Practice and Principles of Isotopic Measurements in Organic Geochemistry

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Preface

These notes have been compiled from teaching materials developed by John Hayes between 1980 and 1995. Since their goals are to introduce basic concepts and practices, they are not grossly outdated in spite of their antiquity. Where necessary or desirable, minimal revisions indicate more recent data and practices. The sections include:

Practice and Principles of Isotopic Measurements in Organic Geochemistry. This review originally appeared as pp. 5-1 to 5-31 in W. G. Meinschein (ed.) *Organic Geochemistry of Contemporaneous and Ancient Sediments*, a volume published in 1983 by the Great Lakes Section of the Society of Economic Paleontologists and Mineralogists. That book was intended to provide the notes for a short course offered in connection with the 1983 National Meeting of the Geological Society of America, but the short course drew inadequate enrollment and was cancelled. The original document included the introduction and sections 1 and 2 reprinted here, as well as an earlier version of section 3.

Primary Standards of Stable Isotopic Abundances. Presented here as section 3, this material was revised and expanded frequently in the years after 1983, notably for use in a short course sponsored by Finnigan MAT in Beijing in 1986 and for frequent use in subsequent classes at Indiana University.

Limits on the Precision of Mass Spectrometric Measurements of Isotope Ratios. Originally prepared for class use some time in the 1980s, this text (Section 4) explains how shot noise, or "ion-beam noise," or "ion statistics" fundamentally limit the precision that can be attained in any measurement based on integration of an electrical current.

Uncertainties in Blank-Corrected Isotopic Analyses. These notes (Section 5) were prepared for use by workers in the Biogeochemical Laboratories at Indiana University during the 1980's (accordingly, their presentation is far from "publishable"). They deal with the common problem in which an analytical result reflects the composition of a sample and, in addition, the magnitude and isotopic composition of an analytical blank.

Further Notes, July 2002. Section 6 elaborates on notes inserted in earlier sections.

Introduction

The modern era of precise isotopic measurements and exploration of natural variations in isotopic abundances began with the work of Nier and Gulbransen (1939), who observed that the relative abundance of carbon-13 was greater in inorganic material (carbonates) than in organic material. Later, Harold Urey and his students at the University of Chicago greatly extended the precision and scope of these measurements. The measurement of oxygen-isotopic abundances in carbonates was introduced for paleotemperature studies (Urey *et al.*, 1951; Epstein *et al.*, 1951), and Harmon Craig (1953) published his landmark study of the biogeochemistry of the stable isotopes of carbon. Wickman (1956) provided an early estimate of the division of sedimentary carbon between organic and inorganic materials. Much earlier, West (1945) published the first report of carbon isotopic studies in petroleum geochemistry, an accomplishment that has received too little recognition.

In view of the growing importance of isotopic studies in organic geochemistry (for major reviews, see Degens, 1969; and Deines, 1980) it is the goal of this review to provide the reader with an introduction to the practical and theoretical bases of isotopic geochemistry.

1. Mass Spectrometric Analyses and Sample Handling

1.1 *The Nature of the Problem*

Because the equilibrium or kinetic characteristics of one isotopic species usually differ from those of another by only a few percent, the variations in isotopic abundances imposed by those differences are small. Highly precise analytical techniques are required, and it has been found convenient to *compare* samples, measuring isotopic *differences*, rather than to attempt absolute measurements of isotopic abundances.

Obtaining short-term high precision is not a problem, but getting long-term stability can be extremely difficult. More graphically, when absolute measurements are employed, a single sample may yield isotope ratios of 0.0100886 ± 0.0000007 today and 0.0100251 ± 0.0000008 tomorrow. In contrast, if that sample is compared to an arbitrary reference sample on both days, the *differences* observed between the sample and the reference will probably be constant. This stability will be observed because the same instrumental fluctuations that have affected the "absolute" ratio of the unknown sample will also have affected the "absolute" ratio of the reference sample. Provided everything is compared to the same reference (either directly or indirectly), very small *differences* between isotope ratios can be accurately measured. This technique of differential comparison has been a cornerstone of precise isotopic analysis since it was introduced around 1950 in Harold Urey's laboratories at the University of Chicago (McKinney *et al.*, 1950).

Sample Preparation. Differential measurement of isotopic abundances requires that each sample be compared to a standard (*i.e.*, a reference point) which differs from the sample, if at all, *only* in its isotopic composition. This is often convenient and simple. For example, precise comparisons of isotopic compositions of carbonates can be made by comparing mass spectra of samples of carbon dioxide prepared from each material.

The mechanism of comparison is neither so obvious nor so simple when isotopic compositions of organic materials are of interest, though there is nothing fundamentally wrong with comparing isotopic compositions of organic materials directly. For example, the difference in carbon-isotopic compositions of two samples of benzene (C_6H_6, molecular weight = 78) might be measured by comparison of the (mass 79)/(mass 78) ion-current ratios, and if both samples were not available at the same time, each might be satisfactorily compared to an intermediate standard. The impracticality of this simple approach becomes clear, however, when it is recognized that (*i*) the measurement would be invalid if the hydrogen-isotopic compositions of the samples differed significantly, (*ii*) an isotopic standard would be required for each compound of interest if indirect comparisons were to be made, (*iii*) comparisons between different compounds would be difficult and subject to systematic errors, and (*iv*) it would be impossible to make isotopic measurements on species that could not be readily volatilized to yield ion beams that were both intense and stable.

Many advantages are gained by converting organic materials to "common denominator" forms especially suitable for mass spectrometry and isotopic analysis. For example, the gases H_2, N_2, and O_2 are ideal for hydrogen, nitrogen, and oxygen isotopic analyses. They are volatile. Because they contain only a single element, ion-current abundances in their mass spectra carry

information only about isotope ratios for that element. Their constituent atoms do not easily participate in exchange reactions. There is, unfortunately, no analogous form of carbon. Carbon tetrafluoride, CF_4, would be a logical choice because fluorine has no isotopes and quantitative details of the mass spectrum therefore depend only on carbon isotopic abundances. Carbon dioxide is easier to prepare, but contains exchangeable atoms of an element other than carbon.

The isotopic composition of the substance employed in the differential measurement (*i.e.*, the compound used in isotope-ratio mass spectrometry) must faithfully represent that of the parent material. Fulfillment of this requirement for isotopic fidelity is assured if all of the element to be analyzed isotopically is converted to the form used for analysis, but quantitative yields in preparative organic reactions are rare. Fortunately, combustion can easily be driven to completion. It may be unusual (some would say perverted) to think of combustion as a preparative reaction, but it offers a remarkable combination of fundamental appeal (ease of obtaining quantitative yield) and experimental convenience. Carbon dioxide may be theoretically inferior to CF_4 as a material for isotopic mass spectrometry, but it's far easier to prepare in high yield. Further, as noted in Fig. 1, the remaining products of combustion can also be utilized in isotopic analyses, though water must first be converted to H_2.

1.2 *Methods of Sample Preparation*

Techniques of Combustion can be either "static" (the sample and oxidant are held together in a restricted volume) or "dynamic" (the sample and oxidant are moved through a series of catalyst beds by a carrier gas).

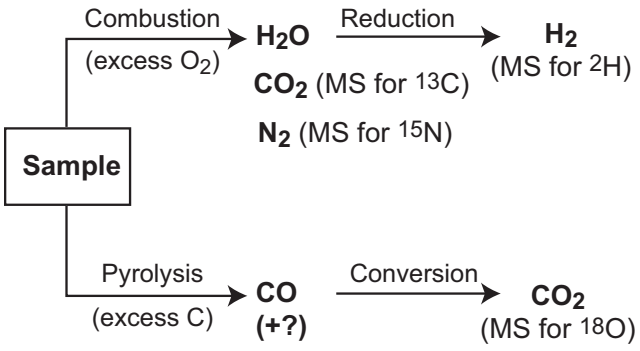


Figure 1. Schematic representation of sample-preparation procedures.

Static techniques offer many advantages and are well established for analyses of carbon and nitrogen isotopes. Systems in use in geochemical laboratories (Wedeking *et al.*, 1983; Stuermer *et al.*, 1978) utilize an approach introduced by Frazer and Crawford (1963). A milligram or less of organic material, together with a few hundred milligrams of cupric oxide and a few milligrams of silver foil, is sealed in an evacuated quartz tube. The tube is heated to a temperature near 850°C and pyrolytic decomposition of the cupric oxide yields O_2 which rapidly attacks the organic material. If the tube is cooled slowly, all nitrogen present in the initial organic material will be converted to N_2, all sulfur will be trapped as $CuSO_4$, and, of course, all carbon will be in the form of CO_2. With care, water can be recovered in quantitative yield (Frazer and Crawford, 1963), but several laboratories have found that hydrogen isotopic analyses are quite variable. Using the static technique, large numbers of samples can be conveniently combusted using batches of tubes, but each tube must be individually opened and the products of combustion isolated and purified by cryogenic distillations.

Dynamic techniques involve more complicated systems, and only one sample can be processed at a time, but offer several advantages and are well established even for hydrogen-isotopic measurements. Procedures utilizing no carrier gas whatever offer the lowest blanks (DesMarais, 1978) but require cryogenic techniques for separation and purification of the products of combustion. Procedures in which oxygen serves as the carrier gas have high capacity, but the fact that oxygen is partly condensable at liquid-nitrogen temperature makes careful control of the system mandatory. Recovery of N_2 from streams of O_2 is difficult, and such systems are not generally applicable. The widely-utilized "LECO" total organic carbon analyzer can, however, be successfully adapted to the preparation of CO_2 for isotopic analysis of total organic carbon in sediments (Wedeking *et al.*, 1983).

Commercially-developed dynamic systems (*e.g.*, Pella and Colombo, 1973) for the elemental analysis of organic materials can be very successfully adapted to the preparation of samples for isotopic analysis. In such systems, combustion, conditioning of the products (*i.e.*, reduction of nitrogen oxides, scavenging of interferents), and separation and purification of N_2, CO_2, and H_2O, are frequently integrated in a single automated sequence. The products can be trapped from the carrier-gas stream as it leaves the instrument (Hayes and Santrock, unpublished results). An accurate elemen-

tal analysis is also obtained. Such systems are generally suitable only for the combustion of milligram samples of pure organic material, however.

The process of combustion can be directly coupled with chromatographic separation of components of natural gas or of more complex molecular species (Matthews and Hayes, 1978; Vogler et al., 1981; Hayes, 1983). At present, such systems offer the best route to the precise determination of the isotopic compositions of individual organic compounds.

As noted, nitrogen isotopic analyses can be accomplished using N_2 recovered from products of combustion. Water, the combustion product carrying sample-derived hydrogen, cannot be directly used for isotopic measurements because the hydrogen atoms are so easily exchangeable with the sorbed water molecules inevitably present on the walls of all vacuum systems. To circumvent this problem, the hydrogen in the water is always converted to H_2 gas for mass-spectrometric analysis. While many metals are electropositive enough to reduce water to H_2, procedures that are both efficient and convenient are extremely rare. The reagent chosen must not be too volatile for convenient use in a vacuum system; must not form adherent oxide layers leading to passivation; must not dissolve and retain the H_2 (a common problem with many metals); and must not tend to retain a portion of the H as hydroxyl groups. The earliest satisfactory procedure was described by Bigeleisen *et al.* (1952), who used uranium metal as a reductant. More recently, a batchwise process utilizing zinc as the reductant has been described by Coleman *et al.* (1982).

Oxygen isotopic analyses. The determination of the abundances of oxygen isotopes in organic material cannot, of course, be based on combustion in the presence of excess oxygen. Direct methods for elemental analysis of oxygen in organic material are pyrolytic. The organic material is degraded at very high temperature and the oxygen-containing fragments are further degraded to products suitable for analysis. The persistence of residual amounts of water can be avoided by selective removal of hydrogen, and this is the principle of successful nickel-bomb techniques (hydrogen gas diffuses freely through nickel at temperatures near 1000°C). Pyrolysis of cellulose in a sealed nickel tube yields most cellulose-derived oxygen in the form of CO and CO_2, and it has been shown (Brenninkmeijer and Mook, 1981) that the oxygen-isotopic composition of cellulose can be determined by conversion of all of that oxygen to the form of CO_2 for mass-spectrometric analysis. In more recent studies, Wedeking and Hayes (unpublished) have shown (*i*) that the recovery of oxygen in such procedures is not absolutely quantitative, but that the losses involve no measurable isotopic fractionation and are thus not consequential, and (*ii*) that the procedure can be adapted to the analysis of oxygen in materials other than cellulose, even in the presence of pyrite and other common sedimentary trace minerals.

The Unterzaucher (1940) technique for elemental analysis of oxygen in organic materials employs a different principle of operation. Products of pyrolysis are equilibrated with elemental carbon in order to produce CO in quantitative yield. This procedure is very well established for elemental analysis of all kinds of organic matter, but is troubled by a significant blank (of little significance in elemental analysis, but crucial in isotopic analyses because it is coupled with troublesome memory effects) and yields a product not suitable for isotopic analysis (CO can only be trapped by procedures that also trap N_2, which is inevitably present as a contaminant and interferes disasterously in mass spectrometric measurements). Nevertheless, Taylor and Chen (1970) have earlier shown that this technique can be adapted for detection of excess oxygen-18 introduced as an isotopic tracer, and Santrock and Hayes (1983) have recently shown that quantitative modeling of a well-controlled system (Pella and Colombo, 1972) allows high-precision analyses to be obtained.

While other techniques of sample preparation have been described in connection with the detection of oxygen isotopic tracers (*e.g.*, Rittenberg and Ponticorvo, 1956), repeated attempts (reviewed by Brenninkmeijer, 1983) to improve the precision of these procedures to levels suitable for the measurement of natural variations in isotopic abundances have been unsuccessful.

Intramolecular isotopic analyses. The determination of isotopic abundances at specific positions within organic molecular structures can be of great interest. Combustion is useless in such investigations, its convenience and efficiency being matched by its lack of selectivity. Special degradative techniques must be devised to produce CO_2 quantitatively from the positions of interest. Investigations of this type have included pyrolytic degradations of two-carbon molecules (Meinschein *et al.*, 1974; DeNiro and Epstein, 1977), the use of specific reactions capable of selective but quantitative attack at specific functional groups (Abelson and Hoering, 1961; Vogler and Hayes, 1979, 1980), and more complicated schemes involving multistep degradative sequences (Monson and Hayes, 1982). Extraordinarily detailed

information can be obtained, but the quantities of material required are large enough that the approach has thus far been utilized only with modern biological materials.

1.3 *Instrumentation*

Isotope-ratio mass spectrometers. The distinguishing characteristics of modern isotope-ratio mass spectrometers are (*i*) very high efficiency of ionization and very intense ion beams (*ii*) multiple-collector systems allowing simultaneous collection of two or more ion beams, and (*iii*) dual – or even triple – inlet systems designed to allow rapid exchange of one sample gas for another in the ion source of the instrument. All of these features, unique to isotope-ratio instruments, are of special importance.

If R represents an ion-current ratio, E the efficiency [(molecular ions at collector)/(molecules introduced)], and N the number of molecules required for a mass spectrometric measurement, the following equation specifies the maximum attainable precision of ratio measurement (where σ_R represents the standard deviation in the measurement of R):

$$\frac{\sigma_R}{R} = \frac{1+R}{\sqrt{ENR}} \qquad (1.1)$$

(Hayes *et al.*, 1977; also derived in section 4 of these notes). A ten-fold improvement in the precision of measurement will, thus, require a 100-fold increase in the product EN, the number of ions formed and collected. There is an inescapable requirement for a large number of ions (ion beams of 10^{-9} A and larger are common in isotope-ratio mass spectrometry; these are three orders of magnitude more intense than those commonly encountered in organic mass spectrometry). If sample requirements (N) are to be minimized, efficiency of ionization (E) must be maximized. Efficiencies approaching 10^{-3} ions/molecule are typical of modern instruments. These are very much higher than efficiencies in organic instruments, which must minimize the residence time of sample molecules in the heated ion source if degradation of thermally labile molecules is to be minimized. Modern instruments provide precisions (σ_R/R) of 10^{-5} and better. [Equation 1 is based on the assumption of a "perfect" signal-processing system, absolutely free of any noise sources, and thus defines a theoretical limit. Peterson and Hayes (1978) have discussed in detail the capabilities of real systems and have shown how closely they can approach this limit.]

Equation 1.1 was derived assuming that both ion beams were collected simultaneously. The procedure is obviously economical of time and sample molecules – no ions are "wasted" while the detector measures one beam at a time. A more fundamental advantage involves elimination of a noise source that would otherwise be crippling, namely variations in ion beam intensity over the course of a measurement. The first example of multiple collection involved dual collectors (Straus, 1941), and isotope-ratio mass spectrometry was stalled at that point for decades. Efficient isotopic analysis of carbon dioxide, however, requires simultaneous measurement of three ion beams, and instruments with three and more collectors and multiple signal-processing pathways are now relatively common.

The specialized techniques utilized in acquiring and processing data from isotope-ratio mass spectrometers have been summarized by Mook and Grootes (1973). [For more recent notes on this subject, see section 6] Particular problems arise in the case of hydrogen. The mass-3 ion beam in the spectrum of H_2 is due not only to HD, the species of interest, but also to H_3, a product of ion-molecule collisions occurring in the ion source of the mass spectrometer. A correction must be determined and applied. The relevant procedures have recently been evaluated, and a new alternative introduced, by Schoeller *et al.* (1983).

Special inlet systems. The great instrumental contribution of Urey's Chicago group was the dual inlet system allowing quick change-over of samples (McKinney *et al.*, 1950; see also Halsted and Nier, 1950). Contributions from a second family of noise sources – those associated with longer-term variations in ion source performance, mass spectral background, and inlet contaminants – are thus minimized. The application of modern vacuum technology to the design of clean inlet systems has yielded a further advance.

Sample Requirements. Many variations in natural abundance can be observed and satisfactorily measured if the precision of ion-current ratio measurement (σ_R / R) is 10^{-4}. Equation 1.1 correctly indicates that samples less than one nanomole in size should allow measurements of this quality if E is 10^{-4} or greater. Unfortunately, practical sample requirements are much higher. Operation of most practical inlet systems requires at least 100 nanomoles of gas, though dilution of samples with helium (Schoeller and Hayes, 1975) can significantly reduce that requirement.

Future developments. An approach to elimination of inlet systems and consequent attainment of the sensitivities noted above has been demonstrated by

Matthews and Hayes (1978), who directly connected a mass spectrometer and a gas chromatograph by way of an in-line combustion oven and gas-purification system. Precisions great enough to allow measurement of natural variations in isotopic abundances were obtained using samples as small as 20 nanomoles in spite of the facts that the GC-MS interface system transmitted only 10% of the sample gas and that the ion source had an efficiency of only 10^{-8} ions/molecule under the conditions of the experiment. Very great improvements in this approach are obviously possible.

2. Rudiments of Isotopic Chemistry

2.1 *Elementary Calculations*

Notation of isotopic abundances. Absolute isotopic abundances are commonly noted in terms of *atom percent*. For example,

$$\text{atom percent } {}^{13}\text{C} = \left(\frac{{}^{13}\text{C}}{{}^{12}\text{C} + {}^{13}\text{C}} \right) 100 \qquad (2.1)$$

A term more convenient in many calculations is *fractional abundance*

$$\text{fractional abundance of } {}^{13}\text{C} = {}^{13}F = \left(\frac{{}^{13}\text{C}}{{}^{12}\text{C} + {}^{13}\text{C}} \right) \qquad (2.2)$$

(in mathematical expressions dealing with isotopes, it is convenient to use left superscripts to designate the isotope of interest, thus avoiding confusion with exponents and retaining the option of defining subscripts). Isotope ratios are also measures of the absolute abundance of isotopes; they are usually arranged so that the more abundant isotope appears in the denominator

$$\text{“carbon isotope ratio”} = \frac{{}^{13}\text{C}}{{}^{12}\text{C}} = {}^{13}R \qquad (2.3)$$

Interconversion between fractional abundances and isotope ratios is straightforward

$${}^{13}R = \frac{{}^{13}F}{1 - {}^{13}F} \qquad {}^{13}F = \frac{{}^{13}R}{1 + {}^{13}R} \qquad (2.4)$$

Oxygen provides an example of the expressions applicable to elements with more than two isotopes:

$${}^{18}R = \frac{{}^{18}\text{O}}{{}^{16}\text{O}} = \frac{{}^{18}F}{1 - {}^{17}F - {}^{18}F} \qquad (2.5)$$

$${}^{18}F = \frac{{}^{18}\text{O}}{{}^{16}\text{O} + {}^{17}\text{O} + {}^{18}\text{O}} = \frac{{}^{18}R}{1 + {}^{17}R + {}^{18}R} \qquad (2.6)$$

It is clear that ${}^{18}F$ is a function not only of ${}^{18}R$, but also ${}^{17}R$, and it would appear that it is not possible to calculate ${}^{18}F$ with perfect accuracy given only ${}^{18}R$. In many cases, however, ${}^{17}R$ and ${}^{18}R$ are functionally related (Santrock *et al.*, 1985) and an accurate, though laborious, calculation can be made. A useful approximation has been introduced by Wedeking and Hayes (1983), who noted that variations in the ratio ${}^{17}R$ /${}^{18}R$ are much smaller than variations in ${}^{17}R$ alone, and rewrote equation 2.6 as

$${}^{18}F = \left(1 + \frac{{}^{17}R}{{}^{18}R} + \frac{1}{{}^{18}R} \right) \qquad (2.7)$$

Insertion of a representative value for ${}^{17}R/{}^{18}R$ then allows convenient calculation of ${}^{18}F$ with good accuracy over a considerable range. Similar approximations will, no doubt, serve with other polyisotopic elements, and are of interest because the calculation of fractional abundances is often required.

The delta notation. Because most isotopic measurements *are* differential measurements, and because the interesting isotopic differences between natural samples usually occur at and beyond the third significant figure of the isotope ratio, it has become conventional to express isotopic abundances using a differential notation. The “uninteresting” (*i.e.*, unchanging) portions of the isotopic abundance are, in this way, not around to clutter the report, confuse the reader’s mind, or tax the investigator’s memory. To provide a concrete example, it is far easier to say – and remember – that the isotope ratios of samples A and B differ by one part per thousand than to say that sample A has 0.3663 %^{15}N and sample B has 0.3659 %^{15}N. The notation that provides this advantage is indicated in general form below. This means of describing isotopic abundances was first used by Urey (1948) in an address to the American Association for the Advancement of Science and first formally defined by McKinney *et al.* (1950).

$$\delta^{\text{A}}\text{X}_{\text{std}} = 1000 \left(\frac{{}^{\text{A}}R_{\text{sample}} - {}^{\text{A}}R_{\text{std}}}{{}^{\text{A}}R_{\text{std}}} \right) = \left(\frac{\Delta R}{R} \right) 10^3 \qquad (2.8)$$

As noted in the second portion of the equation, this expression amounts to nothing but a relative difference expressed in parts per thousand. [See note in section 6 regarding the factor 10^3 and concluding that the definition favored by later authors, in which δ is defined simply as $\Delta R/R$, ought to be preferred.] More formally, it defines an explicit relationship between the abundance of isotope A of element X in a given sample and its abundance in a particular standard (designated by a subscript, here shown in general form as *std*). Values of $\delta^{A}X_{std}$ are usually expressed in parts per thousand. The corresponding symbol, ‰, is called "permil" (from the Latin *per mille* by analogy with *per centum*, percent). In the Russian literature, delta values have often been reported in percent. This has occasionally caused confusion, and care must be taken to multiply such values by 10 for comparison to values reported in permil.

The name or pronunciation given to δ might obviously be "delta" except that a colloquial, shortened version ("del") can occasionally be heard. In mathematics, del (or *nabla*) refers specifically to ∇, the gradient vector operator. Anyone tempted to foster confusion by referring to the isotopic δ as del rather than delta should first consider the limerick on this subject by Harmon Craig [see section 6].

2.2 *Mass-Balance Calculations*

Master equation. Mass-balance calculations are of general importance in isotopic studies. Examples include (*i*) the calculation of isotopic abundances in pools derived by the combination of isotopically-differing materials, (*ii*) isotope-dilution analyses, and (*iii*) the correction of experimental results for the effects of blanks. A single master equation is relevant in all of these cases. Without approximation, we can write

$$n_T F_T = n_1 F_1 + n_2 F_2 + \ldots \qquad (2.9)$$

where the *n* terms represent molar quantities of the element of interest and the *F* terms represent fractional isotopic abundances. The subscript T refers to total sample derived by combination of subsamples 1, 2, ... *etc.* The same equation can be written in approximate form simply by replacing the *F* values with delta values. The magnitude of the error introduced by the approximation depends on the element, but even for carbon, errors will be less than 0.02 permil for most calculations involving only natural materials. Because delta is based on *R* instead of *F*, however, accurate treatment of any calculation involving highly enriched or depleted materials usually requires use of the exact form of the equation.

Isotope dilution. In isotope-dilution analyses, sample 1 might represent a material that could be sampled but not quantitatively isolated (say, total body water) while sample 2 would represent an isotopic spike. All of the isotopic abundances would be known, as would the value of n_2, but it would be of interest to determine n_1. In such cases, the standard equation for isotope-dilution analyses can be derived simply by substituting $n_T = n_1 + n_2$. Exact solution for n_1 yields

$$n_1 = n_2 \frac{F_2 - F_T}{F_T - F_1} \qquad (2.10)$$

Blank corrections. When a sample has been contaminated during its preparation by contributions from an analytical blank, the isotopic abundance actually determined during the mass spectrometric measurement is that of the sample plus the blank. Using T to represent the sample prepared for mass spectroscopic analysis and s and b to represent the sample and blank, we can write

$$n_T F_T = n_s F_s + n_b F_b \qquad (2.11)$$

Substituting $n_s = n_T - n_b$ and rearranging yields

$$F_T = F_s - \frac{n_b (F_s - F_b)}{n_T} \qquad (2.12)$$

an equation of the form $y = a + bx$. If multiple analyses are obtained, plotting F_T (or δ_T) *vs.* $1/n_T$ will yield the accurate (*i. e.*, blank-corrected) value of F_s (or δ_s) as the intercept. A method for assessment of the uncertainties in F_s or δ_s is demonstrated in section 5 of these notes.

2.3 *Isotope Effects*

General terms. Figure 2 schematically illustrates the difference between an *isotope effect* – a physical phenomenon – and an isotopic *fractionation* – an observable quantity. As noted in Figure 3, an *equilibrium* isotope effect will cause the heavy isotope to accumulate in a particular component of a system at equilibrium. The rule (or regularity) for such effects is that "the heavy isotope goes preferentially to the chemical compound in which the element is bound most strongly" (Bigeleisen, 1965). Thus, ^{13}C accumulates in the bicarbonate ion in the example shown (Deuser and Degens, 1967; Wendt, 1968) and, when CO_2 is equilibrated with H_2O and oxygen atoms are exchanged (by way of carbonate intermediates), ^{18}O is concentrated in the CO_2 (Friedman and O'Neill, 1977).

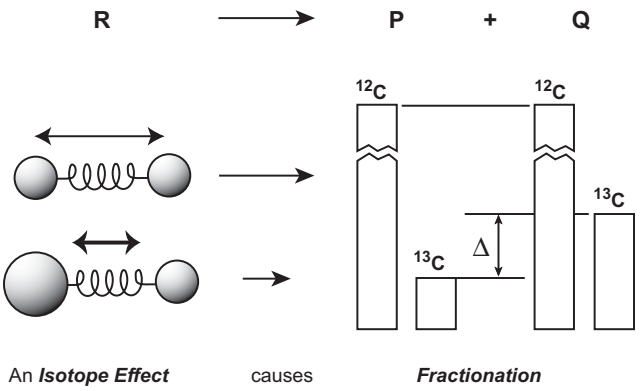


Figure 2. Schematic representation of the relationship between an isotope effect (a physical phenomenon) and the occurence of isotopic fractionation (an observable quantity).

Vapor-pressure isotope effects provide an example of a second kind of equilibrium isotope effect. They are, for the most part, *normal* (species containing the lighter isotope are more volatile; for numerous examples, see Friedman and O'Neil, 1977), though *inverse* vapor-pressure isotope effects in which species containing the heavy isotope are more volatile are frequently encountered when D replaces H at molecular positions not affected by polar interactions in the condensed phase. Thus, CH_3ND_2 is less volatile than CH_3NH_2, but CD_3NH_2 is more volatile than CH_3NH_2 (for a review of this subject, see Hopfner, 1969).

A *kinetic isotope effect* (KIE) is said to occur when the rate of a chemical reaction is sensitive to atomic mass at a particular position in one of the reacting species. If the sensitivity to isotopic substitution exists at the position at which chemical bonding changes during the reaction, the kinetic isotope effect is described as *primary*. A *secondary* kinetic isotope effect is one in which the sensitivity to isotopic substitution occurs at an atomic position not directly involved in the reaction itself. A *normal* KIE is one in which the species containing the lighter isotope reacts more rapidly. Almost all primary KIEs involving elements heavier than H are normal; some secondary KIEs and some primary KIEs involving H are *inverse*. Kinetic isotope effects are intensively studied for the information that they can provide about mechanistic details of reaction pathways, and the field has been the subject of many excellent reviews (Rock, 1975; Cleland *et al.*, 1977; Melander and Saunders, 1980).

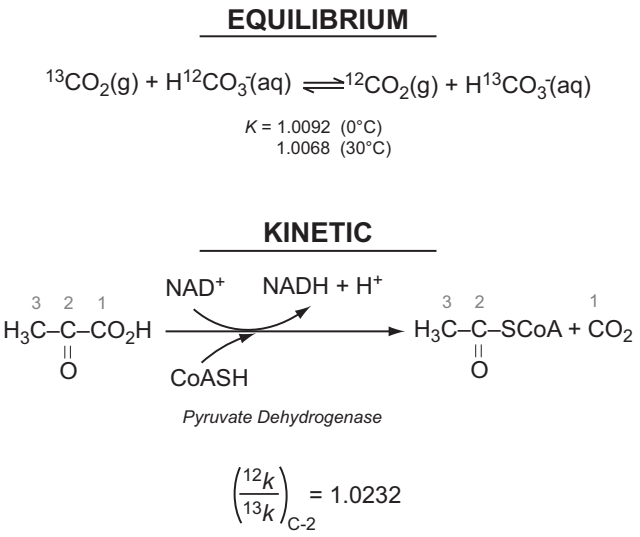


Figure 3. Examples of equilibrium and kinetic isotope effects.

An isotope effect is not directly observable. Its existence must be inferred from its effect on isotopic abundances. As noted in Figure 2, the presence of an isotope effect in a reacting system is likely to lead to an isotopic *fractionation*, an observable effect generally described in terms of enrichment or depletion of the heavy isotope. A useful and memorable colloquial usage has developed in the description of relative isotopic abundances. Substances enriched in the heavy isotope are said to be "heavy," those depleted are said to be "light." These terms are inherently relative, not absolute, and care must be taken that the point of comparison is clear whenever these terms are used.

Fractionation factors. As noted in Figure 3, the magnitude of an equilibrium isotope effect can be represented by an equilibrium constant. Alternatively, a *fractionation factor* can be reported. Virtually without exception, fractionation factors refer to equilibrium constants of (sometimes hypothetical) exchange reactions in which a single atom is exchanged between two species. The equilibrium constant for such a reaction is identically equal to the ratio of isotope ratios for the exchanged positions, and the fractionation factor is usually presented in this simplified form. For the exchange reaction shown in Figure 3, for example, the fractionation factor is given by

$$\alpha_{HCO_3^-/CO_2} = \frac{(^{13}C/^{12}C)_{HCO_3^-}}{(^{13}C/^{12}C)_{CO_2}} \qquad (2.13)$$

and is numerically equal to the equilibrium constant. The use of fractionation factors cuts across the three communities commonly involved in isotopic studies, namely the geochemists, the biochemists, and the physical chemists. Fortunately, all use the term to describe a

ratio of isotope ratios. Most geochemists have adopted a reasonably uniform system of subscripting and, virtually without exception, assign the symbol α to the fractionation factor. Helpful discussions are provided by Friedman and O'Neill (1977) and by Fritz and Fontes (1980). There is no established convention regarding placement of components in the numerator and denominator. A notation like that shown in eq 2.13, in which the numerator and denominator are specified by a subscript attached to α, is therefore preferred.

The magnitude of a kinetic isotope effect can be most simply represented in terms of a ratio of rate constants. When the rate constant pertaining to the species containing the light isotope is placed in the numerator, the numerical value of the ratio is greater than unity if the KIE is normal. For the example shown in Figure 3, the species containing carbon-12 at position 2 reacts 1.0232 times more rapidly that the species containing carbon-13 at that position. This would be termed "a 2.3% isotope effect." Theoretical maximum values for kinetic isotope effects have been reported by Bigeleisen and Wolfsberg (1958). For replacement of protium by deuterium, the primary isotope effect can be as large as 18-fold, or 1700%; for replacement of carbon-12 by carbon-13, 25%; or for replacement of oxygen-16 by oxygen-18, 19%. Replacement of H by D can yield secondary kinetic isotope effects as large as 100%; secondary effects for heavier atoms rarely exceed 1%.

2.4 *Isotopic Fractionations*

The relationship between the magnitude of an isotope effect and the isotopic fractionation that it might cause can be complex. The first thing to note, however, is that even the largest isotope effect possible will not cause any fractionation if the reaction with which it is associated occurs quantitatively. In fact, if we imagine a cascade of reactions in which reactant A irreversibly yields product B, which in turn yields product C, and so on until product Z is finally obtained, it can happen that almost every reaction in the cascade has a large isotope effect but that no fractionation will be observed between A and Z when the system is at steady state. The only requirements for this seemingly unlikely event are that no isotope effect is associated with A → B and that each successive product has no possible fate other than being carried forward in the reaction sequence. Then, as in a pipeline, everything that goes in – including neutrons – will eventually have to come out. It would be said, in such a case, that the isotope effects in the reactions linking B to Z "Were not *expressed*."

Closed systems. An isotopic fractionation will, however, always be observed when a reaction has an isotope effect and the formation of product is not quantitative. Figure 4 depicts a "closed system" in which the famous chemical reaction R → P is occurring. The system is termed "closed" because no material crosses its boundaries. As the reaction proceeds (irreversibly) from onset to completion, the fractional yield of P varies from 0.0 to 1.0 and the isotopic compositions of the components of the system follow the curves plotted in the graph shown in Figure 4. The first-formed product is depleted in the heavy isotope but, at completion (*i. e.*, at yield = 1.0), the isotopic composition of the pooled product (represented by curve P) must match that of the starting material. The preferential utilization of light reactant species enriches the residual reactant in the heavy isotope (see curve R). The isotopic fractionation between the increment of product forming at any instant and the residual reactant is fixed by the magnitude of the isotope effect. As a result, the curve denoting the isotopic composition of successively formed product increments (P') is separated from curve R by a constant difference.

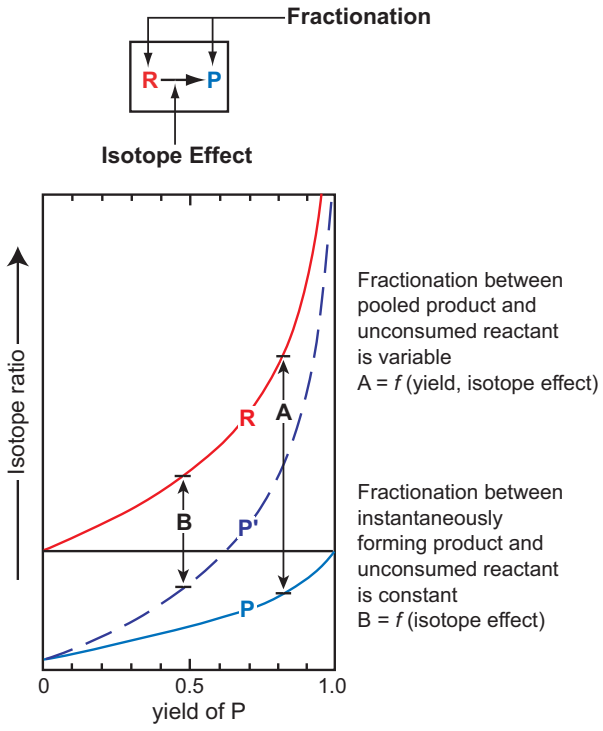


Figure 4. Schematic representation of a closed system and the isotopic fractionations occurring within it as a reaction proceeds to completion. Curve P' represents the isotopic composition of the instantaneously-forming product, and P represents the isotopic composition of the pooled product.

The precise functional relationships between isotopic compositions and yields have been determined by simultaneous solution of the integrated rate equations and are summarized in the landmark review by Bigeleisen and Wolfsberg (1958). In exact form, they write (where h and 1 designate heavy and light isotopes, and isotope ratios place the heavy isotope in the numerator)

$$\frac{^{h}k}{^{l}k} - 1 = \frac{\ln\left(R_{rf} / R_{ro}\right)}{\ln\left(\frac{(1-f)(1+R_{ro})}{1+R_{rf}}\right)} \qquad (2.14)$$

where f is the fractional yield ($f \rightarrow 1$ as the reaction proceeds) and the subscripts designate initial reactant (ro) and reactant remaining when the fractional yield = f (rf). Given R_{ro} (the initial isotopic composition of the reactant) and $^{h}k/^{l}k$ (the isotope effect), this equation can be solved to yield R_{rf} as a function of f.

Alternatively, observation of the isotopic composition of the reactant can be used to determine the isotope effect. A useful approximate form has been described by Mariotti *et al.* (1981)

$$\varepsilon = \frac{\delta_{rf} - \delta_{ro}}{\ln(1-f)} \qquad (2.15)$$

where $\varepsilon = [(^{h}k/^{l}k) - 1]10^{3}$. If δ_{rf} is plotted as a function of $\ln(1-f)$, the value of ε will be given by the slope of the line.

The isotopic composition of the pooled product is given in exact form (Bigeleisen and Wolfsberg, 1958) by

$$\left(\frac{^{h}k}{^{l}k} - 1\right)\ln(1-fc) = \ln\left(1 + \frac{R_{ro} - R_{pf}}{R_{ro}} \times \frac{fc}{1-fc}\right) \qquad (2.16)$$

where the subscript pf designates the product accumulated when the fractional yield = f and c is a correction factor [$c = (1 + R_{ro})/(1 + R_{pf})$] allowing for finite abundance of the heavy isotope. Manipulation of this equation allows (*i*) evaluation of the isotope effect given the isotopic compositions of reactant and products or (*ii*) prediction of isotopic fractionations given the isotope effect.

The corresponding approximate form presented by Mariotti *et al.* (1981) is

$$\delta_{pf} = \delta_{ro} - \frac{\varepsilon(1-f)\ln(1-f)}{f} \qquad (2.17)$$

The coefficient for ε on the right-hand side of this equation approaches –1 as f approaches 0. At very low yields, therefore, $\delta_{pf} \rightarrow \delta_{ro} + \varepsilon$. The isotope effect can again be evaluated from the slope of a straight line, in this case when δ_{pf} is plotted as a function of $(1-f)\ln(1-f)/f$.

Rayleigh distillation. Fractionations like those depicted in Figure 4 can also occur in some circumstances when an equilibrium isotope effect accompanies the reversible interconversion of R and P. It is necessary only that P be removed from the system as it is formed, usually by incorporation in a separable or nonreactive phase. Geochemists describe such processes as Rayleigh distillations. An example is provided by the loss of precipitation from a cloud of atmospheric water vapor. In this case, due to vapor-pressure isotope effects, the first-formed product (the first water to condense and fall from the cloud as rain or snow) will be heavier than the "reactant" (the bulk of the water vapor in the cloud), and the residual reactant will, accordingly, be lighter. Successive increments of precipitation will always be heavy relative to the residual water vapor, but will be increasingly lighter as the water enriched with the heavy isotopes (of both H and O) is preferentially removed from the cloud. As a result, precipitation near the equator (the major area of cloud formation) is heavy relative to that at high latitudes (where clouds are depleted in the heavy isotopes). Because the product is heavy relative to the reactant, the curves representative of this particular phenomenon bend down rather than up, but the functional relationships governing isotopic compositions in this and other Rayleigh distillations are the same as those describing fractionation by a kinetic isotope effect in a closed system. The parameter ε is related to the equilibrium fractionation factor in this case by

$$\alpha_{P/R} = R_{pe}/R_{re} = 1 + 10^{-3}\varepsilon \qquad (2.18)$$

where the e subscripts designate equilibrium values for the isotopic compositions of the reactant and product.

Open systems. Many interesting natural systems are open, not closed. That is, reactant is constantly added and products are constantly withdrawn. A system of this type is schematically depicted in Figure 5. For the special case in which the removal of products exactly compensates for the addition of reactant, the amount of material in the reaction chamber is constant and the calculation of isotopic fractionations is straightforward. A branch point in an enzymatic reaction network provides an example of such an open system, and the functional relationships governing isotopic fractionations

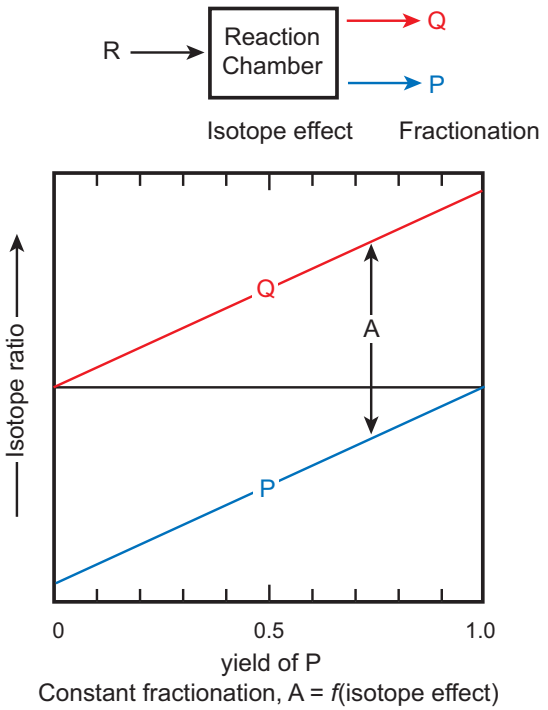


Figure 5. Schematic representation of an open system and the isotopic fractionation occurring within it as a function of the division of R (the input) between two product streams (one of which might be unreacted R leaving the system), P and Q.

have been summarized in that connection by Monson and Hayes (1980).

It is known (*i*) that the isotopic fractionation between the products is controlled by the isotope effects and (*ii*) that material is neither created nor destroyed in the system. Provided only that the reaction chamber is stirred rapidly enough that the isotopic compositions of R, P and Q (see Figure 5) are homogeneous, the treatment of such open systems is independent of whether an equilibrium or kinetic isotope effect is involved. The two conditions outlined above provide two independent equations.

The first equation establishes the isotopic fractionation between the products. For a system at equilibrium, we write

$$R_{pe} = \alpha_{P/R} R_{re} \text{ and } R_{qe} = \alpha_{Q/R} R_{re} \qquad (2.19)$$

The isotopic fractionation observed between the products is then

$$\frac{R_{pe}}{R_{qe}} = \frac{\alpha_{P/R}}{\alpha_{Q/R}} \qquad (2.20)$$

For a system in which the isotopic fractionation is due to a kinetic isotope effect, the same equations are applicable if ${}^{h}k/{}^{l}k$ ratios are substituted for α values. If, as noted in the caption to Figure 5, the product designated here as Q were, in fact, simply unutilized reactant, equations 2.19 and 2.20 (and 2.21 – 2.23) could be simplified by replacement of Q by R' and replacement of $\alpha_{Q/R}$ by unity.

The second equation expresses conservation of mass

$$F_r = f_p F_p + (1 - f_p) F_q \qquad (2.21)$$

and is identical for systems at equilibrium and under kinetic control. The coefficient f_p is the fraction of material leaving the system in the form of P (*i.e.*, the fractional yield of P).

Simultaneous solution of equations 2.20 and 2.21 is straightforward but takes a complicated form because of the necessary distinction between isotope ratios and fractional abundances. Casting the exact result in terms of the delta notation is even more complicated, and approximate forms are often used. Specifically, we can write

$$\delta_p = \delta_r + (1 - f_p)\varepsilon \qquad (2.22)$$

and

$$\delta_q = \delta_r - f_p \varepsilon \qquad (2.23)$$

where $\varepsilon = [(\alpha_{P/R}/\alpha_{Q/R}) - 1]10^3$. These equations yield the graph shown in Figure 5. An exact treatment shows that the lines representing isotopic compositions as a function of the division of R between P and Q (Figure 5) are not perfectly straight, but it is a characteristic of such systems that the fractionation of isotopes between product streams is, for all practical purposes, constant.

Measures of fractionation. It is often necessary to discuss the isotopic contrast between two samples for which delta values are known. A crude expression is sometimes used

$$\Delta_{A-B} = \delta_A - \delta_B \qquad (2.24)$$

but the symbol Δ is not defined in the same way by all authors, and, as noted by Friedman and O'Neill (1977), a single mechanism of fractionation characterized by a specific fractionation factor can yield different values for $\delta_A - \delta_B$. In contrast, the following expression yields α directly and is both simple and exact.

$$\alpha_{A/B} = (\delta_A + 1000)/(\delta_B + 1000) \qquad (2.25)$$

To express the isotopic contrast in parts per thousand, it is best (Friedman and O'Neill, 1977; Fritz and Fontes,

1980) to use the quantity $1000\ln\alpha$, which has further theoretical significance in that the relationship between α and T (absolute temperature) can often be fit to an expression of the form

$$1000\ln\alpha = a + b/T + c/T^2 \qquad (2.26)$$

The following approximations can also be noted

$$1000\ln\alpha \cong (\alpha - 1)1000 \cong \delta_A - \delta_B \qquad (2.27)$$

3. Primary Standards of Stable Isotopic Abundances

Isotope ratios for the presently accepted primary standards are summarized in Table 1. Given a "delta value," reference to these values will allow calculation of an absolute isotopic abundance. Notably, however, the isotope ratios of the standards – which are certainly the most carefully studied materials available – are quite uncertain. For the elements listed in Table 1, the accuracy with which any absolute isotopic abundance can be reported is substantially poorer than the precision with which relative isotopic abundances can be measured.

The question of working standards (*i. e.*, materials for routine laboratory use) has been a thorny one. It is simple enough to prepare a sample of N_2 from air, and the availability of standard water samples from the International Atomic Energy Authority (IAEA) has been good, but other up-to-date standards have been scarce. More recently, however, the IAEA has expanded its list of available standards to cover all elements of interest and a substantial variety of matrices (IAEA, 1995). Earlier, Blattner and Hulston (1978) made a significant contribution to the development of carbonate standards by reporting results from a large intercalibration exercise focused on oxygen isotopes. Harding Iceland Spar, a standard that has been widely used on an informal basis, has also been proposed as a formal standard (Landis, 1983).

More fundamentally, the NBS-19 sample prepared by Friedman *et al.* (1982) has gained acceptance as a new effective primary standard with status equivalent to VSMOW, replacing, in effect, PDB (although the position of the zero point of the scale is not to be changed). Values assigned to the isotopic compositions of this material on the VPDB scales for C and O are: $\delta^{13}C_{VPDB} = +1.95‰$, $\delta^{18}O_{VPDB} = –2.20‰$.

Coplen and Kendall (1982) have prepared two sets of standards in the form of gaseous CO_2. The availability of standards in this directly-usable form is particularly important because variations associated with sample-preparation procedures are avoided. Comparisons of these and many other standards have been reported by Coplen *et al.* (1983), and by Gonfiantini (1984). Particularly notable in the former compilation is a revised assignment for the isotopic composition of NBS-22, a standard petroleum sample. Coplen *et al.* assign δ_{PDB} (NBS-22) = –29.63‰, a substantial change from the previously accepted value of –29.4‰ (Silverman, 1964). There is some reason for concern, however, because yet another intercalibration exercise (Schoell *et al.*, 1983) concluded that the correct isotopic composition for NBS-22 is –29.81‰ *vs.* PDB, and it is reasonable to ask whether this material can be regarded as suitable for calibration of highly precise isotopic analyses.

The need for an abundantly available and certainly homogenous sulfur isotopic standard has been filled by development of the IAEA-S-1 standard (Ag_2S). By analogy with carbon, the zero point of the sulfur isotopic scale is still to be set by the initial standard, troilite from samples of the Canyon Diablo meteorite (abbreviated CDT). The $\delta^{34}S$ value of IAEA-S-1 is –0.30‰ on this scale. Results calibrated by reference to IAEA-S-1 are to be reported *vs.* VCDT.

The practice of "normalizing" the results of isotopic analyses is now well established, and can be illustrated for the case of D/H measurements. Repeated measurements of a secondary standard ("SLAP," Standard Light Antarctic Precipitation) by many laboratories yield an average result of –424.9 ± 6.7‰ on the SMOW scale [reported uncertainty is the standard deviation of a population of 45 measurements, see Gonfiantini (1984) and, for an earlier compilation, Gonfiantini (1978)]. Nevertheless, repeated careful measurements of the absolute isotope ratios of SMOW and SLAP (Hagemann *et al.*, 1970; DeWit *et al.*, 1980; Tse *et al.*, 1980) have established that the true position of SLAP on the SMOW scale is much nearer –428‰ than –425‰. The difference in these values must be due to systematic errors inherent in the procedures employed for conventional differential measurements, and it has been concluded (Gonfiantini, 1978) that it will be useful simply to *define* δ^2H_{SMOW}(SLAP) = –428‰, and to recommend that results of differential analyses be adjusted accordingly (*i.e.*, each laboratory

ought to "stretch" or to "shrink" its hydrogen-isotopic scale so that it obtains –428‰ as the result of analyses of SLAP).

The success of the normalization procedure can be demonstrated by consideration of results of analyses of a third reference water sample, "GISP" (Greenland Ice Sheet Precipitation). Referred only to SMOW by conventional differential measurements, a value of –188.2 ± 2.7‰ [uncertainty is standard deviation of a population of 41 measurements (Gonfiantini. 1984)] is obtained. After normalization, the same set of analyses yielded –189.7 ± 1.1‰ (Gonfiantini, 1984). The second result is presumably more accurate, and obviously more precise.

The defined oxygen isotopic composition of SLAP is –55.5‰ *vs.* SMOW (Gonfiantini, 1978; 1984). A similar process of normalization is to be applied to oxygen isotopic analyses. The standards described by Blattner and Hulston allow an equivalent approach to be taken in the analysis of carbonates.

A second new technique of significance in the definition and calibration of isotopic standards has been introduced by Santrock, Studley, and Hayes (1985), who have reconsidered isotopic calculations and analyses based on the mass spectrum of carbon dioxide. In particular, they have (*i*) incorporated new information regarding natural covariations of ^{17}O and ^{18}O, (*ii*) developed an approach allowing exact calculation of isotopic abundances from observed ion current ratios, and (*iii*) described methods for dealing with samples specifically enriched in a single oxygen isotope.

The first of these innovations was based on the "terrestrial oxygen line" defined and discussed by Matsuhisa *et al.* (1978). This line is defined by a relationship of the form

$$\frac{^{17}R_1}{^{17}R_2} = \left(\frac{^{18}R_1}{^{18}R_2}\right)^a \qquad (3.1)$$

where the subscripts 1 and 2 pertain to any two substances and the exponent a quantifies the relationship between variations in ^{17}O and those in ^{18}O. The value of a varies slightly depending on the chemical mechanisms by which substances 1 and 2 are related. Matsuhisa *et al.* (1978) showed that $a = 0.516$ was broadly representative. Rearrangement of eq. 3.1 yields

$$^{17}R_1 = {}^{18}R_1^a\left(\frac{^{17}R_2}{^{18}R_2^a}\right) \qquad (3.2)$$

This expression shows that, once ^{17}R and ^{18}R are known accurately for any single terrestrial material (*i. e.*, $^{17}R_2$ and $^{18}R_2$ in eq. 3.2), the value of ^{17}R can be calculated for any other material provided that its value of ^{18}R is known. Santrock *et al.* (1985) summarized this relationship by writing

$$^{17}R = {}^{18}R^aK \qquad (3.3)$$

where K is equal to the parenthesized term in eq. 3.2. Writing in 1985, Santrock and coworkers could only estimate the value of K, since there was no single terrestrial material in which the absolute values of both ^{17}R and ^{18}R were then known accurately. The value they provided can now be refined based on the value of $^{17}R_{VSMOW}$ reported by Li *et al.* (1988). The revised, presently best-available value is $K = 0.0093704$.

4. Limits on the precision of mass spectrometric measurements of isotope ratios

Isotope ratios are calculated from observed ion-current ratios. Such observations can be corrupted by noise added to the signal in any part of its path. However, even if the transducer, amplifier, and analog-to-digital converter were perfect (completely free of noise), the precision attainable in all measurements would still be limited by "shot noise," an intrinsic property of all electrical signals. Modern signal-processing components approach perfection closely enough that shot noise is (or should be) the principal noise source in modern isotope-ratio measurement systems. For this reason, and because it will always separate the possible from the impossible in isotopic analysis, our quantitative treatment of precision will be based on a consideration of shot noise only.

The quantity of interest in isotopic measurements is δ, defined as:

$$\delta = \left(\frac{R_1}{R_2} - 1\right) \qquad (4.1)$$

where R is an isotope ratio (*e. g.*, $^{13}C/^{12}C$) and the subscripts denote two different materials which are being compared. The precision with which δ can be determined is best considered in terms of its standard devia-

Table 1. Isotopic Compositions of Primary Standards

Primary Standard	Isotope Ratio	Accepted Value (x 10^6) (with 95% confidence interval)	Notes
Standard Mean Ocean Water (SMOW)			a
	$^2H/^1H$	155.76 ± 0.10	b
	$^{18}O/^{16}O$	2005.20 ± 0.43	c
	$^{17}O/^{16}O$	379.9 ± 1.6	d
PeeDee Belemnite (PDB)			e
	$^{13}C/^{12}C$	11180 ±16	f
	$^{18}O/^{16}O$	2067.2 ± 2.1	g
	$^{17}O/^{16}O$	385.9 ± 1.6	h
Air (AIR)			i
	$^{15}N/^{14}N$	3676.5 ± 8.1	j
Canyon Diablo Troilite (CDT)			k
	$^{34}S/^{32}S$	45004.5 ± 9.3	l

Notes to Table 1:

a. SMOW was defined by H. Craig (1961). Although the initial definition was mathematical and no specific sample of "SMOW" existed, water with an isotopic composition corresponding to Craig's specifications has since been prepared and is distributed by the International Atomic Energy Agency, Vienna. Some confusion exists because it has developed (Coplen and Clayton, 1973) that the isotope ratios (at least $^2H/^1H$) in the water sample being distributed do not perfectly match those prescribed by the initial definition. In practice, the IAEA sample has superseded the initial SMOW. The isotope ratios tabulated here refer to "Vienna SMOW" or VSMOW.

b. In order to estimate the 95% confidence interval, I have assumed that the uncertainty reported (Hagemann *et al.*, 1970) was the standard error of the mean. Essentially identical values (155.75 ± 0.08 and 155.60 ± 0.12, respectively) have been reported by DeWit *et al.* (1980) and by Tse *et al.* (1980).

c. Baertschi (1976) documents a standard deviation of 0.45 $\times 10^{-6}$ for a population of five independent observations.

d. The tabulated value is the result of a new measurement by Li *et al.* (1988).

e. The PDB standard was defined and described by Urey *et al*. (1951). The supply has long since been exhausted. Numerous secondary standards have been defined and carefully compared to PDB or to other well-known materials, and all carbon isotope ratios are still referred to PDB.

f. Computed from $^{13}R_{NBS\text{-}19}$ = 0.011202 (Zhang and Li, 1990) and the definition of VPDB, namely $\delta^{13}C_{VPDB(NBS\text{-}19)}$ = +1.95‰. Zhang and Li (1990) report an uncertainty of ± 28 but obtain it by summing terms which include systematic errors. The result appears to be a significant overestimate. The value of ± 16 chosen here results from adding their error components in quadrature.

g. This number refers to the oxygen *in the mineral*. Isotopic fractionation occurs during the preparation of CO_2 (note: two oxygen atoms) from $CaCO_3$ (note: three oxygen atoms). The value reported here is based on $^{18}R_{VSMOW}$ and the observation that CO_2 equilibrated with VSMOW at 25°C is depleted in ^{18}O by 0.27‰ relative to CO_2 derived from the treatment of VPDB with 100% H_3PO_4 at 25°C (Hut, 1987). Relevant fractionation factors are $^{18}\alpha_{CO2/H2O}$ = 1.0412 (Friedmann and O'Neil, 1977) and $^{18}\alpha_{CO2/calcite}$ = 1.01025 (Friedmann and O'Neil, 1977). In order to estimate the uncertainty in $^{18}R_{VPDB}$, the standard deviations of $^{18}R_{VSMOW}$, $^{18}\delta_{VSMOW\text{-}CO2}$(PDB-$CO_2$), $^{18}\alpha$(CO_2/H_2O, 25°), and $^{18}\alpha$(CO_2/calcite, 25°) have been taken as 2 $\times 10^{-7}$, 0.01‰, 0.0005, and 0.0001, respectively.

h. Calculated from $^{18}R_{PDB}$ by means of eq. 3.3.

i. There is no evidence that N_2 in air is isotopically inhomogeneous (Junk and Svec, 1958; Sweeney *et al.*, 1978).

J. In order to estimate the 95% confidence interval I have assumed that the uncertainty reported by Junk and Svec (1958) was the standard error of the mean.

k. An important early review of sulfur isotopic standards is presented by Ault and Jensen (1962). They cite MacNamara and Thode (1950) as first to investigate meteoritic troilite, which was subsequently adopted as the primary standard. Ault and Jensen note that workers in the Soviet Union utilized the Sikhote-Alin meteorite as their source of meteoritic troilite. Very recently, the use of these meteoritic standards has been criticized by Nielsen (1984), who notes that the materials are not perfectly homogenous isotopically and that there may be a systematic difference between Canyon Diablo and Sikhote Alin.

l. The value specified is that chosen, somewhat arbitrarily, by Jensen and Nakai (1962). The uncertainty reported here is the standard deviation of the mean of five individual values they considered. Nielsen (1984) has noted that corrections to the mass-66 ion current in the spectrum of SO_2 may have been inaccurate (contributions by $^{34}S^{32}S$ have been overlooked), and that $^{34}S/^{32}S$ may be as low as 0.043748, a value recently derived from observations of the spectrum of SF_6.

tion, σ_δ. Assuming only that errors in R_1 are not correlated with errors in R_2, we can write

$$\sigma_\delta^2 = \left(\frac{\partial\delta}{\partial R_1}\right)^2 \sigma_R^2 + \left(\frac{\partial\delta}{\partial R_2}\right)^2 \sigma_R^2 \qquad (4.2)$$

This expression is derived from the standard treatment of the propagation of errors. For example, if we are given $w = f(x, y, z)$, we know that the standard deviation of w will be related to the standard deviations of the quantities from which it is derived by the equation

$$\sigma_w^2 = \left(\frac{\partial w}{\partial x}\right)^2 \sigma_x^2 + \left(\frac{\partial w}{\partial y}\right)^2 \sigma_y^2 + \left(\frac{\partial w}{\partial z}\right)^2 \sigma_z^2 \qquad (4.3)$$

In equation 4.2, we have not specified separate standard deviations for R_1 and R_2 because both ion-current ratios can be assumed to have equal standard deviations (when R_1 and R_2 are nearly equal and are measured sequentially and identically, as is common in isotopic analysis).

The ion-current ratio, R, can, in turn, be expressed as a function of two quantities:

$$R = i_m/i_M \qquad (4.4)$$

where i denotes an ion current (expressed, for example, in amps, or coulombs/sec) and the subscripts m and M are introduced to specify the minor and Major ion beams (for example, masses 45 and 44 in a carbon-isotope ratio measurement). Following the approach of equation 4.3, we can write

$$\sigma_R^2 = \left(\frac{\partial R}{\partial i_m}\right)^2 \sigma_m^2 + \left(\frac{\partial R}{\partial i_M}\right)^2 \sigma_M^2 \qquad (4.5)$$

where σ_m and σ_M denote the standard deviations of the minor- and major-ion currents, respectively.

4.1 The random nature of ion currents

An ion current does have a standard deviation. For concreteness, consider specifically a current of 1.7×10^{-13} amps. This is a bit small by the standards of isotopic mass spectrometry, but it is of interest here because it is almost exactly 10^6 ions/sec (1.06×10^6 ion /sec, to be precise). If you observed this beam for exactly 1 msec you might expect to collect exactly 1060 ions. You would, *on average*. Careful observation would show, however, that the numbers of ions collected in a series of 1-msec intervals varied significantly. A plot of the frequency of observation of various numbers of ions would take the form shown in Figure 6, which is a plot of a normal distribution with a mean of 1060 and a standard deviation of 32.6.

These variable results arise because an ion current derives from a beam of discrete particles distributed randomly with respect to time. If a very small ion current were processed so that individual ions produced audible clicks at a loudspeaker, you would hear (with thanks to e. e. cummings)

tick, tick, tick, tick, tick, tick,tick tick,

not

tick, tick, tick, tick, tick, tick, tick, tick.

Time intervals between ions would follow the Poisson distribution, but collection of large numbers (as in Fig. 6) would always yield count totals following the normal distribution. The standard deviation of the distribution would always be $N^{0.5}$, where N is the average number of ions collected.

Even if you had a relatively large ion beam and, in one second, collected exactly 100,000,000 ions, you could *not* say, "I have measured this ion current with an uncertainty of 1 part in 10^8." The inescapably ran-

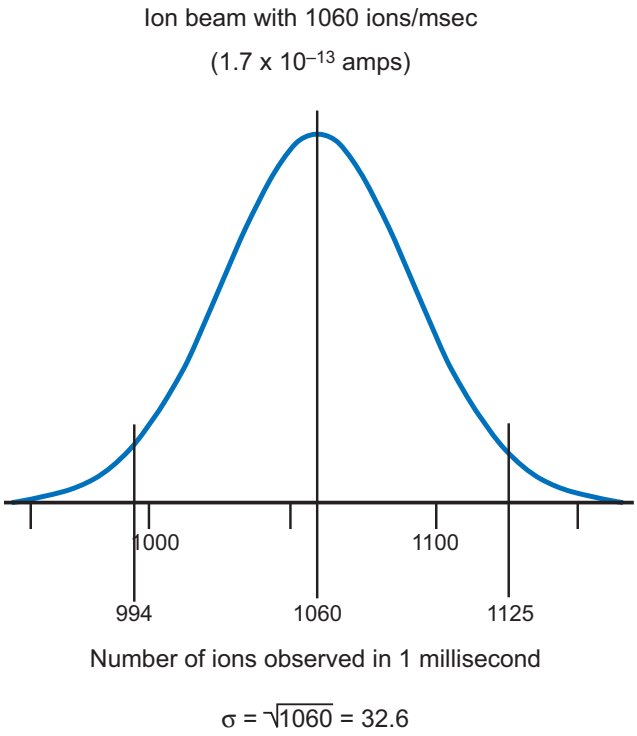


Figure 6. Probability distribution for observing *n* ions during any 1 millisecond interval.

dom distribution of time intervals between ions dictates that you might observe a different total in the next second and that the long-term average might not be exactly 100,000,000 ions/sec. Given the properties of the normal distribution, you know that 95% of your observations will fall within two standard deviations of the true mean and that, therefore, you can be 95% confident that the true value is within 2×10^4 ions/sec of 100,000,000. You could say, "I have measured this ion current with an uncertainty of 1 part in 10^4." In general, if you have collected N ions, the uncertainty will be 1 part in $N^{0.5}$.

4.2 *Sample requirements*

To come up with a quantitative relationship between σ_δ and ion currents or sample sizes, we work backward from equation 4.5, utilizing the relationship explained above. Evaluating the ∂_R/∂_i terms and substituting the results in equation 4.5, we obtain

$$\sigma_R^2 = R^2\left[\left(\frac{\sigma_m}{i_m}\right)^2 + \left(\frac{\sigma_m}{i_m}\right)^2\right] \qquad (4.6)$$

The terms on the right-hand side of this equation involve the squares of the relative standard deviations of the ions currents m and M. These can be recast as follows. An ion current is given by

$$i = Ne/t \qquad (4.7)$$

where i is a current expressed in amperes or coulombs/sec, N is the number of ions collected in a time interval, t (sec), and e, the electronic charge, is the charge (coulombs) carried by a single ion. We note that

$$\sigma_i^2 = \left(\frac{\partial i}{\partial N}\right)^2 \sigma_N^2 = \left(\frac{e}{t}\right)^2 N \qquad (4.8)$$

and that the relative standard deviation of an ion current is given by

$$\left(\frac{\sigma_i}{i}\right)^2 = \frac{1}{N} \qquad (4.9)$$

Equation 4.6 can thus be written as

$$\left(\frac{\sigma_R}{R}\right)^2 = \left(\frac{1}{N_\mathrm{m}}\right) + \left(\frac{1}{N_\mathrm{M}}\right) \qquad (4.10)$$

And we learn that the relative standard deviation of an ion-current ratio is a very simple function of the numbers of ions collected. If we recall that $R = N_\mathrm{m}/N_\mathrm{M}$, we can rewrite equation 4.10 in terms of only the major-beam ion current:

$$\left(\frac{\sigma_R}{R}\right)^2 = \frac{1}{N_\mathrm{M}}\left(\frac{1+R}{R}\right) \qquad (4.11)$$

This is useful because we can relate the number of major-beam ions quite simply to the performance of the mass spectrometer.

The sensitivity of a mass spectrometer can be described in terms of the number of ions collected per input molecule. This is often termed the "efficiency" of the mass spectrometer. To a very good approximation, the efficiency is independent of isotopic composition. That is, we can speak of the number of carbon dioxide ions appearing at the collector per input molecule without worrying about whether we're talking about $^{13}CO_2$ or $^{12}CO_2$. If we denote the efficiency by E and the number of molecules introduced by M, we can always write

$$N_\mathrm{M} = \frac{EM}{1+R} \qquad (4.12)$$

(when R is small, the right-hand side of this equation can be approximated by EM, but if we wished to consider a gas with, for example, $R = 1$, the form given here correctly indicates that only half the molecules would contribute to N_M). Equation 4.11 thus becomes

$$\left(\frac{\sigma_R}{R}\right)^2 = \frac{(1+R)^2}{EMR} \qquad (4.13)$$

To proceed to a master equation relating precision of isotope measurement and sample requirements, we need only fit this expression into equation 4.2.

Simplification of equation 4.2 is aided considerably by the fact that, in most high-precision isotopic measurements, R_1 and R_2 are nearly equal numerically (quite possibly differing only in the fourth significant figure). Thus, when we obtain $\partial\delta/\partial R_2 = -10^3 R^1/(R_2^2)$, we can recognize that this quotient will differ very little from $-10^3/R$, where R (no subscript) is a "generic isotope ratio;" for example, 0.01 for carbon-isotopic measurements. We then can write

$$\sigma_\delta^2 = 2\times10^6\left(\frac{\sigma_R}{R}\right)^2 = 2\times10^6\left(\frac{(1+R)^2}{EMR}\right) \qquad (4.14)$$

Table 2. Theoretical limits on the precision of isotopic measurements.

X	Sample gas	Ratio, *R*	Efficiency (ions/molecule)	Nanomoles of element X required[a] = 0.1‰	= 0.03‰
H	H_2	3/2 = 0.0003	10^{-5}	222	2200
C	CO_2	45/44 = 0.011	10^{-4}	0.31	3.1
N	N_2	29/28 = 0.007	10^{-4}	0.92	9.2
	CO_2	46/44 = 0.004	10^{-4}	1.6	16
O	O_2	33/32 = 0.0007	10^{-4}	8.8	88
	O_2	34/32 = 0.004	10^{-4}	1.6	16

[a]The fact that two moles of H, N, and O are required for each mole of sample gas has been taken into account.

thus producing an equation for the precision attainable in δ as a function of R (*i.e.*, the element of interest), the sample size (expressed in terms of M, a number of molecules), and E, the efficiency of the mass spectrometer. Recasting this expression with M as the dependent variable, we obtain

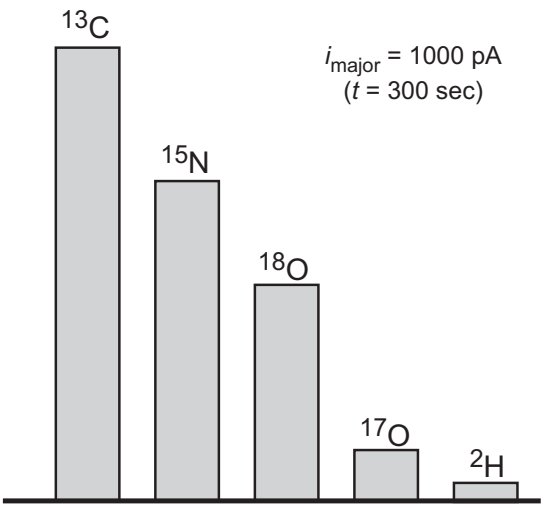
$$M = 2\times10^6 \frac{(1+R)^2}{\sigma_\delta^2 ER} \qquad (4.15)$$

and can derive table 2.

4.3 *Postscript: reality*

The effects of other noise sources are summarized in Figure 7, which is based on signal-to-noise ratios characteristic of typical ion-current-amplifier systems (Peterson and Hayes, 1978). As an example, consider the case of ^{13}C measurements. The figure shows that, if the major ion current is 1000 picoamperes (that is, 10^{-9} A), the beam carrying information about the abundance of ^{13}C will amount to 11.9 pA. If we observed those beams for 300 sec in each of two different samples, the maximal precision (*i.e.*, the precision calculated on the basis described above) obtainable in a measurement of δ would be 0.0095‰. If all components of the signal pathway were at the state of the art, the observed signal to noise ratio (S/N) would be nearly equal to that expected on the basis of ion statistics alone (the table at the base of the figure shows that the observed S/N would be 89% of the theoretical maximum S/N). The corresponding attainable precision for δ would be 0.0106‰, not 0.0095‰.

As minor ion currents become smaller, the effects of noise sources in the signal pathway become more important. In the case of hydrogen-isotope ratio measurements, for example, the same measurement which would be expected to yield a precision of 0.059‰ on the basis of ion-statistical limitations alone is found to yield a "real" precision more than three-fold lower, 0.19‰.



i_{minor}, pA	11.9	7.32	4.08	0.75	0.31
$(\sigma_\delta)_{\sqrt{n}}$, ‰	0.0095	0.012	0.016	0.038	0.059
$\frac{(S/N)_{S/N}}{(S/N)_{\sqrt{n}}}$	0.89	0.84	0.76	0.45	0.31
$(\sigma_\delta)_{S/N}$, ‰	0.0106	0.014	0.021	0.084	0.190

Figure 7. Effects of noise sources on obtainable precision for isotopic measurements.

5. Uncertainties in Blank-Corrected Isotopic Analyses

When a sample has been contaminated during its preparation by contributions from an analytical blank, the isotopic abundance actually determined during the mass spectrometric measurement is that of the sample plus the blank. Using T to represent the sample prepared for mass spectroscopic analysis and s and b to represent the sample and blank, we can write

$$n_T\delta_T = n_s\delta_s + n_b\delta_b \tag{5.1}$$

where n terms represent molar quantities. Substituting $n_s = n_T - n_b$ and rearranging yields

$$\delta_T = \delta_s - \frac{n_b(\delta_s - \delta_b)}{n_T} \tag{5.2}$$

an equation of the form $y = a + bx$. If multiple analyses are obtained, plotting δ_T *vs*. $1/n_T$ will yield the accurate (*i. e.*, blank-corrected) value of δ_s as the intercept.

The coefficients in equation 5.2 can, of course, be most accurately determined by use of statistical techniques (the "least squares" placement of a straight line, more generally referred to as regression). If regression techniques are employed, the $1/n$ values should be treated as precisely known, and point-to-line deviations parallel to the δ axis ought to be minimized. Since all pocket calculators that I know of actually calculate the regression of y on x, the appropriate treatment of blank data will be obtained when δ's are input as y values and $1/n$'s are input as x values.

The slope of the line specified by equation 5.2 is $-n_b(\delta_s - \delta_b)$. Notably, it depends on both the size and the isotopic composition of the blank (n_b and δ_b, respectively). Neither of these quantities can be determined independently unless further information is available. For example, if two different slopes have been obtained by repeatedly carrying two samples of differing isotopic compositions through the same procedure, simultaneous solution of two equations will allow determination of both n_b and δ_b.

Whatever the case, some reasonable means must be employed for estimation of values for n_b and δ_b. This is required because correction of individual analyses is carried out by use of the expression

$$\delta_s = \frac{n_T\delta_T - n_b\delta_b}{n_T - n_b} \tag{5.3}$$

where δ_s is the isotopic composition of some unknown sample (*not* the test material used to develop the regression line and for which, accordingly, the true isotopic composition is already known), n_T is the total molar quantity of CO_2 analyzed isotopically, and δ_T is the isotopic composition of that sample.

The result derived by solution of equation 5.3 is uncertain. Even if all the terms on the right-hand side of the equation are "well known," they all must have some inherent uncertainty, and those uncertainties must cause uncertainties in δ_s. Specifically, n_T will be uncertain because our methods of measurement of the quantities of small gas samples are imperfect. The value of δ_T is uncertain because the mass spectrometer itself can yield only finite precision and because the standardization of the mass spectrometer (*i.e.*, the establishment of the zero point on the PDB scale) may be imperfect. These are, however, the only uncertainties to be considered in δ_T, and the second will not be important if the principal use of the results will be comparisons between samples that have all been analyzed at the same time. Values of n_b and δ_b must be treated as uncertain for two distinct reasons. First, of course, either or both might be more accurately described as an "estimate." Since the size of the blank is usually much smaller than that of the sample, that need not be much of a problem (an example is discussed below). Second, however, n_b and δ_b are probably inherently variable. Even if we did establish their values perfectly at one point in time, it is extremely likely that small variations in procedures, materials, and conditions would cause them to vary at least slightly in subsequent procedures. In assessing the uncertainties in n_b and δ_b it is appropriate to take those variations into account.

Propagation of errors can be treated quantitatively. I take as an example the correction of isotopic analyses of porphyrins that have been purified by TLC. Each equation is followed by corresponding notes.

$$\delta_s = \frac{n_T\delta_T}{n_T - n_b} - \frac{n_b\delta_b}{n_T - n_b} \tag{5.4}$$

This is identical to equation 5.3 but is rewritten in a form more amenable to differentiation:

$$\sigma^2_{\delta_s} = \left(\frac{\partial\delta_s}{\partial n_T}\right)^2\sigma^2_{n_T} + \left(\frac{\partial\delta_s}{\partial\delta_T}\right)^2\sigma^2_{\delta_T} + \left(\frac{\partial\delta_s}{\partial n_b}\right)^2\sigma^2_{n_b} + \left(\frac{\partial\delta_s}{\partial\delta_b}\right)^2\sigma^2_{\delta_b} \tag{5.5}$$

This equation expresses the relationship between the standard deviation in δ_s (*i.e.*, the uncertainty we wish to evaluate) and the standard deviations in the various terms in equations 5.4 and 5.5. Lower-case Greek sigmas (σ) are used to represent standard deviations. The square of a standard deviation is referred to as a "variance."

$$\sigma_{n_T} \approx 0.025\ \mu\text{mol} \tag{5.6}$$

The uncertainty in n_T has been set equal to 65% of the "least count" interval of the baratron readout. At the time these notes were compiled, a one-unit change in the least-significant digit of the baratron readout corresponded to 0.039 mmol C. In the absence of any further information, this is a useful means of assigning an uncertainty to a digital readout. Keep in mind that only 68% of all observations are expected to fall within one standard deviation of the true value, 95% within two standard deviations.

$$\sigma_{\delta_T} \approx 0.03‰ \tag{5.7}$$

The standard deviation of a single isotopic measurement is not the same as the standard deviation reported by the mass spectrometer software. The latter number depends only on the consistency of the various individual measurements of δ (*i.e.*, those deriving from the numerous sample-standard comparisons occurring within a single measurement) that the computer averages in order to derive the δ value it reports. Because it relates only to this internal consistency, it is often referred to as an "internal precision." In contrast, the number which should be inserted here is sometimes termed the "external precision." It can be evaluated by repeated, independent analyses of gas samples having identical isotopic compositions. By choosing a value of 0.03‰, I have estimated that the standard deviation of the population of independent analyses of, for example, repeated doses of standard gas, would be 0.03‰. This does not include allowance for uncertainties in standardization. This value may be a bit high for repeated analyses of large samples, but for the one-micromole quantities considered here, it may even be low.

$$\sigma_{n_b} \approx 0.02\ \mu\text{mol} \tag{5.8}$$

My original estimate of the standard deviation that should be assigned to n_b was 0.03 mmol. In this case, we thought we knew that the blank was 0.086 mmol, so this amounted to assigning a relative uncertainty (and inherent variability) of 35%. By means explained below, I concluded that this estimate was too large, and reduced it to 0.02 mmol.

$$\sigma_{\delta_b} \approx 0.3‰ \tag{5.9}$$

My estimate of variations in the isotopic composition of the blank appears to have been about right.

$$\frac{\partial \delta_s}{\partial n_T} = \frac{(n_T - n_b)\delta_T - n_T\delta_T(1-0)}{(n_T - n_b)^2} - \frac{(n_T - n_b)(0) - n_b\delta_b(1-0)}{(n_T - n_b)^2} \tag{5.10}$$

$$\frac{\partial \delta_s}{\partial \delta_T} = \frac{(n_T - n_b)n_T - n_T\delta_T(0)}{(n_T - n_b)^2} - \frac{(n_T - n_b)(0) - n_b\delta_b(0)}{(n_T - n_b)^2} \tag{5.11}$$

$$\frac{\partial \delta_s}{\partial n_b} = \frac{(n_T - n_b)(0) - n_T\delta_T(0-1)}{(n_T - n_b)^2} - \frac{(n_T - n_b)\delta_b - n_b\delta_b(0-1)}{(n_T - n_b)^2} \tag{5.12}$$

$$\frac{\partial \delta_s}{\partial \delta_b} = \frac{(n_T - n_b)(0) - n_T\delta_T(0)}{(n_T - n_b)^2} - \frac{(n_T - n_b)n_b - n_b\delta_b(0)}{(n_T - n_b)^2} \tag{5.13}$$

$$\frac{\partial \delta_s}{\partial n_T} = \frac{(n_T - n_b)\delta_T - n_T\delta_T + n_b\delta_b}{(n_T - n_b)^2} = \frac{n_b\delta_b - n_b\delta_T}{(n_T - n_b)^2} = \frac{n_b(\delta_b - \delta_T)}{(n_T - n_b)^2} \tag{5.14}$$

$$\frac{\partial \delta_s}{\partial \delta_T} = \frac{(n_T - n_b)n_T}{(n_T - n_b)^2} = \frac{n_T}{(n_T - n_b)} \tag{5.15}$$

$$\frac{\partial \delta_s}{\partial n_b} = \frac{n_T\delta_T - (n_T - n_b)\delta_b - n_b\delta_b}{(n_T - n_b)^2} = \frac{n_T\delta_T - n_T\delta_b}{(n_T - n_b)^2} = \frac{n_T(\delta_T - \delta_b)}{(n_T - n_b)^2} \tag{5.16}$$

$$\frac{\partial \delta_s}{\partial \delta_b} = \frac{(n_T - n_b)n_b}{(n_T - n_b)^2} = \frac{-n_b}{(n_T - n_b)} \tag{5.17}$$

Equations 5.10 – 5.17 show the evaluation of the differentials required by equation 5.5.

$$\sigma_{\delta_s}^2 = \left[\frac{n_b(\delta_b - \delta_T)}{(n_T - n_b)^2}\right]^2 \sigma_{n_T}^2 + \left[\frac{n_T}{n_T - n_b}\right]^2 \sigma_{\delta_T}^2 + \left[\frac{n_T(\delta_T - \delta_b)}{(n_T - n_b)^2}\right]^2 \sigma_{n_b}^2 + \left[\frac{-n_b}{n_T - n_b}\right]^2 \sigma_{\delta_b}^2 \tag{5.18}$$

Equation 5.18 is derived by substitution of the final results of equations 5.14 – 5.17 into equation 5.5.

$$\sigma^2_{\delta_s} = \frac{1}{(n_T - n_b)^2}\left[\left(\frac{n_b(\delta_b - \delta_T)}{n_T - n_b}\right)^2 \sigma^2_{n_T} + n_T^2\sigma^2_{\delta_T}\right] +$$

$$\frac{1}{(n_T - n_b)^2}\left[\left(\frac{n_T(\delta_T - \delta_b)}{n_T - n_b}\right)^2 \sigma^2_{n_b} + (-n_b)^2\sigma^2_{\delta_b}\right] \quad (5.19)$$

This is a general expression for the standard deviation of δ_s as a function of the standard deviations of the terms contributing to δ_s. If you wish to develop a computer program to evaluate these equations, the following test data will be useful.

For n_b = 0.086 μmol and δ_b = –22.0‰

and n_T = 0.768 μmol and δ_T = –26.959‰,

adoption of the standard deviations shown in equations 5.6 – 5.9 (0.02 being used in equation 5.8) yields δ_s = –27.58‰ and a standard deviation for that number of 0.17‰.

The adequacy of a treatment of this kind can be tested (and was in this case) if standards are analyzed along with samples. The individual standard results can be treated as unknowns and the results can be corrected by use of equation 5.3. These corrected results ought, of course, to scatter around the true value determined by reference to the intercept of the initial regression line. You may then proceed as follows.

(*i*) Calculate the difference between each corrected result and the known, true isotopic composition.

(*ii*) Divide each of these differences by the standard deviation assigned to each result. For example, in the specific case noted above, the corrected result of –27.58‰ differed from the true value of –27.43‰ by 0.15‰. Dividing that difference by 0.17‰, the assigned standard deviation, showed that this particular result was 0.85 standard deviations low.

(*iii*) Check to be sure that about 68% of the standard results are less than one standard deviation away from the mean, and that less than 5% are more than two standard deviations away. If you find that almost every result is within less than one standard deviation (and this is what happened in this example when the standard deviation of n_b was set at 0.03 mmol), then one or more of the standard deviations in the model (equations 5.6 – 5.9) should be decreased. If many deviations amount to more than one sigma, you will have to increase some of the input standard deviations.

The tyranny of small numbers and special circumstances can always cause problems. In the porphyrin analyses, for example, the model developed seemed to predict uncertainties accurately for 13 out of 15 standard analyses. The two outliers (3.1 and 3.6σ!) both pertained to large samples. Those samples were, in fact, larger than any of the unknowns. Moreover, the test becomes especially stringent in such circumstances because the large samples lead to very low predicted values for the standard deviation in δ_s. On balance, I concluded that it was fair to use the model within the range of sample sizes where it worked and not to push everything out of shape to accommodate two irrelevant outliers.

What uncertainties should be presented in formal reports of isotopic analyses? I favor the following. In tables, report the result plus or minus two standard deviations (this amounts to the generally accepted standard of 95% confidence limits). That would be –27.58 ± 0.34 in the example given above. When the indicated uncertainty becomes greater than 30 units, I would drop the least significant figure both in it and in the reported number. For example, in a publication I would report this result as –27.6 ± 0.3‰. While the data were under review and consideration, however, I would probably carry the extra significant figure. I would *never* report or carry a standard deviation amounting to more than 100 units. In any report to the outside world, however informal, I would clearly write at the bottom of the table (or someplace where it would be clearly juxtaposed with the numerical results), "indicated uncertainties are plus or minus two standard deviations." Otherwise, many people will assume that the reported uncertainties are merely plus or minus one standard deviation.

6. Further Notes, July 2002

6.1 *Data processing*

The algorithm introduced by Santrock *et al.* (1985) is indeed more accurate than earlier systems of equations. As a result, if the same sample is analyzed at very high precision on two different instruments, one of which uses the Santrock equations and the other some older algorithm, the results can differ significantly. Moreover, if a laboratory has two or more instruments and all do not use the same data-processing algorithms, systematic errors will result if working standards are calibrated on a machine that uses one algorithm and then transferred for use on a machine that uses the other algorithm.

Confronting discrepancies of this kind, isotopic analysts wishing to standardize procedures for measurements of atmospheric trace gases have spelled out algorithms and absolute ratios (*i. e.*, values equivalent to those reported here in Table 1) that should be used by all laboratories in that field (IAEA, 1995). In general, they have chosen antiquated procedures and outdated values, avoiding the Santrock *et al.* (1985) algorithm and the updated absolute ratios reported in Table 1. Presumably these choices have been made in order to provide comparability with all earlier reports.

6.2 *The definition of* δ

The question is whether δ should be defined as $\Delta R/R$ or as $10^3(\Delta R/R)$. American usage overwhelmingly favors the latter and, in doing so, faithfully follows the earliest formal reports. Earlier sections of this document follow this practice.

When this definition of δ is used in the development of further equations relating, for example, to isotopic fractionations in multistep processes, the resulting expressions are cluttered by factors of 10^3. The problem has been pointed out by Professor Graham Farquhar of the Research School for Biological Sciences at Australian National University. Papers from his research group consistently employ the definition of δ in which the thousand-fold multiplier is omitted as an explicit factor. Resulting values of δ and ε can be reported as, for example, –0.0254 (no units) or –25.4‰. This usage embodies the view that the permil symbol (‰) *implies* multiplication by 10^3 and expression of the value in terms of parts per thousand. This same view has been taken by Professor Willem G. Mook of the Centre for Isotope Research, University of Groningen, the Netherlands, in his major new book on isotopic techniques (Mook, 2000). Since this book is freely available *via* the internet, it is particularly likely to shape usage around the world.

The advantages of the $\Delta R/R$ form are substantial. In the future, I plan to use it consistently.

6.3 *The pronunciation of* δ

Confronting a growing trend to refer to δ as "del," Professor Harmon Craig of the Scripps Institution of Oceanography, one of the founders of modern isotopic studies, composed the following limerick. It's claimed that there was no particular target for this missile, least of all anyone at Cornell University.

> The was a young man from Cornell
> Who pronounced every "delta" as "del"
> But the spirit of Urey
> Returned in a fury
> And transferred that fellow to Hell!

References

Abelson P. H. and Hoering T. C. (1961) Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. *Proc. Nat. Acad. Sci.* **47**, 623-632.

Ault W. U. and Jensen M. L. (1962) Summary of sulfur isotopic standards. pp. 16-29 in M. L. Jensen (ed.) *Biogeochemistry of Sulfur Isotopes*, National Science Foundation.

Baertschi P. (1976) Absolute $^{18}0$ content of standard mean ocean water. *Earth & Planetary Sci. Lett.* **31**, 341-344.

Bigeleisen J. (1965) Chemistry of isotopes. *Science* **147**, 463-471.

Bigeleisen J., Perlman M. L. and Prosser H. C. (1952) Conversion of hydrogenic materials to hydrogen for isotopic analysis. *Anal. Chem.* **24**, 1356-1357.

Bigeleisen J. and Wolfsberg M. (1958) Theoretical and experimental aspects of isotope effects in chemical kinetics. *Adv. Chem. Phys.* **1**, 15-76.

Blattner P. and Hulston J. R. (1978) Proportional variations of geochemical ^{18}O scales – an interlaboratory comparison. *Geochim. Cosmochim. Acta* **42**, 59-62.

Brenninkmeijer C. A. M. (1983) Deuterium, oxygen-18 and carbon-13 in tree rings and peat deposits in relation to

climate. Proefschrift, Rijksuniversiteit te Groningen.

Brenninkmeijer C. A. M. and Mook W. G. (1981) A batch process for direct conversion of organic oxygen and water to carbon dioxide for oxygen-18/oxygen-16 analysis. *Int. J. Appl. Radiat. Isot*. **32**, 137-141.

Cleland W. W., OLeary M. H. and Northrop D. B., Eds. (1977) *Isotope Effects on Enzyme-Catalyzed Reactions*, University Park Press, Baltimore, 303 pp.

Coleman M. L., Shepherd T. J., Durham J. J., Rouse J. E. and Moore G. R. (1982) Reduction of water with zinc for hydrogen isotope analysis. *Anal. Chem*. **54**, 993-995.

Coplen T. B. and Clayton R. N. (1973) Hydrogen isotopic composition of NBS and IAEA stable isotope water reference samples. *Geochim. Cosmochim. Acta* **37**, 2347-2349.

Coplen T. S. and Kendall C. (1982) Preparation and stable isotope determination of NBS-16 and NBS-17 carbon dioxide. *Anal. Chem*. **54**, 2611-2612.

Coplen T. B., Kendall C. and Hopple J. (1983) Comparison of stable isotope reference samples. *Nature* **302**, 236-238.

Craig H. (1953) The geochemistry of the stable carbon isotopes. *Geochim. Cosmochim. Acta* **3**, 53-92.

Craig H. (1961) Standard for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science* **133**, 1833-1834.

Degens E. T. (1969) Biogeochemistry of stable carbon isotopes. In *Organic Geochemistry, Methods and Results*. (eds. G. Eglinton and M. T. J. Murphy) p. 304-329. Springer, New York.

Deines P. (1980) The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry, Vol. 1, The Terrestrial Environment* (eds. P. Fritz and J. Ch. Fontes) p. 329-406. Elsevier Scientific, Amsterdam.

DeNiro M. J. and Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**, 261-263.

DesMarais D. J. (1978) Carbon, nitrogen, and sulfur in Apollo 15, 16, and 17 rocks. *Proc. Lunar Planet. Sci. Conf.* 9th, 2451-2467

Deuser W. G. and Degens E. T. (1967) Carbon isotope fractionation in the system CO_2 (gas) – CO_2 (aqueous) – HCO_3. *Nature* **215**, 1033-1035.

DeWit J. C., Van der Straaten C. M., and Mook W. G. (1980) Determination of the absolute D/H ratio of V-SMOW and SLAP. *Geostandards Newsletter* **4**, 33-36.

Epstein S., Buschbaum R., Lowenstam H. and Urey H. C. (1951) Carbonate-water isotopic temperature scale. *Bull. Geol. Soc. Am*. **62**, 417-426.

Frazer J. W. and Crawford R. W. (1963) Modifications in the simultaneous determination of carbon, hydrogen, and nitrogen. *Mikrochim. Acta*, 561-566.

Friedman I. and O'Neil J. R. (1977) Compilation of Stable Isotope Fractionation Factors of Geochemical Interest. U. S. Geological Survey Professional Paper 440-KK.

Friedman I., O'Neil J. and Cebula G. (1982) Two new carbonate stable isotope standards. *Geostandards Newsletter* **6**, 11-12.

Fritz P. and Fontes J. Ch. (1980) Introduction. In *Handbook of Environmental Isotope Geochemistry, Vol. 1, The Terrestrial Environment* (eds. P. Fritz and J. Ch. Fontes), Elsevier Scientific, Amsterdam, p. 1-20.

Gonfiantini, R. (1978) Standards for stable isotope measurements in natural compounds. *Nature* **271**, 534-536.

Gonfiantini, R. (1984) *Report on Advisory Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations.* International Atomic Energy Agency, P. 0. Box 100, A-1400, Vienna, Austria.

Hagemann R., Nier G. and Roth E. (1970) Absolute isotopic scale for deuterium analysis of natural waters. Absolute D/H ratio for SMOW. *Tellus* **22**, 712-715.

Halsted R. E. and- Nier A. O. (1950) Gas flow through the mass spectrometer viscous leak. *Rev. Sci. Instrum*. **21**, 1019-1021.

Hayes J. M. (1983) The state of the art of high-precision stable isotopic analysis in organic materials. Extended abstracts, 31st Ann. Conf. on Mass Spectrom. and Allied Topics, Amer. Soc. Mass Spectrom., in press.

Hayes J. M., DesMarais D. J., Peterson D. W., Schoeller D. A., and Taylor S. P. (1977) High precision stable isotope ratios from microgram samples. In *Advances in Mass Spectrometry,' Vol. 7* (ed. N. R. Daly), p. 475-480. Heyden and Son, London.

Hopfner A. (1969) Vapor pressure isotope effects. *Angew. Chem. Internat. Edit*. **8**, 689-699.

IAEA [International Atomic Energy Association] (1995) *Reference and intercomparison materials for stable isotopes of light elements, Proceedings of a consultants meeting held in Vienna, 1-3 December 1993*. IAEA TECDOC-825, 165 pp. [See www.iaea.org Information relevant to stable isotopic standards and analyses are found in the sections devoted to Isotope Hydrology.]

Jensen M. L. and Nakai N. (1962) Sulfur isotope meteorite standards, results and recommendations. pp. 30-35 in M. L. Jensen (ed.) *Biogeochemistry of Sulfur Isotopes*, National Science Foundation.

Junk G. and Svec H. (1958) The absolute abundance of the

nitrogen isotopes in the atmosphere and compressed gas from various sources. *Geochim. Cosmochim. Acta* **14**, 234-243.

Landis C. P. (1983) Harding Iceland spar: a new $\delta^{18}O - \delta^{13}C$ carbonate standard for hydrothermal minerals. *Isotope Geoscience* **1**, 91-94.

Li Wenjun, Ni Baoling, Jin Deqiu, and Zhang Qingliang (1988) Measurement of the absolute abundance of oxygen-17 in VSMOW. *Kexue Tongbao (Chinese Science Bulletin)* **33**, 1610-1613.

MacNamara J. and Thode H. G. (1950) Comparison of the isotopic constitution of terrestrial and meteoritic sulfur. *Phys. Rev.* **78**, 307-308.

Mariotti A., Germon J. C., Hubert P., Kaiser P., Leto Ile R., Tardieux A. and Tardieux P. (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Pl. Soil* **62**, 413-430.

Matsuhisa Y., Goldsmith J. R. and Clayton R. N. (1978) Mechanisms of hydrothermal crystallization of quartz at 250°C and 15 kbar. *Geochim. Cosmochim. Acta* **42**, 173-182.

Matthews D. E. and Hayes J. M. (1978) Isotope-ratio-monitoring gas chromatog raphy mass spe ctrometry. *Anal. Chem*. **50**, 1465-1473.

McKinney C. R., McCrea J. M., Epstein S., Allen H. A. and Urey H. C. (1950) Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Rev. Sci. Instrum*. **21**, 724-730.

Meinschein W. G., Rinaldi G. G. L., Hayes J. M. and Schoeller D. A. (1974) Intramolecular isotopic order in biologically produced acetic acid. *Biomed. Mass Spec*. **1**, 172-174.

Melander I- and Saunders W. H., Jr. (1980) *Reaction Rates of Isotopic Molecules*, 331 pp. Wiley, New York.

Monson K. D. and Hayes J. M. (1980) Biosynthetic control of the natural abundance of carbon-13 at specific positions within fatty acids in *Escherichia coli*, evidence regarding the coupling of fatty acid and phospholipid synthesis. *J. Biol. Chem*. **255**, 11435-11441.

Monson K. D. and Hayes J. M. (1982) Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochim. Cosmochim. Acta* **46**, 139-149.

Mook, W. G. (2000) Environmental Isotopes in the Hydrological Cycle, Principles and Applications. Available on line from http://www.iaea.org/programmes/ripc/ih/volumes/volumes.htm

Mook W. G. and Grootes P. M. (1973) Measuring procedure and corrections for the high-precision mass-spectrometric analysis of isotopic abundance ratios, especially referring to carbon, oxygen, and nitrogen. *Int. J. Mass Spectrom. Ion Phys.* **12**, 273-298.

Nielsen H . (1984) The standard situation for sulfur isotopes. Annex III in R. Gonfiantini (1984) *op cit.*

Nier A. O. and Gulbransen E. A. (1939) Variations in the relative abundance of the carbon isotopes. *J. Amer. Chem. Soc.* **61**, 697-698.

Pella E. and Colombo B. (1972) Improved instrumental determination of oxygen in organic compounds by pyrolysis-gas chromatography. *Anal. Chem*. **44**, 1563-1571.

Pella E. and Colombo B. (1973) Study of carbon, hydrogen, and nitrogen determination by combustion-gas chromatography. *Mikrochim. Acta*, 697-719.

Peterson D. W. and Hayes J. M. (1978) Signal to noise ratios in mass spectroscopic ion-current measurement systems. In *Contemporary Topics in Analytical and Clinical Chemistry* (eds. D. M. Hercules, G. M. Hieftje, L. R. Snyder, and M. E. Evenson), p. 217-252. Plenum, New York.

Rock P. A., Ed. (1975) *Isotopes and Chemical Principles*, American Chemical Society Symposium Series .11, American Chemical Society, Washington, D. C., 215 pp.

Rittenberg D. and Ponticorvo L. (1956) A method for the determination of the 180 concentration of the oxygen in organic compounds. *Int. J. Appl. Radiat. Isot.* **1**, 208-214.

Santrock J. and Hayes J. M. (1983) A new method for the determination of oxygen-18 in organic materials. Extended abstracts, 31st Ann. Conf. on Mass Spectrom. and Allied Topics, Amer. Soc. Mass Spectrom., in press.

Santrock J., Studley S. A., and Hayes J. M. (1985) Isotopic analyses based on the mass spectrum of carbon dioxide. *Anal. Chem*. **57**, 1444-1448.

Schoell M. and Faber E. (1982) C and H isotopic composition of NBS 22 and NBS 21 standards; an interlaboratory comparison. Informal report, Bundesanstalt fur Geowissenschaften und Rohstoffe, D-3800 Hannover, Federal Republic of Germany.

Schoell M., Faber E., and Coleman M. L. (1983) Carbon and hydrogen isotopic compositions of the NBS 22 and NBS 21 stable isotope reference materials: an interlaboratory comparison. *Org. Geochem*. **5**, 3-6.

Schoeller D. A. and Hayes J. M. (1975) Computer controlled ion counting isotope ratio mass spectrometer. *Anal. Chem.* **47**, 408-415.

Schoeller D. A., Peterson D. W. and Hayes J. M. (1983) Double-comparison method for mass spectrometric determination of hydrogen isotopic abundances. *Anal.*

Chem. **55**, 827-832.

Silverman S. R. (1964) Investigations of petroleum origin and evolution mechanisms by carbon isotope studies. In *Isotopic and Cosmic Chemistry* (eds. H. Craig, S. L. Miller and G. J. Wasserburg), p. 92-102. North Holland, Amsterdam.

Straus H. A. (1941) A new mass spectrograph and the isotopic constitution of nickel. *Phys. Rev.* **59**, 430-433.

Stuermer D. H., Peters K. E. and Kaplan I. R. (1978) Source indicators of humic substances and proto-kerogen. Stable isotope ratios, elemental compositions and electron spin resonance spectra. *Geochim. Cosmochim. Acta* **42**, 989-997.

Sweeney R. E., Liu K. K. and Kaplan I. R. (1978) Oceanic nitrogen isotopes and their uses in determining the source of sedimentary nitrogen. *D. S. I. R. Bulletin (New Zealand)* **220**, 9-26.

Taylor J. W. and Chen I.-J. (1970) Variables in oxygen-18 isotopic analysis by mass spectrometry. *Anal. Chem.* **42**, 224-228.

Tse R. S., Wong S. C., and Yuen C. P. (1980) Determination of deuterium/hydrogen ratios in natural waters by Fourier transform nuclear magnetic resonance spectrometry. *Anal. Chem.* **52**, 2445-7.

Unterzaucher J. (1940) Die mikroanalytische Bestimmung des Sauerstoffes. *Ber. deut. chem. Ges.* **73**, 391-404.

Urey H. C. (1948) Oxygen isotopes in nature and in the laboratory. *Science* **108**, 489-496.

Urey H. C., Lowenstam H. A., Epstein S. and McKinney C. R. (1951) Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and Southeastern United States. *Bull. Geol. Soc. Am.* **62**, 399-416.

Vogler E. A. and Hayes J. M. (1979) Carbon isotopic fractionation in the Schmidt decarboxylation: evidence for two pathways to products. *J. Org. Chem.* **44**, 3682-3686.

Vogler E. A. and Hayes J. M. (1980) Carbon isotopic compositions of carboxyl groups of biosynthesized fatty acids. In *Advances in Organic Geochemistry 1979* (eds. A. G. Douglas and J. R. Maxwell), p. 697-704. Pergamon, London.

Vogler E. A., Meyers P. A. and Moore W. A. (1981) Comparison of Michigan Basin crude oils. *Geochim. Cosmochim. Acta* **45**, 2287-2293.

Wedeking K. W., Hayes J. M. and Matzigkeit U. (1983) Procedures of organic geochemical analysis. In *Earth's Earliest Biosphere: Its Origin and Evolution* (ed. J. W. Schopf). Princeton, in press.

Wedeking K. W. and Hayes J. M. (1983) Exchange of oxygen isotopes between water and organic material. *Isotopic Geoscience*, in press.

Wendt I. (1968) Fractionation of carbon isotopes and its temperature dependence in the systems carbon dioxide gas – carbon dioxide in solution and bicarbonate ion – carbon dioxide in solution. *Earth & Planetary Sci. Lett.* **4**, 64-68.

West S. S. (1945) The relative abundance of the carbon isotopes in petroleum. *Geophysics* **10**, 406-420.

Wickman F. E. (1956) The cycle of carbon and the stable carbon isotopes. *Geochim. Cosmochim. Acta* **9**, 136-153.

Zhang, Q-L. (1989) The square-root formula for O-17 abundance and the atomic weight of oxygen. *Chinese Science Bulletin* **34**, 828-831.

Table 2 first appeared (without a presentation of its derivation) in J. M. Hayes, D. J. DesMarais, D. W. Peterson, D. A. Schoeller, and S. P. Taylor, "High precision stable isotope ratios from microgram samples," pp. 475-480 in N. R. Daly (ed.), *Advances in Mass Spectrometry*, Vol. 7. Heyden and Son, 1977.

A detailed treatment of all factors affecting precision of practical ion-current ratio-measurements is given in D. W. Peterson and J. M. Hayes, "Signal to noise ratios in mass spectroscopic ion-current measurement systems," pp. 217-252 in Vol. 3 of D. M. Hercules, G. M. Hieftje, L. R. Snyder, and M. E. Evenson (eds.) *Contemporary Topics in Analytical and Clinical Chemistry* (1978).

Numerous additional references are cited in both of these works.