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可活化碳-13示踪技术

SUITABLE ACTIVATED CARBON-13 TRACER TECHNIQUES

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摘　要

可活化示踪技术是综合两种新技术的优点而发展起来的高新技术。文章报道了建立这种技术的可能性、可行性及其在医学中的应用。对低能质子引起瞬发γ射线的测量是其出发点，采用不同能量的质子照射石墨靶获得了分析的灵敏度，以质谱分析为基准确定了分析的精确度，通过观测、分析实验现象，推出标定碳-13丰度的基本方程式，体系进行刻度，解决了富集物的测定，其范围可从0.1%到90%以上，呼气样品的测试，计算机处理技术的引入显示了该技术的应用前景和巨大的发展潜力。

SUITABLE ACTIVATED CARBON-13 TRACER TECHNIQUES*

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ABSTRACT

Feasibility and applicability studies of the proton induced gamma ray emission (PIGE) have been performed. The graphite was first bombarded at various proton energies to determine gamma ray yield (and, thus, sensitivities) for the reaction of interest. The accuracy for the determination of ^{13}C abundance was checked, and the precision with which this value and ratios $^{13}C/^{12}C$ may be obtained was established, by repetitive analysis samples. The performance of different standards in this determination was assessed. The mathematical treatment was developed for the determination of ^{13}C abundance in tracer studies, and to derive the equations that govern this method of analysis from first principles, to arrive finally at a simple expression by virtue of the observed regularities. The system was calibrated by measuring the gamma ray yield form the ^{12}C (p, γ) ^{13}N and ^{13}C (p, γ) ^{14}N reaction as a function of known ^{13}C enrichment. Using this experimentally determined calibration curve, unknown materials can be assayed. This technique is applicable to the analysis of samples with ^{13}C enrichments between 0.1% and 90%. The samples of human breath natural samples were analyzed against graphite and Cylinder CO_2 standards. Relative standard deviations were $<20\%$. Then if a 25% change is conservatively assumed observable in ^{13}C abundance, an increase in ^{13}C per cent isotopic abundance from the natural 1.11% (average) to only 1.39% may be ascertained. Finally, PIGE is compared with more classical techniques for analysis of ^{13}C tracer experiments. Ease and speed are important advantages of this technique over mass spectrometry, and its error is compatible with the natural variation of biological results.

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INTRODUCTION

The usefulness of carbon tracer techniques is well established in biomedical and ecological studies as a mean of understanding the metabolism of natural substances in living organisms. Mass spectrometry has been the classical technique in the analysis of stable tracer experiments because it is very sensitive and precise. However, it is a destructive technique requiring unavoidable and tedious sample preparation steps, since the sample must be converted into gas. Furthermore, in the combustion process, there is always the question as to how truly representative the gas of the original sample. From this viewpoint nuclear analytical methods offer a useful alternative because the samples here can generally analyzed immediately without any special preparation. It is to this area that our efforts have been devoted. This paper will discuss the feasibility. its applicability as a conventional method offered by the nuclear reaction ^{12}C (p, γ) ^{13}N and ^{13}C (p, γ) ^{14}N to estimate ^{13}C in breath samples, and the prominent characterization and the salient feature of in the proton induced gamma-ray emission (PIGE) methods for ^{13}C tracer techniques.

1 EXPERIMENTAL

Table 1 lists the nuclear reaction and parameters usable for the selective determination of ^{12}C and ^{13}C, together with their main characteristics [1].

Table 1 Main characteristics of nuclear reaction suitable for selective determination of carbon-12 and carbon-13

Nuclear reaction used	Q MeV	Proton energy keV	corresponding half-widths keV	Maximun cross-section mb	prominent gamma-rays MeV
^{12}C (p, γ) ^{13}N	1.944	456.8±0.5	39.5±1	0.127	2.366
		1698±5	72±9	0.035	3.509
^{13}C (p, γ) ^{14}N	7.550	554±1	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 6.442 2.728 2.144

The principle of the nuclear analytical method lies in the detection of prompt gamma rays produced by the nuclear reaction of Table 1 during proton irradiation of the samples. The intensities of lines characteristic of the above-mentioned nuclide are proportional to their concentrations in the bombarded material.

The experiment was performed using the 2×1.7 MV Tandem accelerator. The prompt gamma rays produced during nuclear reactions with ^{12}C and ^{13}C were measured with ORTEC's GEM-13190 HpGe detector, 75 mm×75 mm NaI(Tl) detector and BGO hexagonal detector with an energy resolution of 18% for ^{137}Cs source. The intensities of the photo peaks of interest were calculated in each of the spectra with the help of the program MMCA-EM8 on PC computer. The analytical system was calibrated by measuring the gamma rays yield from the ^{12}C (p, γ) ^{13}N and ^{13}C (p, γ) ^{14}N reaction as a function of known ^{13}C enrichment of barium carbonate. The various ^{13}C enrichments of $BaCO_3$ used are listed in Table 2. The barium carbonate was pressed directly into 2 mm thick of 12 mm diameter, and tight mounted onto the target holder which was positioned at zero degrees relative beam axis. The target holder allows up to ten samples to be analyzed. Several cross reference normalization measurements were made using natural graphite.

Table 2 Targets of the enriched barium carbonate

Target	^{13}C Enrichment/%
$BaCO_3$	10.0
$BaCO_3$	26.57
$BaCO_3$	58.45
$BaCO_3$	73.00
$BaCO_3$	85.00

2 RESULTS AND DISCUSSION

2.1 Yields and sensitivities

The natural graphite was irradiated at various proton energies to determine gamma-ray yields for the reactions of interest. The results obtained are presented in Table 3.

Table 3 Gamma-ray yields from the reaction ^{12}C (p, γ) ^{13}N, ^{13}C (p, γ) ^{14}N and related analytical parameters

Determination	Units	Reacting nuclide	Proton bombarding energy/MeV			
			0.6	0.7	0.8	0.9
Yield	cpm/μA	^{12}C	5460.8	4559.6	3126.1	4094.2
		^{13}C	1009.4	1070.5	1074.4	1069.2
Sensitivity	cpm/ (nA)*	^{12}C	5.52	4.61	3.16	4.14
		^{13}C	91.1	96.6	96.9	96.5
Detection limit**	ppt*	^{12}C	3.69	4.79	6.09	4.69
		^{13}C	0.109	0.113	0.112	0.110

* 1ppt=1 part per thousand

* * Detection limit= (Minimum determinable activity per μA) / (sensitivity). Assume minimum determinable activity given by 3 times the square root of the background in the energy window of interest.

Table 3 shows little energy-dependence of the gamma-ray yields for ^{12}C and ^{13}C. During elevation of bombarding energy, the background under the ^{12}C peak increases, and ^{13}C peaks become less clearly defined and inconveniently close to interfering radiation. Therefore bombardment with 0.6 MeV protons yields satisfactory sensitivities for ^{12}C and ^{13}C, without suffering significant interference. In this case, only one resonance in ^{12}C and ^{13}C is excited, thus minimizing the complexity of the spectrum and reducing the general background.

2.2 Accuracy and nature of the standard

Classical studies on differences in ^{13}C isotopic abundance among common natural substances indicate a range of variation of 4.5%[2]. Therefore, the accuracy of the present method had to be checked against previous mess spectrometry [MS] determinations, taken as " true" results for this purpose. The mean ^{13}C abundance was obtained for $BaCO_3$, by PIGE, with reference to a " true" graphite standard, and compared with a " true" results obtained by MS for same $BaCO_3$ sample. The result is presented in Table 4.

Table 4 The comparison of MS and PIGE in the determination of ^{13}C abundance

Method	reference standard values	^{13}C abundance in $BaCO_3$
MS (MAT-251)	1.108	1.084
PIGE	1.108	1.089

The relative accuracy error was only −0.4% in this test.

The accuracy depends also on the nature of samples and standard. In general, the best results may be obtained when standard and sample have similar stopping power characteristics. A comparison of all the substances used in this study is made in Table 5, whose figures are percent deviations between the proton induced gamma-ray yield ratio Y (^{13}C) /Y (^{12}C) for a given substance (R_0) and the analogous ratio (R) for each of others; i. e., 100 (R_0-R) /R_0 in each comparison. It may be assumed that ^{13}C abundance variation among biological and organic substances is small; thus, the small deviations within each other may be attributed to similar stopping power characteristics between them. In other words, a known biological tissue can be an effective standard for an unknown of analogous nature.

Table 5 Comparison between carbon isotopic yield ratios for several substances

Substance	Benzamide	Urea	Graphite	Cylinder CO_2	Glucose	Human Breath (Woman)	Human Breath (Man)
Benzamide	0	−9.7	−9.7	−1.7	−4.3	−1.6	−5.9
Urea	+8.8	0	−0.8	+7.3	+4.9	−5.7	+3.4
Graphite	+8.8	+0.8	0	+7.3	+4.9	−5.6	+0.4
Cylinder CO_2	+1.7	−7.8	−7.9	0	−2.5	−14.0	−4.2
Glucose	+4.1	−5.1	−5.2	+2.5	0	−11.1	−1.5
Human Breath (Woman)	+13.7	+5.4	+5.3	+12.2	+9.9	0	+8.6
Human Breath (Man)	+5.5	−3.5	−3.6	+4.0	+1.5	−9.5	0

2.3 Calibration of isotopic carbon abundance

The main difficulty with PIGE method is, perhaps, the complication introduced in its mathematical treatment by the significant kinetic energy degradation that charged particles suffer as they penetrate matter, and, therefore, the nuclear reaction cross-section varies with sample depth; in consequence, the usually significant variations among stopping power of different substance must be taken into account to avoid systematic errors in the results. The stopping power (S) may be calculated by the basic principle discussed by some authors [3,4], and the stopping power of a complex target, containing several elements, may be obtained by Bragg's rule. To calculate stopping power for various organic compounds of biological significance, a program has been written with FORTRAN-77 Language. The stopping power for standard and organic compounds were calculated and plotted in Fig. 1. also, parameters of the nuclear reaction used in the determinations of carbon isotopic ratio are given in Table 1. Finally, some observation may be drawn from these data. First, the stopping power of the organic substances at the proton energies of interest are very high (340~480 MeV/g · cm^2), and thus, the depth in such targets at which the particles reach the resonance (and induce maximum reaction yields) are close together. Second, though these substance are quite heterogeneous chemically, their stopping powers differ only by 29% at the most, for proton energy considered; thus the stopping powers of biological samples (essentially formed by complex combination of the these and analogous substances) may be assumed to have similar characteristics and values. Third, the curves appear to be remarkably parallel in semilogarithmic scale, i. e. the ratio of stopping power for any two organic substances seems to approach a constant throughout this

range; this should be especially true within the relatively narrow resonance energy spans.

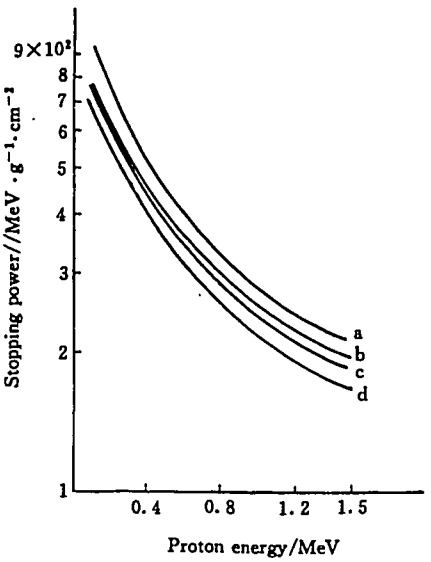

Fig. 1 Proton stopping power for several organic substances
(a) cholesterol, palmitic acid
(b) alanine, glutamic acid, benzamide
(c) starch, histidine, urea
(d) systine, graphite

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 6 for the case of benzamide. The calculations involving urea and graphite standard give analogous results, and interesting conclusions may be drawn from all of these data. First, as expected from inspections of Fig. 1, the stopping power ratio S'/S is very nearly a constant (depending only on the substances) for all resonance energies relevant to 0.6 MeV proton irradiation; in particular, for urea and benzamide the variation of this ratio is $<0.4\%$. Second, the validity of this conclusion may be extended to bombarding up to 1 MeV, for the same standard, since the largest variation is still comparable to the normal counting statistical error. Moreover, despite the gross structural differences between graphite and substances included in the comparison, the variation of S'/S is observed to also be remarkably small in this case: less than 0.6% for 0.6 MeV proton bombardments.

Table 6 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	0.8821	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
Graphite	1.1228	1.1175	0.23
Urea	1.9863	0.9886	0.21

* Maximum percent deviation from average of data for two resonances.

It is reasonable to assume that these conclusions also hold for dry biological samples. Because of te above observations about Fig. 1. Thus, for an adequate standard the above conclusion may be expressed mathematically by

$$(S'/S)_{457keV} = (S'/S)_{554keV} = b \tag{1}$$

Where S and S' are the stopping power (at the indicated resonance energies) of a given unknown (dry) biological sample and of adequate standard, respectively, and b is a constant that only depends on the nature of these two substance. In addition, because these resonances have narrow energy spans, as is typically the case, it may be further assumed that Equation (1) is valid throughout each of these spans; that as

$$(S'/S)_E = b \qquad \text{for} \qquad E_i - \varepsilon_i \leqslant E \leqslant E_i + \varepsilon_i \tag{2}$$

Where i characterizes any one of the resonance of Equation (2) and $2\varepsilon_i$ its corresponding span, somewhat larger than 2Γ.

When a thick target is bombarded with charged particles. The prompt gamma-ray count rate resulting from a given nuclear reaction may be expressed by

$$Y = \frac{aceN_0I}{M}\int_{E_0-\varepsilon}^{E_0+\varepsilon}\frac{\sigma(E)}{S(E)}dE \tag{3}$$

Where a is isotopic abundance of the nuclide to be determined. M the corresponding element's atomic weight, c its concentration in the target of stopping power $S(E)$, I the beam intensity, N_0 is Avogadro's number, and e counting efficiency; the reaction cross-section σ (E) [or rather the ratio σ (E) / S (E)] is integrated over the span 2 ε of the resonance E_0.

When nuclear reaction are induced simultaneously in two different nuclides in the same target, each of these processes is governed by an expression analogous to Equation (3); for example, Y (13) and Y (12) express the yields of the reaction ^{13}C (p, γ) ^{14}N and ^{12}C (p, γ) ^{13}N, respectively. Taking the ratio R of the two corresponding experimental ields gives

$$R=\frac{Y(13)}{Y(12)}=\frac{a_{13}c_{13}e_{13}M_{12}}{a_{12}c_{12}e_{12}M_{13}}\times\frac{\int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}}[\sigma_{13}(E)/S]\mathrm{d}E}{\int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}}[\sigma_{12}(E)/S]\mathrm{d}E} \tag{4}$$

Where sub indexes 13 and 12 correspond to each of the two reactions (whose resonances are, thus, E_{13} and E_{12} etc.) taking place in a target of stopping power S.

Equation (4) is essentially based on absolute parameters. It is usually very convenient to make calculation in relative numbers by means of the comparator method; in it, a standard (comparator) that contains known abundances and concentrations, respectively, of the isotopes and elements sought in the unknown sample is bombarded for comparison in the same conditions as the sample. The ratio R' of te yield for the same two reaction of equation (4), but occurring in this standard, would then be

$$R'=\frac{Y'(13)}{Y'(12)}=\frac{a'_{13}c'_{13}e_{13}M_{12}}{a'_{12}c'_{12}e_{12}M_{13}}\times\frac{\int_{E_{13}-\epsilon_{13}}^{E_{13}+\epsilon_{13}}[\sigma_{13}(E)/S']\mathrm{d}E}{\int_{E_{12}-\epsilon_{12}}^{E_{12}+\epsilon_{12}}[\sigma_{12}(E)/S']\mathrm{d}E} \tag{5}$$

Where paraters of standard are primed, particularly S', since the standard is generally a different subsence from the sample. Clearly, counting efficiencies, atomic weights and excitation functions are same as in Equation (4) and (5); Considering that the resonance energies E_{13} and E_{12} of Equation (4) and (5) are among for which Equations (1) and (2) are vaild, the value $bS=S'$ can be substituted in the former equations. Then, dividing the Equatin (4) by Equation (5) gives

$$\frac{R}{R'}=\frac{(a_{13}/a_{12})(c_{13}/c_{12})}{(a'_{13}/a'_{12})(c'_{13}/c'_{12})} \tag{6}$$

It is useful to define the dimensionless ratios

$$F=\frac{a_{13}}{a_{12}}\qquad F'=\frac{a'_{13}}{a'_{12}}\qquad C=\frac{c_{13}}{c_{12}}\qquad C'=\frac{c'_{13}}{c'_{12}} \tag{7}$$

Equation (7) now reduces to

$$\frac{R}{R'} = \frac{F}{F'}\frac{C}{C'} \tag{8}$$

As only one element, carbon, is involved in carbon tracer experiments, it is $C_{13}C_{12}$ and $C'_{13}C'_{12}$ and, therefore, $C=C'=1$ in the Equation (7), even if the carbon concentration of the sample is different form that of the standard (i.e. $C_{13}\neq C'_{13}$ or $C_{12}\neq C'_{12}$). As the ratio $^{13}C/^{12}C$ is equal to the ratio a (^{13}C) / a (^{12}C) of the fractional isotopic abundance for ^{13}C and ^{12}C, it may be immediately obtained by solving Equation (8)

$$[\text{Ratio } ^{13}\cdot\ \cdot\ \cdot\ ] = \frac{a(^{13}C)}{a(^{12}C)} = F = \frac{F'}{R'}R \tag{9}$$

Where it appears in terms of known or easily measured parameters, such as the ^{13}C /^{12}C abundance ratio F' for the stand, and experimental yield ratios R' and R, between the reaction ^{13}C (p, γ) ^{14}N and ^{12}C (p, γ) ^{13}N, for standard and sample, respectively.

As a (^{13}C) $+a$ (^{12}C) $=1$, the percent abundance [$^{12}C\%$] and [$^{13}C\%$] (in isotope per 100 carbon element nuclei) may be rapidly derived from Equation (9):

$$F+1 = \frac{a(^{13}C)+a(^{12}C)}{a(^{12}C)} = \frac{1}{a(^{12}C)}$$

this is,

$$a(^{12}C) = \frac{1}{F+1} \tag{10}$$

and

$$a(^{13}C) = 1 - a(^{12}C) = \frac{F}{F+1} \tag{11}$$

From Equations (10) and (11),

$$[^{12}C\%] = \frac{100}{F+1}, \qquad [^{13}C\%] = \frac{100F}{F+1} \tag{12}$$

An application of Equations (8) and (12) to the determination of ^{13}C isotopic abundance in breath test samples is satisfactory. Table 7 lists ^{13}C isotopic abundance experimentally obtained for natural samples of urea, glucose, benzamide, human breath sample and barium carbonate by measuring their yield ratios $R=Y$ (^{13}C) $/Y$ (^{12}C), and comparing with those (R') for several standards; Equations (9) and (12) were used in the calculations, for which the $^{13}C/^{12}C$ ratio of all the standards was the natural abundance ratio $F'=1.108/98.892=0.0112$. A measure of accuracy of the determination is given by the percent deviations between the average $^{13}C\%$ abundance found, and the

natural value (1.11%) expected for the samples. Because of Equation (9) and (12) and of the definition of standard deviation, these precision values are independent of the nature of the standard used to determination the yield ratios; moreover, this extends here to the $^{13}C\%$ abundance results because $F<1$ in Equation (12). Consequently, no detailed listing of results calculated against other standard is made. It is clear from Table 7 that the overall accuracy of this method is quite compatible with the statistical variation of the results and, consequently, sufficient and acceptable. Therefore, the mathematical arguments and the basic Equation (8) and its consequences [Equation (12)] are corrected.

Table 7 Accuracy of ^{13}C abundance determination

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

2.4 Determination of carbon-13 enrichment

Typical HpGe, NaI (Tl) and BGO data are shown in Fig. 2～Fig. 7 for a natural abundance target and for a target highly enriched in ^{13}C, respectively, it is seen by comparing the spectra in Fig. 2～Fig. 4 that the " ^{12}C peak" in NaI (Tl) spectrum is composed predominantly of 2.366 MeV gamma ray from the ^{12}C (p, γ) ^{13}N reaction. For higher ^{13}C enrichments, as in Fig 5～Fig. 7 the " ^{12}C peak" in the NaI (Tl) spectrum is composed almost entirely of the 2.313 MeV gamma ray from the ^{13}C (p, γ) ^{14}N reaction, with the 2.370 MeV and the double escape peak of the 3.378 MeV gamma rays also making significant contributions to the ^{12}C yield. In the HpGe spectra, prominent full energy, single escape, and double escape peaks are seen, and makes these assays more accurate. On the other hand, because of nature and size of the detector, in the NaI (Tl) spectra these spectra show only a strong full energy and single escape peak. The double escape peak appears as a broadening on the low energy side of the single escape peak. In the BGO spectra the full energy, the single escape peak and the double escape peak cannot be resolved. It is noted the change in the vertical scale for these spectra, and implying that the used of a large NaI (Tl) detector is necessary when large batches of samples have to be

examined quickly.

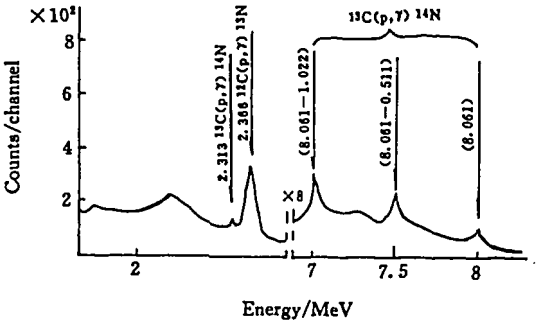

Fig. 2 Type HpGe spectrum for 600 keV proton incident on infinitely, natural graphite carbon target

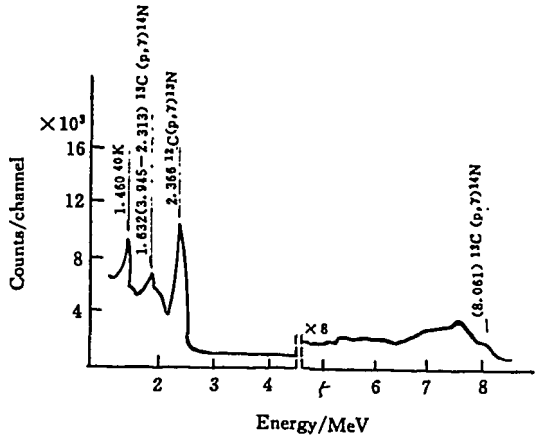

Fig. 3 Type NaI (Tl) spectrum for 600keV proton incident on infinitely, natural graphite carbon target

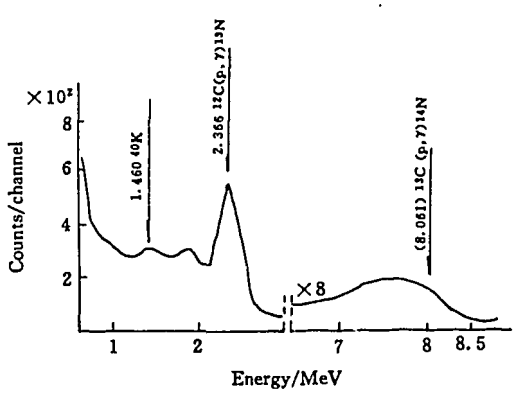

Fig. 4 Type BGO spectrum for 600 keV proton incident on infinitely, natural graphite carbon target

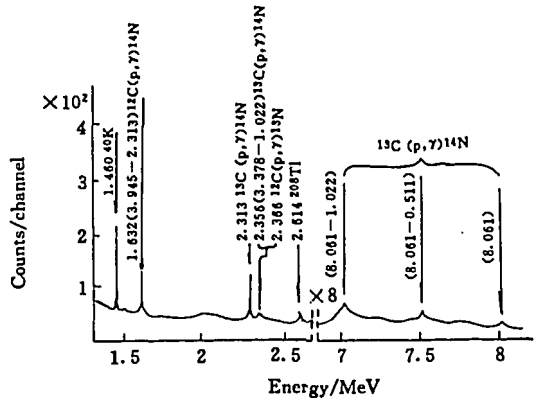

Fig. 5 Type HpGe spectrum for 600keV proton incident on infinitely, highly enriched carbon target

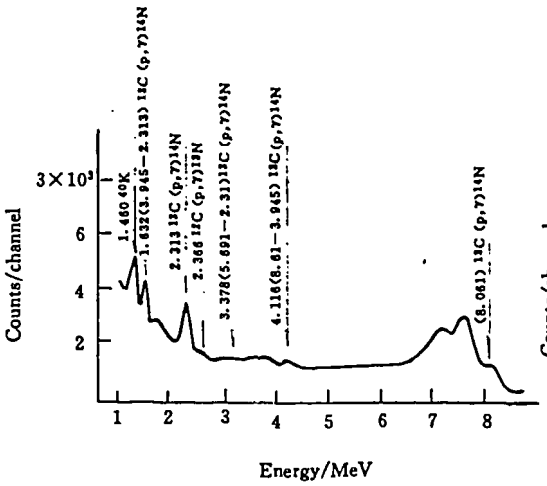

Fig. 6 Type NaI (Tl) spectrum for 600 keV proton incident on infinitely, highly enriched carbon target

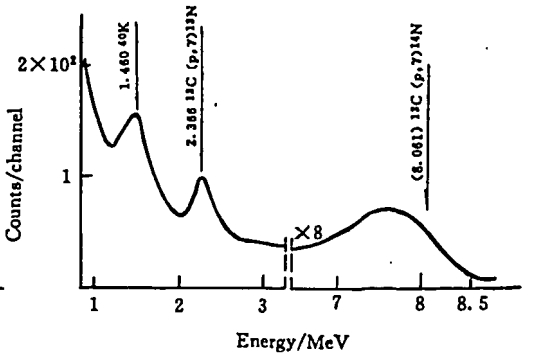

Fig. 7 Type BGO spectrum for 600keV proton incident on infinitely, highly enriched carbon target

It has been shown from Table 1 that the lower resonance in both ^{12}C and ^{13}C are particularly attractive for determining carbon isotopic abundance. The 457 keV resonance in ^{12}C populates the level in ^{13}N at 2.366 MeV; The 554 keV resonance in ^{13}C populates the 8.061 MeV excited state in ^{14}N. The decay of ^{13}N results is only the 2.366 MeV gamma rays. The decay of ^{14}N results is not only the 8.061 MeV gamma ray, but also a 2.313 MeV gamma ray, a 2.370 MeV gamma ray, and a 3.378 MeV gamma ray which has a double escape peak at 2.356 MeV. Fig. 8 shows the 2.3 MeV region of a HpGe spectrum for 600 keV protons incident on an infinitely thick, highly enriched (85% ^{13}C) carbon target. The 2.366 MeV gamma ray is from the ^{12}C (p, γ) ^{13}N reaction, the other gamma rays are from the ^{13}C (p, γ) ^{14}N reaction. The " FE" stands for a full energy peak and " P-2" stands for a double escape peak. These 2.3 MeV gamma rays from ^{14}N interfere with the 2.366 MeV gamma ray from ^{13}N. If a high resolution HpGe detector is used as the gamma rays spectrometer, 2.313 MeV gamma ray from ^{14}N can be resolved from the 2.366 MeV gamma ray from ^{13}N. However, when a NaI (BGO) detector is used, these two rays cannot be resolved.

As can be seen from Table 1, the width of the ^{12}C resonance at $E_p = 457$ keV is 39 keV. Thus, the line associated with the 2.366 MeV gamma ray has a natural width of 39 keV. Consequently, this gamma ray cannot be resolved

from the 2.370 MeV gamma rays and the double escape 2.356 MeV gamma ray resulting from the decay of ^{14}N. The 2.370 MeV gamma ray results from a transition from the resonance state at 8.061 MeV to an excited state at 5.691 MeV. The ^{13}C resonance at $E_p=554$ keV has a natural width of 32 keV, so the line associated with the 2.370 MeV gamma ray has a natural width of 32 keV. However, the 2.356 MeV double escape gamma ray, having a full energy of 3.378 MeV, results from a transition between the 5.691 MeV state and the 2.313 MeV state. Since these are bound states, the line shape associated with this gamma ray transition is sharp the observed line width being determined by the detector resolution. Fig. 8 shows the 2.3 MeV region of a typical HpGe spectrum. All of the gamma rays discussed are labeled, showing explicitly the difficulty in stripping out peaks in this area. If a NaI (Tl) detector, with its poorer resolution, were used, none of the peaks in Fig. 8 would be resolved.

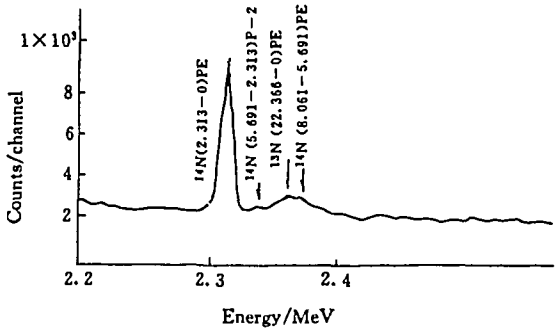

Fig. 8 The 2.3 MeV region of a HpGe spectrum

Thus, when one attempts to measure the abundance of carbon in a target, the carbon-13 is determined very simply and reliably using the 8.061 MeV gamma ray due to the ^{13}C (p, γ) ^{14}N reaction does produce a clean line with no interference, it is, therefore, convenient to use the area under the peak associated with this gamma ray as a measure of the amount of ^{13}C present. For the carbon-12, the experimenter does not get a pure ^{12}C signal since the characteristic 2.366 MeV line is not isolated in the gamma ray spectrum of a carbon target bombarded with 600 keV protons, certain gamma rays from ^{14}N interfere with the 2.366 MeV gamma ray from ^{13}N. These gamma rays, as pointed out above, being in reality a complex mixture of several lines. Fig. 9, 10 gives calibration curves established with NaI (Tl), BGO and HpGe detector. The isotopic contents measurable range from 0.1% to 90.0% for ^{13}C. The analysis

can be performed on a few μg, which means that these process are especially useful for the examination of micro-samples such as biophysics. From Fig. 9, 10 these curves show clearly that although the HpGe detector, as expected, is apparently the more suitable for such analysis, the Nal (BGO) detector can be useful for some isotopic measurements. A point to emphasize in this respect is that while the former instrument is more selective because of its resolution, the latter, owing to its efficiency, reduces the measurement time by a factor of 10 to 20. This is a serious advantage when large batches of samples must be examined quickly at costs competitive enough to warrant the use of these techniques in place of mass spectrometry.

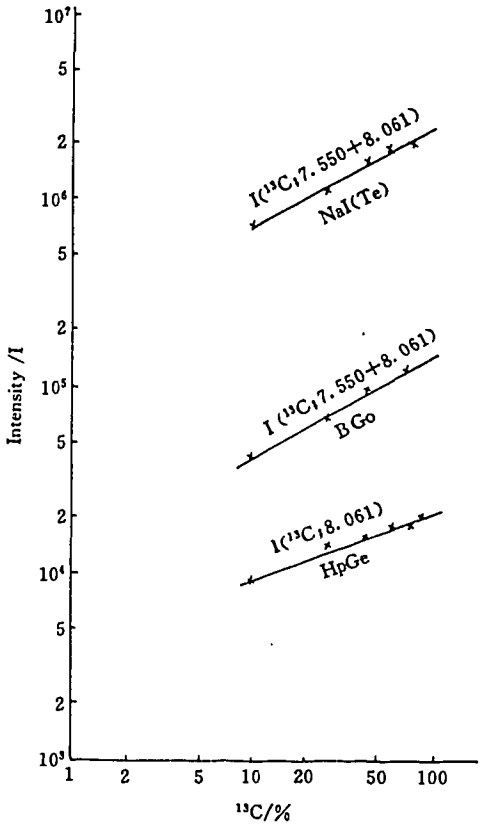

Fig. 9 Intensity of characteristic ^{13}C line versus isotopic abundance of this element in barium carbonate.
$E_p=600$ keV $I=0.5$ μA

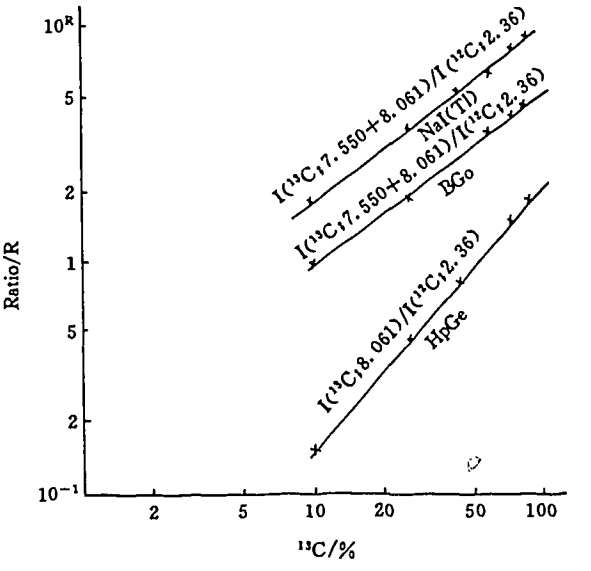

Fig. 10 Ratio of the characteristic ^{12}C and ^{13}C line intensities versus isotopic abundance of this element in barium carbonate.
$E_p=600$ keV $I=0.5$ μA

2.5 Determination of ^{13}C in breath samples

CO_2 breath tests with ^{13}C labeled compounds have been shown to be useful

or potentially useful in the diagnosis of several important diseases [5~9]. Mass spectrometry has been the classical technique in the analysis of stable tracer experiments because it is very sensitive and precise. However, the samples for mass spectrometry must be converted into a gas, nuclear analysis is not limited: solid, liquid or gaseous specimens may be used. As far as breath test, the use of a gas cell allows the analysis more simple. Our results show that the proton-induced gamma ray emission method is capable of meeting of these requirements. The combination of enriched stable nuclide tracer studies with the proton-induced gamma ray emission method has recently grown into an ideal and reliable technique, especially in the field of biology and medicine.

Human exhaled CO_2 was collected and converted into a solid $BaCO_3$ which directly pressed into 2 mm thick disks 1.0 cm in diameter. Each sample was bombarded three successive times with about 0.5 μA beam of 0.6 MeV protons. ^{13}C abundance was determined for human breath natural samples by comparison with the two standards. The typical spectra for breath sample and some results are presented in Fig. 11 and Table. 8.

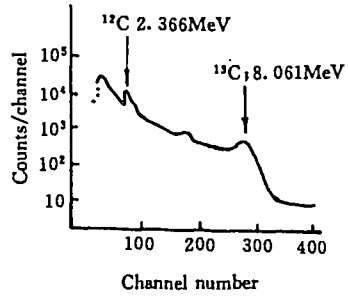

Fig. 11 Typical NaI (Tl) spectra of human breath samples under 0.6 MeV proton bombardment

Table 8 Determinations of ^{13}C in human breath samples by 0.6 MeV proton

Sample	Standard	Average ^{13}C abundance/%	Standard deviation/%	Absolute error/%*
Human breath (woman)	Graphite	1.17±23	19.5	+5.5
	Cylinder CO_2	1.18±23	19.6	+6.5
Human breath (man)	Graphite	1.04±13	12.4	−6.1
	Cylinder CO_2	1.01±15	14.7	−8.8

* All samples had the natural abundance of ^{13}C, that is, 1.108%

The standard deviations indicated in Table 8 are taken to represent the precision of these determinations. It may be observed that the precision values for breath samples is <20% and these precision values are independent of the nature of the standard used to determine the sample, while the absolute error (accuracy) is generally below these values, as expected. Then, if a 25% change is conservatively assumed observable in ^{13}C abundance, an increase from its natural 1.11% value to 1.39% will be detected. In breath test, ^{13}C abundance range of variation is about 1.14%~1.18% and, therefore, satisfactorily meets

the needs of measuring accuracy for breath test. The isotopic abundance for ^{13}C in breath samples was the natural values and, thus, the minimum that can possibly be encountered in most stable tracer experiments; they can only increase with administration of stable tracers to living organisms, with corresponding improvements in counting statisics and, therefore, in precision in actual practice.

A clinically acceptable breath test must meet the requirement that the information gained should be commensurate with the cost of the test, and the test can indeed provide useful, consistent, and predictable information about the patient's condition [7]. There are several parameters that characterized on the breath test can be calculated from the original data for the time course curves, i. e., the peak time (T_p); the peak values (δ_{max}); the elimination rate constant (λ); the elimination half-times ($T_{1/2}$); the absorption rate constant (K); the 2 h recoveries (R) and the Ω values, i. e., the product of λ and 2 h recovery used as a working parameters of the breath test for clinical purposes. To speeding and accurate calculate these parameters above, a program has been written with True Basic Language, which to operate on IBM compatible microcomputers. As soon as determining time course curves for individual human subject, the program calculates all parameters and giving the graphical output showing the orginal and fitted data. A successful application of this program to the calculation of the parameters for the breath test is described in detail elsewhere. Some results will be summarized here, however, for the sake of completeness and comporison. An example of this program calculations is given in Table 9, which original data was obtained experimentally by Xia Zangqin using mass spectrometry [8]. As shown in Table 9, for the patients with liver diseases, among the seven parameters mentioned above were changed, especially the difference of δ_{max}, λ, $T_{1/2}$, R, Ω were significant between the normal adult and patients. All this, to provide an optimum parameters, and predictable information about the patient's condition.

Table 9 Changes of parameters in breath test

subjects	I_p/h	δ_{max}	K	λ	$T_{1/2}$/h	R/%	Ω
normal adult	0.410	21.722	6.962	0.763	0.908	37.744	28.802
chronic persistent hepatitis	0.409	17.753	9.123	0.563	1.230	37.242	20.985
compensated cirrhosis	0.448	11.879	1.264	0.467	1.484	27.267	12.735
decompensated cirrhosis	0.498	6.067	3.926	0.050	13.848	21.963	1.094

Determination of ^{13}C in breath (samples) (Table 8) and comparison between carbon isotopic yield ratios for several substances (Table 5) indicate that there are no significant matrix effects due to the standards, and implying that the choice of a standard is not critical. Also, provided the conclusion of the stopping powers of biological samples have similar values and characteristics for 600 keV protons. ^{13}C abundance in this work has the value (1.10%+0.08%) for the average ratio of $^{13}C/^{13}C+^{12}C$ in natural human breath samples investigated. The agreement between the present results and the reported natural values (1.11%) for $^{13}C/^{13}C+^{12}C$ in natural samples, (by mass spectrometry) supports the proton-induced gamma ray analysis for carbon tracer techniques. Speed and simplicity are important advantages of this technique over mass spectrometry, and its widely applied not only, once more, made stable nuclide become essential tool in biology and medicine research, but also helped us to be moving in to a new era of human studies.

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