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Tritium AMS for Biomedical Applications

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Abstract

We are developing ^3H -AMS to measure ^3H activity of mg-sized biological samples. LLNL has already successfully applied ^{14}C AMS to a variety of problems in the area of biomedical research. Development of ^3H AMS would greatly complement these studies. The ability to perform ^3H AMS measurements at sensitivities equivalent to those obtained for ^{14}C will allow us to perform experiments using compounds that are not readily available in ^{14}C -tagged form. A ^3H capability would also allow us to perform unique double-labeling experiments in which we learn the fate, distribution, and metabolism of separate fractions of biological compounds.

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Introduction

We are developing ^3H AMS to measure ^3H activity of mg sized biological samples. Presently, most biological samples are assayed for ^3H content using decay counting techniques. While the technique can be used on small samples, ^3H decay counting lacks the sensitivity and specificity needed for very low-specific activity materials. ^3He in-growth techniques for the measurement of ^3H at low concentrations has the sensitivity and specificity required but needs relatively large samples. When compared to decay counting techniques, ^3H AMS promises a 100- to 1000-fold improvement in detection sensitivity for assaying mg sized biological samples.

Motivation

Motivation to develop ^3H AMS comes from our results in applying ^{14}C AMS to biomedical and environmental tracer research. As has been reported^[1,2], LLNL has used ^{14}C AMS for research in carcinogenesis, mutagenesis, elemental metabolism, immunoassays, dermal transport, and general pharmacokinetics. Application of AMS to the measurement of ^{14}C -tagged compounds has created great interest in the international cancer and toxicological research community. Development of a ^3H AMS capability is expected to have similar impact for two significant reasons. First, ^3H is the most widely used radioisotope in biomedical research. The number of commercially available ^3H -tagged compounds far exceeds the number of commercially available ^{14}C -tagged compounds. ^3H AMS measurement capability will make possible experiments using compounds that are simply unavailable in ^{14}C -tagged form. Second, a ^3H AMS capability, when used in conjunction with our present ^{14}C AMS capability, will also allow us to perform double-labeling experiments in which we concurrently learn the fate, distribution, and metabolism of separate compounds. This would allow study of combined or synergistic effects of combinations of different chemicals.

Current Status

The status of ^3H AMS can be sub-divided into two different areas: 1) spectrometer performance and 2) efforts to prepare samples. Current spectrometer performance, as demonstrated in Figure 1, is more than

adequate for preliminary ^3H biomedical work. $^3\text{H}:^1\text{H}$ ratios as high as 10^{-9}

Figure 1. Measured $^3\text{H}:^1\text{H}$ ratios from a sequentially diluted water sample compared to ratios determined from decay counting. The dashed line represents our current sensitivity limit.

and as low as 10^{-14} have been measured from a sequentially diluted water sample. Measured ratios agreed with ratios determined from decay counting to accuracy's approaching 10%.

Our efforts to develop a ^3H AMS sample preparation technique for complex biological samples have met with mixed success. As has been done for ^{14}C biomedical AMS[3], it is necessary with ^3H AMS to develop a one- or two-step technique in which organic samples are reduced into a form suitable for the ion source. Our initial attempt at sample preparation was to directly reduce samples on titanium powder at 850°C in a simple single tube system. Although this technique worked well enough to produce the data shown in Figure 1, this method often produced samples with ^1H ion currents that were less than 10% of ^1H ion currents obtained from commercially available titanium hydride. In addition, samples produced by this method sometimes showed poor sample-to-sample $^3\text{H}:^1\text{H}$ reproducibility. Reproducibility was especially severe for biological samples where it was approximately $\pm 50\%$.

Although our ion currents were low and sample reproducibility was poor, we have conducted a single test experiment. In this experiment we fed a single laboratory mouse a known quantity of ^3H -tagged 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx). MeIQx is a known carcinogen found in cooked meat^[4]. After approximately one hour, the animal was sacrificed and aliquots of its blood, liver, kidneys, and lungs were analyzed for ^3H content using both AMS and decay counting techniques. Results of that comparison are shown in Figure 2. As can be seen, the agreement between ^3H

Figure 2. ^3H : ^1H ratios of various mouse tissues obtained from ^3H AMS compared to ratios determined from decay counting. See text for details.

AMS and ^3H decay counting on biological samples leaves something to be desired. We are currently trying to determine the source of this disagreement.

Recently we have begun to investigate an alternative sample preparation technique. In this new method (which we have only tried on water), water samples are reduced on zinc and the resulting hydrogen gas is

adsorbed into titanium. To prepare samples using this technique, a 9 mm (OD) Vycor tube is loaded with 20-25 mg of titanium powder and a second, smaller 6 mm (OD) culture tube containing 50 mg of Zinc powder. The Vycor tube is then purged with nitrogen and loaded with a 5 micro-liter pipette filled with the water sample of interest. The tube is then temporarily sealed with a rubber septum, placed in a liquid nitrogen bath to freeze the water, evacuated (using a needle stuck through the rubber septum), and flame sealed. After flame sealing, the tube is placed in a muffle furnace at 550°C for 9 hours, cooled to 450°C over a period of 4 hours, kept at 450°C for 8 hours, and then quenched by turning off the furnace and opening the door. Water samples prepared by this method have given ^1H ion currents nearly equal to ^1H ion currents from commercially available titanium hydride. Samples produced by this new method have also shown better reproducibility than our previous method.

Future Plans

While our $^3\text{H}:^1\text{H}$ sensitivity is adequate for pilot biomedical ^3H experiments, our present spectrometer is far from ideal. In the present configuration, ^1H ion currents and ^3H counts are sequentially measured by a very slow process that involves inserting and withdrawing a low energy faraday cup while making large changes in either the magnetic field or bounce voltage of the low energy spectrometer. This process is time consuming and can result in large systematic errors in the measured data. For samples that have $^3\text{H}:^1\text{H}$ ratios of between $10^{-13}:1$ and $10^{-9}:1$, it can take as long as 15 minutes to obtain a single measurement to a non-statistically limited accuracy of 15%. In the next six months we plan to modify the vacuum tank of the low energy injection magnet so that we can add an off-axis faraday cup. As the change will allow us to quickly switch between measurement of ^1H ion currents and injection of ^3H ions into the accelerator, this modification should significantly reduce measurement time and systematic errors.

Next, we need to continue development of sample preparation methods. Development a one- or two-step technique in which ^3H samples are reduced to a form suitable for the ion source has proved to be a very difficult coupled organic/inorganic chemistry problem. As discussed above,

one of the techniques that we have tried works poorly while another shows promise. This latter technique, however, has yet to be tried on biological samples. To make use of this method, we are considering using cupric oxide to combust our biological samples into water and other gases, and then transferring the water into our reduction stage.

Finally, while our initial success with the application of AMS to the measurement of ^{14}C -tagged compounds has created great interest in the international cancer and toxicological research community, expanded use of ^3H and ^{14}C AMS in biomedical research relies on the development of small and relatively inexpensive instrumentation to provide routine AMS measurement capability. As the difference between radiologically significant concentrations of an isotope and the natural background concentration of that isotope can be several orders of magnitude, biomedical research spectrometers may be simpler and optimized differently than from those designed to measure radioisotopes at or below natural concentrations. We are currently studying several different ^3H and/or ^{14}C spectrometer schemes that would easily fall within the financial, resource, and space limits of a typical hospital or research institution.[5,6]

Conclusion

We believe the successful development and demonstration of ^3H AMS will have a major impact on biomedical research. Such technology is critically important for evaluating effects of exposure to environmental levels of toxic agents and for monitoring effects of pharmaceuticals at low doses.

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