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HIGHLY SENSITIVE ^{14}C AND ^{3}H QUANTIFICATION OF BIOCHEMICAL SAMPLES USING ACCELERATOR MASS SPECTROMETRY

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INTRODUCTION

Accelerator Mass Spectrometry (AMS) is an isotope ratio mass spectrometer that quantifies low levels of rare isotopes with half-lives between 10 and 10^8 years. Typical sensitivities are 10^6 atoms in a milligram-sized sample. AMS was originally developed for use in the geosciences as a tool to carbon date archaeological artifacts, but has applications in many fields. In the biosciences, the extreme sensitivity of AMS is used to trace nutrients, toxins and therapeutics in humans and animals using less than μg/kg doses containing between 1-100 nCi of ^{14}C [1, 2]. This sensitivity is used to reduce sample size, reduce chemical exposures to environmental or physiological levels, reduce radiation exposures to subjects, and/or reduce radioactive (and "mixed") waste. Compared to decay counting, AMS provides for a much higher measurement throughput for low activity samples. For example, a milligram-sized sample containing 1 dpm of ^{14}C can be measured to 3% precision in several seconds. That same sample would require approximately 1 week of decay counting to obtain similar precision.

^{14}C-AMS

Recently, the feasibility of ^{14}C-AMS at lower terminal voltages was demonstrated, resulting in a much smaller system, with its subsequent reduction in cost and increased ease in operation [3, 4]. We recently completed construction of an AMS facility dedicated to the quantification of ^{14}C for biomedical samples [5, 6]. The spectrometer is centered around a 1-MV tandem accelerator and uses a copy of our high current ion source to generate intense ion beams from solid graphite samples [7]. This AMS system is located in a 110-m^2 room and is approximately 20% the size and cost of our large 10 MV FN tandem AMS spectrometer. Typical throughput is 105 ^{14}C samples per 8-hour day at 3-5% precision. Last year, we quantitated ^{14}C in over 7500 samples and we have capacity for approximately 15,000 samples per year. System sensitivity is 0.4 attomol ^{14}C/mg carbon (0.9 μBq ^{14}C/mg C). The dynamic range extends 4-5 orders in magnitude and may be extended through careful isotope dilution. This spectrometer is available through collaborations with our National Center for Research Resources National Resource for Biomedical Accelerator Mass Spectrometry, as well as through a contract service (for details, see: http://www.llnl.gov/bioams/).

Carbonaceous biochemical samples for AMS quantitation are first combusted to CO_2, followed by reduction to graphite. Septa-sealed vials are used, along with commercially available disposable materials, to eliminate sample cross contamination, to minimize complex handling and to keep per sample cost low [8]. Samples containing

between 0.25 and 10 mg total carbon can be reduced to graphite in approximately 4 hours in routine operation. A single technician can prepare approximately 150 samples per 8-hour day. This method has been exported to major collaborators.

^{3}H-AMS

A ^{3}H-AMS capability will have significance in the biomedical research community for two reasons. Firstly, ^{3}H is one of the most widely used radioisotopes in biomedical research, and compounds can be easily labeled through ^{3}H exchange. Often the compound is only available in a ^{3}H-labeled form. Secondly, a ^{3}H-AMS capability can be used in conjunction with ^{14}C-AMS to perform unique low-level dual-labeling experiments (9, 10). Such experiments enable us to study the interaction of two independent, co-administered compounds. Another advantage to dual labeling is that different moieties of a single organic compound can be studied with two separate radio-labels. For example, one radio-label can be used to study the fate and distribution of the compound, whereas the other tracer can be used to examine the effect that the compound has on a specific process, such as DNA synthesis.

When compared to the conventional decay counting techniques, biomedical ^{3}H-AMS delivers at least a 1000-fold improvement in detection sensitivity for assaying mg-sized biological samples for ^{3}H content [9-12]. We have also expanded the spectrometer's capabilities to include the quantification of tritium [13]. System sensitivity of hydrogen isotope ratios for milligram-sized samples was measured at 4 x 10^{-16} ^{3}H/^{1}H (430 μBq ^{3}H/mg H). Solid TiH_2 targets are routinely prepared from biochemical compounds containing 1-10 mg hydrogen [12]. We can quantitate approximately 60 typical biochemical samples per 24-hour day at 10% precision.

DIRECTLY COUPLED QUANTITATION

Analysis systems that are compatible with the direct input of biochemical separation instrumentation such as liquid chromatography, capillary electrophoresis or other instruments would allow real-time and automated sample preparation, leading to increased resolution, minimal handling and the ability to do molecule-specific tracing of small samples. This approach involves the direct introduction of carbon as CO_2 and hydrogen as H_2 into the ion source. This sample form is more efficient for the small samples common to biochemical research and will allow the direct interfacing of separation instrumentation to AMS. The online conversion of a sample to CO_2 gas with the CO_2 fed directly into an ion source for trace isotope analysis is required for ultimate sensitivity, but with a cost in precision and throughput.

We have purchased a gas-accepting ion source from National Electrostatics Corporation (Middleton, WI) and plan to install it onto our 1-MV AMS spectrometer to use in addition to our current ion source. We performed ion–optics calculations and designed a beam line to transport both carbon and hydrogen isotopes, while matching the phase space of the ion beam to the acceptance of the accelerator. We will link nanoLC and CE instrumentation to this ion source through online combustion furnaces to provide directly coupled AMS quantitation of ^{14}C and ^{3}H from biochemically separated compunds. This source will also accept solid samples and we will quantify ^{3}H/^{1}H isotope ratios from solid TiH_2 targets without employing isotope switching, thereby increasing our measurement thoughput for this isotope.

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