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“Clumped-isotope” geochemistry—The study of naturally-occurring, multiply-substituted isotopologues

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Abstract

Clumped isotope geochemistry is concerned with the state of ordering of rare isotopes in natural materials. That is, it examines the extent to which rare isotopes (D, ^{13}C , ^{15}N , ^{18}O , etc.) bond with or near each other rather than with the sea of light isotopes in which they swim. Abundances of isotopic ‘clumps’ in natural materials are influenced by a wide variety of factors. In most cases, their concentrations approach (within ca. 1%, relative) the amount expected for a random distribution of isotopes. Deviations from this stochastic distribution result from: enhanced thermodynamic stability of heavy-isotope ‘clumps’; slower kinetics of reactions requiring the breakage of bonds between heavy isotopes; the mass dependence of diffusive and thermo-gravitational fractionations; mixing between components that differ from one another in bulk isotopic composition; biochemical and photochemical fractionations that may reflect combinations of these simpler physical mechanisms; and, in some cases, other processes we do not yet understand. Although clumped isotope geochemistry is a young field, several seemingly promising applications have already emerged. Most importantly, it appears that proportions of ^{13}C – ^{18}O bonds in carbonate minerals are sensitive to their growth temperatures, independent of bulk isotopic composition. Thus, ‘clumped isotope’ analysis of ancient carbonates can be used as a quantitative paleothermometer that requires no assumptions about the $\delta^{18}\text{O}$ of waters from which carbonates grew. This approach has been used to reconstruct marine temperatures across the Phanerozoic (reaching back to the Silurian), terrestrial ground temperatures across the Cenozoic, thermal histories of aqueously altered meteorites, among other applications. Clumped isotope geochemistry is also placing new constraints on the atmospheric budget and stratospheric photochemistry of CO_2 , and should be capable of placing analogous new constraints on the budgets of other atmospheric gases. Finally, this field could be extended to encompass sulfates, volatile hydrocarbons, organic moieties and other materials.

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1. Introduction

For most of the history of stable isotope geochemistry it has focused, knowingly or implicitly, on the concentrations of isotopic species containing one rare isotope. For example, when discussing the ^{18}O content

of meteoric water, one generally means the relative abundances of $\text{H}_2\ ^{18}\text{O}$ vs. $\text{H}_2\ ^{16}\text{O}$; the ^{18}O contained in other isotopic variants of water (e.g., HD^{18}O and $\text{D}_2\ ^{18}\text{O}$) is neglected or deduced based on a few simple and generally unverified assumptions.

There are good reasons for this neglect. Species containing two or more rare isotopes typically constitute 10’s of parts per million or less of a given population of

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molecules (Table 1). Therefore, they generally have no meaningful impact on bulk isotopic compositions (an exception is the δD of water, which can be measurably impacted by variations in D_2O abundance; (Polyakov et al., 2005; Rolston and Gale, 1982). Moreover, until recently it was not clear how any of the species containing two or more rare isotopes could be analyzed in natural materials with meaningful precision, nor what purpose such data would serve if they could be obtained.

However, there are also good reasons to think that these rare, neglected isotopic species could be an untapped resource for geochemistry. First, they are more diverse than their isotopically normal and singly-substituted relatives. For example, of the twelve naturally occurring isotopic variants of CO_2 , eight contain two or more rare isotopes (Table 1). Second, just as singly-substituted molecules have physical and chemical properties distinct from isotopically normal molecules, each of the species containing multiple isotopes is in some sense unique (i.e., in bond vibration frequencies, zero point energies, near-infra-red absorption spectra, etc.). Therefore, these multiply-substituted species should exhibit diverse and distinctive fractionations, and studies of their distributions in nature might reveal new and useful information. Finally, these species, whatever their ultimate usefulness, constitute one of geochemistry's last large, unmapped realms. The urge to discover new things demands that it be explored.

This *Frontiers* article provides an overview of the recent development of a field of geochemistry concerned with measurements of abundances in natural materials of molecules containing two or more rare isotopes. The methods of these measurements and their applications to-date are detailed in a series of papers and abstracts published over the last three years (Schauble et al., 2003, 2006; Eiler and Schauble, 2004; Wang et al., 2004; Affek et al., 2005, in press; Guo and Eiler, 2005; Affek and Eiler, 2006; Ghosh et al., 2006a,b, 2007; Eiler et al., 2006; Eiler, 2006a,b; Came et al., in press; Guo and Eiler, submitted for publication). These studies involved a large number of authors from several different institutions; therefore, sections of this paper written in the active voice use plural pronouns in recognition of the contributions of these colleagues. Rather than systematically reviewing all of the published work, our purpose here is to provide a broader context of related prior research, an overview of the physical and chemical principles that are common to all work on such isotopic species, a description of the state of this nascent field, and a preview of the likely directions of its future growth. Our subject makes use a

Table 1
Stochastic abundances of isotologues of common gases

Mass ^a	Isotopologue	Relative abundance
N_2 ^b		
28	$^{14}N_2$	99.30%
29	$^{15}N^{14}N$	0.73%
30	$^{15}N_2$	13.4 ppm
O_2 ^c		
32	$^{16}O_2$	99.50%
33	$^{17}O^{16}O$	756 ppm
34	$^{18}O^{16}O$	0.40%
35	$^{17}O_2$	0.144 ppm
36	$^{18}O^{17}O$	1.52 ppm
	$^{18}O_2$	4.00 ppm
CO_2 ^d		
44	$^{12}C^{16}O_2$	98.40%
45	$^{13}C^{16}O_2$	1.11%
	$^{12}C^{17}O^{16}O$	748 ppm
46	$^{12}C^{18}O^{16}O$	0.40%
	$^{13}C^{17}O^{16}O$	8.4 ppm
	$^{12}C^{17}O_2$	0.142 ppm
47	$^{13}C^{18}O^{16}O$	44.4 ppm
	$^{12}C^{17}O^{18}O$	1.50 ppm
	$^{13}C^{17}O_2$	1.60 ppb
48	$^{12}C^{18}O_2$	3.96 ppm
	$^{13}C^{17}O^{18}O$	16.8 ppb
49	$^{13}C^{18}O_2$	44.5 ppb

^a Nominal cardinal mass in AMU.

^b Assuming a $^{15}N/^{14}N$ ratio equal to atmospheric N_2 .

^c Assuming $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios equal to the VSMOW standard.

^d Assuming $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios equal to the VSMOW standard and $^{13}C/^{12}C$ ratio equal to the PDB standard.

variety of unfamiliar terms; please see the accompanying “Jargon Box” for their definitions.

2. Background

2.1. The chemistry of isotopes

Stable isotope geochemistry is rooted in the landmark discoveries in physical chemistry made during the 1920s and 30s. The most relevant of these was that isotopic substitution of a heavy for a light isotope (e.g., D for H) in a chemical bond reduces the vibration frequencies of that bond and, therefore, its zero-point energy (Urey, 1947; Bigeleisen and Mayer, 1947; Fig. 1). For this reason, isotopic substitution influences thermodynamic stabilities of molecules and rates of many kinetically-controlled reactions.

Bonds between two heavy isotopes (e.g., the D–D bond in doubly substituted molecular hydrogen) are slower-vibrating and lower in energy than equivalent

Box 1

The jargon of clumped isotope geochemistry

Isotopologues are two or more variants of a molecule that differ in the isotopic identity of one or more of their constituent atoms. For example, $^{14}\text{N}_2$, $^{15}\text{N}^{14}\text{N}$ and $^{15}\text{N}_2$ are the three naturally-occurring isotopologues of molecular nitrogen. We also use this term to refer to isotopic variants of organic moieties or mineral structural units (e.g., the isotopologues of the carbonate ion include $^{12}\text{C}^{16}\text{O}_3^{-2}$, $^{13}\text{C}^{16}\text{O}_3^{-2}$, $^{12}\text{C}^{18}\text{O}^{16}\text{O}_2^{-2}$, etc.).

Multiply-substituted isotopologues are isotopologues that contains two or more rare isotopes. This term is precise but sufficiently awkward that we also use the term clump as a short-hand for these species (i.e., they are produced by ‘clumping’ two rare isotopes together).

Isotopomers are structural variants of an isotopologue that differ in the symmetrically non-equivalent location of an isotopic substitution. For example, $^{14}\text{N}-^{15}\text{N}-^{16}\text{O}$ and $^{15}\text{N}-^{14}\text{N}-^{16}\text{O}$ (where ‘-’ denotes the location of a bond between two isotopes) are isotopomers of one another; both are nitrous oxide isotopologues containing one ^{15}N atom, but in two different and symmetrically non-equivalent positions within the molecule. $^{18}\text{O}-^{12}\text{C}-^{16}\text{O}$ and $^{16}\text{O}-^{12}\text{C}-^{18}\text{O}$ are not isotopomers of one another because they are symmetrically equivalent and thus constitute a single kind of CO_2 isotopologue. The analytical methods that form the basis of most of this paper are unable to distinguish between isotopomers, and so we focus our discussion on relative abundances of isotopologues (although the principles that guide the work presented here could be extended to the study of naturally occurring, multiply-substituted isotopomers, assuming one could develop a sufficiently precise method for their analysis).

Stochastic distribution refers to a state in which all the stable isotopes in a given population of molecules are randomly distributed among all possible isotopologues. For example, in a population of water molecules in which $[\text{D}]$ is the fraction of all hydrogen atoms that are deuterium (i.e., $[\text{D}] = \text{D}/(\text{D} + \text{H})$) and $[\text{O}^{16}]$ is the fraction of all oxygen atoms that are ^{16}O (i.e., $[\text{O}^{16}] = ^{16}\text{O}/(^{16}\text{O} + ^{17}\text{O} + ^{18}\text{O})$), then the stochastic abundance of D_2^{16}O is equal to: $[\text{D}]^2 \cdot [\text{O}^{16}]$. We use the stochastic distribution as a reference frame and focus our attention on the causes and significance of deviations from that reference frame.

Δ_i values measure the difference, in per mil, between the measured abundance of isotopologue i (or all isotopologues of mass i) and the abundance of that isotopologue expected for the stochastic distribution for that sample. A key concept is that absolute abundances of multiply-substituted isotopologues vary with bulk isotopic composition, but Δ_i values do not. Rather, they measure the propensity of rare isotopes to clump together more (for positive Δ_i values) or less (for negative Δ_i values) than would occur by random chance.

bonds between a heavy and a light isotope (e.g., H–D) which are, in turn, slower vibrating and lower in energy than equivalent bonds between two light isotopes (e.g., H–H; Fig. 1). It is commonly assumed that the change in bond energy associated with double heavy isotope substitution (i.e., the difference in energy between the H–H bond and the D–D bond) is exactly twice that associated with heavy-isotope substitution of only one atom (i.e., the difference in bond energy between the H–H bond and the H–D bond); this assumption is sometimes called the ‘rule of the geometric mean’ or simply the ‘rule of the mean’ (Bigeleisen, 1955).

The rule of the mean implies that there is no energetic preference for grouping heavy isotopes into bonds with

each other as opposed to distributing them into a larger number of bonds with light isotopes (i.e., a system containing a H_2 and a D_2 has the same total vibrational energy as a system containing two HD’s). Stated in terms of classical thermodynamics, the rule of the mean assumes that compounds containing two or more isotopologues are ideal; i.e., there is no enthalpy of mixing associated with mixing two or more isotopologues of the same compound (Bigeleisen, 1955). However, the rule of the mean is more of a simplifying approximation than a rule. Its statistical-mechanical justification arises from an approximation to the series-expansion form of the equation for the partition function (the statistical mechanical variable that describes the

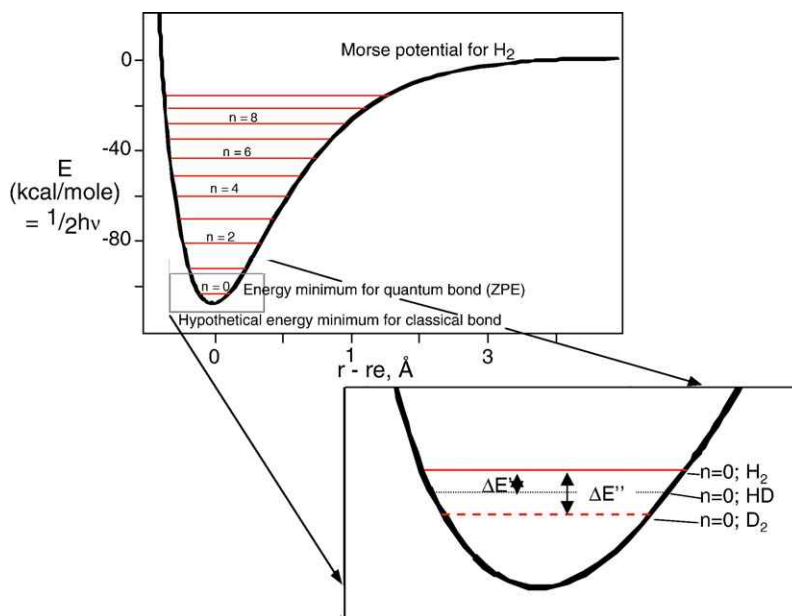


Fig. 1. Illustration of the energy content of a molecular hydrogen bond (E , in kcal/mol) as a function of bond distance, ($r-r_e$, in angstroms, where r_e is the minimum-energy bond distance). The solid curve represents the Morse potential for H_2 . Horizontal lines indicate the energy levels corresponding to the quantum states of bond vibration, $n=0, 1, 2, \dots$. The lowest accessible quantum state, $n=0$, is referred to as the zero point energy, or ZPE. The inset illustrates the effect of isotopic substitution on the energy of the $n=0$ quantum state. Note that the zero point energy is reduced with a single heavy isotope substitution and reduced further with a second heavy isotope substitution. Distances $\Delta E'$ and $\Delta E''$ indicate these energy changes. Based on (Urey, 1947; Bigeleisen and Mayer, 1947).

vibrational, rotational and translational energies of molecules) and is not accurate except at the high temperature limit (Urey, 1947; Bigeleisen, 1955). In fact, decreases in vibrational energy associated with double heavy-isotope substitution often slightly exceed twice that of a single heavy-isotope substitution. Departures from the rule of the mean arise from the higher-order terms to the series expansion form of the equation describing partition functions (Urey, 1947; Bigeleisen, 1955); alternatively, one can think of these departures as a consequence of the fact that there is a small but observable enthalpy of mixing associated with mixtures of two or more isotopologues of most compounds. One can reach a more intuitive understanding of these departures by considering some of the structural features of molecules that might lead to preferential concentration of isotopes into one atomic site vs. another. For example, in N_2O (N–N–O) the bonding environment for the terminal N differs from that for the central N, which leads to a difference in ^{15}N content between the two sites. Similarly, the vibration energies of C–O bonds differ slightly between the ^{18}O –C– ^{16}O and ^{16}O –C– ^{16}O isotopologues of CO_2 , and this difference gives rise to a preferential partitioning of ^{13}C into the former at the expense of the latter (this example is discussed in greater detail below).

For our purposes, the important thing about the failures of the rule of the mean is that there is a thermodynamic driving force that often promotes ‘clumping’ of heavy isotopes into multiply-substituted isotopologues at the expense of singly-substituted isotopologues. This effect can be described in terms of the equilibrium constant for a homogeneous isotope exchange reaction involving only isotopologues of a single molecule, such as:



Such reactions are also sometimes referred to as ‘isotopic disproportionation reactions’ (e.g., Polyakov et al., 2005). If the stochastic distribution prevails, the equilibrium constant for this reaction, K_1 , is a simple function of the symmetry numbers and stoichiometry coefficients of reactant and product molecules: $K_1 = ([H_2] \cdot [D_2])/[HD]^2 = ([H]^2 \cdot [D]^2)/(2 \cdot [H] \cdot [D])^2 = 0.25$. But, because D_2 is slightly more stable than one would predict based on the rule of the mean, there is a thermodynamic propensity to drive Reaction (1) to the right, raising K_1 . This driving force is balanced by the effect of isotopic distribution on the entropy of the system, such that the random distribution of rare isotopes among all possible isotopic species is increasingly

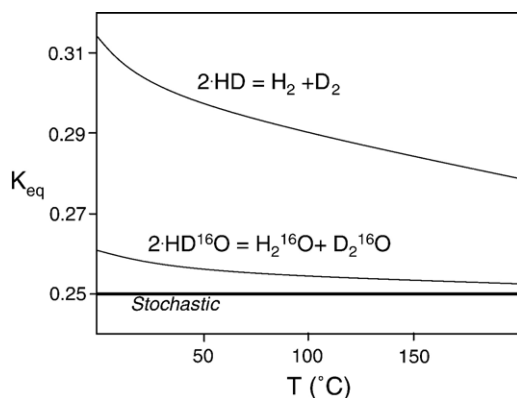
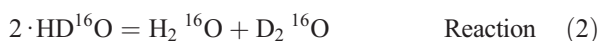


Fig. 2. Temperature dependence of the equilibrium constants for the homogeneous isotope exchange reactions, Reactions (1) and (2). The heavy horizontal line indicates the value of the equilibrium constant that corresponds to a stochastic distribution (0.25). Note that thermodynamic driving forces promote higher-than-stochastic values of K_{eq} , corresponding to larger proportional abundances of multiply-substituted isotopologues (D_2 and D_2O) at ambient temperatures, and a gradual approach toward the stochastic distribution with increasing temperature. Equilibrium curves are taken from (Richet et al., 1977).

avored with increasing temperature. And, the enthalpies of isotope exchange reactions are temperature dependent (generally decreasing in absolute value with increasing temperature). These two factors lead to a temperature dependence of equilibrium constants for homogeneous isotope exchange reactions, such that K_1 is significantly more than 0.25 at room temperatures and approaches 0.25 with increasing temperature. Reaction (1) and a similar homogeneous isotope exchange reaction involving isotopologues of water:



are among the few that were explored by experiments and statistical thermodynamic theory (e.g., Pyper et al., 1967) prior to our recent work on such problems (Fig. 2; significant previous work has also been done on similar reactions for hydrogen sulfide and ammonia; see Polyakov et al., 2005; Richet et al., 1977 for reviews and primary references).

2.2. Anthropogenic isotopic enrichments and labeling experiments

There are several uses of isotopes in engineering and the natural sciences that can be thought of as inadvertent clumped-isotope geochemistry. Isotopic enrichment procedures used for nuclear weapons, energy and medicine yield materials that must contain abundances of multiply-substituted isotopologues many orders of

magnitude greater than those found in nature, simply because their exceptionally high concentrations of rare isotopes increase the probability that two or more will form bonds with one another. Similarly, many branches of the natural sciences use isotopically enriched materials as tracers for movement of atomic and molecular species. Examples include labeled nutrients fed to microbial communities (Chang et al., 2005) or isotopically labeled dopants in chemical diffusion experiments (Elphick et al., 1991). These studies generally involve mixing of materials that contrast strongly in their abundances of multiply substituted isotopologues. In Section 4 of this paper we illustrate the effects of such mixing on abundances of clumped isotopic species, and discuss how these effects might be exploited.

2.3. Physical chemistry experiments on enriched materials

Multiply substituted isotopologues are of interest to physical and organic chemistry because of their distinctive kinetics in unidirectional reactions (e.g., photolysis or enzymatically controlled reactions). There is a rich history and high current rate of experimental studies of such isotope effects, generally conducted on materials that are artificially enriched in rare isotopes. The purpose of such work is to probe the physical mechanisms behind poorly-understood chemical reactions. A recent example is the study of the isotope effects that accompany ozone

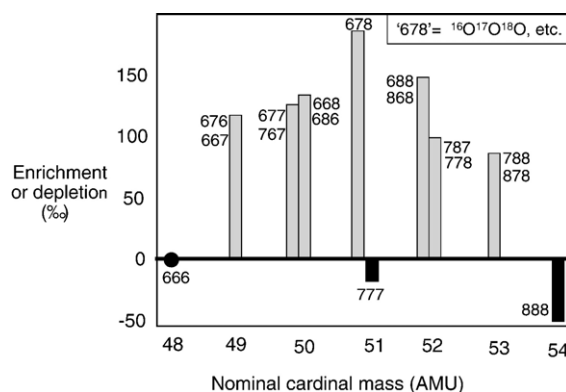


Fig. 3. Enrichments or depletions in relative abundances of isotopologues of ozone, in units of per mil, normalized to a unit change in $^{16}O_3$, produced during photochemical experiments on O_2-O_3 mixtures (Mauersberger et al., 1993). Note that all asymmetric isotopologues of ozone are anomalously enriched, and all symmetric isotopologues are relatively depleted. These results are representative of a large body of experimental work on the kinetic isotope effects accompanying gas-phase reactions, which collectively constitute the largest single source of observational data on the chemical properties of multiply-substituted isotopologues.

production and consumption by photochemical reactions in O₂-rich atmospheres (Mauersberger et al., 1993). In this study, Mauersberger et al. demonstrate that the distinctive ‘mass independent’ enrichment in ¹⁸O and ¹⁷O in atmospheric ozone (Mauersberger, 1981) reflects enhanced production of the asymmetric isotopologues (¹⁸O¹⁶O₂, ¹⁷O¹⁶O₂, ¹⁸O₂ ¹⁶O, ¹⁸O₂ ¹⁷O, etc.; Fig. 3). This observation underlies theoretical advances in our understanding of the physical chemistry responsible for this isotope effect (Gao and Marcus, 2001).

2.4. Half-steps towards applied clumped-isotope geochemistry

The previous research on multiply-substituted isotopologues summarized above constitutes a broad and important body of work in physical and biochemistry. But, it is not geochemistry in the full sense of the word because it does not involve the analysis and interpretation of these rare species in nature. There have been several previous studies that more closely approached, but did not quite achieve, a real ‘clumped isotope’ geochemistry.

The largest body of such work involves attempts to measure isotopic compositions of specific atomic sites within larger molecules or condensed phases; e.g., analyses of the oxygen isotope composition of hydroxyl groups in hydrous minerals (Hamza and Epstein, 1980), position-specific carbon isotope analysis of organic molecules (Monson and Hayes, 1982), or measurements of the distribution of ¹⁵N between central and terminal positions in N₂O (Brenninkmeijer and Rockmann, 1999). These are not clumped isotope geochemistry because they consider only singly-substituted isotopic species. However, they are motivated by an understanding that rare isotopes are not randomly distributed within molecules, organic moieties and mineral structures, and this is a key concept behind clumped isotope geochemistry.

It has been shown that conventional measurements of δ¹³C in CO₂ potentially contain small but observable systematic errors due to the failure to account correctly for the fraction of ¹³C contained in ¹³C¹⁸O¹⁶O (Zyakun, 2003). While this study recognized that natural variations in this multiply-substituted isotopologue could exist, it did not attempt to measure these variations. Similarly, (Kaiser et al., 2003) presented theoretical arguments for the consequences of photochemical reactions on the abundance of atmospheric ¹⁵N¹⁵N¹⁶O. This study also did not present measurements of these species (and, in fact, concluded that such measurements would likely contain little distinctive information).

Mroz et al. (1989) presented what are, to the best of our knowledge, the first attempted measurements of a

‘clumped’ isotopic species at natural abundances. They examined the mass spectrum of methane from Antarctic air, finding abundances of 20 AMU species (presumably some combination of ¹²CD₄ and ¹³CHD₃) up to 500 times higher than expected for the stochastic distribution of methane. This report was immediately followed by publication of a model demonstrating that such extreme enrichments are inconsistent with the experimentally-constrained atmospheric lifetimes of these species (Kaye and Jackman, 1990). This inconsistency has never been satisfactorily resolved, but presumably reflects some combination of analytical error in the atmospheric measurements and/or misconceptions about the budget of atmospheric methane (e.g., unrecognized anthropogenic sources of ¹²CD₄).

2.5. The relationship between ‘clumped isotope’ and ‘mass independent’ isotope geochemistry

On first glance, clumped isotope geochemistry might appear to be a subset of mass-independent isotope geochemistry—the field of isotope geochemistry concerned with the study of the mass dependence of isotopic fractionations in systems having three or more isotopes (e.g., ¹⁸O, ¹⁷O and ¹⁶O). The most obvious point of similarity between these two fields is that both report data using ‘Δ’ values. In the case of clumped isotope measurements, Δ values refer to deviations from a stochastic distribution, whereas mass-independent isotope geochemistry use Δ values to denote deviations from a specific mass-dependant fractionation law. However, beyond this superficial similarity in nomenclature, the fields have little in common. Mass-independent isotope geochemistry generally considers bulk isotope compositions of materials, and thus primarily reflects variations in the relatively abundant singly-substituted isotopologues. In contrast, clumped isotope geochemistry is concerned with the organization, or state of ordering, of isotopes among all possible isotopologues, independent of bulk isotopic composition. In this sense, clumped isotope geochemistry is just as different from mass-independent isotope geochemistry as it is from conventional isotope geochemistry. Also, because most natural processes of isotopic fractionation have a simple mass dependence, mass-independent isotope geochemistry generally focuses on the search for rare anomalies that signify unusual processes (e.g., the signatures of photochemical reactions on Δ¹⁷O values of atmospheric O₃, O₂ and CO₂). This is because the strongest mass-independent isotopic fractionations stem from symmetry-based isotope effects that occur only in a few species and environments (e.g., Mauersberger, 1981; Mauersberger et al., 1993). In

contrast, essentially all processes of isotopic fractionation (diffusion, isotope exchange reactions, irreversible reactions, etc.) lead to distinctive clumped isotope variations and so clumped isotope geochemistry is applicable to a wider variety of problems (note however, that studies attempting to characterize subtle variations in the slopes of mass-dependent fractionations more closely resemble clumped isotope geochemistry in this regard). Perhaps the most meaningful similarity between these two fields is in the ways in which they can be used: both add dimensionality and specificity to isotopic measurements that complement conventional isotopic data and enhance our ability to recognize the processes responsible for isotopic fractionation.

3. Analytical approaches—real and imaginable

The basic demands of clumped-isotope analysis are: high abundance sensitivity (because the target species make up small fractions of the analyzed gas; e.g., Table 1); high precision (typically 10^{-5} ; because isotope signals of interest are often less than 10^{-3}); high sample purity or excellent mass resolving power (because interferences can easily lead to small variations of apparent abundances of rare species); and, most importantly, integrity of the original bonds in the analyte (because re-distribution of isotopes among isotopologues during analysis will render the measurements meaningless). Our recent work demonstrates that gas-source isotope ratio mass spectrometry can meet these demands for some compounds of interest (Eiler and Schauble, 2004; Guo and Eiler, 2005; Affek and Eiler, 2006; Ghosh et al., 2006a; Eiler, 2006b). In this section, we review the principles and limitations of this approach, and then discuss possible alternatives that should be explored in the future.

3.1. Gas-source mass spectrometry

Gas-source isotope ratio mass spectrometry is an attractive analytical approach to clumped isotope geochemistry for two reasons: (1) the multiple-Faraday collection arrays used by such instruments can achieve exceptionally high precision in measured isotope ratios (as good as ca. 10^{-5} to 10^{-6} ; e.g., Severinghaus et al., 1998); and (2) analyses are routinely made on molecular ions, which potentially retain information on the distribution of isotopologues. Disadvantages include: analytes must be gasses at ambient conditions; noise levels in the Faraday-based collectors used in these instruments generally preclude meaningful analyses of the lowest-abundances species (e.g., $^{13}\text{C}^{18}\text{O}_2$; Table 1); the mass-resolving power

of such instruments (typically corresponding to $M/\Delta M$ values of ca. 500) is insufficient to discriminate among isotopologues having the same cardinal mass (e.g., $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{17}\text{O}$), or between analyte molecules and some isobaric interferences; and, analyte molecules typically undergo fragmentation and recombination during ionization, possibly leading to redistribution of isotopes among the various analyzed isotopologues. Experiments on mixtures of isotopically labeled gases demonstrate that this last factor can be reduced to negligible levels for CO_2 (Eiler and Schauble, 2004), CO (Guo and Eiler, 2005) and O_2 (unpublished data) but it might be limiting for other species.

The only instruments that have been used to make published, high-precision clumped-isotope measurements are a pair of modified Thermo-Finnegan 253's housed in the Caltech laboratories for stable isotope geochemistry (Schauble et al., 2003, 2006; Eiler and Schauble, 2004; Wang et al., 2004; Affek et al., 2005, in press; Guo and Eiler, 2005; Affek and Eiler, 2006; Ghosh et al., 2006a,b, 2007; Eiler et al., 2006; Eiler, 2006a,b; Came et al., in press; Guo and Eiler, in press). These instruments have collector arrays consisting of eight Faraday cups, four of which are registered through 10^{12} Ohm resistors. We suspect other instruments of broadly similar design and capability could be similarly effective at such measurements. Most published analyses using these instruments have been made using a dual-inlet system for sample introduction, which maximizes the amount of gas available for measurement and assures stable analytical conditions for relatively long times (tens of minutes or longer). A smaller number of published measurements have been made using a micro-volume inlet, which maintains acceptably high source pressures with ca. 1 μm -sized samples (Guo and Eiler, in press), but at the cost of poorer signal stability and shorter analytical durations. We also imagine these measurements could be made in a similar way using techniques of carrier-gas mass spectrometry (Matthews and Hayes, 1978), although it likely will be difficult to achieve 10^{-5} -level external precisions.

Because clumped-isotope species make up little of most natural gases (ca. 10^{-5} and less; Table 1) yet often must be analyzed with unusually high precision (of order 10^{-5}), extraordinarily large samples and/or long counting times often are required. Fig. 4 illustrates the shot-noise limits to precision in measurements of Δ_{47} of CO_2 (primarily a measure of the departure of $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ abundances from the stochastic distribution; see the end of this section for a more complete definition) as a function of the integrated charge collected for the major isotopologue of CO_2 ($^{12}\text{C}^{16}\text{O}_2$). Precisions approaching

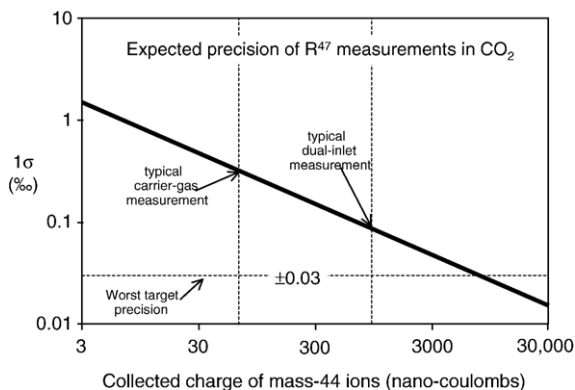


Fig. 4. Shot-noise limit to the external precision of a measurement of the mass-47 to mass-44 ratio in natural CO_2 , in units of per mil, as a function of the integrated charge of mass-44 collected during a mass spectrometric analysis. The heavy solid line indicates the precision calculated according to the methods of (Merritt and Hayes, 1994). The horizontal dashed line indicates a typical ‘target’ precision for applications, and the vertical dashed lines indicate the amounts of charge typically collected during dual-inlet and carrier-gas mass spectrometric analyses (assuming standard analytical protocols). One to two orders of magnitude more charge than is commonly collected must be obtained before a clumped isotope analysis becomes usefully precise.

a few hundredths of per mil (the ‘price of entry’ for many applied problems) are not reached until one collects ca. 10x the amount of charge normally collected in a dual inlet measurement. Exceptional precisions of ca. 0.01‰ or better (required for high-precision carbonate paleothermometry; see Section 5, below) demand nearly 100x the amount of charge collected for a typical dual inlet measurement.

Finally, even nominally pure gases prepared on conventional vacuum lines can contain significant (i.e., ppb and higher) abundances of volatile trace gases (e.g., organics, organic halides, and sulfides), some of which can undergo fragmentation and/or recombination reactions in the mass spectrometer source to produce isobaric interferences (e.g., the $^{12}\text{C}^{35}\text{Cl}^+$ fragment from methyl chloride interferes with $^{13}\text{C}^{18}\text{O}^{16}\text{O}^+$ in CO_2). This problem calls for the most stringent sample purification procedures, often involving repeated cryogenic separations, gas chromatography, and/or exposure to reactive ‘getters’ (such as silver phosphate to remove sulfide contamination) (Eiler and Schauble, 2004; Affek and Eiler, 2006; Eiler, 2006b; Guo and Eiler, in press).

3.2. Possible alternatives

Spectroscopic methods could form an ideal basis for measurements of multiply-substituted isotopologues be-

cause isotopologues have unique near-infra-red spectroscopic features that could be used to discriminate them from their relatives — even those having identical cardinal mass (Perevalov et al., 2006). And, such methods can be exceedingly sensitive, potentially permitting analysis of isotopologues containing three or more rare isotopes (e.g., $^{13}\text{C}^{18}\text{O}^{17}\text{O}$; Table 1). Spectroscopic measurements of stable isotope composition have achieved precisions on the order of 10^{-4} for singly-substituted isotopologues (Bergamaschi et al., 1994). However, we are not aware of any evidence that these methods could generate precision on the order of 10^{-5} for the less abundant multiply-substituted isotopologues. Nevertheless, progress on this front would constitute a genuine breakthrough in our ability to study multiply-substituted isotopologues, and even existing instruments and methods might be adequate for study of multiply-deuterated water or methane (which likely exhibit relatively large departures from the stochastic distribution).

Modern thermal ionization mass spectrometers employ multiple-Faraday collector arrays and routinely achieve precisions in isotope ratio measurements in the range of 10^{-5} and 10^{-6} (Caro et al., 2003). Moreover, many species can be analyzed as molecular ions, and thus potentially preserve information regarding the distribution of isotopes among the various possible isotopologues of those molecules. For these reasons, this approach is an attractive possibility for studying the clumped isotope geochemistry of solids that cannot be easily converted into gases (e.g., sulfides, oxides, silicates and halides). However, thermal ionization occurs at high temperatures from a condensed substrate, and may promote substantial isotopic redistribution during analysis. This exchange will have to be minimized and/or carefully controlled in order to make precise and meaningful measurements.

Secondary ion mass spectrometry yields high relative abundances of molecular ions from many materials and can, in some cases, achieve precisions for isotope ratio analysis on the order of 10^{-4} (Kita et al., 2007); therefore, it potentially provides a means of making in-situ measurements of clumped isotope species in solids. However, this approach faces considerable challenges. Most fundamentally, while molecular ions are emitted from solids during ion sputtering, an unknown, but likely large proportion of them form by fragmentation/recombination rather than through direct emission of dimers and higher-order clusters containing the same interatomic bonds that existed in the pre-sputtered sample. This phenomenon will have to be understood and controlled before sputtering could be used as a quantitative analytical tool for clumped isotope geochemistry. Furthermore, ion

probe measurements seem unlikely to ever achieve 10^{-3} and better precisions for low-abundance multiply-substituted molecular ions, and thus this approach, even if physically feasible, may be limited to isotopically enriched, synthetic materials, elements that exhibit strong departures from the stochastic distribution (e.g., H) and elements having sub-equal abundances of two or more isotopes (and thus relatively high abundances of clumped isotopic species; e.g., Cl).

3.3. Precise definition of the Δ_i value

Clumped isotope analyses use values of Δ_i to denote the excess or deficit of isotopologue i relative to the amount expected if a material conforms to the stochastic distribution. This is a straightforward concept, but it is not obvious on inspection just how a Δ_i value relates to the properties one can measure in a gas sample. In this section we explain in detail how this is done for the most commonly measured value of Δ_i —the Δ_{47} value for CO_2 (which largely reflects variations in abundance of $^{13}\text{C}^{18}\text{O}^{16}\text{O}$). This example is adapted from more detailed discussions of this subject found in references (Eiler and Schauble, 2004; Wang et al., 2004; Affek and Eiler, 2006). The Δ_{47} value of CO_2 can be calculated as:

$$\Delta_{47} = \left[\left(\frac{R^{47}}{R^{47*}} - 1 \right) - \left(\frac{R^{46}}{R^{46*}} - 1 \right) - \left(\frac{R^{45}}{R^{45*}} - 1 \right) \right] \times 1000$$

where R^{47} , R^{46} , R^{45} are abundance ratios of masses 47, 46 and 45 relative to mass 44; i.e., the 47/44, 46/44, 45/44 ratios, respectively. These R^i values are determined by comparison with a standard having an accepted composition (this is usually CO_2 gas of known bulk isotopic composition that has been intensely heated to drive its isotopic distribution to the stochastic distribution). R^{47*} , R^{46*} , R^{45*} are the corresponding ratios that would occur in the sample if it had a stochastic distribution. These are calculated using the equations:

$$R^{45*} = R^{13} + 2 \cdot R^{17}$$

$$R^{46*} = 2 \cdot R^{18} + 2 \cdot R^{13} \cdot R^{17} + (R^{17})^2$$

$$R^{47*} = 2 \cdot R^{13} \cdot R^{18} + 2 \cdot R^{17} \cdot R^{18} + R^{13} \cdot (R^{17})^2$$

where R^{13} , R^{17} and R^{18} are the abundance ratios $^{13}\text{C}/^{12}\text{C}$, $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for the sample. R^{13} and R^{18} are derived from the measured $\delta^{13}\text{C}_{\text{PDB}}$ and $\delta^{18}\text{O}_{\text{VSMOW}}$ values of the sample and R^{17} is calculated from R^{18} by assuming that the sample lies on a specific mass-dependent fractionation line in dimensions of R^{17}

vs. R^{18} . Note that this approach requires iteration if the Δ_{47} value is more than a few per mil different from 0 because R^{13} , R^{17} and R^{18} values initially calculated based on measured $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values using standard assumptions will contain small errors.

4. Fractionating processes—principles and examples

The processes that lead to familiar fractionations of bulk isotopic compositions (e.g., diffusion, exchange equilibria, evaporation, photolysis, etc.) generally also lead to fractionations of relative abundances of multiply-substituted isotopologues. For example, just as the difference in vapor pressures between HD^{16}O and H_2^{16}O leads to fractionations of D/H ratios during evaporation of water (Horita and Wesolowski, 1994), differences between the vapor pressures of D_2^{16}O and H_2^{16}O [Polyakov et al., 2005 and references therein] must lead to fractionations of these species from one another during evaporation of water. The important thing, in the context of our discussion, is to identify and understand fractionating processes that result in departures from the stochastic distribution, because it is these departures that convey new information distinct from that learned by conventional measurements of bulk isotopic composition. The following paragraphs review the principles and systematics of some of the more common and potentially useful clumped isotope fractionations.

4.1. Thermodynamically-controlled fractionations

As was explained in Section 2, chemical systems at equilibrium are generally expected to group heavy isotopes into bonds with each other in abundances greater than expected for a stochastic distribution, and do so more strongly with decreasing temperature (e.g., Fig. 2). Recent theoretical and experimental studies have examined, at 10^{-5} levels of precision, the size and temperature-dependence of homogeneous isotope exchange equilibria analogous to Reactions (1) and (2), but for isotopologues of N_2 , O_2 , CO , NO , CO_2 , N_2O (Schauble et al., 2003; Eiler and Schauble, 2004; Wang et al., 2004; Guo and Eiler, 2005) and carbonate ion groups in calcite, aragonite, magnesite, dolomite, and carboxylic acids (Ghosh et al., 2006a, 2007; Schauble et al., 2006; Eiler, 2006a; Came et al., in press). Data we intend to publish soon will extend these calculations and measurements to include sulfates, compounds of uranium, methane and dissolved inorganic carbon (i.e., $\text{CO}_{2\text{aq}}$, H_2CO_3 , HCO_3^- , CO_3^{2-}). In this section we consider some of the general trends revealed by these theoretical calculations and experimental data.

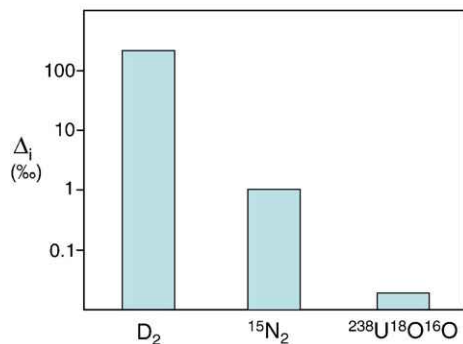


Fig. 5. Values of Δ_i (i.e., enrichments relative to the stochastic distribution, in per mil) expected for representative multiply substituted isotopologues in thermodynamically equilibrated populations of H_2 , N_2 and UO_2 at 298 K. Note that values of Δ_i for the clumped isotopic species are relatively large (ca. 100‰) for molecules with low reduced mass, moderate (ca. 1‰) for those with reduced mass comparable to common atmospheric gases, and small (ca. 0.02‰) for molecules with high reduced mass. The value of Δ_i for D_2 was taken from (Richet et al., 1977), that for N_2 is from (Wang et al., 2004), and that for $^{238}U^{18}O^{16}O$ was calculated by E. Schauble (personal communication).

For bonds of generally similar type, the equilibrium constants for homogeneous equilibria involving clumped isotopic species vary inversely with reduced mass (Fig. 5). This phenomenon reflects the fact that zero-point-energy effects scale with bond vibration frequency, which in turn depend strongly on reduced mass (Urey, 1947; Bigeleisen and Mayer, 1947).

Most thermodynamically-controlled deviations from the stochastic distribution exhibit a simple temperature dependence: Δ_i values of the multiply-substituted species closely approach 0 at temperatures in excess of ca. 500 K, and increase with decreasing temperature following a trend that is nearly linear with T^{-2} at temperatures of interest to most problems (Fig. 6A). However, some equilibria of this kind have more complex temperature dependences. For example, Δ_i values of $^{15}N_2^{16}O$, $^{15}N_2^{17}O$ and $^{15}N_2^{18}O$ in thermodynamically equilibrated populations of N_2O molecules vary non-linearly with T^{-2} and exhibit inflection points (i.e., changes in the sign of $\partial\Delta_i/\partial T$) and negative Δ_i values (Fig. 6B). These phenomena are indirect consequences of differences in stability between the isotopomers of singly-substituted N_2O molecules (e.g., $^{15}N^{14}N^{18}O$ vs. $^{14}N^{15}N^{18}O$).

Finally, there are several second-order effects on the sizes of Δ_i values for multiply-substituted isotopologues resulting from homogenous isotope exchange equilibria: Within a given molecule type (e.g., CO_2), the highest Δ_i values are observed for isotopologues that have bonds between heavy isotopes, and smaller Δ_i values for

isotopologues that have two or more heavy isotopes in non-adjacent positions. For example, even though $^{12}C^{18}O_2$ (structure: $^{18}O-^{12}C-^{18}O$) has a higher molecular mass than $^{13}C^{18}O^{16}O$ (structure: $^{18}O-^{13}C-^{16}O$), the Δ_i value of the former is only about half that of the latter at any given temperature (Wang et al., 2004). Also, among broadly similar types of bonds there are systematic correlations between Δ_i values and bond order. For example, clumping of ^{13}C with ^{18}O in compounds containing C–O bonds is strongest at any given temperature for CO (triple bond), weaker for CO_2 (double bond) and weakest for compounds containing CO_3^{-2} (average bond order 4/3) (Schauble et al., 2006). Finally, for molecules of a given type, there may be very weak dependences of Δ_i values involving one given bond on the nature of other bonds in that molecule. For

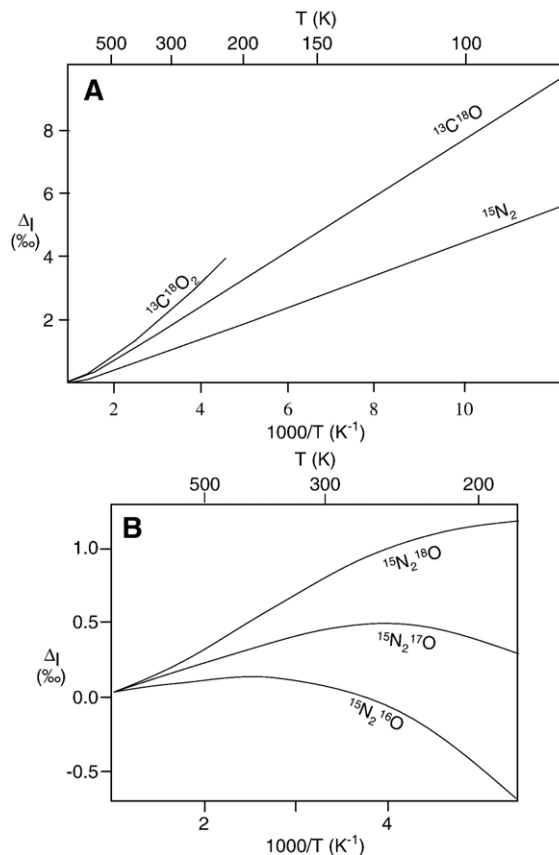


Fig. 6. Temperature dependences of Δ_i values, in units of per mil, for representative multiply-substituted isotopologues of common molecular gases, as calculated by reduced partition function theory (all are taken from Wang et al., 2004). Panel A shows calculated trends for molecules that show a typical ca. T^{-2} temperature dependence of Δ_i values. Panel B shows calculated trends for example species showing anomalous temperature dependences of Δ_i values.

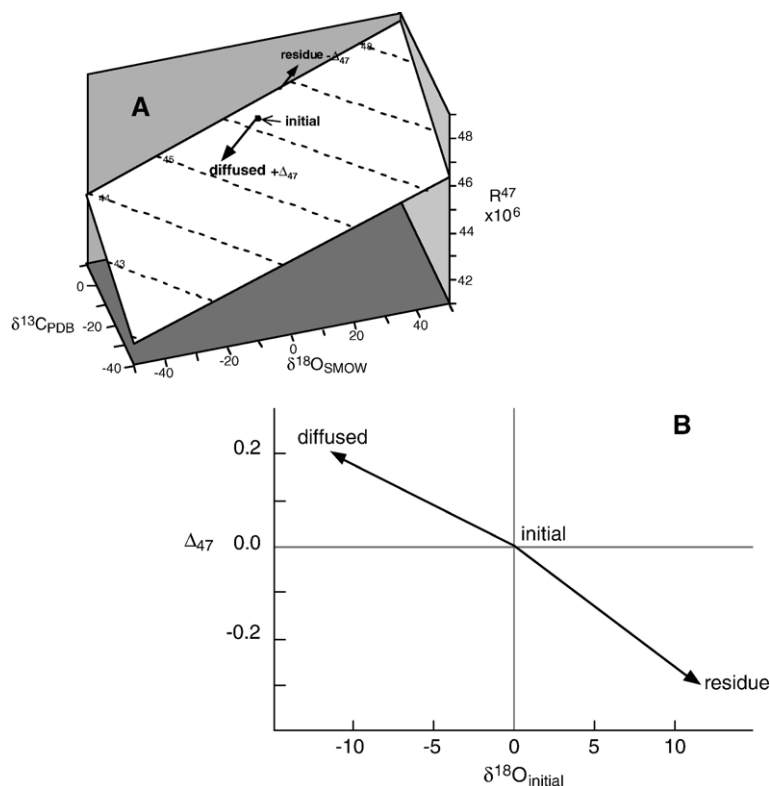


Fig. 7. Illustration of a typical diffusive fractionation of multiply-substituted isotopologues. The three-dimensional panel (A) shows the surface of the stochastic distribution for CO_2 in dimensions of $\delta^{13}\text{C}_{\text{PDB}}$ vs. $\delta^{18}\text{O}_{\text{VSMOW}}$ vs. R^{47} (the abundance ratio of mass-47 isotopologues to mass 44 isotopologues). Deviations above and below that surface correspond to positive and negative values of Δ_{47} , respectively. Dashed lines show contours of constant R^{47} value. The solid arrow in this panel shows the vector followed by Knudsen diffusion fractionation (calculated following Gibbs, 1928). Note that the diffusive vector is closer to horizontal than is the surface of the stochastic distribution (which is almost, but not exactly, flat over this range of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values). The two-dimensional panel (B) projects the Knudsen diffusion vector into dimensions of Δ_{47} (departure from the stochastic distribution) vs. $\delta^{18}\text{O}$, where we assume the initial gas (before diffusive fractionation) has values of 0 for both variables.

example, formation of $^{13}\text{C}\text{--}^{18}\text{O}$ bonds in carbonate ions is predicted to vary slightly at any given temperature with the identity of the cation to which the carbonate ion bonds (e.g., $\Delta(^{13}\text{C}^{18}\text{O}^{16}\text{O}_2^{-2})$ in calcite is predicted to be 0.004‰ greater than in dolomite at 298 K) (Schauble et al., 2006).

4.2. Vapor pressure isotope effects

Vapor-pressure isotope effects are thermodynamically-controlled differences in isotopic composition between vapor and a condensed phase. In thermodynamically equilibrated system containing a vapor and a condensed phase, relative abundances of the various possible isotopologues in each phase can be described by the homogeneous isotope exchange equilibria discussed in the preceding section. For example, K_{eq} for Reaction (2) ($'K_2'$) in water vapor is the same in vapor only and vapor+liquid systems. In this sense, clumped isotope

abundances in systems containing a condensed phase and a vapor are just a special case of equilibrium fractionations and follow all the rules and general trends outlined above.

However, it is important to note that the homogeneous equilibria that control clumped isotope abundances need not have identical equilibrium constants in the condensed and vapor phases (e.g., K_2 for water vapor is greater than that for liquid water at any given temperature; Polyakov et al., 2005), and differences between the two might, in some circumstances, lead to distinctive clumped isotope fractionations during phase changes. For example, if liquid water is able to continuously maintain equilibrium with respect to the D_2^{16}O -forming homogeneous isotope exchange reaction but the vapor does not (e.g., because collision frequency is too low to promote efficient intermolecular exchange), then H_2O irreversibly evaporated from the liquid could ‘inherit’ the $\Delta_{\text{D}_2^{16}\text{O}}$ value of the

liquid, lower than that expected for equilibrated vapor. One can imagine using such an isotope effect to complement the information content of conventional stable isotope data, much as the ‘deuterium excess’ (a measure of the deviation from the meteoric water line) has been used in studies of water vapor and meteoric precipitation.

4.3. Kinetic fractionations associated with unidirectional reactions

Many natural systems are influenced by unidirectional reactions that manifest kinetically-controlled isotopic fractionations arising from differences in reaction rate between isotopologues. Common examples of such processes include: photo-oxidation of methane (Saueressig et al., 2001) and other gas-phase reactions (Mauersberger et al., 1993), biosynthesis (e.g., photosynthesis; Guy et al., 1993), and various hydration, oxidation, sorption and solvation reactions involved in chemical weathering of minerals (Cole and Chakraborty, 2001).

Bulk isotopic fractionations associated with many kinetically controlled reactions have been studied extensively, but rates of reaction for multiply-substituted isotopologues are generally only well known for photolytic and other gas-phase reactions (e.g., Mauersberger et al., 1993; Saueressig et al., 2001). With a few exceptions (Methane and N₂O; Kaiser et al., 2003; Mroz et al., 1989; Kaye and Jackman, 1990), these data have not been considered in light of their implications for clumped isotope analyses of natural materials. We are aware of no data that usefully (i.e., at per-mil and better levels of precision) constrain relative reaction rates for multiply-substituted isotopologues in condensed-phase reactions. Until such data become available, transition-state theory can provide some guidance as to the directions and orders of magnitude of clumped-isotope fractionations expected to accompany unidirectional, condensed-phase reactions. Briefly, given a detailed description of the atomic-scale structure of reactants and transition states in chemical reactions, one can predict the relative rates of reaction for different isotopologues based on the effects of isotopic substitution on zero-point energies of those reactants and transition states (much as zero point energies can be used to predict equilibrium isotope fractionations; (Urey, 1947; Bigeleisen and Mayer, 1947). In the near future, we intend to publish studies using this approach to model isotopic fractionations accompanying hydration of CO₂, dissociation of carbonic acid, and reaction of carbonate with phosphoric acid (W. Guo, pers. com.).

4.4. Diffusion

Isotopic fractionations caused by diffusion-limited transport are well known for gas- and liquid-phase processes (e.g., exchange of CO₂ across leaf stomata or through leaf water; Jahne et al., 1987; Gibbs, 1928). Diffusion rates generally have mass-dependences that lead to fractionations of isotopologues between diffused and residual reservoirs that lead to deviations from a stochastic distribution; therefore, diffusion can lead to changes in Δ_i values (Eiler and Schauble, 2004).

Diffusive fractionations of Δ_i values are somewhat counter-intuitive. For example, CO₂ diffused through a small orifice (i.e., Knudsen diffusion) is 11‰ lower in $\delta^{13}\text{C}$, but 0.49‰ higher in Δ_{47} , than residual gas (Eiler and Schauble, 2004). Similar effects occur for gas-phase interdiffusion and are expected for liquid-phase diffusion. These effects can be understood in the following way: The stochastic distribution for CO₂ can be depicted as a two-dimensional surface in the three-dimensional space of $\delta^{18}\text{O}$ vs. $\delta^{13}\text{C}$ vs. the abundance of mass-47 isotopologues (mostly ¹³C¹⁸O¹⁶O; Fig. 7). This surface is curved, but for much of its area (and all of that relevant to natural materials), it is very nearly a flat plane. That plane has a slope that is steeper than the vector that describes the diffusive fractionation in these dimensions. Therefore, when a gas is fractionated by diffusion, the diffused portion moves along the vector of the diffusive fractionation toward lower $\delta^{13}\text{C}$, lower $\delta^{18}\text{O}$ and higher Δ_{47} , whereas the residual portion moves along that vector toward higher $\delta^{13}\text{C}$, higher $\delta^{18}\text{O}$ and lower Δ_{47} .

4.5. Fractionations caused by gravitational potential and thermal diffusion

The kinetic theory of gases predicts that a static gas phase (i.e., one not subjected to convective mixing) in a gravitational or thermal gradient will develop isotopic gradients characterized by high abundances of heavy molecules in regions that are cold and have a low gravitational potential energy, and *visa versa* (Gibbs, 1928; Grachev and Severinghaus, 2003).

The kinetic theory of gases also predicts that gravitational separation should lead to distinctive variations in Δ_i values of multiply substituted species (Gibbs, 1928). Gravitational fractionations are proportional to absolute mass difference; e.g., fractionation of ¹⁵N₂ from ¹⁴N₂ (2 AMU per molecule mass difference) should be twice as strong as fractionation of ¹⁵N¹⁴N from ¹⁴N₂ (1 AMU per molecule mass difference). Thus, for a given increase in bulk $\delta^{15}\text{N}$ (almost entirely controlled by ¹⁵N¹⁴N), one should see a 2x larger increase in ¹⁵N₂

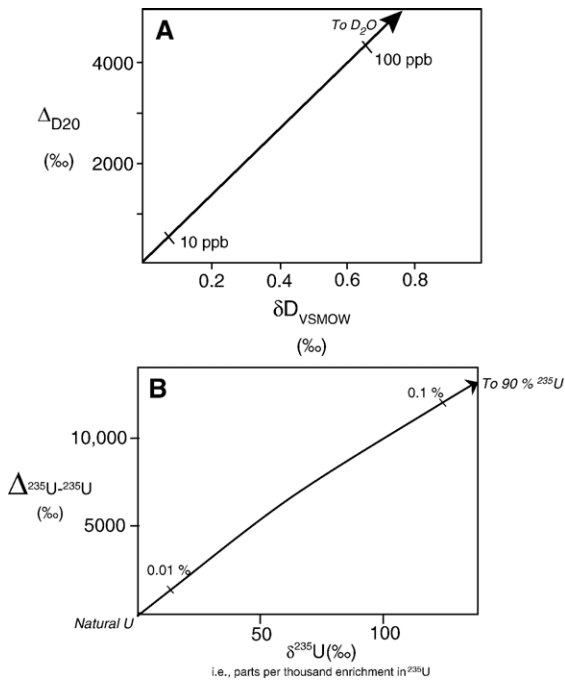


Fig. 8. Effects of mixing between materials of natural isotopic composition and highly enriched materials, in dimensions of a Δ_i value for a multiply-substituted isotopologue vs. a δ value describing a bulk isotopic composition. Panel A illustrates the effects of mixing between water vapor having the isotopic composition of the VSMOW standard and pure D_2O , as might occur if ‘heavy water’ mixed with natural atmospheric water vapor. Panel B illustrates the effects of mixing between natural uranium metal and weapons-grade uranium (assumed 90 at. % ^{235}U), as could occur during ‘blending’ or other mixing processes. Note that in both cases relatively subtle variations in bulk composition and small mixing ratios of the enriched end member lead to remarkably large enrichments in Δ_i values for multiply substituted isotopologues. This reflects the fact that the stochastic distribution is generally curved in dimensions of isotopologue abundance vs. bulk composition (e.g., $[D_2]$ vs. $[D]$) whereas mixing lines in those dimensions are straight. One implication of these trends is that clumped isotopic analyses could greatly enhance the sensitivity for detecting enriched materials (e.g., by factors of ca. 100 for weapons-grade U, and of ca. 6000 for heavy water), assuming clumped isotopic species can be measured with precision comparable to bulk isotopic compositions (which is true in at least some cases, given sufficient material and effort; e.g., Eiler and Schauble, 2004; Affek and Eiler, 2006; Ghosh et al., 2006a,b; Came et al., in press).

concentration. But, the stochastic distribution predicts that $[^{15}N_2]$ should increase as the square of $[^{15}N]$. Thus, gas at the bottom of a non-convecting, gravitationally fractionated column should have a relative deficit in $\Delta^{15}N_2$ (and the gas at the top of that column a relative $\Delta^{15}N_2$ excess). For example, gravitational fractionation of nitrogen gas in a static column 10 km long at 273.15 K and under a gravitational acceleration of 9.81 ms^{-2} leads to enrichments of 43.2‰ in $^{14}N^{15}N$ and 86.4‰ in $^{15}N_2$,

but 1.71‰ lower $\Delta^{15}N_2$, at the bottom of the column relative to the top. However, for environments of interest to natural applications, the predicted change in $\Delta^{15}N_2$ is negligible: for a 100 m column, similar to the thickness of glacial firn, the contrast in $\Delta^{15}N_2$ between top and bottom is only 0.0002‰—likely un-measurable (note $\Delta^{15}N_2$ is not linearly proportional to column length because of curvature in the relationship between $[^{15}N]$ and $[^{15}N_2]$ for the stochastic distribution).

Isotopic fractionations caused by thermal gradients are usually calculated using empirical factors derived from laboratory study of gas mixtures subjected to extreme temperature gradients (because theoretical estimates of such fractionations cannot easily account for interactions between molecules other than ideal gases; Grachev and Severinghaus, 2003). Fractionations in a temperature gradient have been measured for both the $^{15}N_2/^{14}N_2$ and $^{15}N^{14}N/^{14}N_2$ ratio, but the overlapping data are insufficiently precise to yield a meaningful prediction of fractionations in $\Delta^{15}N_2$ across thermal gradients (see Grachev and Severinghaus, 2003 for a review and primary references). If we turn to the theoretical mass-scaling law for fractionations in a thermal gradient (which predicts that α varies as $(M_1 - M_2)/(M_1 + M_2)$; (Grachev and Severinghaus, 2003, and references therein)), then we predict that a thermal gradient sufficient to create a 1‰ increase in $\delta^{15}N$ will lead to a -0.035% decrease in $\Delta^{15}N_2$. This is small but measurable. Because thermal fractionations lead to larger Δ_i changes for a given change in bulk composition than do gravitational fractionations, clumped isotope measurements may be able to distinguish gravitational from thermal fractionations in gases trapped in glacial ice and therefore refine the interpretation of such data as constraints on past climate change (Severinghaus et al., 1998).

4.6. Mixing

Generally speaking, one cannot tell whether the bulk isotopic composition of a given material (e.g., the δD value of water vapor) reflects isotopic fractionation that changed it from some previous, different composition (e.g., by condensation of rain from vapor), or mixing between two isotopically distinct reservoirs to yield an intermediate hybrid (e.g., mixing water vapor from air masses that originally differ in isotopic composition). There are instances in which ancillary information might be used to help make this distinction (e.g., under some cases, one can tell fractionations of water-vapor-isotopes due to condensation from isotopic variations due to mixing by plotting the water-vapor mixing ratio vs. δD value). But, these are special cases — there is no

universal ‘finger print’ of bulk isotopic compositions that result from mixing.

However, because two-component mixing follows linear vectors in isotopic composition space but the stochastic distribution is generally a curved surface in that space, mixing can lead to large, predictable and interpretable deviations from the stochastic distribution. Subtle (per mil level) mixing effects of this kind have been observed experimentally for CO₂ (Eiler and Schauble, 2004), and much more extreme ones are expected for mixing involving anthropogenically enriched materials. For example, accidental or intentional release of ‘heavy water’ (nominally D₂O) to the atmosphere, or ‘blending’ of weapons grade uranium (ca. 90% ²³⁵U) with natural uranium (ca. 0.72% ²³⁵U), assuming each end member conforms to the stochastic distribution, will produce mixtures that have positive Δ_i values for clumped isotopic species (Fig. 8). For these cases, mixing produces changes in Δ_i values hundreds or thousands of times greater than changes in bulk isotopic composition for a given mixing ratio of end members. Thus, clumped isotope analyses could provide greatly increased sensitivity for detecting enriched components and a means of distinguishing moderate anthropogenic enrichments vs. mixtures between natural and highly enriched components.

5. Demonstrated uses

Clumped isotope geochemistry is a new discipline with few practitioners. So, while it seems likely to us that the field will grow and diversify, at present there are just a few applications with demonstrated ‘staying power’. In this section, we briefly review these emerging areas of applied research. We refer readers to the primary publications of these studies for details (Schauble et al., 2003, 2006; Eiler and Schauble, 2004; Wang et al., 2004; Affek et al., 2005, in press; Guo and Eiler, 2005; Affek and Eiler, 2006; Ghosh et al., 2006a, b, 2007; Eiler et al., 2006; Eiler, 2006a; Eiler, 2006b; Came et al., in press; Guo and Eiler, in press); here we focus on methods, goals and principles of interpretation shared by them all.

5.1. Carbonate paleothermometry

Perhaps the most useful recognized application of clumped isotope geochemistry is a carbonate paleothermometer based on the formation of carbonate ion groups containing both ¹³C and ¹⁸O (Ghosh et al., 2006a,b, 2007; Eiler et al., 2006; Schauble et al., 2006; Came et al., in press; Guo and Eiler, in press). At

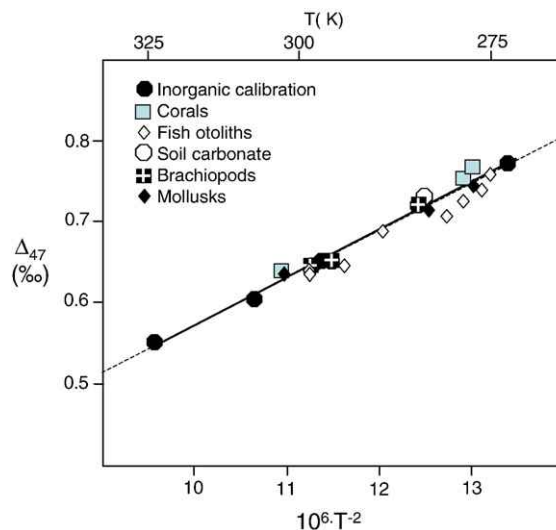
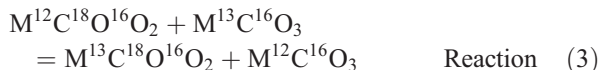


Fig. 9. Dependence of the Δ₄₇ value of CO₂ produced by phosphoric acid digestion of carbonate on the growth temperature of that carbonate. Data are shown for inorganic calcite grown under controlled conditions in the laboratory (Ghosh et al., 2006a), various biogenic carbonates collected in nature and having known or estimated growth temperatures (Ghosh et al., 2006a, 2007; Came et al., in press), and a natural soil carbonate collected from a location with known mean annual temperature. See the legend for further details. The line is fitted to the data for inorganic calcite from (Ghosh et al., 2006a) (this line is solid where interpolated and dashed where extrapolated). Note that all carbonates approximately conform to the inorganic trend (excepting a ca. 0–2 °C offset for the data for otoliths—possibly attributable to systematic error in estimated growth temperatures of free-living fishes; Ghosh et al., 2007).

thermodynamic equilibrium, carbonate minerals are expected to contain abundances of ¹³C¹⁸O¹⁶O₂⁻² ionic groups defined by the homogeneous equilibrium:



(where M is a metal such as Ca; Schauble et al., 2006). This reaction is analogous to those for molecular hydrogen and water discussed above (i.e., Reactions (1) and (2)). The equilibrium constant for Reaction (3) (K₃) approaches the stochastic distribution at high temperature, and increases, driving the reaction to the right (forming super-stochastic abundances of ¹³C–¹⁸O bonds) with decreasing temperature. Thus, by analyzing a carbonate mineral for its relative abundances of all the reactant and product isotopologues that appear in Reaction (3), one could directly constrain the carbonate mineral growth temperature (assuming equilibrium growth and an appropriate calibration of the temperature dependence of K₃). We refer to this approach as ‘carbonate clumped isotope thermometry’.

There is a critical difference between carbonate clumped isotope thermometry and conventional oxygen-isotope carbonate-water paleothermometry (McCrea, 1950; Epstein et al., 1953; Kim and O'Neil, 1997; Veizer et al., 1999): conventional carbonate-water paleothermometry is based on a heterogeneous equilibrium that fractionates ^{18}O between carbonate and water. Therefore, temperature is only rigorously constrained if the $\delta^{18}\text{O}$ values of both phases are known. However, for much of the geological (and meteoritical) record, we have few direct constraints on the oxygen isotope compositions of waters from which carbonates grew. For this reason, many applications of the carbonate-water thermometer are in some sense compromised by assumptions regarding the isotopic composition of water. In contrast, the carbonate clumped isotope thermometer is based on a homogeneous equilibrium, and all of the information needed to reconstruct the equilibrium constant for that reaction is preserved within the carbonate mineral itself.

In practice, because existing methods of precise clumped isotope analysis require gas as an analyte, the carbonate clumped isotope thermometer is actually calibrated by comparing the Δ_{47} value of CO_2 (which is dominated by variations in $^{13}\text{C}^{18}\text{O}^{16}\text{O}$) released from carbonate by phosphoric acid digestion to the growth temperature of that carbonate (Ghosh et al., 2006a). This calibration includes a significant but apparently well-behaved acid-digestion fractionation (much as for measurements of $\delta^{18}\text{O}$ in carbonate by phosphoric acid digestion; Swart et al., 1991). Ongoing theoretical and experimental research on this effect suggests it is a consequence of a kinetic isotope effect associated with dissociation of H_2CO_3 to release CO_2 gas (W. Guo, pers. com.). Whatever its cause, it varies little with reaction temperature (ca. 1 ppm per $^\circ\text{C}$) and appears to be indistinguishable among various carbonate types. We have experimentally or empirically calibrated the carbonate clumped isotope thermometer for synthetic inorganic calcite (Ghosh et al., 2006a), natural inorganic calcite from a soil (previously unpublished), aragonitic otoliths (Ghosh et al., 2007), aragonitic corals (Ghosh et al., 2006a), aragonitic mollusks and calcitic brachiopods (Came et al., in press) (Fig. 9). Ongoing research is extending this calibration to include foraminifera and synthetic aragonite and dolomite. The uniformity of the results to-date suggests that vital effects and other material-specific isotopic fractionations (a common complexity to conventional stable isotope thermometry) are relatively unimportant for this system. This uniformity is expected based on theoretical models of isotopic clumping in various kinds of carbonate minerals

(Schauble et al., 2006). Nevertheless, it will be important to continue expanding the breadth of this calibration to include other carbonate types.

Given the best external precisions achieved for analyses of Δ_{47} in CO_2 from carbonate (ca. $\pm 0.005\%$; Came et al., in press) and the temperature sensitivity implied by the data in Fig. 9, the carbonate clumped isotope thermometer has uncertainties in temperature of ca. ± 1 $^\circ\text{C}$ at earth-surface temperatures (more at higher temperatures).

Perhaps the most useful thing about the carbonate clumped isotope thermometer is its lack of dependence on the isotopic composition of water (or any other phase with which carbonate might co-exist). Moreover, this thermometer is based on a thermodynamically controlled process that apparently differs little among various kinds of biological and inorganic carbonates, and so can be used equally well in many different sorts of materials (although some caution is required on this point until we gain more experience with it in applied use). In this respect, it is potentially broader in its use than the various empirical thermometers developed for Cenozoic marine climate records (e.g., alkenone abundances or various biodiversity or physiognomic temperature proxies). For these reasons, the carbonate clumped isotope thermometer can be particularly useful for reconstructing temperatures in times and locations where the isotopic composition of water is unknown and the various empirical thermometers are inapplicable or uncalibrated. Examples include pre-Cenozoic marine sediments, terrestrial sediments and meteorites.

Another important characteristic of the carbonate clumped isotope thermometer is that it provides a means of rigorously constraining the $\delta^{18}\text{O}$ of ancient waters. That is, once one has determined the growth temperature of a carbonate using the clumped isotope approach, one can then combine the $\delta^{18}\text{O}$ value of that carbonate (measured at the same time and on the same material as the clumped isotope measurement) with the known temperature-dependent fractionation of oxygen isotopes between carbonate and water (McCrea, 1950; Epstein et al., 1953; Kim and O'Neil, 1997) to calculate the $\delta^{18}\text{O}$ value of the water from which carbonate grew. There are many problems for which the $\delta^{18}\text{O}$ of water is of equal or greater importance than carbonate growth temperature (e.g., water-rock reaction in the crust; aqueous alteration of meteorites; some aspects of terrestrial climate change and paleoaltimetry; e.g., Ghosh et al., 2006b). We expect that clumped isotope thermometry will play an important role in advancing such problems.

It is important to establish how well the carbonate clumped isotope thermometer resists post-depositional

resetting through diagenesis, burial metamorphism, weathering and other processes. These sorts of complications affect all methods of paleothermometry to greater or lesser degrees, and generally can be understood only through applied study in a wide range of settings (because experiments at laboratory time-scales generally fail to capture the complexity of the relevant mechanisms, especially at low temperatures). Experience to-date with soil carbonates of varying age and burial depth (Eiler et al., 2006), Precambrian limestones (Eiler, 2006a and unpublished), marbles (Ghosh et al., 2006a) and Phanerozoic fossils (Came et al., in press) suggests that the carbonate clumped isotope thermometer preserves primary temperatures through subsequent heating over geological time scales up to temperatures of ca. 200 °C, provided that carbonate does not undergo dissolution and reprecipitation. This last clause is key: if carbonate dissolves and re-precipitates at any temperature, the relatively rapid kinetics of oxygen exchange between water and dissolved inorganic carbon (Kim and O'Neil, 1997) will promote isotopic re-distribution. For this reason, carbonate clumped isotope temperatures should be determined only after one has established the primary character of the sample through textural, structural and/or trace-metal studies (e.g., Veizer et al., 1999).

5.2. Budgets of atmospheric gases

Clumped isotope measurements can contribute to the study of atmospheric gases because they provide new, independent constraints on gas budgets that often suffer from under-constraint (e.g., concentration and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of atmospheric CO_2 are insufficient to uniquely define its budget). Moreover, it is relatively straightforward to extract and analyze atmospheric gases without redistribution of isotopes, so this is among the simplest uses of clumped isotope geochemistry from a purely practical standpoint.

We have significant experience with Δ_{47} measurements of atmospheric CO_2 , including field experiments, regional studies and nominally 'clean' background air (Eiler and Schauble, 2004; Affek et al., 2005, in press; Affek and Eiler, 2006). To-date, such data have been used to constrain the mixing ratios of combustion products in urban air (Affek and Eiler, 2006) and have resulted in two unexplained but potentially important observations: (1) the Δ_{47} value of CO_2 in nominally clean air varies seasonally with surprisingly high amplitude (ca. 0.2‰) and in a way that is not consistent with purely thermodynamic control (i.e., Δ_{47} values are lower in the winter than in the summer, opposite the

direction predicted for equilibrium) (Affek et al., 2005, in press). We suspect this seasonal trend reflects the trade-off of respiration and photosynthesis and kinetic controls on the Δ_{47} value of respired CO_2 . However, this hypothesis awaits laboratory and field tests. And, (2) the Δ_{47} value of stratospheric CO_2 rises strongly (by more than 0.5‰) with increasing photochemical exchange of CO_2 with $\text{O}(\text{1D})$ (as measured by the $\Delta^{17}\text{O}$ value of CO_2) (Affek et al., 2005). This phenomenon is opposite to the expected trend because the $\text{CO}_2 + \text{O}(\text{1D})$ exchange reaction is highly energetic and should promote isotopic 'scrambling' (driving CO_2 toward the stochastic distribution). Nevertheless, the observations are clear and appear to indicate something counter-intuitive about the photochemical processing of stratospheric CO_2 . We are currently examining this issue further through laboratory photochemical studies.

We can imagine many uses of clumped isotope geochemistry analogous to those summarized above. Particularly attractive targets include: Studies of multiply-deuterated methane and molecular hydrogen, which could precisely define the residence times of these gases with respect to atmospheric photo-oxidation (Mroz et al., 1989; Kaye and Jackman, 1990); studies of $^{18}\text{O}_2$ and $^{18}\text{O}^{17}\text{O}$, which could help de-convolve the competing effects of photosynthesis, respiration and photochemistry on the isotopic budget of O_2 ; and studies of $^{15}\text{N}_2$, which could potentially constrain the N_2 budget.

5.3. Mechanisms of isotopic fractionation

As was mentioned above, there is a long history of mechanistic studies of the physical and biochemistry of isotopically substituted species through laboratory and field experiments on artificially enriched materials. The advent of precise clumped isotope measurements suitable for work on natural abundances of these species creates the possibility that studies of this kind could be performed on natural systems. Such data could be used to relate physical, chemical and biosynthetic processes that are well-understood from laboratory studies on enriched materials to natural processes and budgets, or to discover new and unexpected mechanisms of isotopic fractionation through exploration of the clumped isotope compositions of natural things. For example, it has been suggested that vital effects controlling the bulk C and O isotope composition of some biogenic carbonates reflect fractionation between seawater and animal body water combined with equilibrium partitioning during precipitation of carbonate from body water (Adkins et al., 2003). Clumped isotope geochemistry could test this hypothesis because it predicts that all biogenic

carbonates, regardless of the impact of vital effects on their bulk isotopic compositions, will have clumped-isotope compositions consistent with equilibrium at their temperatures of growth.

Our work to-date has provided several instances in which clumped-isotope measurements revealed mechanistically significant information (although some, such as the anomalous Δ_{47} enrichments in stratospheric CO_2 cited above (Affek et al., 2005), will require additional experimental work before it is fully understood). The simplest example is a study of the stable isotope systematics of CO_2 in car exhaust, which demonstrated that its oxygen isotope and clumped isotope composition reflects equilibration with co-emitted water vapor at ca. 200 °C (presumably the temperature at which the cooling exhaust stream ‘quenches’ its internal isotope exchange equilibria; (Affek and Eiler, 2006)).

5.4. Future Challenges

At present, clumped isotope geochemistry is more noteworthy for its novelty and promise than for its accomplishments, and there are a number of pressing challenges that must be met before it can advance.

Most importantly, the community of practitioners of this field must grow. Spread of the analytical approaches and applied work described here to other labs will spur technical and conceptual innovation, diversify the family of problems that are addressed, and more quickly fill in the many gaps in our knowledge of clumped isotope chemistry and distributions in nature. Clumped isotope analyses can be performed in any modern stable isotope laboratory, but are sufficiently challenging that they are unlikely to become widespread until technical innovation makes them simpler, less time consuming and more reliable. Automation of sample preparation devices will help in this regard.

A second fundamental advance that is sorely needed is development of analytical instruments that are more suitable to the needs of clumped isotope measurements. These analyses are currently made on commercially-available gas-source isotope ratio mass spectrometers that were only modestly modified for this purpose. These instruments have relatively poor mass resolving power, preventing mass-resolution of the most common trace contaminants, and are insufficiently sensitive for analyzing exceedingly rare species, such as triply-substituted isotopologues. A major step forward in analytical capabilities would result from development of a gas source mass spectrometer with enhanced mass resolving power

($M/\Delta M$ of ca. 2000–5000 would be sufficient) and the capability to use ion counters (e.g., electron multipliers) to collect some species.

The scope of clumped isotope geochemistry could also be greatly expanded by developing a class of instruments for near-infra-red absorption spectroscopy that are optimized for precise isotope ratio measurements of the clumped isotopic species. Even if such measurements required purified samples and highly controlled conditions, this would allow for resolved analysis of species having the same cardinal mass (e.g., $^{12}\text{C}^{17}\text{O}^{18}\text{O}$ from $^{13}\text{C}^{18}\text{O}^{16}\text{O}$, or $^{17}\text{O}^{17}\text{O}$ from $^{18}\text{O}^{16}\text{O}$)—something that will likely remain impossible for magnetic sector mass spectrometers.

Existing thermal ionization mass spectrometers may be suitable for clumped isotope analysis of some solids, and we strongly encourage that this be explored by one of the existing appropriately equipped laboratories.

Even barring creation of new classes of analytical instruments, the scope of clumped isotope geochemistry could be expanded significantly by developing new methods for extracting analyte gases from currently inaccessible materials. For example, we have attempted several approaches to extracting CO from organic matter (all unsuccessful) and have plans to prepare SO_2 from sulfates for clumped isotope analysis. Such methodological innovations face significant hurdles because a successful technique must not redistribute isotopes during extraction of analyte gas and, if it fractionates isotopologues from one another it must do so reproducibly (e.g., as for phosphoric acid digestion of carbonates Ghosh et al., 2006a).

Finally, even if we restrict ourselves to the analytes that have been demonstrated to be reliably measurable (CO_2 from carbonates and air), there are several pressing needs. First, our theoretical understanding of equilibrium fractionations (i.e., the equilibrium constants for homogeneous isotope exchange equilibria) and fractionations arising from simple physical processes (e.g., diffusion) are relatively sound, but only a few such fractionations have been studied by experiment (Schauble et al., 2003; Eiler and Schauble, 2004; Ghosh et al., 2006a, 2007; Came et al., in press). The foundation of this field will not be firm until we have thoroughly examined more of these fractionations through direct observation. Our understanding of kinetic isotope effects associated with unidirectional, condensed-phase reactions is even poorer, and demands focused and detailed studies of clumped isotope fractionations accompanying processes such as photosynthesis, respiration and other metabolic processes, and a-biological processes such as air-sea gas exchange.

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