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International Journal of Mass Spectrometry 229 (2003) 217–224

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

What factors can limit the precision of isotope ratio measurements in the isotopomer pool?[☆]

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Received 12 January 2003; accepted 24 July 2003

Abstract

Mono- and diisotopomers differ by quantity of atoms of the polyisotopic element (PIE). The PIE isotope abundance in a monoisotopomer pool is estimated by the ratios of quantities of isotopically different PIE-bearing molecules. As in the case of conventional isotope analysis in a monoisotopomer, if the abundance of one of the two PIE isotopes in a diisotopomer is a small quantity, two main species of diisotopomer molecules are used, not taking into account the quantity of molecules with two atoms of a minor isotope. Assessment of this isotope abundance measurement has demonstrated that such method permits to get a satisfactory result only at homogeneous isotope distribution in the both PIE sites of the diisotopomer molecule. At non-homogeneous isotope PIE distribution in the diisotopomer molecule the estimation of isotope content by the conventional method may have a hidden error whose value depends on the ways of formation or transformation of the diisotopomer pool.

In the case of oxygen as PIE, the homogeneity of oxygen isotope distribution in the pool of CO_2 (oxygen diisotopomer) can be disrupted by: (a) different sources of two oxygen sites in CO_2, (b) oxygen isotope fractionation of CO_2 during molecular efflux or influx from/to reservoirs, (c) mixtures of two or more CO_2 pools with different oxygen isotope ratios. The estimation of $\delta^{18}O$ deviations between results of the conventional oxygen isotope measurements of non-homogeneous CO_2 pool and this pool after its oxygen isotope homogenization (where oxygen isotope content is valid) has shown that hidden errors can be over 3‰.

Keywords: Conventional isotope measurement; Diisotopomers; Hidden errors of measurement; Isotope fractionation; Carbon dioxide isotopomers; Oxygen isotopes

1. Introduction

Atoms of the polyisotopic element (PIE) being present in a molecule form a pool of molecules that are of identical chemical structure and elemental composition but different mass because of isotopic substitution and are designated as isotopomers (isotopic isomers).

Mono- and polyisotopomers are distinguished by the quantity of PIE atoms: the first ones are molecules with only one atom of PIE in their structure, and the second ones have two and more atoms of PIE. The pools of molecules involving the ${}^{p}A$ and ${}^{q}A$ isotopes of element A are to be found as the ${}^{p}AB$ and ${}^{q}AB$ monoisotopomer molecules or the ${}^{p}A_nB$, ${}^{p}A_{n-1}{}^{q}AB$, ${}^{p}A_{n-1}{}^{q}AB, \ldots,$ ${}^{p}A{}^{q}A_{n-1}B$, ${}^{q}A_nB$ *n*-isotopomer molecules.

For instance, the simplest known isotopomers are: *diisotopomers*: H_2O, H_2S (by hydrogen), N_2, N_2O (by nitrogen), O_2, CO_2 (by oxygen), CH_3COOH, C_2H_4 (by carbon), CH_2Cl_2, $C_2H_2Cl_2$, CCl_2F_2 (by chlorine); *triisotopomers*: NH_3, CH_3Br (by hydrogen), O_3 (by oxygen), $HCCl_3$, CH_3CCl_3 (by chlorine); *tetraisotopomers*: CH_4, CH_3OH, C_2H_4 (by hydrogen), CCl_4, C_2Cl_4 (by chlorine), etc.

The analysis of isotope abundance ratios of PIE occurring in a monoisotopomer pool consists in the determination of the ratios of isotopically different molecule quantities with atoms of this element. The main sources of errors in mass spectrometric measurement of the above molecule quantities are instrumental factors (mass discrimination effects in ion source and mass analyzer, incomplete separation of analyzed isotopically different molecules, background conditions of the instrument, etc.). To exclude or minimize the

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[☆] A brief abstract of this work was presented at the 3rd International Conference "Applications of Stable Isotope Techniques to Ecological Studies" (Flagstaff, AZ, USA, April 30–May 1, 2002).

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doi:10.1016/S1387-3806(03)00342-7

effect of instrumental factors on the accuracy of measured isotopic ratios of PIE in monoisotopomer pool, the method of comparison of isotopic composition of analyzed sample and the standard has been proposed [1,2].

In case of polyisotopomer, the quantity of isotopically different molecules in its pool significantly increases as compared with the pool of monoisotopomer. So, the molecule pool of diisotopomer possessing a diisotopic element will contain three isotopically different kinds of molecules, i.e., the molecules possessing two light isotopes, one light and one heavy isotope, and two heavy isotopes, respectively. In accordance with conventional practice, under minor abundance of one of the two isotopes, for example, ^{15}N as compared with ^{14}N for nitrogen, or ^{18}O as compared with ^{16}O for oxygen, the measurements are made subject to only two most intensive peaks in their mass spectra of diisotopomer molecules. To measure nitrogen or oxygen isotope composition in the pools of diisotopomers N_2O or CO_2 the quantities of $^{14}N_2O$ and $^{14}N^{15}NO$ or $C^{16}O_2$ and $C^{16}O^{18}O$, respectively, are used. Minor quantities of molecules possessing two heavy isotopes (^{15}N or ^{18}O, respectively) are not taken into consideration. Errors determined by instrumental factors, as in case of monoisotopomer, may be excluded or significantly reduced when isotope measurements are carried out relative to standard samples. A question arises to what extent neglecting of the quantity of molecules possessing the two least abundant isotopes may affect the accuracy of determination of abundance ratios of isotopic atoms of diisotopic element in the course of analysis of diisotopomer pool.

Since the oxygen isotope content of CO_2 (oxygen diisotopomer) can be used to elucidate the atmospheric carbon dioxide sources and fluxes, it was important to detect causes restricting the precision of CO_2 oxygen isotope measurements.

This presentation is aimed at evaluating possible deviations of the quantitative parameters of the oxygen isotope content in the pool of CO_2 (diisotopomer) measured by conventional methods (that exclude the analysis of minor quantity of $C^{18}O_2$ particles) in comparison with its actual oxygen isotope content. The factors that evoke such deviations have to be also considered.

2. Theoretical part

2.1. Monoisotopomers

Precision mass spectrometric measurements of the stable isotope abundance ratios in monoisotopomer pools were initially developed and described in [3–5]. It should be noted that in the first work [5] the light-to-heavy isotope abundance ratios (e.g., $R_s = [^{32}S]/[^{34}S]$ or $R_c = [^{12}C]/[^{13}C]$) were used as quantitative characteristics of isotope composition of elements. In our case, such presentation of isotope composition of polyisotopic element proved to be convenient to create a mathematical formalism reflecting distribution of isotopes in the analyzed pool of isotopically different molecules.

The analysis of isotope abundances of the polyisotopic element A occurring in a monoisotopomer consists in the determination of the ratio of isotopically different molecular quantities with the element A in the monoisotopomer pool, i.e.,

$$R = \frac{[^{p}A]}{[^{q}A]} \tag{1}$$

where $[^{p}A]$, $[^{q}A]$ are quantities of molecules containing ^{p}A and ^{q}A isotopes of the analyzed element A ($q > p$).

The equation describing the isotope distribution in the molecule pool of monoisotopomer can be written as

$$R - R_0 = 0 \tag{2}$$

where R is a variable, R_0 is the abundance ratio of the ^{p}A- and ^{q}A-bearing molecules in the pool of the tested monoisotopomer.

2.2. Polyisotopomers

The presence of no less than two PIE atoms in the molecule drastically changes the methodology of isotope abundance measurements. Polyisotopomers are characterized by probabilistic (homogeneous) or nonprobabilistic (non-homogeneous) isotope distribution of PIE in each of its sites in the molecule [6]. If the $R_0 = [^{p}A]/[^{q}A]$ value represents of the isotope abundance ratios for all sites of element A in the molecule (homogeneous isotope distribution), the isotopic equation generally characterizing a pool of n-isotopomer molecules may be given as

$$(R - R_0)^n = 0 \tag{3}$$

The solutions of Eq. (3) will have the values $R_1 = R_2 = \cdots = R_n = R_0$. At non-homogeneous distribution of ^{p}A and ^{q}A atoms in a polyisotopomer molecule the isotopic equation characterizing this n-isotopomer pool may be written as

$$(R - R_1)(R - R_2)\cdots(R - R_n) = 0 \tag{4}$$

Different values of R ($R_1 \neq R_2 \neq \cdots \neq R_n$) will be the solutions of Eq. (4), respectively. For the sake of simplicity, let us consider the homogeneous distribution of ^{p}A and ^{q}A isotopes of the element A in the diisotopomer pool. Abundances of these isotopes are equal to a and b, respectively (where $a + b = 1$ by definition). The isotopic composition of the element A is represented by the value $R = a/b$. If the element A has the same isotope abundance ratios for sites 1 and 2 in the molecule (i.e., $R_1 = R_2 = R_0 = a/b$), Eq. (3) characterizing the distribution of isotopes in this isotopomer pool can be formulated as

$$\left(R - \frac{a}{b}\right)^2 = 0, \tag{5}$$

where a and b are abundances of isotopes ^{p}A and ^{q}A of the analyzed element A.

If $b \neq 0$, then the Eq. (5) will be written as

$$b^2R^2 - 2abR + a^2 = 0 \tag{6}$$

Since $a+b = 1$, the values a^2 and b^2 reflect the probabilities of finding one of the isotopes $^{\mathrm{p}}\mathrm{A}$ or $^{\mathrm{q}}\mathrm{A}$ by two positions of the element A in the diisotopomer molecule, and $2ab$ is the probability of their simultaneous finding by these positions. Previously Zyakun and Schidlowski [7] discussed that the values b^2, $2ab$, and a^2 have to be proportional to peak intensities in the mass spectrum of diisotopomer: $I_2 = k \times b^2$ is the peak intensity determined by the quantity of molecules having in two sites only isotope $^{\mathrm{q}}\mathrm{A}$; $I_1 = k \times 2ab$ is the peak intensity corresponding to the quantity of molecules having $^{\mathrm{p}}\mathrm{A}$ or $^{\mathrm{q}}\mathrm{A}$ isotopes simultaneously by two positions; $I_0 = k \times a^2$ is the peak intensity corresponding to the quantity of molecules having only isotope $^{\mathrm{p}}\mathrm{A}$ by two sites in the molecule of diisotopomer (k is the coefficient of proportionality). Normalizing the intensities of the I_2, I_1, I_0 peaks by I_2, we obtain a set of values in Eq. (7): 1, I_1/I_2 and I_0/I_2, or 1, $2a/b$, and a^2/b^2.

$$R^2 - (I_1/I_2)R + I_0/I_2 = 0 \tag{7}$$

It is quite obvious that the solution of Eq. (7) with the values 1, I_1/I_2 and I_0/I_2 will be $R_0 = a/b$, i.e., the ratio of abundances of isotopic atoms $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ of the element A in diisotopomer.

In case of non-homogeneous isotope distribution in two-site molecules with a different probability of finding isotopes $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ by each of two positions in the pool of diisotopomer (i.e., $R_1 \neq R_2$), Eq. (4) can be presented as

$$(R - R_1)(R - R_2) = 0 \tag{8}$$

or

$$R^2 - (R_1 + R_2)R + R_1R_2 = 0 \tag{9}$$

The values 1, $(R_1 + R_2)$ and R_1R_2 are the normalized intensities of the molecular ion peaks in the mass spectrum of diisotopomer with the non-homogeneous $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ isotope distribution in the molecules. Therefrom it follows that the quantity of isotopically different molecules in the isotopomer pool is determined by the ratios of the $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ abundances for the individual element A sites of molecule. One should remember that the data obtained from solution of the corresponding mathematical Eqs. (7) and (9) may evidence only the existence of the element A sites in the molecule, which enable the finding of isotope atoms in the analyzed pool with the same or different probability but not identification of their sites in the molecule directly.

2.3. Isotope measurements

In the case of a diisotopomer by element A, the $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ isotope contents may be determined by three known methods: (a) *conventional practice* (by measuring the quantities of two isotopically distinctive molecular species, one of which contains two atoms of a predominant isotope ($^{\mathrm{p}}\mathrm{A}$) and the other one has one atom of a minor isotope ($^{\mathrm{q}}\mathrm{A}$) along with the major ($^{\mathrm{p}}\mathrm{A}$) isotope); (b) *atomic isotope abundance ratios* (by the determination from the sums of quantities of molecules bearing the light ($^{\mathrm{p}}\mathrm{A}$) and heavy ($^{\mathrm{q}}\mathrm{A}$) isotopes); (c) *average isotope abundance ratios* (by the calculation of the $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ isotope ratio average for all sites in the diisotopomer).

Are there any differences between the results of isotopic measurements of the element A in the diisotopomer pool by the above methods?

As already noted, in the *conventional practice* the ratios of only two peak intensities in the diisotopomer mass spectrum determined by the most abundant and isotopically different molecules (I_0/I_1) is commonly measured that not taking into account a small quantity (I_2) of molecules bearing two minor abundance isotope atoms of the analyzed element. As a result, the ambiguity in distribution of the element A isotope atoms in the diisotopomer pool remains, because there is an unlimited number of diisotopomer pools in whose mass spectra the values I_0/I_1 are equal, while I_2 may be arbitrary. Although the *atomic isotope abundance ratios* are conventionally measured by the amounts of three isotopically different diisotopomer molecules, the ambiguity of the element A isotope characteristics obtained is also present. Really, the ratios of the isotope atom amount sums may be absolutely equal at different probability detection of isotope atoms in each of the two sites of element A in the diisotopomer molecule. The uniqueness of the element A isotope distribution in the diisotopomer pool can be only obtained by a method based on the *average isotope abundance ratios* by using the respective isotopic equation whose parameters are determined by the quantities of three isotopically different molecules in the diisotopomer pool.

Let us quantitatively compare the data on the isotope content of the element A in the polyisotopomer pool obtained by the above approaches by the example of a diisotopomer. In the case of *homogeneous* distribution of element A isotopes in diisotopomer, the isotope content is characterized by values $R_1 = R_2 = R_{\mathrm{av}}$, where R_1 and R_2 are isotope abundance ratios of the element A for sites 1 and 2 in the diisotopomer, respectively. R_{av} is an average of values R_1 and R_2. The equation describing the homogeneous isotope distribution of the diisotopomer molecule pool can be written as

$$R^2 - 2R_{\mathrm{av}}R + (R_{\mathrm{av}})^2 = 0 \tag{10}$$

Peak intensities I_2, I_1, and I_0 in the mass spectrum of this diisotopomer are proportional to the values 1, $2R_{\mathrm{av}}$ and $(R_{\mathrm{av}})^2$ (see Eq. (10)). The *conventional practice* of the $^{\mathrm{p}}\mathrm{A}$ and $^{\mathrm{q}}\mathrm{A}$ isotopic analysis of the element A in diisotopomer relies on the two most intensive peaks of the diisotopomer mass spectrum as

$$R_{\mathrm{c}} = \frac{I_0}{I_1} \tag{11}$$

or

$$R_c = \frac{(R_{av})^2}{2R_{av}} = \frac{R_{av}}{2} \tag{12}$$

Using the peak intensities I_2, I_1, and I_0 in the mass spectrum of diisotopomer, the *atomic isotope ratio* can be calculated as

$$R_a = \frac{2I_0 + I_1}{I_1 + 2I_2} \tag{13}$$

or

$$R_a = \frac{2(R_{av})^2 + 2R_{av}}{2R_{av} + 2} = R_{av} \tag{14}$$

Consequently, in case of *homogeneous* distribution of the element A isotopic atoms in a diisotopomer the *atomic* and *conventional* values R_a and R_c are equal or proportional to the *average* isotopic value R_{av}.

For the *non-homogeneous* isotope distribution of the element A in the molecule, the diisotopomer pool is characterized by two values of isotopic ratios $R_1 \neq R_2$ of the element A in sites 1 and 2, respectively. The equation describing the isotope distribution of the non-homogeneous diisotopomer pool would be presented as

$$(R - R_1)(R - R_2) = 0 \tag{15}$$

or

$$R^2 - (R_1 + R_2)R + R_1R_2 = 0 \tag{16}$$

where R_1 and R_2 are the solutions of Eq. (16).

The I_2, I_1, and I_0 peak intensities in the mass spectrum of the diisotopomer are proportional to the values 1, $(R_1 + R_2)$ and R_1R_2 (see Eq. (16)), respectively. If $R_{av} = (R_1 + R_2)/2$ and $\Delta R = (R_1 - R_2)/2$, then $R_1 = R_{av} + \Delta R$ and $R_2 = R_{av} - \Delta R$. The Eq. (16) may be rearranged to

$$R^2 - 2R_{av}R + (R_{av})^2 - (\Delta R)^2 = 0 \tag{17}$$

Determination of the ${}^{p}A/{}^{q}A$ isotopic ratios for diisotopomer pool with non-homogeneous isotope distribution by *conventional practice* would be calculated as

$$R_c = \frac{[(R_{av})^2 - (\Delta R)^2]}{2R_{av}} = \frac{[R_{av} - (\Delta R)^2/R_{av}]}{2} \tag{18}$$

The *atomic isotope ratios* for non-homogeneous isotope distribution in the two-site molecule pool are determined as

$$R_a = \frac{[2((R_{av})^2 - (\Delta R)^2) + 2R_{av}]}{2R_{av} + 2} = R_{av} - (\Delta R)^2/(R_{av} + 1) \tag{19}$$

The value ΔR reflects deviations of the isotope abundance ratios of the element A by its individual sites in the diisotopomer molecule from R_{av}. According to Expressions (18) and (19) it follows that if the value ΔR is presented by real numbers then the ratios of R_c and R_a will have the lower values than R_{av} (i.e., $R_a < 2R_c < R_{av}$) and if the value ΔR will be presented by imaginary numbers then the R_c and R_a values will be higher than R_{av} (i.e., $2R_c > R_a > R_{av}$). In view of the fact that the *average isotope abundance ratio* (R_{av}) is found from the solutions of the particular isotopic equation, it is considered as the unique isotopic characteristic of the diisotopomer pools.

3. Conventional isotopic measurements and their hidden errors

As pointed out above, the isotope abundance ratios of PIE measured by *conventional method* are proportional to the *average* isotope abundance ratios only in case of homogeneous ${}^{p}A$ and ${}^{q}A$ isotope distribution in the diisotopomer pool. In case of non-homogeneous isotope distribution in a diisotopomer, the isotope abundance ratios determined by the *conventional* method could be more or less than the *average* isotope abundance ratios of two PIE sites of the diisotopomers.

There are the principal factors that cause the disturbance of homogeneous isotope distribution in a polyisotopomer pool: (1) different sources of PIE in polyisotopomer molecules, (2) the kinetic isotope effects concomitant to chemical and physical processes, (3) isotope fractionation during disintegration of a polyisotopomer due to electron ionization and other impacts on the molecular structure, (4) a mixture of several homogeneous polyisotopomers.

3.1. Different sources of element A in diisotopomer

The factor, which may be the reason of a disturbed homogeneity of isotope distribution in the polyisotopomer pool, is different sources of the element A for its sites in the molecule. An example may be carbon dioxide obtained at low-temperature oxidation of CO using iodine pentoxide (I_2O_5) [8]. Schematically, the oxygen transfer in this reaction is:

$$CO + I_2O_5 \rightarrow CO_2$$

In this case, one oxygen atom in CO_2 comes from CO (R_1) and the other one—from I_2O_5—oxidant (R_2). If $R_1 \neq R_2$, the CO_2 pool will be non-homogeneous by oxygen isotopes.

Let us consider two pools of CO_2, where total contents of their atoms ${}^{16}O$ and ${}^{18}O$ are the same values each and the R_{av} is their isotopic characteristic, yet in one of the pools homogeneous isotope distribution ($R_1 = R_2 = R_{av}$) is observed, while in the other one—non-homogeneous (${}^{*}R_1 \neq {}^{*}R_2$ or ${}^{*}R_1 = R_{av} + \Delta R$ and ${}^{*}R_2 = R_a - \Delta R$). In case of homogeneous and non-homogeneous oxygen isotope distributions in both sites of the CO_2 molecules measured by the *conventional method* (i.e., the registration of the ratios of the m/z 44 and 46 peak intensities), the different isotopic characteristics (r_c and r_{av}) were obtained (Table 1). A disparity value depends on a degree of the oxygen isotope non-homogeneity of the CO_2 molecular pool. As seen from Table 1, if one

Table 1
Deviation of the *m/z* 46 and 44 peak intensity ratios for homogeneous and non-homogeneous oxygen isotope distribution in both oxygen sites of CO_2[a,b]

Oxygen isotope distribution	
Non-homogeneous	Homogeneous
1. Sources of the CO_2 oxygen atoms: $^*R_1 = [\mathrm{SMOW}]$ and $^*R_2 = [\mathrm{PDB}]$	
$^*R_1 = R_{\mathrm{av}} + \Delta R = 485.0645 + 13.6388 = 498.7033$	$R_1 = R_{\mathrm{av}} = 485.0645$
$^*R_2 = R_{\mathrm{av}} - \Delta R = 485.0645 - 13.6388 = 471.4257$	$R_2 = R_{\mathrm{av}} = 485.0645$
$(m/z\ 46) : {}^*R_1 + {}^*R_2 = 970.129$	$(m/z\ 46)$: $R_1 + R_2 = 2R_{\mathrm{av}} = 970.129$
$(m/z\ 44) : {}^*R_1 \times {}^*R_2 = 235101.5523$	$(m/z\ 44)$: $R_1 \times R_2 = (R_{\mathrm{av}})^2 = 235287.569$
$r_{\mathrm{c}} = ({}^*R_1 + {}^*R_2)/2{}^*R_1 \times {}^*R_2 = 2.0632 \times 10^{-3}$	$r_{\mathrm{av}} = R_{\mathrm{av}}/(R_{\mathrm{av}})^2 = 2.06158 \times 10^{-3}$
$\delta^{18}O_{\mathrm{c\text{-}av}} = (r_{\mathrm{c}}/r_{\mathrm{av}} - 1) \times 1000 = 0.786‰$	
2. Sources of the CO_2 oxygen atoms: $^*R_1 = [\mathrm{SMOW}]$ and $^*R_2 = [\mathrm{Atmosphere}]$	
$^*R_1 = R_{\mathrm{av}} + \Delta R = 488.8825 + 9.8208 = 498.7033$	$R_1 = R_{\mathrm{av}} = 488.8825$
$^*R_2 = R_{\mathrm{av}} - \Delta R = 488.8825 - 9.8208 = 479.0617$	$R_2 = R_{\mathrm{av}} = 488.8825$
$(m/z\ 46) : {}^*R_1 + {}^*R_2 = 977.765$	$(m/z\ 46)$: $R_1 + R_2 = 2R_{\mathrm{av}} = 977.765$
$(m/z\ 44) : {}^*R_1 \times {}^*R_2 = 238909.70$	$(m/z\ 44)$: $R_1 \times R_2 = (R_{\mathrm{av}})^2 = 239006.10$
$r_{\mathrm{c}} = ({}^*R_1 + {}^*R_2)/2{}^*R_1 \times {}^*R_2 = 2.04631 \times 10^{-3}$	$r_{\mathrm{av}} = R_{\mathrm{av}}/(R_{\mathrm{av}})^2 = 2.04548 \times 10^{-3}$
$\delta^{18}O_{\mathrm{c\text{-}av}} = (r_{\mathrm{c}}/r_{\mathrm{av}} - 1) \times 1000 = 0.406‰$	

[a] Total oxygen isotope content in the homogeneous CO_2 pool is equal to one in non-homogeneous CO_2 pool.

[b] The isotopic equations of non-homogeneous and homogeneous oxygen isotope distribution in the CO_2 pools are $R^2 - ({}^*R_1 + {}^*R_2)R + {}^*R_1 \times {}^*R_2 = 0$ and $R^2 - (2R_{\mathrm{av}})R + (R_{\mathrm{av}})^2 = 0$, respectively.

oxygen atom of CO_2 has the isotopic content equal to the PDB standard and the other one—to the SMOW standard, the deviation ($\delta^{18}O_{\mathrm{c\text{-}av}}$) between results of the *conventional* oxygen isotope measurements of the non-homogeneous CO_2 pool and the pool after its oxygen isotope homogenization is about 0.786‰. If the isotope content of one oxygen atom is identical to atmospheric oxygen and the other one—to oxygen of marine water (SMOW), the above difference is about 0.406‰. Actually, the deviations presented above restrict the measurement accuracy of oxygen isotope content in some CO_2 pools by the *conventional* method.

Thus, if the *conventional* method is used for measuring the isotope ratios, the actual value of oxygen isotope content in the CO_2 pool can be obtained only in case of homogeneous distribution of oxygen isotope atoms. At non-homogeneous distribution of oxygen isotopes in CO_2 their distribution should be homogenized in two sites or the quantity of three CO_2 forms isotopically distinct by oxygen be registered.

3.2. Kinetic isotope effects

The second factor disturbing the homogeneity of distribution of isotope atoms in the polyisotopomer pool is a difference in reaction rates for isotopically distinct molecules (the kinetic isotope effect during the diffusion efflux/influx of molecules from/to reservoir, consumption of diisotopomer molecules in chemical and biochemical processes, isotope fractionation during disintegration of a polyisotopomer due to electron ionization and other impacts on the molecular structure etc.) With incomplete utilization of the molecule pool in reactions or processes, its remaining part will show a change in abundance ratios of isotopically distinct molecules relative to their initial values.

Let us consider the change in the oxygen isotope characteristics of the carbon dioxide pool during the molecule efflux from reservoir and influx to a certain reservoir. The analysis will use a ^{12}C-cluster of CO_2 molecules isotopically different by ^{16}O and ^{18}O, i.e., $^{12}C^{16}O_2$, $^{12}C^{16}O^{18}O$, and $^{12}C^{18}O_2$. An equation reflecting the distribution of oxygen isotopic atoms in this case can be written as

$$^{48}I \times R^2 - {}^{46}I \times R + {}^{44}I = 0 \tag{20}$$

where ^{44}I, ^{46}I and ^{48}I are intensities of the *m/z* 44 ($^{12}C^{16}O_2{}^+$), 46 ($^{12}C^{16}O^{18}O_2{}^+$), and 48 ($^{12}C^{18}O_2{}^+$) peaks in the mass spectrum of CO_2 pool, respectively.

At homogeneous distribution of oxygen isotopes in the initial CO_2 pool the solutions of Eq. (20) will be $R_{1(0)} = R_{2(0)}$.

At a non-collinear and mass-dependent efflux of CO_2 from the reservoir, the quantity of molecules containing ^{16}O will decrease along with CO_2 run-off. Depending on the share of carbon dioxide efflux (*f*), the contents of $^{12}C^{16}O^{18}O$ and $^{12}C^{18}O_2$ relative to $^{12}C^{16}O_2$ (i.e., $^{44}I_{\mathrm{fe}} = 1.0$, $^{46}I_{\mathrm{fe}}$ and $^{48}I_{\mathrm{fe}}$) will be found by expressions

$$^{46}I_{\mathrm{fe}} = {}^{46}I_0(1 - f)^{1/\alpha 1 - 1} \tag{21}$$

$$^{48}I_{\mathrm{fe}} = {}^{48}I_0(1 - f)^{1/\alpha 2 - 1} \tag{22}$$

In case of CO_2 influx into the reservoir, the quantities of $^{12}C^{16}O^{18}O$ and $^{12}C^{18}O_2$ relative to $^{12}C^{16}O_2$ (i.e., $^{44}I_{\mathrm{fi}} =$

1.0, $^{46}I_{\mathrm{fi}}$ and $^{48}I_{\mathrm{fi}}$) will be calculated as

$$^{46}I_{\mathrm{fi}} = \frac{^{46}I_0(1-(1-f)^{1/\alpha 1})}{f} \tag{23}$$

$$^{48}I_{\mathrm{fi}} = \frac{^{48}I_0(1-(1-f)^{1/\alpha 2})}{f} \tag{24}$$

where $\alpha 1 = ([^{12}C^{16}O^{18}O]/[^{12}C^{16}O_2])^{1/2}$ and $\alpha 2 = ([^{12}C^{18}O_2]/[^{12}C^{16}O_2])^{1/2}$ are fractionation coefficients of isotopically different molecules of carbon dioxide.

At efflux/influx of CO_2 from/to the reservoir the parameters in Eq. (20) will have the following quantitative values, respectively:

$$^{48}I_f \times R^2 - {}^{46}I_f \times R + {}^{44}I_f = 0 \tag{25}$$

Solutions of isotopic Eq. (25) are $R_{1(f)}$ and $R_{2(f)}$. The *conventional method* of a measurement of isotope values ($R_{c(f)}$) and *average isotope abundance ratios* ($R_{av(f)}$) for two sites in the diisotopomer can be described as

$$R_{c(f)} = \frac{2^{44}I_0}{^{46}I_{(f)}} \tag{26}$$

$$R_{av(f)} = \frac{(R_{1(t)} + R_{2(f)})}{2} \tag{27}$$

A difference between the oxygen isotope abundance ratios in CO_2 obtained by the *conventional* method and their actual values can be found by use of the Expression (28):

$$\delta^{18}O_{\text{c-av}} = \left(\frac{R_{c(f)}}{R_{av(f)}} - 1\right) 1000‰ \tag{28}$$

As can be seen from Table 2, a difference between the values characterizing oxygen isotope content in the CO_2 pool obtained by the *conventional* method of measurement and its actual value increases with CO_2 efflux from the model reservoir. The above difference can vary from 0.3 to 3.4‰ at 20 and 90% of the CO_2 efflux from the reservoir, respectively.

Thus, in the case of isotopic studies by the *conventional method*, the provision of accurate measurements of the isotope abundance ratios in the diisotopomer pool participating in diffusion processes it requests that isotope distribution of analyzing element in diisotopomer should be homogenized.

3.3. Mixture of homogeneous diisotopomer pools

Two model pools of A_2M molecules with homogenous distribution of ^pA and ^qA isotopes mix together in the ratio of $x/(1-x)$, where x and $(1-x)$ are the parts of the 1st and 2nd diisotopomer pools in their mixture with R_1 and R_2 oxygen isotope abundance ratios, respectively.

Let the isotopic equation of the 1st diisotopomer pool will be represented as Eq. (29), and the 2nd diisotopomer pool—as Eq. (30). Expressing the parameters of Eqs. (29) and (30) in the same quantitative dimension, the first equation is multiplied by $(R_2)^2$ and the second one—by $(R_1)^2$ (values which show the amounts of the most abundant diisotopomer molecules in mixed pools).

$$R^2 - 2 \times R_1 \times R + (R_1)^2 = 0 \quad \text{by} \quad (R_2)^2 \tag{29}$$

$$R^2 - 2 \times R_2 \times R + (R_2)^2 = 0 \quad \text{by} \quad (R_1)^2 \tag{30}$$

Taking into account of the inputs x and $(1-x)$ of mixed pools, the isotopic equation of a mixture can be written as Eq. (31):

$$\begin{aligned}&[x(R_2)^2 + (1-x)(R_1)^2]R^2 - 2[xR_1(R_2)^2 \\ &\quad + (1-x)R_2(R_1)^2]R + (R_1)^2(R_2)^2 = 0\end{aligned} \tag{31}$$

where R_1 and R_2 are isotope characteristics for the 1st and 2nd mixed homogeneous pools of A_2M molecules.

Table 2
Alternative calculation of f-related changes in the $^{16}O/^{18}O$ ratios of carbon dioxide model reservoir by the conventional approach (R_c) using two most intensive peaks in the CO_2 mass spectra and by the average values of the oxygen isotope ratios ($R_{av} = (R_1 + R_2)/2$) of both oxygen sites in CO_2

f	$^{48}I_f$ [a]	$^{46}I_f$ [b]	$R_{1(f)}$ [c]	$R_{2(f)}$ [c]	$R_{av(f)}$ [d]	$R_{c(f)}$ [e]	$\delta^{18}O_{\text{c-av}}$ (‰)
0.0	4.4931	4239.34	471.98	471.56	471.77	471.772	0.006
0.1	4.5132	4249.17	476.45	465.00	470.73	470.680	0.056
0.2	4.5359	4260.18	477.80	461.40	469.60	469.464	0.290
0.3	4.5618	4272.71	478.10	458.50	468.30	468.087	0.457
0.4	4.5918	4287.21	478.80	454.80	466.80	466.504	0.633
0.5	4.6276	4304.42	479.40	450.70	465.05	464.639	0.885
0.6	4.6717	4325.59	479.05	446.80	462.93	462.365	1.210
0.7	4.7293	4353.03	479.00	441.40	460.20	459.450	1.630
0.8	4.8116	4392.00	478.00	434.80	456.40	455.373	2.250
0.9	4.9557	4459.43	475.30	425.10	450.20	448.488	3.410

The f values reflect the progressive efflux of CO_2 from the model reservoir. $\delta^{18}O_{\text{c-av}} = (R_{c(f)}/R_{av(f)} - 1)1000‰$.

[a] $^{48}I_f = {}^{48}I_0(1-f_f)^{1/\alpha 2-1}$.

[b] $^{46}I_f = {}^{46}I_0(1-f_f)^{1/\alpha 1-1}$, where $^{48}I_0 = 4.4931$; $^{46}I_0 = 4239.34$; and $^{44}I_0 = 10^6$; $\alpha 1 = 1.0224747$; $\alpha 2 = 1.0444659$.

[c] $R_{1(f)}$ and $R_{2(f)}$ are solutions of the next isotopic equation. $^{48}I_f \times R^2 - {}^{46}I_f \times R + {}^{44}I_f = 0$.

[d] $R_{av(f)} = (R_{1(f)} + R_{2(f)})/2$.

[e] $R_{c(f)} = 2 \times 10^6/{}^{46}I_f$.

The distribution of ^{p}A and ^{q}A isotopes of element A in two sites of the diisotopomer mixture pool formed will be determined by the new $^{*}R_1$ and $^{*}R_2$ values:

$$^{*}R_{1,2} = \frac{R_1 \times R_2[xR_2 + (1-x)R_1]}{x(R_2)^2 + (1-x)(R_1)^2} \pm \Delta^{*}R \tag{32}$$

where

$$\Delta^{*}R = \frac{i[x(1-x)]^{1/2}(R_2 - R_1)}{[x(R_2)^2 + (1-x)(R_1)^2]}$$

The *average* isotope ratios of the two sites of element A in the diisotopomer molecule mixed pool can be given as

$$^{*}R_{\mathrm{av}} = \frac{R_1 R_2[xR_2 + (1-x)R_1]}{[x(R_2)^2 + (1-x)(R_1)^2]} \tag{33}$$

If $x \neq 1$ and $R_1 \neq R_2$, then $\Delta^{*}R$ will be presented by an imaginary value or $(\Delta^{*}R)^2 < 0$. The isotopic characteristics $^{*}R_1$ and $^{*}R_2$ of the diisotopomer molecule mixed pool are quantitatively different, i.e., $^{*}R_1 \neq {}^{*}R_2$.

Two important conclusions can be made: (a) a mixture of two diisotopomer pools with homogeneous isotope distribution results in a non-homogeneous pool, (b) the isotopic non-homogeneity of the mixed pool depends on a difference between the isotopic characteristics of mixed diisotopomer pools.

When the element A isotope content in a mixture of two diisotopomers was estimated by the *conventional method* ($r_{\mathrm{c}} = I_1/I_0$), it differed from the actual abundance ratios of isotopes ^{p}A and ^{q}A. This difference for various amounts of mixed pools ($x/(1-x)$) can be determined by the expression:

$$\delta^{18}\mathrm{O}_x = \left(\frac{r_{\mathrm{c}(x)}}{r_{\mathrm{av}(x)}} - 1\right) 1000‰ \tag{34}$$

or taking into account Eq. (31) and Expression (33), we shall obtain:

$$\delta^{18}\mathrm{O}_x = \left(\frac{-[x(1-x)(R_2 - R_1)^2]}{[x(R_2)^2 + (1-x)(R_1)^2]}\right) 1000‰ \tag{35}$$

where R_1 and R_2—the isotope characteristics of mixed pools.

Depending on the values x and $(1-x)$ designated the shares of components in a mixture, the differences ($\delta^{18}\mathrm{O}_x$) in the oxygen isotope content in carbon dioxide estimated by the *conventional method* ($r_{\mathrm{c}(x)} = I_1/I_0$) as compared to the actual content ($r_{\mathrm{av}(x)}$) of isotopes ^{16}O and ^{18}O are given in Table 3. The estimates were made for two pools of CO_2 with homogeneous distribution of oxygen isotopes one of which inherits the oxygen isotope content SMOW standard ($R_1 = [^{16}\mathrm{O}]/[^{18}\mathrm{O}] = 498.7033$), and the second one—PDB standard ($R_2 = [^{16}\mathrm{O}]/[^{18}\mathrm{O}] = 471.4257$). The maximum difference between conventional isotope measurement result and actual isotope ratio value at mixing of these two CO_2 pools at the ratio 1:1 reached $\delta^{18}\mathrm{O} = 0.79‰$.

It is common knowledge that the modern oxygen isotope measurement of CO_2 provides the precision about 0.015‰ [9]. Bearing in mind, that CO_2 pool in the atmosphere can be resulted as mixtures of some oxygen isotopically different CO_2 pools, then the oxygen isotope ratios measured in the atmospheric CO_2 pool by the *conventional method* are not capable of matching the declared accuracy.

Table 3
A difference ($\delta^{18}\mathrm{O}_x$) in the oxygen isotope content in a mixture of two CO_2 pools with homogeneous distribution of ^{16}O and ^{18}O isotopes estimated by the *conventional* method ($r = I_1/I_0$) as compared to the actual isotope content

X	R_{c}	R_{av}	ΔR	$\delta^{18}\mathrm{O}_x$, ‰
0.00	471.43	471.43	0.00	0.000
0.05	472.72	472.65	5.65	0.143
0.10	474.04	473.89	7.82	0.272
0.15	475.33	475.14	9.36	0.388
0.20	476.64	476.41	10.54	0.489
0.25	477.96	477.69	11.47	0.576
0.30	479.29	478.98	12.21	0.649
0.35	480.63	480.29	12.77	0.707
0.40	481.97	481.61	13.19	0.750
0.45	483.32	482.95	13.47	0.778
0.50	484.68	484.30	13.62	0.790

$[x(R_2)^2+(1-x)(R_1)^2]R^2-2[xR_1(R_2)^2+(1-x)R_2(R_1)^2]R+(R_1)^2(R_2)^2 = 0$—the isotopic equation of the two pool CO_2 mixture, where $R_1 = 498.7033$ (SMOW) and $R_2 = 471.4257$ (PDB) are isotope characteristics for the 1st and 2nd mixed homogeneous pools of CO_2 molecules $R_{\mathrm{c}} = R_1 \times R_2/[xR_2 + (1-x)R_1]$—the result of conventional method isotope measurement $^{*}R_{\mathrm{av}} = R_1 R_2[xR_2 + (1-x)R_1]/[x(R_2)^2 + (1-x)(R_1)^2]$—the average isotope ratio of CO_2 pool mixtures $\delta^{18}\mathrm{O}_x = \{-[x(1-x)(R_2 - R_1)^2]/[x(R_2)^2 + (1-x)(R_1)^2]\}1000‰$.

4. Conclusion

The isotope content of polyisotopic element A estimated in the diisotopomer pool by using the *conventional* method reflects the actual isotope abundance ratio only at the homogeneous isotope distribution for all sites of element A in the molecule. At non-homogeneous isotope distribution for the separate sites of element A in a polyisotopomer the isotopic characteristics obtained by the *conventional method* fail to reflect the actual isotope abundance ratios and differ from the *average* isotope ratios determined for the element A sites in the molecule. The value of this difference depends on a degree of non-homogeneity in the distribution of isotope atoms in the polyisotopomer molecule analyzed. Preliminary homogenization of the isotope distribution in all element A sites should be performed or the amounts of all isotopically distinct molecules by this element registered with their further analysis in order to get the actual value of element A isotope content in the polyisotopomer pool with non-homogeneous isotope distribution.

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