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Calculation of hydrogen isotopic fractionations in biogeochemical systems

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Abstract—Hydrogen-isotopic data are often interpreted using mathematical approximations originally intended for other isotopes. One of the most common, apparent in literature over the last several decades, assumes that delta values of reactants and products are separated by a constant fractionation factor: $\delta_p = \delta_r + \varepsilon_{p/r}$. Because of the large fractionations that affect hydrogen isotopes, such approximations can lead to substantial errors. Here we review and develop general equations for isotopic mass balances that include the differential fractionation of each component in a mixture and discuss their use in three geochemical applications. For the fractionation of a single component, the reactant and product are related by $\delta_p = \alpha_{p/r}\delta_r + \varepsilon_{p/r}$, where α and ε refer to the same fractionation. Regression of δ_p on δ_r should give equivalent fractionations based on the intercept and slope, but this has not generally been recognized in studies of D/H fractionation. In a mixture of two components, each of which is fractionated during mixing, there is no unique solution for the three unknown variables (two fractionation factors and the elemental mixing ratio of the two hydrogen sources). The flow of H from CH_4 and H_2O to bacterial lipids in the metabolism of *Methylococcus capsulatus* provides an example of such a case. Data and conclusions from an earlier study of that system (Sessions et al., 2002) are reexamined here. Several constraints on the variables are available based on plausible ranges for fractionation factors. A possible refinement to current experimental procedures is the measurement of three different isotopes, which would allow unique determination of all variables.

1. INTRODUCTION

Recent improvements in analytical methodology have produced a rising tide of interest in hydrogen-isotopic studies. In dealing with the results, most authors have used mathematical approximations originally adopted for ^{13}C and ^{18}O, for example that delta values of two components related by a single fractionation will have a 1:1 relationship: $\delta_a = \delta_b + \varepsilon_{a/b}$, or $\delta_a = \delta_b + 1000\ln\alpha a/b$. Because of the very large fractionations that affect hydrogen isotopes, particularly in many biologic systems, such approximations are inappropriate. In at least one case (Sessions et al., 2002), interpretive errors have resulted. In many others, confusion has or will result from the publication of equations in which approximations are overlooked. Accordingly, we review here the mathematics of isotopic fractionation with specific reference to hydrogen, examining several specific biogeochemical applications. The equations derived and conclusions reached are applicable to all isotopic systems.

2. GENERAL EQUATIONS

Isotopic mass balance in a mixture of components can be described without approximation, regardless of the levels of isotopic enrichment, by

$$F_T = X_1F_1 + X_2F_2 + \ldots X_nF_n \quad (1)$$

where X is the molar fraction of each component of the mixture, and F is the fractional abundance of the rare isotope (Hayes, 1983; Criss, 1999). The subscript T designates the total derived from mixing components 1, 2...n. For the specific case of hydrogen isotopes, X refers to total hydrogen (D + H) and F refers to the fractional abundance of deuterium [= D/(D + H)].

When mixing is accompanied by isotopic fractionation, application of Eqn. 1 is not straightforward. A concrete example is a cell that is synthesizing lipids while using two isotopically distinct sources of hydrogen, e.g., methane and water. Isotopic fractionations will accompany the transfer of H from each of those sources to lipids. Mixing calculations become complicated because there is no form of the fractionation factor (α) that can be used directly to transform values of F. This difficulty leads to a more useful, approximate form of the mass-balance equation

$$R_T = X_1R_1 + X_2R_2 + \ldots X_nR_n \quad (2)$$

where R is the isotope ratio (e.g., D/H). Eqn. 2 is exact in the limit that R (and hence F) approaches zero. Errors increase in proportion to both the absolute isotope ratio (R) and to the differences between isotope ratios. Thus relatively large isotope ratios can be tolerated as long as fractionations are small, as is the case for ^{13}C where R is ~0.01 and the range of R values is generally <0.0005 ($\Delta R/R < 50‰$). Conversely, large fractionations can be tolerated as long as isotopic abundances are low, as for ^{2}H where $R = 0.00015$ and the range of R values can be 0.00008 ($\Delta R/R = 500‰$) or larger. An alternative form of Eqn. 2, in which values of X represent the mole fractions of the reference isotope, e.g. ^{12}C or ^{1}H, is exact for all cases and has been suggested by Criss (1999).

Eqn. 2 can be modified to accommodate fractionations of individual components. If R_n is the isotope ratio of the source

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Table 1. Calibrations between δD values of lipids and environmental water.

Analyte	Derived relationship	α_{slope} [a]	α_{intept} [b]	R^2	Reference
Bulk lipids	$\delta_l = 0.546\delta_w - 141‰$	0.546	0.859	0.618	Sternberg (1988)
Phytoplantonic sterols	$\delta_l = 0.748\delta_w - 199‰$	0.748	0.801	0.965	Sauer et al. (2001)
Palmitic acid	$\delta_l = 0.939\delta_w - 167‰$	0.939	0.833	0.894	Huang et al. (2002)

[a] Fractionation factor calculated from the slope of the derived relationship.
[b] Fractionation factor calculated from the intercept of the derived relationship.

of the *n*th component, then the isotope ratio of that component in the mixture will be $\alpha_n R_n$, where α_n is the fractionation factor. Eqn. 2 then becomes

$$R_T = X_1\alpha_1 R_1 + X_2\alpha_2 R_2 + \ldots X_n\alpha_n R_n \quad (3)$$

A second common modification of Eqn. 2 substitutes delta values (δ) for isotope ratios (*R*). Here we follow the lead of Farquhar et al. (1989) and Mook (2000) who point out that the ubiquitous permil symbol implies a factor of 10^3, which can then be omitted from the definition of delta: $\delta_x = R_x/R_{std} - 1$. This simplification removes factors of 1000 that would otherwise clutter the equations presented below. Substituting in Eqn. 3 gives

$$(\delta_T + 1)R_{std} = X_1\alpha_1(\delta_1 + 1)R_{std} + \ldots X_n\alpha_n(\delta_n + 1)R_{std} \quad (4)$$

which simplifies to

$$\delta_T + 1 = X_1(\alpha_1\delta_1 + \alpha_1) + \ldots X_n(\alpha_n\delta_n + \alpha_n) \quad (5)$$

Using the identity $X_1 + X_2 + \ldots X_n = 1$ then gives

$$\delta_T = X_1(\alpha_1\delta_1 + \alpha_1 - 1) + \ldots X_n(\alpha_n\delta_n + \alpha_n - 1) \quad (6)$$

A third common substitution is to use ε in place of the fractionation factor α, where $\varepsilon = \alpha - 1$ (again assuming that permil units imply a factor of 10^3). Eqn. 6 thus becomes

$$\delta_T = X_1(\alpha_1\delta_1 + \varepsilon_1) + \ldots X_n(\alpha_n\delta_n + \varepsilon_n) \quad (7)$$

where α_n and ε_n represent the same fractionation.

Despite the use of the delta notation, Eqn. 7 does not involve any approximation beyond that inherent in substituting isotope ratios (Eqn. 2) for fractional abundances. Thus for systems containing a natural abundance of D, ^{13}C, ^{15}N, or ^{18}O, Eqn. 7 can be viewed as exact and should be used whenever possible, remembering that values of δ and ε in permil units must be divided by 1000 before insertion into Eqn. 7.

3. SOME COMMON GEOCHEMICAL APPLICATIONS

3.1. Fractionation of a Single Component

For the fractionation of a single component Eqn. 7 simplifies to

$$\delta_P = \alpha\delta_S + \varepsilon \quad (8)$$

where δ_P and δ_S represent the product and source, respectively. As α approaches unity, the frequently-used approximation $\delta_P = \delta_S + \varepsilon$ becomes accurate. This approximation serves for carbon and oxygen isotopes, where values of α are typically between 0.95 and 1.05, but is inappropriate for hydrogen where values of α commonly range from 0.7 to 1.5 and can theoretically be as large as 18 (Bigeleisen and Wolfsberg, 1958).

If δ_P is plotted as a function of δ_S, the magnitude of the fractionation can be determined from the intercept (ε), the slope (α), or both. In fact, their values should be redundant, with $\varepsilon = \alpha - 1$. As a concrete example of this phenomenon, we consider the fractionation of hydrogen isotopes by plants during photosynthesis. Because water is the source of all hydrogen in plants, the isotopic compositions of plant lipids and environmental water are expected to be related simply by Eqn. 8, in which a single fractionation factor represents the net effect of all biosynthetic processes. Several authors have attempted to calibrate this fractionation by measuring values of δD for plant lipids from lakes covering a latitudinal range. Results are summarized in Table 1. In each case the intercept of the regression has been interpreted as the 'true' fractionation factor. However, there is significant disagreement between the fractionations implied by the slope (α) and intercept ($a - 1$) of each of the regressions. The fractionations should be equivalent, and the fact that they are not implies that the relationship is not as simple as has been assumed. Agreement between the slope and intercept improves as the correlation coefficient for each regression increases, suggesting that the estimate of Sauer et al. (2001) is the closest to representing a single process. As with any statistically significant correlation, the derived equations can still be used to predict values of δD for environmental water based on those of sedimentary lipids, but they should not be interpreted as representing a single fractionation between water and lipid.

3.2. Fractionation of One Component in a Two-Component Mixture

If two components are being mixed but isotopic fractionation affects only one of them, Eqn. 7 can be reduced to

$$\delta_T = X_1(\alpha_1\delta_1 + \varepsilon_1) + (1 - X_1)\delta_2 \quad (9)$$

A common application of Eqn. 9 arises in the analysis of organic hydrogen in samples containing exchangeable H (Schimmelmann et al., 1999; Wassenaar and Hobson, 2000). After equilibrating such a sample with water of known D/H ratio, the exchangeable hydrogen will have a new isotopic composition offset from that of the water by some fractionation factor, while C-bound H will retain its original isotopic composition. Using the notation of Schimmelmann (1991) and Wassenaar and Hobson (2000), Eqn. 9 can be rewritten as

$$\delta_T = f_e(\alpha_{e/w}\delta_w + \varepsilon_{e/w}) + (1 - f_e)\delta_n \quad (10)$$

Table 2. Hypothetical values for parameters in Eqn. 11 showing that it does not have a unique solution.

Experimental data[a]		
δ_T (‰)	δ_1 (‰)	δ_2 (‰)
−240	0	−200
−280	−100	−200
−320	−200	−200
Possible solutions[b]		
X_1	α_1	α_2
0.40	1.000	0.750
0.45	0.889	0.818
0.50	0.800	0.900
0.60	0.667	1.125
etc.		

[a] Hypothetical data chosen to follow the form of Eqn. 11.
[b] Possible values for the variables in Eqn. 11, all of which satisfy the experimental data listed above.

where f_e is the fraction of exchangeable hydrogen, and the subscripts e, n, and w refer to exchangeable, nonexchangeable, and water H, respectively.

Several recent reports have dealt with methodologies for determining both f_e and δ_n. Unfortunately, exact formulations (eqns. 1–4 in Schimmelmann et al., 1999; eqns. 1–3 in Wassenaar and Hobson, 2000) and approximate formulations (eqns. 1 and 2 in Schimmelmann, 1991; eqns. 2–3 in Chamberlain et al., 1997; eqn. 1 in Hobson et al., 1999; eqn. 4 in Wassenaar and Hobson, 2000) have both been reported, leading to the potential for considerable confusion. In all cases, calculations based on Eqn. 10 (above) or eqn. 4 in Schimmelmann et al. (1999) will give the most accurate results.

3.3. Fractionation and Mixing of Two Components

A more general expression reflecting the potential fractionation of both components is

$$\delta_T = X_1(\alpha_1\delta_1 + \varepsilon_1) + (1 - X_1)(\alpha_2\delta_2 + \varepsilon_2) \quad (11)$$

The equation can be written using isotope ratios rather than delta values as

$$R_T = X_1\alpha_1R_1 + (1 - X_1)\alpha_2R_2 \quad (12)$$

Eqns. 11 and 12 are equivalent, and provide identical accuracy. They describe a situation that is encountered, for example, when studying hydrogen isotopic fractionations in heterotrophic organisms, all of which must have at least two potential sources of hydrogen, i.e., organic substrates and water (Hobson et al., 1999; Sessions et al., 2002; Valentine et al., 2004).

Eqn. 12 contains terms in which X and α are multiplied, and so is nonlinear. As a result, multiple solutions for (X_1, α_1, α_2) exist. This is true no matter how many parallel experiments are conducted with differing values for R_1 and R_2. This fact is demonstrated in Table 2, which provides three sets of hypothetical isotopic compositions. All conform to Eqn. 11 and thus represent data that might be collected in an experiment designed to provide three equations with only three unknowns. The bottom half of Table 2 lists four sets of values for the unknown coefficients X_1, α_1, α_2. All are exact solutions for the three equations defined by the top half of the table. They are merely examples, and there is an infinite number of such solutions. Accordingly, the approach taken by Sessions et al. (2002) to provide a unique solution must be invalid. To redress that fault, we first consider alternative approaches, then, in section 3.5 below, reconsider the conclusions reached by Sessions et al. (2002).

The set of (X_1, α_1, α_2) solutions for Eqns. 11 and 12 has a single degree of freedom, i.e., fixing the value of any one of the three parameters uniquely defines the other two. If none of the three parameters can be estimated independently, the simplest approach to this problem lies in reducing the number of free variables by reparameterizing Eqn. 12 as

$$R_T = p_1R_1 + p_2R_2 \quad (13)$$

where $p_1 = X_1\alpha_1$ and $p_2 = (1 - X_1)\alpha_2$. Eqn. 13 is linear and has a unique solution, thus both parameters (p_1, p_2) can be determined directly by the regression of R_T on R_1 or R_2. Although values for the original variables (X_1, α_1, α_2) still cannot be uniquely determined, several useful constraints can be recognized based on the properties of the physical quantities being studied. These include

1. The value of X_1 must lie between zero and one. Thus p_1 provides a minimum estimate for α_1, and p_2 provides a minimum estimate for α_2.

2. Both fractionation factors must have values >0. Thus if either p_1 or $p_2 = 0$, it can be concluded that $X_1 = 0$ or $X_1 = 1$, respectively.

3. Fractionation factors for most natural systems vary over limited ranges, even for hydrogen isotopes. For example, fractionation between water and organic hydrogen generally ranges between 0.900 and 0.650 (Estep and Hoering, 1980; Sessions et al., 1999). These ranges can be used to further constrain possible values of X_1. The constraints placed on X_1 by assumed ranges for α_1 and α_2 should be overlapping but not identical.

The form of Eqn. 13 leads to two additional considerations regarding experimental design. First, both parameters (p_1, p_2) can be calculated if the isotopic composition of only one hydrogen source is varied, and that of both hydrogen sources is known. This provides significant analytical convenience, since it is easy to change the isotopic composition of water by the addition of D_2O, but difficult to change the isotopic composition of an organic substrate.

Second, the slope of any regression can be calculated with smaller uncertainty than the intercept, except in the particular case where the intercept value lies near the centroid of the independent variable data. Thus for experiments with only one hydrogen source (section 3.1 above), it is generally more accurate—and never less accurate—to estimate a fractionation factor from the slope of the regression than from the intercept.

3.4. Experiments Using Three Isotopes

In principle, the problem can be solved by measuring abundances of three or more isotopes, e.g., protium, deuterium, and tritium. Isotope effects for most chemical reactions vary with isotopic mass following the relationship

$$^3\alpha = (^2\alpha)^m \quad (14)$$

Table 3. Values of the coefficients p_1 and p_2 for lipids from *Methylococcus capsulatus,* reproduced from Sessions et al. (2002).

Lipid	p_1[a]	p_2[b]
squalene	0.220	0.601
4-methyl sterol + diplopterol	0.247	0.578
4,4-dimethyl sterol	0.253	0.582
hopanol	0.238	0.583
3-methyl hopanol	0.280	0.578
16:1 fatty acid (AS)[c]	0.305	0.626
16:1 fatty acid (PL)[c]	0.278	0.653
16:0 fatty acid (AS)	0.322	0.650
16:0 fatty acid (PL)	0.327	0.646

[a] Equivalent to $f_m\alpha_{l/m}$ in table 4 of Sessions et al. (2002).
[b] Equivalent to $(1 - f_m)\alpha_{l/w}$.
[c] AS = acetone soluble fraction (neutral lipids); PL = phospholipid fraction.

The left superscripts 2 and 3 indicate fractionation factors affecting the D/H and T/H ratios, respectively. The constant m is related only to the masses of the isotopes involved (Bigeleisen, 1965), and for the hydrogen system has a theoretical value of 1.442 (Swain et al., 1958; Cleland, 2003). In contrast, the variable X is a characteristic of each hydrogen source, and its value does not vary between isotopes. Thus for an experiment in which both D/H and T/H ratios are measured, we can write two mass balance equations

$$^2R_T = X_1(^2\alpha_1)(^2R_1) + (1 - X_1)(^2\alpha_2)(^2R_2) \quad (15a)$$

$$^3R_T = X_1(^2\alpha_1)^m(^3R_1) + (1 - X_1)(^2\alpha_2)^m(^3R_2) \quad (15b)$$

which can be parameterized as

$$^2R_T = {}^2p_1 \cdot {}^2R_1 + {}^2p_2 \cdot {}^2R_2 \quad (16a)$$

$$^3R_T = {}^3p_1 \cdot {}^3R_1 + {}^3p_2 \cdot {}^3R_2 \quad (16b)$$

The p coefficients can be determined by linear regression, and algebraic combination of the two equations then gives

$$\left(^2\alpha_1\right)^{m-1} = {}^3p_1/{}^2p_1 \quad (17)$$

and

$$\left(^2\alpha_2\right)^{m-1} = {}^3p_2/{}^2p_2 \quad (18)$$

Unfortunately, sample requirements for precise measurement of T/H ratios are thousands of times larger than those required for measurement of D/H ratios. Still, the approach does offer one potential route to determining hydrogen-isotopic fractionation factors in organisms that obligately use two or more hydrogen sources.

3.5. Interpretation of Data from *Methylococcus capsulatus*

In an earlier report regarding the methanotrophic bacterium *Methylococcus capsulatus*, Sessions et al. (2002) systematically varied δ_1 and δ_2 (corresponding to δ_{CH4} and δ_{H2O}) and measured values of δ_T (corresponding to δ_{lipid}) in multiple, parallel cultures. To interpret the results, values for p_1 and p_2 (termed a and b in Sessions et al., 2002) were calculated by multivariate regression. This part of the analysis is correct, and the data are reproduced in Table 3. Next, starting from a version of Eqn. 11 in which values of α were set to 1.0 while values of ε were allowed to vary, Sessions et al. (2002) calculated values for X_1 (termed f_m). That approximation is invalid and the results must be discarded. Here we reinterpret the data in Table 3 in light of the equations developed above.

Given values of p_1 and p_2, X_1 can be determined only if either α_1 or α_2 is known:

$$X_1 = p_1/\alpha_1 \quad (19)$$

and

$$X_1 = \left(\alpha_2 - p_2\right)/\alpha_2 \quad (20)$$

Then, given X_1, the remaining α value can be calculated. If neither α value is known precisely, as is the case here, combination of Eqns. 19 and 20 yields the relationship which must prevail between the fractionation factors:

$$\alpha_1 = p_1\alpha_2/\left(\alpha_2 - p_2\right) \quad (21)$$

Two families of curves result and are shown in Figure 1. One represents the relationship that must prevail between α_1 and α_2 for isoprenoidal products, the other for fatty acids. For any given (α_1, α_2), there is a corresponding X_1 which is also shown in Figure 1.

The measured isotopic compositions require that α_1 and α_2 are related inversely. If the lipid-water fractionations are similar to those observed in plants (Sessions et al., 1999), then

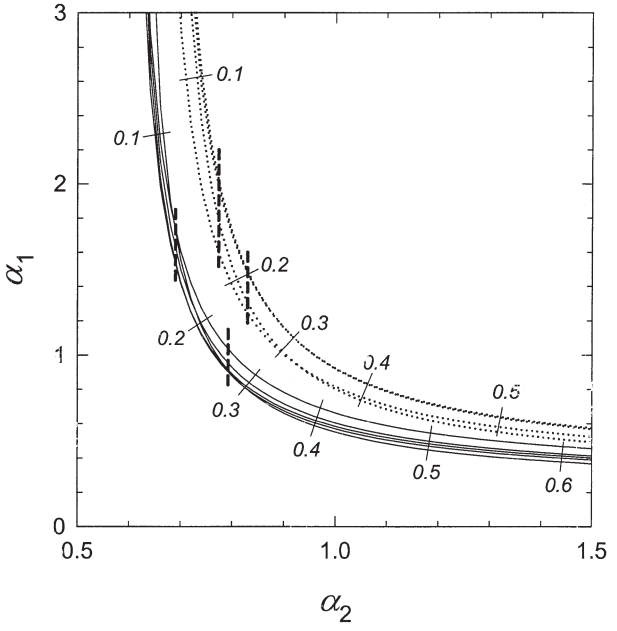

Fig. 1. Relationships between α_1 and α_2, equivalent to $\alpha_{lipid/methane}$ and $\alpha_{lipid/water}$, respectively. The lines derive from Eqn. 21 and the values of p_1 and p_2 specified in Table 3. The solid lines pertain to the polyisoprenoidal compounds (squalene, sterols, hopanols). The dotted lines pertain to the fatty acids. The heavy, vertical broken lines mark the ranges of α_2 observed in plants (Sessions et al., 1999). Different ranges are shown because the polyisoprenoidal compounds are generally depleted in D ($0.690 \le \alpha_2 \le 0.792$) relative to the fatty acids ($0.774 \le \alpha_2 \le 0.830$). The italicized numbers and tick marks normal to the fractionation lines mark values of X_1.

values of α_1, the lipid-methane fractionation factor, are probably near 1.3. This would indicate that, when H was removed from methane, protium flowed preferentially to fates other than lipid biosynthesis, leaving deuterium to be concentrated in the lipids. In these circumstances, $X_1 \approx 0.2$, i.e., only ~20% of lipid hydrogen is derived from CH_4. Conversely, if the lipid-methane fractionation approached 0.570, the intermolecular isotope effect associated with methane uptake by the methane monooxygenase enzyme in vitro (Wilkins et al., 1994), fractionations between lipids and water must have been >1.0. Values of X_1 would be near 0.5.

The first of these alternatives is more likely. It is based on observed water-to-lipid fractionations. The fractionation used to provide a boundary condition in the second alternative pertains to methane uptake rather than to biosynthesis, and takes no account of fractionations downstream from methane oxidation. If the former interpretation is correct, then the data are consistent with the earlier conclusion (Sessions et al., 2002) that X_1 remains approximately constant for all lipids, during both exponential and stationary phases of growth.

4. CONCLUSIONS

Mathematical approximations originally developed for use in manipulating carbon- and oxygen-isotopic data can introduce large errors when used for D/H data. In systems with only one hydrogen source, the reactant and product delta values must be linearly related, and the slope and intercept of that relation should indicate equivalent fractionations. In systems with two or more hydrogen sources, in which both sources have been fractionated from their original isotopic composition, it is not possible to uniquely determine both fractionation factors and the relative contributions of each hydrogen source to the mixture from measurements of D/H ratios alone. This is true even when the isotopic compositions of all hydrogen sources are varied independently. Experiments in which the relative abundances of three isotopes are measured have the ability to solve this problem.

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