CALIBRATION METHOD IN ISOTOPIC CARBON ANALYSIS*

Zhang Weicheng (张维成) and Li Liwu (李立武)

(Institute of Modern Physics, Academia Sinica, Lanzhou 730000, China)

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ABSTRACT

A very simple basic equation for the comparator technique is derived for the determination of $^{13}C/^{12}C$ ratio in biological samples by proton induced gamma- ray emission. On the basis of this treatments a new series of experiments has been made at a 2×1.7 tandem accelerator that proved conclusion about the method.

Keywords: PIGE $^{13}C/^{12}C$ ratio   Biological samples   Computer calculation Isotope measurement   Stopping power

1 INTRODUCTION

Stable isotopes have been more extensively used as tracers in biology and medicine. Carbon tracer technique is well established in biomedical and ecological studies for understanding the pharmacological metabolism of natural and synthetic substances in living organisms. CO_2- breath tests with ^{13}C labeled compounds have been useful or potentially useful in the diagnosis of several serious diseases[1- 4]. Mass- spectrometry, neutron activation analysis and nuclear magnetic resonance have been used for determination of stable isotope ratios and abundance for tracer experiments. It was recently shown[5,6], however, that proton induced gamma- ray emission (PIGE) provides a reliable alternative method for biological samples. Ease and speed are important advantages of this technique over mass- spectrometry and nuclear magnetic resonance.

The main difficulty of PIGE method is, perhaps, due to energy degradation of the protons travelling in a target, the nuclear- reaction cross section varying with depths. The reaction yield is a function of the depth and should be integrated over the range of the particles. In our PIGE experiments to determine ^{13}C in breath test samples interesting regularities were found and theoretical treatment of the problem was drastically simplified. The purpose of this paper is to derive the equations that govern this method of analysis from first principles, and arrive finally at a simple expression based on the observed regularities.

2   STOPPING POWER

As charged particles readily lose their energy in matter, the nuclear reaction cross section varies with depth. In consequence, the variations of stopping powers (between samples and standards) must be taken into account to avoid systematic errors. The stopping powers (S) may be calculated by the basic principles[7,8], and can be obtained by Bragg's rule for a target consisting of several elements.

$$S = \Sigma f_i S_i \qquad (1)$$

Where S_i is the stopping power of an element and f_i is its weight fraction in the mixture or compound. The proton stopping powers in graphite and some organic compounds were calculated and plotted in Fig.1. The parameters of the nuclear reactions used in determination of carbon isotopic ratio are given in Table 1, and stopping powers for these resonance energies are listed in Table 2. From these data one may find that, firstly, stopping powers of the organic substances at the proton energies of interest are very high (340 to 480 MeV/(g/cm^2)) and the depths at which the resonance occur are close together to each other. Secondly, these substances are chemically quit heterogeneous and their stopping powers differ by only 29% at the most for the proton energies considered. Thus, the stopping powers of biological samples (essentially formed by complex combinations of these analogous substances) may be assumed to have similar characteristics and values. Thirdly, the curves appear to be remarkably parallel in semilogarithmic scale, i.e., the ratio of stopping powers for any two organic substances seems to approach a constant throughout this range. This is especially true within the narrow energy spans of the resonances.

Fig.1   Proton stopping powers for several
organic substances
a) Cholsterol, Palmitic acid
b) Alanine, Glutamic acid, Benzamide
c) Starch, Histidine, Urea
d) Cystine, Graphite

To prove the last point, stopping power ratios S'/S of standards to organic samples were calculated. An example of these calculations is given in Table 3 with benzamide as the standard. Calculations with urea and graphite standards give similar results. From all these data it is interesting to find that, as expected from Fig.1, S'/S
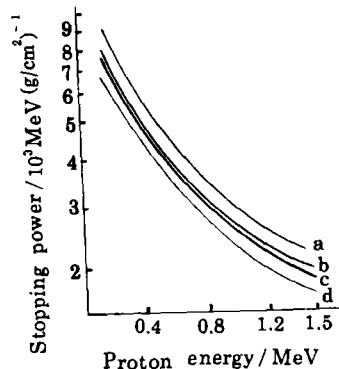
is very nearly a constant (depending only on the substances involved) for all two resonance energies relevant to 0.6 MeV proton bombardments. For urea and

Table 1

Main characteristics of nuclear reactions suitable for selective determination of ^{12}C and ^{13}C

Nuclide considered	Nuclear reaction used	Resonance energy/ keV	Corresponding half–width/ keV	Maximum cross–section/ mb	Energy of prompt γ –rays/ MeV
^{12}C	$^{12}C(p,\gamma)^{13}N$	456.8 ± 0.5	39.5 ± 1	0.127	2.366
		1698 ± 5	72 ± 9	0.035	3.059
^{13}C	$^{13}C(p,\gamma)^{14}N$	554 ± 2	32.5 ± 1	1.44	8.061, 4.116, 3.378, 2.370, 2.313, 1.632
		1747.6 ± 0.9	0.075 ± 0.050	340	9.172 7.028 6.444 2.928 2.144

Table 2

**Stopping powers of several substances at resonances
for proton reactions with ^{12}C and ^{13}C**　　　　$MeV/(g/cm^2)$

Substance	Basic chemical formula	^{12}C(457 keV)	^{13}C (554 keV)
Carbohydrate: Starch	$(CH_2O)x$	419.8	370.4
Lipids: Cholesterol	$C_{27}H_{46}O$	478.6	420.5
Palmitic acid	$C_{16}H_{32}O_2$	481.3	422.8
Amino acids: Alanine	$C_3H_7O_2N$	435.8	383.8
Glutamic acid	$C_5H_9O_4N$	418.5	369.1
Histidine	$C_6H_9O_2N_3$	423.1	372.7
Cystine	$C_6H_{12}O_4N_2S_2$	386.9	341.1
Standards: Graphite	C	377.7	334.6
Urea	CH_4ON_2	430.0	378.2
Benzamide	C_7H_7ON	424.1	373.9
Barium carbonate	$BaCO_3$	204.0	182.7

Table 3

**Stopping power ratios between benzamide (S') and other organic
substances (S) at resonance energies**　　　　%

Substance	Ratios S'/S		Maximum deviation* /
	^{12}C (457 keV)	^{13}C (554 keV)	%
Starch	1.0102	1.0094	0.04
Cholesterol	0.8861	0.8892	0.18
Palmitic acid	0.8812	0.8843	0.18
Alanine	0.9732	0.9742	0.05
Glutamic acid	1.0134	1.0130	0.02
Histidine	1.0024	1.0032	0.04
Cystine	1.0961	1.0962	0.01
Craphite	1.1228	1.1175	0.23
Urea	0.9863	0.9886	0.21

* Maximum percent deviation from average of data for two resonance

benzamide the ratio varies within 0.4 %. The validity of this conclusion may well be extended to impinging protons of up to 1 MeV, for the same standards, since the largest variation of the constant is still comparable to the normal counting statistics. Moreover, despite the structural differences between graphite and the substances under investigation, the S'/S variation is also remarkably small, less than 0.6 % for 0.6 MeV proton bombardments.

It is reasonable to assume that these conclusions hold for dry biological samples. Thus, for an adequate standard the above conclusions may be expressed by

$$(S'/S)_{457 \text{ keV}} = (S'/S)_{554 \text{ keV}} = b \qquad (2)$$

where S and S' are the stopping powers (at the indicated resonance energies) of a biological sample (dry) and of an adequate standard, respectively, and b is the constant that depends only on the nature of the two substances. In addition, because these resonances have narrow energy spans, as is typically the case, it may be further assumed that Eq.(2) is valid throughout each of these spans; that is

$$(S'/S)_E = b \qquad \text{for} \qquad E_i - \varepsilon_i \leqslant E \leqslant E_i + \varepsilon_i \qquad (3)$$

where i characterizes any one of the resonances of Eq.(2) and $2\varepsilon_i$ its corresponding span, somewhat larger than 2Γ.

3 BASIC EQUATION TO DETERMINE CARBON ISOTOPIC RATIO AND ABUNDANCE

When a thick target is bombarded with charged particles, the prompt gamma ray count rate resulting from a given nuclear reaction can be expressed by

$$Y = (aceN_0 I/M) \int_{E_0 - \varepsilon}^{E_0 + \varepsilon} [\sigma(E)/S(E)] \, dE \qquad (4)$$

where a is isotopic abundance of the nuclide to be determined, M the corresponding atomic weight, c its concentration in the target, I the beam intensity, N_0 Avogadro's number, and e the counting efficiency. The reaction cross section $\sigma(E)$ or rather the ratio $\sigma(E)/S(E)$, is integrated over the 2ε span of the resonance.

When nuclear reactions are induced simultaneously in two different nuclides in the same target, each of these processes is governed by an expression analogous to Eq.(4). For example, $Y(13)$ and $Y(12)$ express the yields of the reactions $^{13}C(p, \gamma)^{14}N$ and $^{12}C(p, \gamma)^{13}N$, respectively. Taking the ratio R of the two corresponding experimental yields gives

$$R = Y(13)/Y(12) = (a_{13} c_{13} e_{13} M_{12} / a_{12} c_{12} e_{12} M_{13}) \times$$

$$\int_{E_{13} - \varepsilon_{13}}^{E_{13} + \varepsilon_{13}} [\sigma_{13}(E)/S] \, dE \bigg/ \int_{E_{12} - \varepsilon_{12}}^{E_{12} + \varepsilon_{12}} [\sigma_{12}(E)/S] \, dE \qquad (5)$$

where subindexes 13 and 12 correspond to each of the two reactions.

Eq.(5) is essentially based on absolute parameters. It is usually much convenient to do the calculations with the comparator method in which a standard (comparator) that is known in the isotopic abundances and the elemental concentrations, as far as the samples under investigation concerned, is bombarded for comparison in the same conditions. The ratio R' of the yield for the two reactions occurring in this standard would then be

$$R' = Y'(13)/Y'(12) = (a'_{13}c'_{13}e_{13}M_{12}/a'_{12}c'_{12}e_{12}M_{13}) \times$$
$$\int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}} [\sigma_{13}(E)/S']dE / \int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}} [\sigma_{12}(E)/S']dE \tag{6}$$

where parameters of standard are primed, particularly S', since the standard is generally a different substance from the sample. Clearly, counting efficiencies, atomic weights and excitation functions are the same as in Eq.(5), and one derives

$$R/R' = [Y(13)/Y(12)]/[Y'(13)/Y'(12)] = [(a_{13}/a_{12})(C_{13}/C_{12})]/[(a'_{13}/a'_{12})(C'_{13}/C'_{12})]$$
$$\times \{ \int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}} [\sigma_{13}(E)/S]dE \times \int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}} [\sigma_{12}(E)/S']dE\} / \tag{7}$$
$$\{ \int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}} [\sigma_{13}(\dot{E})/S']dE \times \int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}} [\sigma_{12}(E)/S]dE\}$$

Considering that the resonance energies E_{13} and E_{12} of Eq.(7) are among those for which Eq.(2, 3) are valid, the value $bS = S'$ can be substituted in Eq (7), i.e.

$$R/R' = [(a_{13}/a_{12})(C_{13}/C_{12})]/[(a'_{13}/a'_{12})(C'_{13}/C'_{12})] \times \{ \int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}} [\sigma_{13}(E)/S]dE$$
$$\times \int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}} [\sigma_{12}(E)/bS]dE\} / \{ \int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}} [\sigma_{13}(E)/bS]dE$$
$$\times \int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}} [\sigma_{12}(E)/S]dE\} = [(a_{13}/a_{12})(c_{13}/c_{12})]/[(a'_{13}/a'_{12})(c'_{13}/c'_{12})] \tag{8}$$

It is useful to define the dimensionless ratios

$$F = a_{13}/a_{12} \qquad F' = a'_{13}/a'_{12} \qquad C = c_{13}/c_{12} \qquad C' = c'_{13}/c'_{12} \tag{9}$$

then
$$R/R' = (F/F') \cdot (C/C') \tag{10}$$

As only one element, carbon, is involved in carbon tracer experiments, it is apparent that $c_{13} = c_{12}$ and $c'_{13} = c'_{12}$, therefore $C = C' = 1$, even if the carbon concentration of the sample is different from that of the standard (i.e., $c_{13} \neq c'_{13}$ or $c_{12} \neq c'_{12}$. And one immediately obtains from Eq.(10)

$$[\text{Ratio } ^{13}C/^{12}C] = a(^{13}C)/a(^{12}C) = F = (F'/R')R \tag{11}$$

As $a(^{12}C) + a(^{13}C) = 1$, the percentage abundances $[^{12}C \%]$ and $[^{13}C \%]$ (in isotope nuclei per 100 carbon element nuclei) may be rapidly derived from Eq.(11):

$$F + 1 = [a(^{13}C) + a(^{12}C)]/a(^{12}C) = 1/a(^{12}C)$$

i.e.
$$a(^{12}C) = 1/(F+1) \tag{12}$$

and
$$a(^{13}C) = 1 - a(^{12}C) = F/(F+1) \tag{13}$$

From Eq. (12, 13) one obtains:

$$[^{12}C \%] = 100/(F+1); \quad [^{13}C \%] = 100F/(F+1) \quad \quad (14)$$

4 EXPERIMENTAL TEST

A successful application of Eq.(10, 14) to the determination of ^{12}C and ^{13}C isotopic abundances and ratios in breath test samples is described in detail elsewhere[9]. Some of the results are summarized as follows. Table 4 lists the isotope ^{13}C abundances determined from urea, glucose, benzamide and human breath samples and barium carbonate. The yield ratios $R = Y(^{13}C)/Y(^{12}C)$ were measured and compared with R' of several standards. Eq.(11, 14) were used in the calculations. The $^{13}C/^{12}C$ ratio of all the standards was the natural abundance ratio $F' = 1.108/98.892 = 0.011204$. The accuracy of the determinations is given by the deviations between the average ^{13}C abundance and the natural value (1.11 %) expected for the samples. The precision values are independent of the nature of the standards. It is clear from Table 4 that the overall

Table 4

Accuracy of ^{13}C abundance determinations   %

Sample	Standard	Average ^{13}C abundance	Error
Urea	Graphite	1.11 ± 0.06	0
Glucose	Graphite	1.18 ± 0.22	6.3
Benzamide	Graphite	1.08 ± 0.21	-2.7
Human breath samples	Graphite	1.105 ± 0.09	-0.5
Barium carbonate	Graphite	1.09 ± 0.20	-1.8

accuracy of this method is quite compatible with the statistical variation of the results. We believe that the method described in this paper can be of much help to relevant studies.

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