Theoretical estimation of the equilibrium distribution of clumped isotopes in nature

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Abstract

Clumped (or multiply substituted) isotope geochemistry, as an emerging field, has displayed an array of unique and effective geological and atmospheric applications. In this study, we introduce a new method to facilitate the calculation of concentrations of multiply substituted isotopologues at equilibrium conditions, especially for the calculation of Δ_i ("i" refers to a specific isotopologue) and Δ_{mass} ("mass" refers to isotopologues with the same mass number). The proposed *exact* method has equivalent accuracy and can be solved by similar level of effort compared to that of Wang et al. (2004). However, the *approximate* method proposed here can handle molecules with isotopomers (e.g., $^{14}N^{15}N^{16}O$ vs. $^{15}N^{14}N^{16}O$) and can estimate Δ_{mass} of any kind of molecule, whether the Δ_i values of singly-substituted isotopologues are small or large. The accuracy and convenience of the proposed method are illustrated through several examples (i.e., CO_2, N_2O and CH_4).

Higher-order anharmonic corrections to Δ_i have been studied carefully. Our results suggest that such corrections are significant, especially for isotopologues involving hydrogen atoms. To obtain the most precise Δ_i or Δ_{mass} value, including those higher-order anharmonic corrections into the calculation is recommended. Such corrections to Δ_i are on the order of percent and are similar to anharmonic corrections for the β factor. The difference is that higher-order anharmonic corrections usually reduce β values, but would either reduce or increase Δ_i values. For example, the corrections can reduce $\Delta_{^{13}CDH_3}$ by five percent but increase $\Delta_{^{18}O^{13}C^{16}O}$ by two percent at 300 K. The choice of the frequency scaling factor in the Δ_i calculation is also found similar to the calculation of β factors. The scaled Δ_i value will approximately equal the un-scaled Δ_i times the scaling factor, suggesting that the uncertainty in the frequency scaling factor (usually on the order of percent) could possibly cause large errors in the calculation of Δ_i. The best way to reduce such errors is to use high-level theoretical methods to reduce the uncertainty of the scaling factor, or to choose frequency scaling factors carefully.

Using the method proposed, the equilibrium Δ_{47} of CO_2 is found to be roughly independent of $\delta^{13}C$ and $\delta^{18}O$ for most geologic samples, suggesting the effects of bulk isotope compositions on Δ_{47} are negligible. We find that the sum of Δ_i for all of the singly substituted isotopologues will be equal to zero approximately, enabling us to determine the stochastic concentrations of isotopologues from experiments.
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1. INTRODUCTION

Stable isotope geochemistry, for a long time, has focused on bulk isotope composition, which is characterized by the concentration of one rare isotope (e.g., $\delta^{18}O$ or $\delta^{34}S$).

Multiply-substituted isotopologues containing two or more rare isotopes, however, had not been considered extensively in geochemistry field until the work of Eiler and his colleagues (e.g., Eiler and Schauble, 2004; Wang et al., 2004; Affek and Eiler, 2006; Ghosh et al., 2006a,b; Schauble et al., 2006; Affek et al., 2007; Came et al., 2007; Eiler, 2007; Ghosh et al., 2007; Guo and Eiler, 2007; Affek et al., 2008; Guo et al., 2009; Huntington et al., 2009; Yeung et al., 2009; Eagle et al., 2010). Similarly, Ma et al. (2008) studied multiply substituted isotopologues in meth-

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ane (CH_4), in which the behavior of the ^{13}C–D species ($^{13}CDH_3$) was studied by integrating IR spectral data and statistical mechanics. Multiply substituted isotopologues are surely to exist in many molecules and compounds, e.g., nitrous oxide (N_2O), water (H_2O), sulfur dioxide (SO_2), sulfate (SO_4^{2-}), and quartz (SiO_2). Analytical techniques that can measure these multiply substituted isotopologues and the theoretical understanding of their formation are lacking.

Wang et al. (2004) proposed the first theoretical framework for multiply substituted isotopologues. They laid a framework for predicting, recognizing and interpreting the abundance variations of clumped isotopologues at equilibrium. Importantly, a new proposed variable "Δ_i" was introduced to describe the abundance of an isotopologue "i" of interest in excess of that expected for a stochastic distribution of isotopes. The magnitude of Δ_i was demonstrated to be temperature dependent. However, at present Δ_i cannot be measured directly. Instead, another variable Δ_{mass}, which refers to isotopologues with the same cardinal molecular mass, is used in experiments. For example, Δ_{47} of CO_2 includes three isotopologues ($^{18}O^{13}C^{16}O$, $^{18}O^{12}C^{17}O$ and $^{17}O^{13}C^{17}O$), which all have a mass of 47, while $\Delta_{^{18}O^{13}C^{16}O}$ (i.e., Δ_i) only refers to $^{18}O^{13}C^{16}O$. If Δ_i or Δ_{mass} can be measured precisely enough, then formation temperatures can be estimated directly without having to assume isotopic equilibrium with another compound. This approach has many advantages over the traditional method of equilibrium oxygen isotope paleo-thermometry, which requires that the $\delta^{18}O$ of two materials are in equilibrium and their $\delta^{18}O$ can be analyzed or determined independently. This condition is often difficult to meet for geologic samples.

The definition of Δ_i given by Wang et al. (2004) is composition-oriented and relies upon a calculated stochastic concentration of the isotopologue of interest (see Eq. (19) in their paper). This definition leads to a complicated calculation procedure when dealing with polyatomic molecules. Here, we suggest that the definition of Δ_i by Wang et al. (2004) can be transformed equivalently to a new definition based on the free energy differences between isotopologues (i.e., partition function ratios). The advantages of the new definition of Δ_i are as follows: (1) the equilibrium concentration of any isotopologue can be calculated directly, (2) either exact or approximate Δ_i values can be calculated, depending on the targeted accuracy and (3) our approximate method can deal with Δ_i and Δ_{mass} values of molecules containing isotopomers (e.g., $\Delta_{^{15}N^{14}N^{16}O}$, $\Delta_{^{14}N^{15}N^{16}O}$ and Δ_{47} of N_2O), which are important properties that the previous approximate methods have difficulty to handle.

As an emerging field within stable isotope geochemistry, the number of theoretical and computational studies on clumped-isotopes is increasing rapidly. Several important issues and pitfalls must be clarified for the calculation of Δ_i or Δ_{mass}. Wang et al. (2004) and Schauble et al. (2006) checked the effects of anharmonicity of ^{13}C–^{18}O clumping and found that the harmonic oscillator approximation has sufficient accuracy for clumped isotope calculations in many small molecules. However, this approximation might not hold for other clumped systems, especially those

involving hydrogen atoms because of the large anharmonicity that is commonly associated with it (e.g., Bigeleisen and Mayer, 1947; Liu et al., 2010). Another common issue is the choice of frequency scaling factors. Schauble et al. (2006) chose scaling factors by comparisons with experimental data. Ma et al. (2008) and Guo et al. (2009) used scaling factors recommended by other researchers. However, many choices are available for frequency scaling factors. Each theoretical method has different uncertainties (Irikura et al., 2009). A specific theoretical level can have several slightly different recommendations (e.g., Scott and Radom, 1996; Merrick et al., 2007; Irikura et al., 2009; Alecu et al., 2010). The frequency scaling factors for a specific theoretical level are also often different when dealing with different types of compounds. Therefore, the effect of scaling factors on the calculation of Δ_i should be carefully checked.

According to the definition of equilibrium Δ_{mass} in Wang et al. (2004), the Δ_{mass} value depends on the formation temperature and on the bulk isotope compositions (in terms of δ, e.g., $\delta^{18}O$) of the studied samples. However, people usually assume the effects of the bulk isotope compositions on Δ_{mass} are very small, and directly use a unique calibration to estimate the formation temperature. Huntington et al. (2009) examined the dependence of Δ_{47} on $\delta^{18}O$ and $\delta^{13}C$ experimentally but used another Δ_{47} definition. Guo et al. (2009) theoretically studied the dependence of kinetic Δ_{47} on $\delta^{18}O$ and $\delta^{13}C$ and found that the dependence was very weak. Here, we provide a more rigorous theoretical analysis of the influence of bulk isotope compositions on the equilibrium Δ_{mass}.

2. THEORETICAL METHODS

For equilibrium isotope fractionations, the fractionation factor (α) is equal to the ratio of the β factors for two compounds (i.e., $\alpha = \beta_a/\beta_b$). The β factor, defined in Richet et al. (1977), is an isotopic fractionation between a compound and a reference material which is an ideal atomic gas (e.g., dissociated and non-interacting atoms of O or C as described in Schauble (2004)). Following this idea, we introduce a monatomic reaction treatment for clumped-isotope systems.

If an isotope exchange reaction occurs between VY_n and the ideal gaseous atomic materials V and Y, then

$$VY_n + V' + kY' \leftrightarrow V'Y'_kY_{n-k} + V + kY \qquad (1)$$
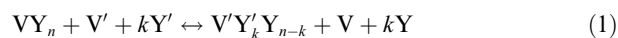
where V and V', Y and Y' denote different isotopes, n is the total Y in VY_n and k is the number of isotopes exchanged. The equilibrium constant of this isotope exchange reaction (K_1) can be conventionally obtained from the partition functions ratios f'_k (Bigeleisen–Mayer equation or Urey Model; Bigeleisen and Mayer, 1947; Urey, 1947):

$$K_1 = f'_k = \frac{s}{s'_k} \cdot \prod_i^l \frac{u'_{ik}}{u_i} \cdot \frac{e^{-u'_{ik}/2}}{1 - e^{-u'_{ik}}} \cdot \frac{1 - e^{-u_i}}{e^{-u_i/2}} \qquad (2)$$

where s is the symmetry number, l is the number of degrees of freedom for vibration modes ($l = 3N - 5$ for a linear molecule and $l = 3N - 6$ for a non-linear molecule, N is the number of atoms in the molecule), u_i is equal to $hc\omega_i/k_BT$, h is the Planck constant, c is the speed of light, ω_i is

the ith normal vibration mode, k_B is the Boltzmann constant and T is the temperature in Kelvin. The superscript ($'$) and subscript (k) denote the V$'$ and Y$'$ rare isotopes, respectively.

The equilibrium concentration of $V'Y'_k Y_{n-k}$ in reaction (1) is

$$[V'Y'_k Y_{n-k}] = f'_k \cdot [VY_n] \cdot \left(\frac{[V']}{[V]}\right)\left(\frac{[Y']}{[Y]}\right)^k \tag{3}$$

According to the definition of β (Richet et al., 1977; Schauble, 2004),

$$\frac{[V']}{[V]} = \left(\frac{V'}{V}\right)_{VY_n} \bigg/ \left({}^{V'}\beta_{VY_n}\right) \tag{4a}$$

$$\frac{[Y']}{[Y]} = \left(\frac{Y'}{Y}\right)_{VY_n} \bigg/ \left({}^{Y'}\beta_{VY_n}\right) \tag{4b}$$

Substituting Eqs. (4a) and (4b) into Eq. (3), we get

$$\frac{[V'Y'_k Y_{n-k}]}{[VY_n]} = \frac{f'_k}{{}^{V'}\beta_{VY_n} \cdot \left({}^{Y'}\beta_{VY_n}\right)^k} \cdot \left(\frac{V'}{V}\right)_{VY_n} \cdot \left(\frac{Y'}{Y}\right)^k_{VY_n} \tag{5}$$

According to the definition of Δ_i (Wang et al., 2004),

$$\Delta_i = 1000 \cdot (R_{i\text{-e}}/R_{i\text{-r}} - 1) \tag{6}$$

where the ratio $R_{i\text{-e}}$ is the abundance of a specific isotopologue i divided by the abundance of the isotopologue containing no rare isotopes, evaluated at thermodynamic equilibrium. $R_{i\text{-r}}$ is the same ratio but it is for the random (stochastic) distribution. For the clumped isotopologue $[V'Y'_k Y_{n-k}]$,

$$R_{i\text{-e}} = \frac{[V'Y'_k Y_{n-k}]}{[VY_n]} \tag{7a}$$

$$R_{i\text{-r}} = \frac{s}{s'_k} \cdot \left(\frac{V'}{V}\right)_{VY_n} \cdot \left(\frac{Y'}{Y}\right)^k_{VY_n} \tag{7b}$$

where the symmetry numbers (s) are added to include the isotopologues with equivalent structural positions into the final random concentration. Combining Eqs. (5), (6), (7a) and (7b), we obtain

$$\Delta_{[V'Y'_k Y_{n-k}]} = 1000 \left(\frac{(s'_k/s) \cdot f'_k}{{}^{V'}\beta_{VY_n} \cdot \left({}^{Y'}\beta_{VY_n}\right)^k} - 1\right) \tag{8}$$

The exact β value of a molecule includes contributions from all of isotopologues in it. Using VY_n as an example, if there are V, V$'$, Y and Y$'$ isotopes in this molecular system, then the exact ${}^{V'}\beta$ and ${}^{Y'}\beta$ are

$$^{V'}\beta_{VY_n} = \frac{\sum_{p=0}^{n}\sum_{j=1}^{t_p} f'_{jp}\left(\frac{[Y']}{[Y]}\right)^p}{\sum_{p=0}^{n}\sum_{j=1}^{t_p} f_{jp}\left(\frac{[Y']}{[Y]}\right)^p} \tag{9a}$$

$$^{Y'}\beta_{VY_n} = \frac{\sum_{p=0}^{n} p \cdot \sum_{j=1}^{t_p}\left(f'_{jp}\frac{[V']}{[V]} + f_{jp}\right)\left(\frac{[Y']}{[Y]}\right)^{p-1}}{\sum_{p=0}^{n}(n-p) \cdot \sum_{j=1}^{t_p}\left(f'_{jp}\frac{[V']}{[V]} + f_{jp}\right)\left(\frac{[Y']}{[Y]}\right)^p} \tag{9b}$$

where the prime superscript in f'_{jp} refers to heavy isotope V$'$, p refers to heavy isotope Y$'$, t_p represents the t isotopomers

when p are fixed and j represents the jth isotopomer. See Appendix A for the full derivation.

As defined, the β factor is a value greater than 1. Therefore, the monatomic [V$'$]/[V] and [Y$'$]/[Y] ratios must be smaller than $(V'/V)_{VY_n}$ and $(Y'/Y)_{VY_n}$, respectively, according to Eqs. (4a) and (4b). In most geological isotope systems, the low abundance of rare isotopes (e.g., D, ^{18}O, ^{17}O or ^{13}C) leads to small bulk isotope ratios. For example, ^{18}O/^{16}O ≈ 0.002 and ^{17}O/^{16}O ≈ 0.0004 in natural abundance, resulting in even smaller concentration ratios of $[^{18}\text{O}]/[^{16}\text{O}]$ and $[^{17}\text{O}]/[^{16}\text{O}]$ in a hypothetical equilibrium. Therefore, Eqs. (9a) and (9b) can be approximated into simple forms by ignoring all of the terms multiplied either [V$'$]/[V] or [Y$'$]/[Y]:

$$^{V'}\beta_{VY_n} \approx f'_0/f_0 = f'_0 \tag{10a}$$

$$^{Y'}\beta_{VY_n} \approx \sum_{j=1}^{t_1} f_{j1} \bigg/ (n \cdot f_0) = \sum_{j=1}^{t_1} f_{j1} \bigg/ n \tag{10b}$$

where f_0 denotes the f function without isotope exchange, obviously, $f_0 = 1$. f'_0 denotes no isotope exchange for Y but there is an isotope exchange on the V atom of VY_n. In this situation, $t_0 = 1$ (there are no isotopomers for VY_n or $V'Y_n$). f_{j1} represents the partition function ratio of the jth isotopomer, which only contains one rare isotope Y$'$, to the one containing no rare isotope. Galimov (1971) suggested equations similar to Eqs. (10a) and (10b) for carbon isotope studies in biogeochemistry. Similar approximate treatments were also used by other researchers in studies of oxygen isotopes in clay minerals and hydrogen isotopes in organic matter (Méheut et al., 2007; Wang et al., 2009).

If β factors can be approximated by Eqs. (10a) and (10b), then we can obtain approximate Δ_i values for any isotopologue. The approximation method in Wang et al. (2004) becomes inaccurate when the Δ_i values of singly substituted isotopologues are large, e.g., in N_2O or deuterium-bearing clumped-isotope systems.

3. APPLICATION EXAMPLES

3.1. Exact and approximate calculations of Δ_i

First, we will show how to calculate Δ_i exactly by using CO_2 as an example. From Eqs. (A1a) and (A1b), we obtain these relationships (see Appendix A for details):

$$\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{CO_2} = \frac{\sum_{p=0}^{2}\sum_{q=0}^{2-p} f'_{pq}(x_1)^p(x_2)^q}{\sum_{p=0}^{2}\sum_{q=0}^{2-p} f_{pq}(x_1)^p(x_2)^q} \cdot x_3 \tag{11a}$$

$$\left(\frac{^{18}\text{O}}{^{16}\text{O}}\right)_{CO_2} = \frac{\sum_{p=0}^{2}\sum_{q=0}^{2-p} p \cdot (f'_{pq}x_3 + f_{pq})(x_1)^p(x_2)^q}{\sum_{p=0}^{2}\sum_{q=0}^{2-p}(2-p-q) \cdot (f'_{pq}x_3 + f_{pq})(x_1)^p(x_2)^q} \tag{11b}$$

$$\left(\frac{^{17}\text{O}}{^{16}\text{O}}\right)_{CO_2} = \frac{\sum_{p=0}^{2}\sum_{q=0}^{2-p} q \cdot (f'_{pq}x_3 + f_{pq})(x_1)^p(x_2)^q}{\sum_{p=0}^{2}\sum_{q=0}^{2-p}(2-p-q) \cdot (f'_{pq}x_3 + f_{pq})(x_1)^p(x_2)^q} \tag{11c}$$

where x_1, x_2 and x_3 are the $[^{18}\text{O}]/[^{16}\text{O}]$, $[^{17}\text{O}]/[^{16}\text{O}]$ and $[^{13}\text{C}]/[^{12}\text{C}]$ ratios, respectively, and represent the concentration ratios of the ideal monatomic materials O and C in

equilibrium with CO_2. The left sides of Eqs. (11a–c) are the bulk isotope composition of CO_2. In this example calculation, we use the VSMOW (Vienna standard mean ocean water) and PDB (Pee Dee Belemnite) standards as these compositions: $^{18}O/^{16}O = 2005.2 \times 10^{-6}$, $^{17}O/^{16}O = 379.9 \times 10^{-6}$ (IUPAC, 2003) and $^{13}C/^{12}C = 11237.2 \times 10^{-6}$ (Craig, 1957). The bulk isotope compositions do not have large effects on the value of Δ_i and Δ_{mass} (see Section 4.1). Therefore, using any bulk isotope compositions that is similar to those of natural samples will make little difference to the results.

Using the Bigeleisen–Mayer equation (B.M. equation hereafter, Bigeleisen and Mayer, 1947; Urey, 1947) coupled with quantum chemistry calculations, we can obtain all of the f and f' functions in Eqs. (11a–c) (details are given at below). With known f and f' functions and $(^{18}O/^{16}O)_{CO_2}$, $(^{17}O/^{16}O)_{CO_2}$ and $(^{13}O/^{12}O)_{CO_2}$ bulk isotopic ratios, solving Eqs. (11a-c) for the unknown variables x_1, x_2 and x_3 is straightforward. Once x_1, x_2 and x_3 are obtained, the $^{13}\beta$, $^{18}\beta$ and $^{17}\beta$ factors can be obtained through Eqs. (9a) and (9b). Consequently, all of the Δ_i values of the CO_2 system can be calculated precisely from Eq. (8).

To calculate the f and f' functions by the B.M. equation, the harmonic frequencies of every isotopologue are needed. Many quantum chemistry software packages can provide these harmonic frequencies. Here, the Gaussian03 program package (Frisch et al., 2004) is used. The Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid DFT (Density Functional Theory) method and the Møller–Plesset perturbation (MP) method are used for the calculations. The DFT method deals with electron correlation corrections in a partially empirical way (Lee et al., 1988; Becke, 1993), and the MP theory includes electron correlation corrections through Rayleigh–Schrödinger perturbation

theory (RS-PT), most commonly to the second order (MP2) (e.g., Møller and Plesset, 1934). In order to avoid using scaling factors, we chose the B3LYP and MP2 methods with large basis sets (i.e., B3LYP/6-311 + G(3df,2p) or MP2/aug-cc-pVTZ) to obtain accurate harmonic frequencies directly. The frequency results are shown in Table 1. Even without scaling, our calculated frequencies generally agree with the experimental harmonic frequencies (Gray and Robiette, 1979; Majcherova et al., 2005; Vlasova et al., 2006). Because the B3LYP/6-311 + G(3df,2p) level seems to overestimate frequency numbers by a few percent (i.e., 1–3%) for molecules without hydrogen atoms, and underestimate those of H-containing molecules by a similar order, we chose not scale the results of B3LYP/6-311 + G(3df,2p) for all of the cases. We also calculated the f and f' functions using the zero-point energy (ZPE) model, which includes anharmonic corrections through the direct use of accurate zero point energies (see Appendix B for details).

The calculated results of Δ_i are shown in Table 2 (at 300 K), and our results are comparable with those of Wang et al. (2004). The difference between our ZPE-E results and their results is very small, and it may be a result of different numerical calculation processes. The Δ_i results of the B.M. equation differ slightly from the anharmonic method (i.e., the ZPE model). Anharmonic effects will be discussed in detail in Section 4.2.

Our exact method has one less equation to be solved than the exact method of Wang et al. (2004) because of the relative concentration being used directly. For instance, the concentration of $^{16}O^{12}C^{16}O$ is not needed in our method because it will be canceled during the calculation. If needed, the concentration can be obtained through the following equation:

Table 1
Calculated and experimental harmonic frequencies (corrected for anharmonicity).

$^{16}O^{12}C^{16}O$	ω_1 (cm^{-1})	ω_2^* (cm^{-1})	ω_3 (cm^{-1})	ZPE (cm^{-1})
Experimental[a]	1353.670	672.840	2396.232	2531.403[f]
Wang et al.[b]	1348.479	670.315	2387.185	2525.394
B3LYP/6-311 + G(3df,2p)	1374.088	678.762	2414.272	–
MP2/aug-cc-pVTZ	1326.072	658.807	2401.922	–
$^{15}N^{15}N^{16}O$				
Experimental[c]	1280.613	581.253	2209.576	2302.703[f]
Wang et al.[b]	1275.062	576.848	2201.112	2301.072
B3LYP/6-311 + G(3df,2p)	1313.218	601.015	2268.811	–

$^{12}CH_4$	ω_1 (cm^{-1})	ω_2^* (cm^{-1})	ω_3^+ (cm^{-1})	ω_4^+ (cm^{-1})	ZPE (cm^{-1})
Experimental[d]	3025.5	1582.7	3156.8	1367.4	9725.1[f]
Ma et al.[e]	2991.55	1547.83	3135.36	1365.17	9794.40[g]
B3LYP/6-311 + G(3df,2p)	3029.59	1559.64	3131.79	1341.83	9638.56

ω_2^* is doubly degenerate.
ω_3^+ and ω_4^+ are triply degenerate.
[a] Majcherova et al. (2005).
[b] Originally from Zúñiga et al. (1999) and Zúñiga et al. (2001) and also used in Wang et al. (2004).
[c] Vlasova et al. (2006).
[d] Gray and Robiette (1979).
[e] Ma et al. (2008).
[f] Calculated by Eq. (B3) without G_0 term (see Appendix B).
[g] Calculated by ZPE = $1/2 \sum_i \omega_i$.

Table 2
The Δ_i results of CO_2 at 300 K using different methods.

	Wang et al. results	This study – using Wang et al.'s data				This study – DFT[*]		This study – MP2^{+}	
		ZPE-E[a]	ZPE-A[b]	B.M.-E[c]	B.M.-A[d]	B.M.-E[c]	B.M.-A[d]	B.M.-E[c]	B.M.-A[d]
$^{16}O^{13}C^{16}O$	−0.004	−0.004	0.000	−0.004	0.000	−0.004	0.000	−0.004	0.000
$^{16}O^{12}C^{17}O$	−0.006	−0.006	0.000	−0.006	0.000	−0.006	0.000	−0.006	0.000
$^{16}O^{12}C^{18}O$	−0.011	−0.011	0.000	−0.011	0.000	−0.011	0.000	−0.011	0.000
$^{16}O^{13}C^{17}O$	0.489	0.489	0.499	0.477	0.487	0.488	0.498	0.480	0.490
$^{17}O^{12}C^{17}O$	0.085	0.087	0.099	0.087	0.099	0.093	0.105	0.081	0.092
$^{16}O^{13}C^{18}O$	0.938	0.936	0.951	0.917	0.932	0.935	0.951	0.921	0.936
$^{17}O^{12}C^{18}O$	0.168	0.167	0.184	0.172	0.189	0.183	0.201	0.160	0.177
$^{17}O^{13}C^{17}O$	1.074	1.075	1.091	1.052	1.067	1.079	1.095	1.051	1.067
$^{18}O^{12}C^{18}O$	0.333	0.330	0.353	0.340	0.363	0.362	0.384	0.316	0.339
$^{17}O^{13}C^{18}O$	1.608	1.605	1.627	1.579	1.600	1.618	1.639	1.572	1.593
$^{18}O^{13}C^{18}O$	2.219	2.216	2.243	2.180	2.207	2.240	2.267	2.166	2.192

[*] DFT represents the B3LYP/6-311 + G(3df,2p) level.
[+] MP2 represents the MP2/aug-cc-pVTZ level.
[a] ZPE-E is the results calculated by ZPE model exactly.
[b] ZPE-A is the results calculated by ZPE model approximately.
[c] B.M.-E is the results calculated by B.M. equation exactly.
[d] B.M.-A is the results calculated by B.M. equation approximately.

$$[^{12}C^{16}O^{16}O] \cdot \sum_{p=0}^{2} \sum_{q=0}^{2-p} (f'_{pq} \cdot x_3 + f_{pq})(x_1)^p(x_2)^q = 1 \quad (12)$$

We also calculate Δ_i approximately by Eqs. (10a) and (10b) (denoted as "ZPE-A" or "B.M.-A" in Table 2). The results of the approximation methods deviate from the results of the exact methods by about 1–2%, which is similar to the effects of anharmonicity and the scaling factor. For example, $\Delta_{^{16}O^{13}C^{18}O}$ deviates from its exact result (0.936‰) by about 0.015‰ at 300 K. Such small differences are already acceptable for many cases, and the approximate results are always larger than the exact results, suggesting a possibility to improve the approximate results by using scaling treatments (e.g., scaling down the results by 1%). With increase in temperature, the differences between the exact methods and approximate methods rapidly decrease (see Appendix C).

3.2. Exact and approximate calculations of Δ_{mass}

We have only discussed how to calculate Δ_i. However, the Δ_{mass} value (such as Δ_{47} for CO_2) is the one actually measured in experiments. With the present technology, it is difficult to distinguish isotopologues of the same cardinal mass, such as $^{18}O^{13}C^{16}O$, $^{17}O^{13}C^{17}O$ and $^{18}O^{12}C^{17}O$. Here, we use the Δ_{47} of CO_2 as an example to show how to calculate Δ_{mass} exactly and approximately.

Generally, Δ_{mass} and Δ_i are related by

$$\Delta_{mass} = \sum_i m_i \Delta_i \quad (13)$$

where m_i is the relative abundance fractions of the isotopologues "i" when it is in stochastic distribution (Wang et al., 2004; Schauble et al., 2006; Guo et al., 2009). According to the Δ_{47} definition by Wang et al. (2004):

$$\Delta_{47} = 1000 \times \left(\frac{R^{47}}{R_r^{47}} - 1 \right)$$

$$= 1000 \times \left(\frac{\frac{[^{18}O^{13}C^{16}O]+[^{18}O^{12}C^{17}O]+[^{17}O^{13}C^{17}O]}{[^{16}O^{12}C^{16}O]}}{\frac{[^{18}O^{13}C^{16}O]_r+[^{18}O^{12}C^{17}O]_r+[^{17}O^{13}C^{17}O]_r}{[^{16}O^{12}C^{16}O]_r}} - 1 \right) \quad (14)$$

We obtain

$$\Delta_{47} = m_{^{18}O^{13}C^{16}O} \cdot \Delta_{^{18}O^{13}C^{16}O} + m_{^{18}O^{12}C^{17}O} \cdot \Delta_{^{18}O^{12}C^{17}O} + m_{^{17}O^{13}C^{17}O} \cdot \Delta_{^{17}O^{13}C^{17}O} \quad (15)$$

where

$$m_{^{18}O^{13}C^{16}O} = \frac{[^{18}O^{13}C^{16}O]_r}{[^{18}O^{13}C^{16}O]_r + [^{18}O^{12}C^{17}O]_r + [^{17}O^{13}C^{17}O]_r}$$

$$m_{^{18}O^{12}C^{17}O} = \frac{[^{18}O^{12}C^{17}O]_r}{[^{18}O^{13}C^{16}O]_r + [^{18}O^{12}C^{17}O]_r + [^{17}O^{13}C^{17}O]_r}$$

$$m_{^{17}O^{13}C^{17}O} = \frac{[^{17}O^{13}C^{17}O]_r}{[^{18}O^{13}C^{16}O]_r + [^{18}O^{12}C^{17}O]_r + [^{17}O^{13}C^{17}O]_r}$$

and "r" represents the random distribution.

Eq. (13) suggests that accurate Δ_{mass} values can be determined as long as Δ_i is accurate. If the bulk isotope ratios (($^{18}O/^{16}O$), ($^{17}O/^{16}O$) and ($^{13}C/^{12}C$)) of CO_2 are the same as the VSMOW and PDB standards (see the previous section), then we can obtain ms as $m_{^{18}O^{13}C^{16}O} = 0.96726$, $m_{^{18}O^{12}C^{17}O} = 0.03270$ and $m_{^{17}O^{13}C^{17}O} = 0.00004$. Using our exact Δ_i in Table 2, we obtain $\Delta_{47} = 0.911‰$ at 300 K, compared to 0.914‰ in Wang et al. (2004). Using approximate Δ_i values, we obtain $\Delta_{47} = 0.926‰$ at 300 K and $\Delta_{47} = 0.056‰$ at 1000 K approximately. A scaling treatment on approximate Δ_i values may be a practical way to improve the results of Δ_{mass}.

3.3. Approximate calculation of Δ_i when containing isotopomers

In many situations, the molecules are large and have many isotopomers. The complexity, in terms of numerous isotopologues, may prohibit the use of cumbersome calculation procedures. The approximate method proposed in this study is a practical way to handle such situations.

We use N_2O as an example to show how to approximate the Δ_i values of molecules containing isotopomers. Although N_2O is just a small molecule, the procedure is the same for large molecules.

N_2O has two isotopomers containing one ^{15}N isotope ($^{15}N^{14}NO$ and $^{14}N^{15}NO$). The approximate β factors of N_2O can be calculated from Eqs. (10a) and (10b):

$$^{18}\beta \approx f(^{14}N^{14}N^{18}O) \tag{16a}$$

$$^{17}\beta \approx f(^{14}N^{14}N^{17}O) \tag{16b}$$

$$^{15}\beta \approx \frac{f(^{14}N^{15}N^{16}O) + f(^{15}N^{14}N^{16}O)}{2}. \tag{16c}$$

The β factor for molecules with isotopomers is the average value of the f functions of the related isotopologues (e.g., Eq. (16c)). According to Eq. (8), we can calculate any Δ_i values of N_2O. For example, $\Delta_{^{14}N^{15}N^{16}O}$ is obtained from:

$$\Delta_{^{14}N^{15}N^{16}O} = 1000\left(\frac{f(^{14}N^{15}N^{16}O)}{^{15}\beta} - 1\right). \tag{17}$$

By using the frequency data of Wang et al. (2004) and our methods, the exact and approximate Δ_i values of N_2O at 300 K were calculated (Table 3). The results are in good agreement with the previous calculations (Table 3). The Δ_{47} of N_2O (including three isotopologues – $^{15}N^{15}N^{17}O$, $^{14}N^{15}N^{18}O$ and $^{15}N^{14}N^{18}O$) is calculated approximately as 0.376‰ at 300 K, compared to 0.371‰ of Wang et al. (2004).

4. DISCUSSION

4.1. The relationship between equilibrium Δ_{mass} and bulk isotope composition

Δ_{mass} is dependent on the equilibration temperature and the bulk isotope composition (e.g., Eq. (15)). The potential influence of the bulk isotope composition on Δ_{mass} should be investigated to see if composition-dependent corrections to inferred temperatures could improve their accuracy. Huntington et al. (2009) checked the dependence of Δ_{47} on $\delta^{18}O$ and $\delta^{13}C$ experimentally, but they used another Δ_{47} definition. The dependence of equilibrium Δ_{mass} on the bulk isotope composition is theoretically analyzed here.

Using CO_2 as an example and the frequency data from Wang et al. (2004), the Δ_{47} values are calculated using $\delta^{18}O$ and $\delta^{13}C$ isotopic compositions varying from $-40‰$ to $40‰$ (Fig. 1), which is a range spanning most geologic samples. The $\delta^{17}O$ values are obtained by $\delta^{17}O = 0.516 * \delta^{18}O$ assuming a typical mass-dependent isotope fractionation relationship (e.g., Boering et al., 2004). Fig. 1 shows that Δ_{47} varies slightly with $\delta^{18}O$ and $\delta^{13}C$. Δ_{47} has a negative correlation with $\delta^{18}O$ and a positive correlation with $\delta^{13}C$. However, such variations are very small, e.g., less than 0.002‰ at 300 K (equivalent to less than 0.5 °C in temperature reconstruction).

Because the concentration and Δ_i value of isotopologue $^{18}O^{12}C^{17}O$ in CO_2 is far less than $^{18}O^{13}C^{16}O$ and the concentration of $^{17}O^{13}C^{17}O$ is negligible, Eq. (15) can be further approximated to

$$\Delta_{47} \approx m_{^{18}O^{13}C^{16}O} \cdot \Delta_{^{18}O^{13}C^{16}O}$$

$$\approx \Delta_{^{18}O^{13}C^{16}O}\left/\left(1 + \frac{^{17}R_{ref} \cdot (1 + \delta^{18}O)^{0.516}}{^{13}R_{ref} \cdot (1 + \delta^{13}C)}\right)\right. \tag{18}$$

where $^{17}R_{ref} = 379.9 \times 10^{-6}$ and $^{13}R_{ref} = 11237.2 \times 10^{-6}$.

In Eq. (18), $\Delta_{^{18}O^{13}C^{16}O}$ is almost independent of the isotopic composition (see Section 2). Because the ratio

Table 3
The Δ_i results of N_2O at 300 K using different methods.

	Wang et al. results	This study – Wang et al.'s data				This study – DFT[*]	
		ZPE-E[a]	ZPE-A[b]	B.M.-E[c]	B.M.-A[d]	B.M.-E[c]	B.M.-A[d]
$^{14}N^{15}N^{16}O$	22.807	22.806	22.807	23.421	23.422	23.731	23.732
$^{15}N^{14}N^{16}O$	−22.809	−22.808	−22.807	−23.423	−23.422	−23.733	−23.732
$^{14}N^{14}N^{17}O$	−0.002	−0.001	0.000	−0.001	0.000	−0.002	0.000
$^{15}N^{15}N^{16}O$	0.073	0.067	0.069	0.034	0.036	0.060	0.062
$^{14}N^{15}N^{17}O$	23.116	23.112	23.114	23.720	23.722	24.040	24.042
$^{15}N^{14}N^{17}O$	−22.729	−22.731	−22.729	−23.341	−23.339	−23.640	−23.637
$^{14}N^{14}N^{18}O$	−0.003	−0.003	0.000	−0.003	0.000	−0.003	0.000
$^{15}N^{15}N^{17}O$	0.454	0.446	0.449	0.408	0.411	0.454	0.457
$^{14}N^{15}N^{18}O$	23.398	23.399	23.403	23.995	23.999	24.320	24.324
$^{15}N^{14}N^{18}O$	−22.657	−22.655	−22.651	−23.265	−23.262	−23.553	−23.549
$^{15}N^{15}N^{18}O$	0.801	0.797	0.802	0.752	0.756	0.815	0.820

[*] DFT represents the B3LYP/6-311 + G(3df,2p).
[a] ZPE-E is the results calculated by ZPE model exactly.
[b] ZPE-A is the results calculated by ZPE model approximately.
[c] B.M.-E is the results calculated by B.M. equation exactly.
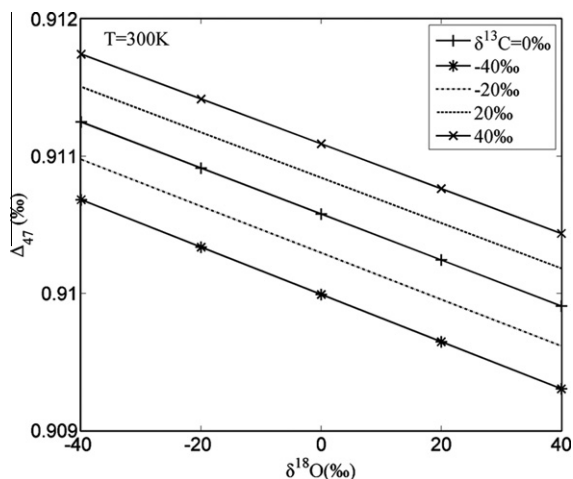[d] B.M.-A is the results calculated by B.M. equation approximately.

Fig. 1. The value of Δ_{47} varies with $\delta^{13}C$ and $\delta^{18}O$ at 300 K. Δ_{47} increases with increasing $\delta^{13}C$ but decreases as $\delta^{18}O$ increases. Although Δ_{47} varies with δ, the magnitude of variation is very small, consistent with a uniform Δ_{47}–T relationship in natural samples.

$(^{17}R_{ref}/^{13}R_{ref})$ is only 0.034, any changes caused by variations of $\delta^{18}O$ and $\delta^{13}C$ will be minimal. Therefore, a roughly independent Δ_{47}–T relationship could be expected in nature. However, for artificial samples with extreme isotopic compositions and some new clumped isotope systems (e.g., those with hydrogen atoms), the corrected Δ_{mass}–T relationships should be incorporated in thermometric calculations.

Note that another Δ_{47} definition (i.e., $\Delta_{47} = 1000 \times (R^{47}/R_r^{47} - R^{46}/R_r^{46} - R^{45}/R_r^{45} + 1)$) has been used (e.g., Eiler, 2007; Huntington et al., 2009). The difference between the Δ_{47} values using these two definitions is small. At roughly natural C- and O-isotope abundances, we find the relationship between them is

$$\Delta_{47}(\text{Eiler, 2007}) \approx 1.016 \times \Delta_{47}(\text{Wang et al., 2004}) \quad (19)$$

4.2. The effect of anharmonic corrections

Much like the definition of Δ_i in Eq. (6), we define Δ_i' as $\Delta_i' = 1000 \times \ln(R_{i\text{-e}}/R_{i\text{-r}})$. Generally, Δ_i and Δ_i' are approximately equal to each other because the ratio of $R_{i\text{-e}}/R_{i\text{-r}}$ is close to 1 and the approximation $\ln(1 + x) \approx x$ is true when x is small. Here, we will only discuss the anharmonic correction to Δ_i' instead of Δ_i. Δ_i' can be calculated using

$$\Delta'_{[V'Y'_k Y_{n-k}]} = 1000 \ln \left(\frac{(s'_k/s) \cdot f'_k}{V' \beta_{VY_n} \cdot \left(Y' \beta_{VY_n}\right)^k} \right) \quad (20)$$

Taking $\Delta'_{^{18}O^{13}C^{16}O}$ of CO_2 as an example,

$$\Delta'_{^{18}O^{13}C^{16}O} = 1000 \ln \left(\frac{1}{2} \frac{f'_1}{^{13}\beta \cdot {}^{18}\beta} \right) \approx 1000 \ln \left(\frac{f_{836}}{f_{636} \cdot f_{826}} \right) \quad (21)$$

where f_{836}, f_{636} and f_{826} are the partition function ratios of $^{18}O^{13}C^{16}O$ to $^{16}O^{12}C^{16}O$, $^{16}O^{13}C^{16}O$ to $^{16}O^{12}C^{16}O$ and

$^{18}O^{12}C^{16}O$ to $^{16}O^{12}C^{16}O$, respectively. Each f can be expressed as a product of two parts: the harmonic part and the anharmonic correction part (i.e., $f = f_{Harm} \times f_{Corr}$, see Eq. (B6) in Appendix B for the detail of f_{Corr}). In this way, Δ_i' can also be separated into harmonic and anharmonic parts: $\Delta_i' = \Delta'_{i\text{-h}} + \Delta'_{i\text{-anhc}}$. The anharmonic correction part of Δ_i' is (see Appendix B)

$$\Delta'_{^{18}O^{13}C^{16}O-anhc} = 1000 \left(\frac{1}{4} \frac{hc}{k_b T} \sum_{i \leqslant j}^{l} \left(\chi_{ij}^{636} + \chi_{ij}^{826} - \chi_{ij}^{836} - \chi_{ij} \right) \right) \quad (22)$$

where χ_{ij}^{636}, χ_{ij}^{826}, χ_{ij}^{836} and $\chi_{i,j}$ are the anharmonic constants of $^{16}O^{13}C^{16}O$, $^{18}O^{12}C^{16}O$, $^{18}O^{13}C^{16}O$ and $^{16}O^{12}C^{16}O$. Finding experimental data for minor isotopologues is difficult, therefore, we use the Darling–Dennison approximation rule (e.g., Richet et al., 1977) to obtain anharmonic constants of minor isotope species approximately:

$$\chi'_{ij} = \chi_{ij} \frac{\omega'_i \omega'_j}{\omega_i \omega_j} \quad (23)$$

where the prime superscript denotes the molecule containing heavy isotopes. This approximation is only suitable for simple molecules (e.g., Darling and Dennison, 1940).

The harmonic frequencies in Eq. (23) are obtained by a calculation at the B3LYP/6-311 + G(3df,2p) level. For the anharmonic constants of the major isotopologue, we use the values provided in Majcherova et al. (2005). Then, we can obtain the pure anharmonic contribution part to Δ_i'. Fig. 2 shows the anharmonic correction to $\Delta'_{^{18}O^{13}C^{16}O}$ and its temperature dependence. This correction is in good agreement with the results in Table 2.

Ma et al. (2008) studied the doubly-substituted methane isotopologue $^{13}CDH_3$ at a harmonic theoretical level. Here, we check the anharmonic contribution to $\Delta_{^{13}CDH_3}$ of methane (CH_4). Without anharmonic corrections, our harmonic results agree well with those of Ma et al. (2008). However,
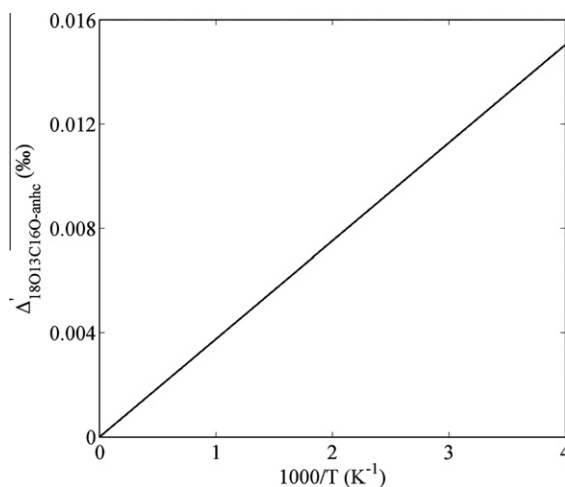

Fig. 2. The anharmonic correction to $\Delta'_{^{18}O^{13}C^{16}O}$ varies with temperature, raising $\Delta'_{^{18}O^{13}C^{16}O}$ by about 0.0125‰ at room temperature. Our results in Table 2 closely match previous calculations (Wang et al., 2004) very well.
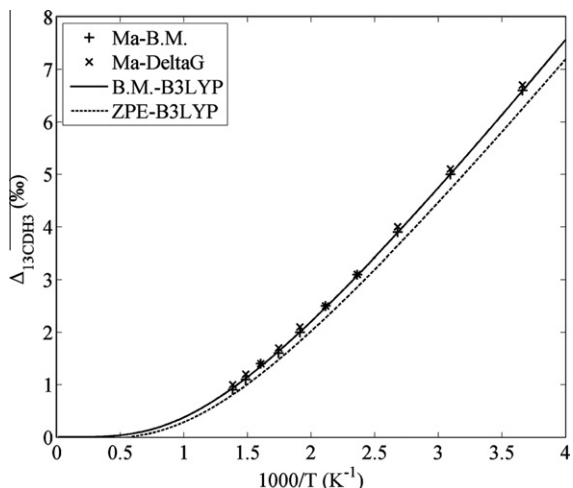
Fig. 3. The anharmonic correction to $\Delta_{^{13}CDH_3}$ varies with temperature. Our harmonic results agree well with Ma et al. (2008). The anharmonic correction reduces $\Delta_{^{13}CDH_3}$ by about 0.2‰ from 0 to 450 °C.

our results with the anharmonic correction reduce $\Delta_{^{13}CDH_3}$ by approximately 0.2‰ (i.e., reduce by about 5%) from 0 to 450 °C (Fig. 3).

The calculated β factors in this study ($^{13}\beta = 1.1263$ and $^{D}\beta = 12.7984$) are in good agreement with the β factors ($^{13}\beta = 1.1262$ and $^{D}\beta = 12.8201$) provided by Richet et al. (1977), also with anharmonic corrections. The difference between our $^{D}\beta$ values and their results will bring about 0.01‰ error in $\Delta_{^{13}CDH_3}$ calculation (estimated by the proportional projection method, see Schauble et al. (2006) for details).

From Eqs. (10a) and (10b), we know that the β factor is a function of f. For general cases, β factors will become smaller after applied anharmonic corrections (see Appendix B and Liu et al., 2010). However, anharmonic corrections can either reduce or increase Δ_i. For example, they can reduce $\Delta_{^{13}CDH_3}$ by 5% (i.e., 0.309‰) but increase $\Delta_{^{18}O^{13}C^{16}O}$ by 2% (i.e., 0.019‰) at 300 K.

Generally, anharmonic corrections can change β factors and Δ_i by similar orders. For example, they reduce $^{13}\beta$, $^{D}\beta$ or $\Delta_{^{13}CDH_3}$ of CH_4 by about 5% (i.e., 5‰, 149‰ and 0.309‰), respectively. For CO_2 and N_2O, the corresponding corrections on β factors and Δ_i values are about 2% at 300 K.

These theoretical analyses of the anharmonic effects on Δ_i indicate that the corrections are on the order of percents of Δ_i and β. We recommend including anharmonic corrections for the Δ_i calculation. Clumps involving hydrogen atoms will show even larger effects.

4.3. The effects of frequency scaling factor

Pure harmonic frequencies are needed for the partition function ratio (or RPFR) calculation if using the B.M. equation. *Ab initio* quantum chemistry calculations are an effective approach to provide such harmonic frequencies (Hehre et al., 1986). However, when the theoretical level used is insufficient, frequency scaling factors may be needed

to correct the frequency results. Previous studies (e.g., Liu and Tossell, 2005; Schauble et al., 2006; Méheut et al., 2007, 2009; Li et al., 2009; Schauble, 2011) suggested that the choice of frequency scaling factors only bring small effects on the calculation of isotope fractionation factor (α) when α is not very large. For multiply substituted isotopologues, we need to know whether the Δ_i calculation is sensitive to frequency scaling factors or not.

As a first-order approximation, we find the following relationship (see Appendix D for details):

$$\Delta'_{i-s} \approx S \cdot \Delta'_i \quad (24)$$

where Δ'_{i-s} is the Δ'_i value after the scaling treatment and S is the frequency scaling factor. Eq. (24) implies that the uncertainties in the scaling factors will be transferred to Δ_i proportionately. Usually, the uncertainty in the frequency scaling factors is on the order of percent, meaning the same order of error in Δ_i. One common way to reduce such errors is to use high-level theoretical methods, whose scaling factors have relatively small uncertainties (e.g., Andersson and Uvdal, 2005; Irikura et al., 2009; Liu et al., 2010). If less precise theoretical levels have to be used, as is usually the case for large systems, then the frequency scaling factors should be chosen carefully.

4.4. Exploring the behavior of singly substituted isotopologues

Many general rules for the behavior of clumped isotopes have been observed (Wang et al., 2004; Schauble et al., 2006; Ma et al., 2008; Guo et al., 2009; Eagle et al., 2010). For example, Wang et al. (2004) found that most singly substituted isotopologues have Δ_i values close to zero; excepting $^{15}N^{14}N^{16}O$ and $^{14}N^{15}N^{16}O$ (see Tables 2 and 3). Schauble et al. (2006) found that carbonate minerals possess very similar Δ_i values for different chemical compositions and structures, and that even carbonate ion in apatite has the same $^{13}C-^{18}O$ clumping (Eagle et al., 2010). Here, the behavior of singly substituted isotopologues is theoretically analyzed.

For singly substituted isotopologue without isotopomers (e.g., $^{18}O^{12}C^{16}O$, $^{14}N^{14}N^{18}O$, etc.), the β factors are approximately equal to f_1/n, where f_1 is the partition function ratio of a molecule containing one rare isotope relative to one without a rare isotope (e.g., $^{18}O^{12}C^{16}O$ to $^{16}O^{12}C^{16}O$) and n is the number of atoms relating to the corresponding β (e.g., $n = 2$ for $^{O}\beta_{CO_2}$; $n = 1$ for $^{C}\beta_{CO_2}$). n in this case is always equal to s/s_1 (symmetry number of non-substituted to one rare isotope substituted). Hence, from Eq. (8):

$$\Delta_1 = 1000 \times \left(\frac{s_1}{s}f_1 \middle/ \beta - 1\right) \approx 1000 \times \left(\frac{s_1}{s}f_1 \middle/ \frac{f_1}{n} - 1\right) = 0 \quad (25)$$

where '1' refers to a singly substituted isotopologue. This result shows that the Δ_i values of singly substituted isotopologues without isotopomers will be close to zero. It implies that this kind of isotopologue is always close to stochastic abundance.

For singly substituted isotopologues with isotopomers (e.g., $^{15}N^{14}N^{16}O$ and $^{14}N^{15}N^{16}O$, $^{13}CH_3CH_2CH_3$ and

$CH_3{}^{13}CH_2CH_3$, etc.), large Δ_i values appear as a result of the large free energy difference among the isotopomers (Galimov, 1971; Wang et al., 2004). However, all these isotopomers have the same mass, and their relative stochastic abundance fractions are inversely proportional to their related symmetry number from Eq. (7b) (i.e., s/s_{j1}). From Eq. (13), their final contribution to the corresponding Δ_{mass} could be obtained as

$$
\Delta_{mass}^{sc} = \sum_{j=1}^{t_1} \frac{s/s_{j1}}{\sum_{j=1}^{t_1} s/s_{j1}} \Delta_{j1} = \frac{1}{n} \sum_{j=1}^{t_1} \frac{s}{s_{j1}} \left(\frac{s_{j1}}{s} \cdot \frac{f_{j1}}{\beta} - 1 \right)
$$

$$
\approx \frac{1}{n} \left(\left(\sum_{j=1}^{t_1} f_{j1} \right) \Big/ \left(\sum_{j=1}^{t_1} f_{j1} \Big/ n \right) - \sum_{j=1}^{t_1} \frac{s}{s_{j1}} \right) = 0 \quad (26)
$$

where n is always equal to $\sum(s/s_{j1})$. Therefore, there is little "net" contribution to Δ_{mass} from singly substituted isotopologues, no matter whether they occur with or without isotopomers. Wang et al. (2004) reached the same conclusion in their numerical calculations of Δ_i for many singly substituted isotopologues.

One application of Eq.(26) is that it justifies the assumption used to determine δ values (e.g., $\delta^{18}O$ and $\delta^{13}C$) in experiments. For example, because $^{16}O^{13}C^{16}O$ and $^{18}O^{12}C^{16}O$ of CO_2 dominate the isotopologues with mass-45 and mass-46 (Eiler, 2007), it is reasonable to assume that R_{45} and R_{46} are in stochastic distribution too (Eiler and Schauble, 2004). This assumption underlies the standard procedure of extracting $\delta^{13}C$ and $\delta^{18}O$ values from mass-spectrometric data (e.g., Craig, 1957).

5. CONCLUSIONS

In this paper, a new method based on statistical mechanics is proposed to calculate Δ_i for clumped isotope systems. The calculations for CO_2, N_2O and CH_4 agree with the previous results. Unlike the method suggested by Wang et al. (2004), the approximate method proposed here can handle molecules with isotopomers (e.g., $^{14}N^{15}N^{16}O$ vs. $^{15}N^{14}N^{16}O$) and can estimate Δ_{mass} for any kind of molecule, no matter whether the Δ_i values of singly-substituted isotopologues are small or large. Because the proposed approximate method appears to consistently overestimate Δ_{mass} by 1 to 2 percent, a scale factor could further improve the results. We find that equilibrium Δ_{mass} is roughly independent of the bulk isotope composition at room temperature and above. The anharmonic corrections to Δ_i are similar to those affecting β factors and are on the order of one to a few percent. The frequency scaling factor can significantly influence Δ_i and we recommend using the most accurate theoretical method available to avoid errors. The net contribution of singly substituted isotopologues to Δ_{mass} is close to zero, which justifies an assumption used in determining the bulk isotopic compositions in mass spectrometric measurements.

ACKNOWLEDGMENTS

APPENDIX A. THE EXACT β CALCULATIONS

From its definition, the exact β should include all of the isotopologues in the molecule of interest, VY_n. Similar to Eq. (3), the equilibrium concentration of any isotopologue can be directly obtained by using its f function and the equilibrium concentrations of $[VY_n]$, $[V']/[V]$ and $[Y']/[Y]$. The bulk isotope ratio $(V'/V)_{VY_n}$ or $(Y'/Y)_{VY_n}$ can be obtained by summing over all of the isotopologues related to their own isotope substitutions::

$$
\left(\frac{V'}{V} \right)_{VY_n} = \frac{\sum_p [V'Y'_p Y_{n-p}]}{\sum_p [VY'_p Y_{n-p}]} = \frac{\sum_{p=0}^{n} f'_p \left(\frac{[Y']}{[Y]} \right)^p}{\sum_{p=0}^{n} f_p \left(\frac{[Y']}{[Y]} \right)^p} \cdot \frac{[V']}{[V]} \quad (A1a)
$$

$$
\left(\frac{Y'}{Y} \right)_{VY_n} = \frac{\sum_p p \cdot \left([V'Y'_p Y_{n-p}] + [VY'_p Y_{n-p}] \right)}{\sum_p (n-p) \cdot \left([V'Y'_p Y_{n-p}] + [VY'_p Y_{n-p}] \right)}
$$

$$
= \frac{\sum_{p=0}^{n} p \cdot \left(f'_p \frac{[V']}{[V]} + f_p \right) \left(\frac{[Y']}{[Y]} \right)^p}{\sum_{p=0}^{n} (n-p) \cdot \left(f'_p \frac{[V']}{[V]} + f_p \right) \left(\frac{[Y']}{[Y]} \right)^p} \quad (A1b)
$$

If $[V'Y'_p Y_{n-p}]$ has t isotopomers, which differ in non-equivalent structural positions of isotopic substitution, such as N_2O containing one ^{15}N that has two isotopomers (i.e., $^{15}N^{14}NO$ and $^{14}N^{15}NO$), then

$$
\left(\frac{V'}{V} \right)_{VY_n} = \frac{\sum_{p=0}^{n} \sum_{j=1}^{t_p} f'_{jp} \left(\frac{[Y']}{[Y]} \right)^p}{\sum_{p=0}^{n} \sum_{j=1}^{t_p} f_{jp} \left(\frac{[Y']}{[Y]} \right)^p} \cdot \frac{[V']}{[V]} \quad (A2a)
$$

$$
\left(\frac{Y'}{Y} \right)_{VY_n} = \frac{\sum_{p=0}^{n} p \cdot \sum_{j=1}^{t_p} \left(f'_{jp} \frac{[V']}{[V]} + f_{jp} \right) \left(\frac{[Y']}{[Y]} \right)^p}{\sum_{p=0}^{n} (n-p) \cdot \sum_{j=1}^{t_p} \left(f'_{jp} \frac{[V']}{[V]} + f_{jp} \right) \left(\frac{[Y']}{[Y]} \right)^p} \quad (A2b)
$$

where t_p represents t isotopomers and j represents jth isotopomer, when p is fixed.

From its definition, β factors can be exactly calculated by the following equation:

$$
^{V'}\beta_{VY_n} = \left(\frac{V'}{V} \right)_{VY_n} \Big/ \frac{[V']}{[V]} = \frac{\sum_{p=0}^{n} \sum_{j=1}^{t_p} f'_{jp} \left(\frac{[Y']}{[Y]} \right)^p}{\sum_{p=0}^{n} \sum_{j=1}^{t_p} f_{jp} \left(\frac{[Y']}{[Y]} \right)^p} \quad (A3a)
$$

$$
^{Y'}\beta_{VY_n} = \left(\frac{Y'}{Y} \right)_{VY_n} \Big/ \frac{[Y']}{[Y]}
$$

$$
= \frac{\sum_{p=0}^{n} p \cdot \sum_{j=1}^{t_p} \left(f'_{jp} \frac{[V']}{[V]} + f_{jp} \right) \left(\frac{[Y']}{[Y]} \right)^{p-1}}{\sum_{p=0}^{n} (n-p) \cdot \sum_{j=1}^{t_p} \left(f'_{jp} \frac{[V']}{[V]} + f_{jp} \right) \left(\frac{[Y']}{[Y]} \right)^p} \quad (A3b)
$$

APPENDIX B. ANHARMONIC EFFECTS

The B.M. equation or Urey model (i.e., Eq. (2)) is based on the harmonic vibration and rigid rotator approximations,

which become imprecise for H/D isotope exchange reactions. A brief introduction about the method with corrections beyond the harmonic level is given here. Readers are referred to Bron and Wolfsberg (1972), Richet et al. (1977) and Liu et al. (2010) for more details.

Anharmonic effects arise from the fact that potential energy is not a simple quadratic function of the internal molecular coordinates. For a polyatomic molecule, an expression for the second-order correction to the vibrational energy is given by (e.g., Wolfsberg et al., 1970; Barone, 2004)

$$E_n = hc \left(G_0 + \sum_i \left(n_i + \frac{1}{2} \right) \omega_i + \sum_{i \leqslant j} \chi_{ij} \left(n_i + \frac{1}{2} \right) \left(n_j + \frac{1}{2} \right) \right) \tag{B1}$$

where G_0 is a constant, n, n_i and n_j are the quantum numbers, ω_i is harmonic frequencies and χ_{ij} is anharmonic constants. The partition function for the vibration is given by

$$Q = \exp \left(- \frac{\sum_n E_n}{k_b T} \right) \tag{B2}$$

The zero-point energy (ZPE) of the vibration is an important contribution to the partition function, especially at low temperature. The ZPE is given by

$$\text{ZPE} = hc \left(G_0 + \frac{1}{2} \sum_i \omega_i + \frac{1}{4} \sum_{i \leqslant j} \chi_{ij} \right) \tag{B3}$$

From this, f'_k can be calculated as

$$f'_k = \frac{s}{s'_k} \cdot \frac{\exp(-\text{ZPE}'_k / k_b T)}{\exp(-\text{ZPE}/k_b T)} \cdot \prod_i^l \frac{u'_{ik}}{u_i} \cdot \frac{1 - e^{-u_i}}{1 - e^{-u'_{ik}}} \tag{B4}$$

where ZPE'_k and ZPE denote the zero point energies of $V'Y'_k Y_{n-k}$ and VY_n, respectively. Here, we call Eq. (B4) the "ZPE model", which includes anharmonic corrections to ZPE. This model is a more accurate treatment than the B.M. equation or the Urey model (e.g., Wang et al., 2004; Liu et al., 2010). Note that there are higher order corrections to the harmonic approximation possible. Here, we only adopt the largest one that is related to the ZPE (e.g., Liu et al., 2010).

The anharmonic correction to the f function can be represented as

$$f'^k_{\text{Corr}} = \exp \left(\frac{hc}{k_b T} \left(G_0 - G'^k_0 \right) + \frac{1}{4} \frac{hc}{k_b T} \sum_{i \leqslant j}^l \left(\chi_{ij} - \chi'^k_{ij} \right) \right) \tag{B5}$$

For non-H/D isotope exchange reactions, the G_0 term can be safely neglected (see Table 7 in Liu et al., 2010), the Eq. (B5) can then be simplified to

$$f'^k_{\text{Corr}} = \exp \left(\frac{1}{4} \frac{hc}{k_b T} \sum_{i \leqslant j}^l \left(\chi_{ij} - \chi'^k_{ij} \right) \right) \tag{B6}$$

which is the formula used by Richet et al. (1977) and Zeebe (2009).

APPENDIX C. ESTIMATING THE ERROR OF THE APPROXIMATE METHOD

In comparing Eqs. (9a) and (9b) with Eqs. (10a) and (10b), the error mainly arises from neglecting those isotope ratios that usually are close to zero. Essentially, the larger those ignored isotope ratios are, the bigger the error will be. A notation d^n is defined to represent the deviation from the exact β factor results:

$$d^n = 1000 \times (\beta_e / \beta_a - 1) \tag{C1}$$

where n represents the different rare isotopes by using their mass numbers, such as $n = 13$ for the ^{13}C case. β_e is an exact β value obtained from Eqs. (9a) and (9b). β_a is an approximate result from Eqs. (10a) and (10b) which is completely independent of the isotope ratios. d^n is in units of per mil.

For the CO_2 system, the unknown variables for the β_e calculation are the isotope ratios $[^{18}O/^{16}O]$ and $[^{13}C/^{12}C]$ of an ideal atomic material O or C, which is in isotopic equilibrium with CO_2. We can set $[^{18}O/^{16}O]$ and $[^{13}C/^{12}C]$ to 2005.2×10^{-6} and $11,237 \times 10^{-6}$, respectively, for general situations. The variable $[^{17}O]/[^{16}O]$ can be obtained from a mass-dependent fractionation relationship. The results are shown in Fig. C1 for different temperatures. The largest errors are from the β factor of ^{18}O (but only less than 0.02‰). In general, these results suggest that the β factors obtained by the approximate method are very close to those from the exact method, suggesting our approximate method is adequate for the CO_2 case.

APPENDIX D. SCALING FACTOR EFFECTS

The reduced partition function ratio (RPFR) may be used to calculate isotope fractionation, which is defined as (Bigeleisen and Mayer, 1947; Urey, 1947)

$$\text{RPFR}'_k = \frac{s'_k}{s} f'_k = \prod_i^l \frac{u'_{ik}}{u_i} \cdot \frac{e^{-u'_{ik}/2}}{1 - e^{-u'_{ik}}} \cdot \frac{1 - e^{-u_i}}{e^{-u_i/2}} \tag{D1}$$
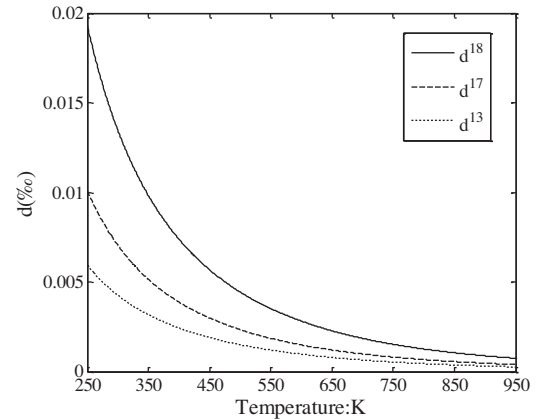

Fig. C1. The error of our approximate method varies with temperature when $[^{18}O/^{16}O]$ and $[^{13}C/^{12}C]$ are fixed to 2005.2×10^{-6} and 11237×10^{-6}, respectively.

According to previous researchers (e.g., Criss, 1991; Méheut et al., 2009), at low temperature, the relationship between the scaled RPFR (i.e., $\text{RPFR}'_{k\text{-s}}$) and the un-scaled one (i.e., RPFR'_k) is

$$\ln \text{RPFR}'_{k\text{-s}} \approx S \cdot \ln \text{RPFR}'_k \qquad (D2)$$

Generally speaking, β factors equal to:

$$\beta = \frac{1}{n}\sum_{j=1}^{n}\text{RPFR}_j \qquad (D3)$$

where RPFR_j represents the reduced partition function ratio of the molecule containing one rare isotope. For high-symmetry molecules, all of these RPFRs are equal, therefore $\beta = \text{RPFR}$ and $\ln \beta_s = \ln \text{RPFR}_s \approx S \times \ln \text{RPFR} = S \times \ln \beta$. For asymmetric molecules, the isotopologue containing one rare isotope might have isotopomers. In these cases, as a first-order approximation,

$$\ln \beta = \ln\left(\frac{1}{n}\sum_{j=1}^{n}\text{RPFR}_j\right) \approx \sum_{j=1}^{n}x_j \Big/ n \approx \sum_{j=1}^{n}\ln \text{RPFR}_j \Big/ n \qquad (D4)$$

where $x_j = \text{RPFR}_j - 1 \approx \ln \text{RPFR}_j$. Substituting Eq. (D2) into Eq. (D4) gives

$$\ln \beta_s \approx \frac{1}{n}\sum_{j=1}^{n}\ln \text{RPFR}_j^S \approx S \cdot \ln \beta \qquad (D5)$$

Substituting Eqs. (D2) and (D5) into Eq. (20), we obtain

$$\Delta'_{i\text{-s}} \approx S \cdot \Delta'_i \qquad (D6)$$

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