneities.

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### 1. Introduction

AMS readily quantifies the amount of radiocarbon-labeled compound in a biological sample with attomole ( $10^{-18}\text{ M}$ ) sensitivity [1,2]. This extreme sensitivity allows substantial reduction of the  $^{14}\text{C}$  radiotracer to near ambient levels, virtually eliminating the need to control for radioactive material. Furthermore, the dose of new pharmaceutical agents can be reduced to sub-pharmacologic levels, making these microdosing clinical studies safer than conventional first-in-human studies conducted at pharmacological doses. Existing approaches for the determination of the total  $^{14}\text{C}$  activity in biomedical samples require two separate aliquots, one to be converted to graphite for the AMS instrument and one for the total carbon measurement. In principle the carbon mass could be estimated by means of pressure measurements in the reduction chamber. However, to prepare graphite targets we are currently using the sealed vial technique [3], which is not suitable for this kind of accurate mass determinations. Furthermore, the use of shared fixed volumes reduction chambers and pressure gauges would increase the potential for cross-contamination, especially for biomedical samples.

In a previous study [4] we evaluated the range of carbon concentration in plasma, whole blood preparations, urine and fecal

homogenate collected from the same individual maintaining a consistent diet over a 200 day period. Carbon concentration varied 6% in plasma and 20% in urine and fecal homogenates. In current studies, we have observed inter-individual variations to be approximately 8% in plasma and up to 30% in urine and fecal homogenates. Such variations prevent the use of reference values and force measurement of total carbon in every sample, thereby introducing the additional risk of inconsistent determination of  $^{14}\text{C}$  concentration and total carbon.

All drawbacks connected to double sampling can be eliminated if a known amount of  $^{13}\text{C}$ -enriched carbon carrier is directly added, without the need for careful blending, to the unknowns in their respective combustion vials. By measuring by AMS the  $^{13}\text{C}/^{12}\text{C}$  and  $^{14}\text{C}/^{13}\text{C}$  ratios of the mix it is possible to simultaneously determine the carbon mass and the  $^{14}\text{C}$  concentration of the original sample.

### 2. Theoretical background

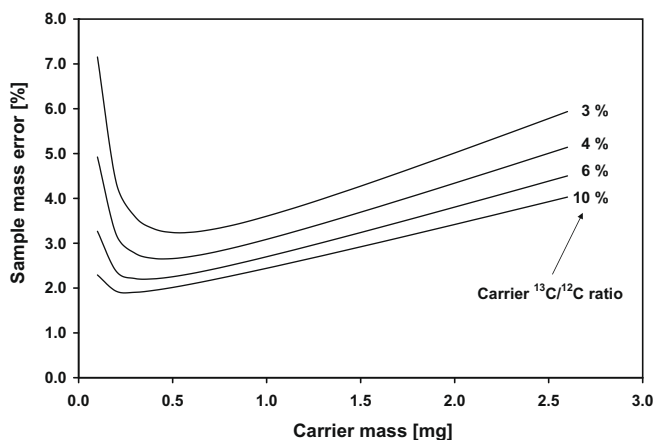
When a carrier (C) is added to a sample (S) producing a mix (M), the number of atoms of each species, not the isotopic ratios, are added. Knowing that the total carbon mass of a substance is

$$M_x = {}^{12}\text{C}_x \cdot 12 + {}^{13}\text{C}_x \cdot 13.00336 \quad (x = \text{C}, \text{S}, \text{M}), \quad (1)$$

it can be demonstrated that the sample mass  $M_S$  is given by

\* Corresponding author. Tel.: +1 206 281 3915.

E-mail address: [uzoppi@acciumbio.com](mailto:uzoppi@acciumbio.com) (U. Zoppi).



**Fig. 1.** Calculated  $1\sigma$  errors that could be achieved in a hypothetical AMS experiment conducted by mixing a 1 mg C sample with different amounts of carrier. Different curves corresponding to carriers with different  $^{13}\text{C}/^{12}\text{C}$  ratios are given. For each curve, the absolute value of the minimum heavily depends on how accurately the carrier mass can be established (1% error has been assumed here).

$$M_S = M_C \cdot \frac{\left(12 + \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_S \cdot 13.00336\right)}{\left(12 + \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_C \cdot 13.00336\right)} \cdot \frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_C - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_M}{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_M - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_S} \quad (2)$$

In addition,

$$\left(\frac{^{14}\text{C}}{^{13}\text{C}}\right)_S = \left(\frac{^{14}\text{C}}{^{13}\text{C}}\right)_M + \frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_C \cdot \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_M - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_S}{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_C - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_M} \cdot \left[ \left(\frac{^{14}\text{C}}{^{13}\text{C}}\right)_M - \left(\frac{^{14}\text{C}}{^{13}\text{C}}\right)_C \right] \quad (3)$$

Please note that all ratios in Eqs. (2) and (3) are absolute ratios, not ratios measured by AMS. The formula for  $(^{14}\text{C}/^{13}\text{C})_S$  is particularly interesting because it does not depend on the amount of carrier used and thus is not affected by its uncertainty. To be able to calculate  $M_S$  and  $(^{14}\text{C}/^{13}\text{C})_S$ , an estimated value for  $(^{13}\text{C}/^{12}\text{C})_S$  is needed. In the case of biological specimens a natural abundance corresponding to  $\delta^{13}\text{C} = -25$  permil can be assumed. The values of  $(^{13}\text{C}/^{12}\text{C})_M$  to be determined by AMS will range from the carrier level for unknowns with very low carbon content down to the natural abundance for larger samples. The  $^{13}\text{C}$  beam intensity is expected to exhibit similar variations introducing potentially significant sample-to-sample changes in space charge effects and terminal loading. Therefore, even though error analysis (see Fig. 1) indicates that  $\sigma(M_S)$  can be reduced by increasing the  $^{13}\text{C}/^{12}\text{C}$  ratio of the carrier, values above 10% are not recommended.

### 3. Proof of principle

To test the accuracy of total carbon mass measurement, 1 g of commercially available glucose (>99%  $^{13}\text{C}$ , molecular weight = 186.1, 41.92% carbon) was mixed with 0.6 g IAEA-C6 sucrose standard (molecular weight = 342.30, 42.11% carbon, 150.6 pMC,  $\delta^{13}\text{C} = -10.8$  permil,  $^{13}\text{C}/^{12}\text{C} = 1.112\%$ ) and dissolved in 6 mL of purified water to produce a carrier with a calculated  $^{13}\text{C}/^{12}\text{C}$  ratio of 6.535% (4.36 pMC,  $\delta^{13}\text{C} = 4815.51$  permil, Carbon content = 44.555 mg C/mL). Furthermore, 30 mg of IAEA-C6 standard material were dissolved in 1 mL of purified water (C concentration of 12.63 mg C/mL) and four different 'unknown' samples were prepared (see Table 1).

**Table 1**

Four 'unknown' samples were prepared by mixing 5  $\mu\text{L}$  of carrier solution ( $^{13}\text{C}/^{12}\text{C} = 6.535\%$ ; carbon content = 44.555 mg C/mL) with different amounts of a IAEA-C6 standard solution (12.63 mg C/mL).

Carrier ( $\mu\text{L}$ )	IAEA-C6 ( $\mu\text{L}$ )	Calculated $^{13}\text{C}/^{12}\text{C}$ (%)
5	25 (0.316 mg C)	3.280
5	50 (0.632 mg C)	2.468
5	75 (0.947 mg C)	2.098
5	100 (1.263 mg C)	1.887

**Table 2**

Comparison between expected and measured total carbon masses and  $^{14}\text{C}$  concentrations for all samples listed in Table 1.

Sample mass (mg C)		$^{14}\text{C}$ concentration (pMC)	
Calculated	Measured	Consensus	Measured
0.316	0.3065 $\pm$ 0.0051 0.3132 $\pm$ 0.0052	152.8	155.6 $\pm$ 1.7 159.7 $\pm$ 1.9
0.632	0.633 $\pm$ 0.010 0.638 $\pm$ 0.010		157.3 $\pm$ 1.2 154.9 $\pm$ 1.1
0.947	0.963 $\pm$ 0.016 0.988 $\pm$ 0.016		149.3 $\pm$ 1.0 147.28 $\pm$ 0.91
1.263	1.265 $\pm$ 0.022 1.313 $\pm$ 0.023		150.9 $\pm$ 1.4 149.73 $\pm$ 0.88

Samples were prepared in duplicate and measured by AMS at Accium BioSciences [3]. Measured ratios were normalized using the procedure described in [5]. Carbon masses and fraction of modern values were then calculated (see Table 2) by applying formulas (2) and (3). Comparison of sample masses measured by AMS with expected values indicates that the largest deviation was about 50  $\mu\text{g}$ , with an average of 16  $\mu\text{g}$ , corresponding to an accuracy of approximately 3%. Values listed in Table 2 further demonstrate that fraction of modern values for the 'unknown' IAEA-C6 samples were also reproduced with 3% accuracy.

### 4. Conclusions

We introduced a novel measurement procedure for the simultaneous determination by AMS of total carbon mass and  $^{14}\text{C}$  concentration in biomedical samples. First test measurements indicate that carbon mass and fraction of modern values can be established with 3% accuracy, thus reducing the final uncertainty due to double sampling of in-homogeneous samples. Further improvement is expected after a more suitable carrier compound is identified. Ideally it should be radiocarbon free, mix well with a range of possible sample matrices producing homogeneous AMS samples and not be easily subject to evaporation, which changes the total carbon concentration.

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