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AND THE APPLICATION TO ENVIRONMENTAL AND BIOLOGICAL STUDIES

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DETECTION OF THE STABLE ISOTOPES OF CARBON USING A 3-MV VAN DE GRAAFF AND THE APPLICATION TO ENVIRONMENTAL AND BIOLOGICAL STUDIES*

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Summary

The proton radiative capture reactions, $^{12}\text{C}(p,\gamma)^{13}\text{N}$ and $^{13}\text{C}(p,\gamma)^{14}\text{N}$, have been studied to determine their applicability for assaying materials for their ^{13}C enrichment. This technique for determining carbon isotopic ratios compares favorably with the more classical analysis techniques for ^{13}C tracer experiments.

Introduction

The usefulness of carbon tracer techniques is well established in biomedical and ecological studies as a means of understanding the metabolism of natural substances in living organisms. To date, radioactive ^{14}C has been the most extensively used carbon tracer because of its easily detected 156 keV beta particle. However, because of its long half-life (5730 years), ^{14}C can be a radiological hazard for humans and other animals. Furthermore, its use in plants and animals is often open to question regarding the influence of its radiation on the very processes being studied. For example, it would be impractical to use a radioactive tracer, such as ^{14}C , to study the potential effects of radioactive wastes released from a nuclear power plant on the local ecological system. With the increasing concern about exposure to radioactivity even at low levels, the use of non-radioactive isotopes is clearly advantageous in environmental studies and clinical medicine. The increasing availability of the rare, stable isotope of carbon, ^{13}C , suggests as a logical alternative the use of ^{13}C if suitable detection methods can be found. The radiative capture reactions, $^{12}\text{C}(p,\gamma)^{13}\text{N}$ and $^{13}\text{C}(p,\gamma)^{14}\text{N}$, have been studied to determine their applicability for assaying materials for their ^{13}C enrichment.

Background

There are several resonance parameters that characterize a radiative capture reaction. Some of these parameters are listed in Table I for the reactions pertinent to this investigation. The strength of the resonance, $\omega\gamma$, is directly related to the maximum cross section, σ_R , which occurs at the resonance energy (second column). By inspecting the values of σ_R , it is possible to deduce the relative abundance of gamma rays from different reactions. It is seen that the ^{12}C resonance at $E_p = 457$ keV is an order of magnitude weaker than the ^{13}C resonance at $E_p = 551$ keV. Despite this difference in relative abundance of gamma rays from the $^{12}\text{C}(p,\gamma)^{13}\text{N}$ and the $^{13}\text{C}(p,\gamma)^{14}\text{N}$ reactions, these two reactions are very convenient for determining the carbon isotopic ratio in matter by using thick targets and 600 keV protons so that both resonances are populated to saturation.³

To experimentally determine the ^{13}C enrichment, the ratio, R , of the yield from the resonance transition from the $^{12}\text{C}(p,\gamma)$ reaction to the yield from the resonance transition from the $^{13}\text{C}(p,\gamma)$ reaction is measured. The theory of resonance reactions⁴ allows one to calculate this yield ratio, R . We have shown⁵ that the yield ratio, R , is a function of the reciprocal of the actual ^{13}C enrichment, e , and that it can be expressed in the form of a straight line as

$$R = \frac{1}{e} a + b \quad (1)$$

By measuring the yield ratio for targets having a known ^{13}C enrichment and performing a weighted least squares fit to the data, a calibration curve is generated, from

TABLE I

Resonance Parameters Characterizing $^{12,13}\text{C}(p,\gamma)^{13,14}\text{N}$,
 $^{14}\text{N}(p,\gamma)^{15}\text{O}$, $^{15}\text{N}(p,\alpha,\gamma)^{12}\text{C}$

Reaction	Proton Energy (keV)	Γ_{lab} (keV)	$\omega\gamma$ (eV)	σ_R (mb)	Excitation Energy (MeV)	Prominent γ Rays (MeV)
$^{12}\text{C}(p,\gamma)^{13}\text{N}^a$	456.8 ± 0.5	39.5 ± 1.0	0.67	0.113	2.366	2.366
$^{13}\text{C}(p,\gamma)^{14}\text{N}^a$	551 ± 1	32.5 ± 1	9.2	1.54	8.061	8.061, 4.116, 3.378 2.370, 2.313, 1.632
$^{14}\text{N}(p,\gamma)^{15}\text{O}^a$	1061.6 ± 1.4	3.9 ± 0.7	0.95	0.681	8.2833	8.283, 5.242, 3.042
$^{15}\text{N}(p,\alpha,\gamma)^{12}\text{C}^b$	429 ± 1	0.9	25	191	12.582	4.439
	897.37 ± 0.29	2.0 ± 0.2	502	824	12.9668	4.439

^aRef. 1.

^bRef. 2.

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which the ^{13}C enrichment of a sample can be extracted.

Experimental Procedure

The experimental arrangement used for these measurements is shown in Fig. 1. Protons from the Los Alamos Scientific Laboratory 3.75 MV Van de Graaff were used to bombard the samples. The four sets of tantalum beam defining slits were used to limit the dimensions of the proton beam to those of the sample. A liquid nitrogen cold trap was placed in front of the target to remove organic vapors. A large volume, high resolution Ge(Li) detector viewed the targets; the absorber (1/8 in. Pb) served to attenuate the high flux of low energy gamma rays and x rays which would have swamped the detector. A stream of water was directed at the back of each target to prevent it from being excessively heated by the proton beam. A PDP-9/L computer was used to control the collection of the data and its analysis.

Fig. 1. Equipment schematic for charged particle activation analysis.

Results

A typical Ge(Li) spectrum is shown in Fig. 2 for a natural abundance carbon target. The signature at 8.061 MeV from the $^{13}\text{C}(p,\gamma)^{14}\text{N}$ reaction, the resonance to ground state transition, is clearly seen. The three peaks (full energy, single and double escape peaks) associated with the 8 MeV line are due to the characteristics of the Ge(Li) detector. Also, the very predominant 2.366 MeV gamma ray from the $^{13}\text{C}(p,\gamma)^{13}\text{N}$ reaction is clearly seen.

Fig. 2. Typical Ge(Li) spectrum for 600 keV protons incident on an infinitely thick, natural carbon target.

A spectrum from an actual biological specimen, enriched kidney tissue, is shown in Fig. 3. Again, the signature of the $^{13}\text{C}(p,\gamma)^{14}\text{N}$ reaction is very clear. However, extracting the yield of the $^{13}\text{C}(p,\gamma)^{13}\text{N}$ reaction is a definite problem for higher ^{13}C enrichments. The yield from the 2.366 MeV gamma ray cannot easily be separated from the yield from two interfering gamma rays from the $^{13}\text{C}(p,\gamma)^{14}\text{N}$ reaction. For higher ^{13}C enrichments, the 2.370 MeV gamma ray and the 2.356 MeV double escape gamma ray, having a full energy 3.378 MeV, cause a serious interference problem with the 2.366 MeV gamma ray. The effects of these additional gamma rays must be taken into account to obtain an accurate assay. These two figures represent extremes in the ^{13}C enrichment. There is no problem in determining the $^{13}\text{C}(p,\gamma)$ response. For low ^{13}C enrichments, there is no problem in determining the $^{13}\text{C}(p,\gamma)$ response; for higher enrichments, ^{13}C enrichments $>3\%$, one must realize the complications and calibrate for them.

Fig. 3. Ge(Li) spectrum for 600 keV protons incident on an infinitely thick, enriched mouse kidney tissue showing the carbon response.

Table II is a list of samples that were assayed and their experimentally determined ^{13}C enrichments. The quoted uncertainties are due to statistical errors only. This list indicates the wide variety of biological samples which can be assayed using charged particle activation analysis. The assay for embryonic protein has an unusually large error, 35%. This is because the protein was in a sodium hydroxide solution, and there was severe interference from the $^{23}\text{Na}(p,\gamma)$ reaction with the $^{13}\text{C}(p,\gamma)$ resonance transition region (8 MeV).

TABLE II

Results of Assay Measurements

Compound	^{13}C Enrichment (%)
Depleted $\text{KC}_2\text{H}_3\text{O}_2$	0.14 $\pm$ 0.05
Natural yeast	1.06 $\pm$ 0.14
Enriched yeast	81.6 $\pm$ 11.6
Embryonic protein	3.36 $\pm$ 1.24
Embryonic lipid	17.5 $\pm$ 2.5
Natural heart tissue	1.07 $\pm$ 0.15
Enriched heart tissue	60.9 $\pm$ 8.2
Enriched kidney tissue	58.5 $\pm$ 7.2
Enriched liver tissue	65.2 $\pm$ 9.6
Enriched muscle tissue	59.1 $\pm$ 7.6

A comparison between the radiative capture technique and mass spectrometry is presented in Table III. These samples were all embryonic partial fractions obtained from pregnant mice to determine how carbon compounds are transmitted between mother and the embryo. The agreement between the two methods is very good for the three protein samples. However, the agreement for the MPS (polysaccharide) samples is not good. There is some question about the homogeneity of the original sample before it was split into two smaller samples.

TABLE III

Comparison Between the Radiative Capture Technique and Mass Spectrometry

Sample	¹³ C Enrichment (%)	
	Radiative Capture	Mass Spectrometry ^a
Protein-8	2.1 ± 1.7	2.0
Protein-24	4.2 ± 1.9	2.3
Protein-48	4.8 ± 1.9	4.0
MPS-8	6.2 ± 3.1	1.8
MPS-48	1.1 ± 0.3	4.3

^aRef. 6.

Discussion

This technique compares favorably with mass spectrometry and nuclear magnetic resonance (NMR).^{5,7} All three techniques require several hundred microgram samples. Mass spectrometry and NMR require about one day for an analysis while the radiative capture technique requires only about two hours. Mass spectrometry and NMR work best for ¹³C enrichments in the range of 1.1% (natural) to approximately 50%. The radiative capture technique can analyze samples whose ¹³C enrichments range from approximately 0.2% to 90%. A distinct disadvantage with mass spectrometry is that it is a destructive technique, requiring unavoidable and tedious sample preparation steps, since the sample must be converted into a gas. Furthermore, in the combustion process, there is always the question as to how truly representative the gas is of the original sample.

The nondestructive proton radiative capture technique is not limited to solid specimens. The embryonic lipid and embryonic protein were originally solutions which were evaporated to dryness. The use of a gas cell allows the analysis of gaseous samples, such as breath. Because this technique is nondestructive, a three-dimensional analysis of solid specimens is possible. The sample can be scanned in the two directions perpendicular to the beam axis by translating the sample; the sample can be scanned in the direction parallel to the beam axis by varying the incident proton beam energy. This allows the spatial distribution of ¹³C throughout the sample to be studied.

A potential use of this technique could be the study of metabolic disorders, such as diabetes.⁸ A diabetic suffers impairment of the carbohydrate metabolism process which leads to an increase in blood sugar levels and to an abnormally slow appearance of CO₂ in the exhaled breath originating from the ingested carbohydrates. To detect diabetes, then, a patient would be fed glucose labeled with ¹³C, followed by a collection of a few breath samples or blood samples, the samples then analyzed using the radiative capture technique.

There are also environmental uses of ¹³C labeled

compounds. For example, the merits of the application of a particular pesticide over a large area could be determined by tracing the distribution of the pesticide through the biosphere. Another use would stem from a legislative requirement that potential polluters label their raw materials in some uniquely identifiable way.⁸

The distillation plant at the Los Alamos Scientific Laboratory also has the capability of producing carbon compounds depleted in ¹³C (1 ppm ¹³C). Plants grown in an atmosphere of this depleted carbon compound can be transplanted into a natural environmental system. The rate at which these plants then assimilate the ¹³C from the environmentally occurring natural carbon compounds (1.1% ¹³C, 98.8% ¹²C) could be studied by observing how the ¹³C - ¹²C ratio approaches the natural value.

Perhaps other stable isotopes would have more environmental uses, notably those of nitrogen, ¹⁴N and ¹⁵N. From Table I, it is seen that the resonance at E_p = 1062 keV in ¹⁴N(p,γ)¹⁵O and the resonance at E_p = 897 keV in ¹⁵N(p,α,γ)¹²C are suitable for an isotopic nitrogen analysis similar to the isotopic carbon analysis. The 429 keV resonance in ¹⁵N(p,α,γ)¹²C is too weak to cause any interference. Figure 4 shows a typical nitrogen spectrum for 1100 keV protons incident on a thick, natural urea target. It is noticed that the peaks forming the signature from both the ¹⁴N(p,γ)¹⁵O reaction and the ¹⁵N(p,α,γ)¹²C reaction are well defined and free from interference, thus making them quite acceptable for yield comparisons. These stable isotopes would be important in tracing nitrogen fertilizers in agricultural studies.⁹

Fig. 4. Typical Ge(Li) spectrum for 1100 keV protons incident on a thick, natural urea target showing the nitrogen response.

The many potential uses of compounds labeled with stable isotopes require that a quick analysis procedure must be perfected that can accommodate samples in many different forms. Our results show that the nondestructive technique presented here is capable of meeting many of these requirements.

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