



## DETERMINATION OF ISOTOPOMERS IN POOLS OF MOLECULES WITH POLYISOTOPIC ELEMENTS

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**Abstract.** Polyisotopic element atoms that are present in a molecule form a pool of isotopomer molecules. Mono- and polyisotopomers are distinguished depending on the quantity of atoms of polyisotopic elements. Methodical approaches have been considered for the quantitative determination of the isotope composition of an element included in mono- and polyisotopic molecules. A possibility of the equally probable (homogeneous) and non-equally probable (non-homogeneous) distribution of isotopes of a polyisotopic element, the atoms of which have different positions in the molecule of polyisotopomer, has been shown. Factors disturbing the homogeneous distribution of isotopes of the element in the polyisotopomer pool have been revealed. When a polyisotopomer is involved in a mass-dependent process or reaction, the homogeneity of isotope distribution of the element is disturbed both in the residual and newly formed pools of polyisotopomer. By the example of CO<sub>2</sub> (polyisotopomer by oxygen) it has been shown that one can judge of the history of the analyzed pool formation by distribution of oxygen isotopes within this pool. The isotope content in the oxygen sites of polyisotopomer is a diagnostic feature of CO<sub>2</sub> involvement in the efflux from a reservoir or influx to the analyzed reservoir from an external source.

### 1. INTRODUCTION

In the course of their study of radioactive elements, Soddy [1] and Fajans [2] first revealed compounds (isotopes) which contained atoms of an element different in weight but having the same nuclear electric charge and close chemical properties. By the example of neon, Thomson [3] demonstrated the existence of isotopes in light elements using a mass spectrograph. Also Aston [4] and Dempster [5] detected and measured isotope abundances of many other elements with the use of a mass spectrograph. Thus, it was shown that there are natural pools of molecules, which contain isotopic atoms of the corresponding polyisotopic element, i.e., *isotopomers*. Mono- and polyisotopomers are distinguished depending on the quantity of atoms of this element in the molecule structure.

*Monoisotopomers* are molecules with only one atom of polyisotopic element in their structure. *Polyisotopomers* are molecules with two and more atoms of polyisotopic element. If polyisotopomer by element A contains another element B, then this polyisotopomer may be *symmetrical* or *asymmetrical* relative to element B. In its turn, element B may be a mono- or polyisotopic element. If it is monoisotopic, then there is only one cluster of molecules isotopically different by element A (a *monocluster* pool of polyisotopomer). If element B is a polyisotopic element, then each of its isotopes will have a corresponding cluster of molecules isotopically different by element A (i.e., a *polycluster* pool of polyisotopomer). Carbon dioxide (CO<sub>2</sub>), for example, as a polyisotopomer by oxygen, will be a symmetrical isotopomer relative to carbon and consist of <sup>12</sup>C- and <sup>13</sup>C-clusters. Polyisotopomer by nitrogen N<sub>2</sub>O will be an asymmetrical isotopomer relative to oxygen and consist of three clusters (<sup>16</sup>O-, <sup>17</sup>O-, and <sup>18</sup>O-clusters). Polyisotopomer tetrafluoroethylene (F<sub>2</sub>CCF<sub>2</sub>) will be a symmetrical and monocluster polyisotopomer by carbon. It should be noted that, according to

the established practice, the isotopic composition of element A is measured by the quantitative analysis of molecules isotopically different by this element, using the cluster of the most widespread isotope of element B. The abundances of isotopic atoms for mono- and polyisotopomers are measured by different methodical approaches. The current report was aimed to analyze the known methods of measuring the abundances of stable isotopes of elements occurring in the pool of mono- and polyisotopomers.

## 2. THEORY

### 2.1. Monoisotopomers

Precision mass spectrometric measurements of the ratios of abundances of stable isotopes in monoisotopomers were initially developed in [6-10]. The analysis of isotope abundances of the polyisotopic element A occurring in a monoisotopomer consists in the quantitative determination of the ratio of isotopically different molecules with the element A in the monoisotopomer pool, i.e.,

$$R = [{}^pA]/[{}^qA] \quad (1)$$

where  $[{}^pA]$ ,  $[{}^qA]$  are quantities of molecules containing  ${}^pA$  and  ${}^qA$  isotopes of the analyzed element A ( $q > p$ ). The equation describing the isotope distribution in the molecule pool of the monoisotopomer can be written as

$$R - R_0 = 0 \quad (2)$$

where  $R$  is a variable,  $R_0$  is the ratio of abundances of isotopically distinctive molecules  ${}^pA$  and  ${}^qA$  in the pool of the tested monoisotopomer.

Modeling the efflux of the monoisotopomer from a reservoir as a first order reaction would give us the ratios of isotopic abundances ( $r_f$ ) in a residual part (1- $f$ ) of the monoisotopomer pool to the initial isotopic ratio ( $r_0$ ) as

$$r_f = r_0(1-f)^{1/\alpha-1} \quad (3)$$

where  $\alpha$  is the discrimination (fractionation) coefficient of the  ${}^qA$  isotope containing monoisotopomer relative to the  ${}^pA$  isotope containing monoisotopomer;  $f$  is the fraction of monoisotopomer which has left the reservoir; and  $r = 1/R = [{}^qA]/[{}^pA]$ .

Analogous to the monoisotopomer efflux, the ratios of the element A isotopic abundances ( $p_f$ ) in the isotopomer pool transferred from a donor reservoir can be expressed as

$$p_f = r_0(1-(1-f)^{1/\alpha})/f \quad (4)$$

If the monoisotopomer containing the element A has three or more isotopic atoms (for instance, oxygen has three isotopes:  ${}^{16}\text{O}$ ,  ${}^{17}\text{O}$  and  ${}^{18}\text{O}$ ), there are three or more isotopically distinctive species of molecules. In this case, the abundances of these molecules make up a set of the isotope abundance ratios of the element A. Using one isotopic molecule species as a reference, the quantities of other two or more isotopically distinct molecules can be compared with the above quantity of molecules. Mass dependent or mass independent fractionation may occur only in the molecule pool of the monoisotopomer bearing the element with more than two isotopes.

## 2.2. Polyisotopomers

The isotope abundances of polyisotopic element in the pool of polyisotopomer were first measured by the analysis of the corresponding monoisotopomers obtained from polyisotopomers. For example, the  $^{13}\text{C}/^{12}\text{C}$  ratios in organic substances (polyisotopomers by the carbon element) were determined using  $\text{CO}_2$  (monoisotopomer by carbon), which was obtained by quantitative oxidation of these substances. It is obvious that the differences in the carbon isotope content of different carbon positions in the polyisotopomer molecule are leveled by this operation. Isotopic variations of the element constituent of polyisotopomer were revealed by specific chemical reactions isolating structures with the analyzed element. For example, in case of amino acids and organic acids it was possible to isolate and measure the carbon isotopic composition of the carboxyl group and the rest part of the molecule separately, using the reactions of decarboxylation [11]. It was shown that amino acids produced by photoautotrophs were characterized by the lower content of isotope  $^{13}\text{C}$  in the alkyl portion of the amino acids as compared with their carboxyl group. On the other hand, amino acids synthesized by heterotrophic organisms have in their carboxyl groups the carbon atoms with the lower content of  $^{13}\text{C}$  isotope relative to the rest part of the amino acids.

Thus, the isotope distribution of the polyisotopic element  $A$  in the polyisotopomer pool may be intramolecularly heterogeneous, i.e., the ratio of abundances of isotopic atoms of the element  $A$  may be distinguished in different sites in the molecule.

Possible finding of isotopic heterogeneity (non-homogeneity) in a polyisotopomer by the quantitative mass spectrometric analysis of the ratios of its isotopically distinctive molecule ions was first proposed in 1974 [12]. Later, this approach was further developed by the examples of analysis of the oxygen isotope composition of  $\text{CO}_2$  as an oxygen diisotopomer [13, 14]. The idea of calculation of isotope distribution of the element  $A$  in the polyisotopomer pool using a set of isotopically distinctive molecules will be considered in details in the next part.

For simplicity of reasoning, let us consider the distribution of isotopes  $^pA$  and  $^qA$  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) and their isotopic composition is represented by value  $R = a/b$ . If the element  $A$  has the same isotope abundances by positions 1 and 2 in the molecule (i.e.,  $R_1 = R_2 = R_0 = a/b$ ), then the equation characterizing the distribution of isotopes in the pool of this isotopomer can be formulated as

$$(R - a/b)^2 = 0, \quad (5)$$

where  $a$  and  $b$  are abundances of isotopes  $^pA$  and  $^qA$  of the analyzed element  $A$ .

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

$$b^2R^2 - 2abR + a^2 = 0 \quad (6)$$

Since  $a + b = 1$ , the values  $a^2$  and  $b^2$  reflect the probabilities of finding one of the isotopes  $^pA$  or  $^qA$  by two positions of the element  $A$  in the molecule, and  $2ab$  is the probability of their simultaneous finding by these positions. Previously Zyakun and Schidlowski [13] discussed that the values  $b^2$ ,  $2ab$ , and  $a^2$  have to be proportional to peak intensities in the mass spectrum of diisotopomer:  $I_2 = k b^2$  is the peak intensity determined by the quantity of molecules having in two sites only isotope  $^qA$ ;  $I_1 = k 2ab$  is the peak intensity corresponding to the quantity of molecules having  $^pA$  and  $^qA$  isotopes simultaneously by two positions;  $I_0 = k a^2$  is the peak

intensity corresponding to the quantity of molecules having only isotope  $^pA$  by two positions in the molecule of diisotopomer ( $k$  is the coefficient of proportionality). Normalizing the intensities of the peaks  $I_2, I_1, I_0$  by  $I_2$ , we obtain a set of values in Eq. (6):  $1, I_1/I_2$  and  $I_0/I_2$ , or  $1, 2a/b$ , and  $a^2/b^2$ . It is quite obvious that the solution of the equation (6) 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  $^pA$  and  $^qA$  of the element  $A$  in diisotopomer.

In case of non-homogeneous isotope distribution in two-site molecules with a different probability of finding isotopes  $^pA$  and  $^qA$  by each of the two positions in the pool of diisotopomer (i.e.,  $R_1 \neq R_2$ ), Eq. (6) can be presented as

$$(R - R_1)(R - R_2) = 0 \quad (7)$$

or

$$R_2 - (R_1 + R_2) R + R_1 R_2 = 0 \quad (8)$$

The values  $1, (R_1 + R_2)$  and  $R_1 R_2$  are normalized intensities of the peaks of molecular ions in the mass spectrum of diisotopomer with different ratios of isotopic atoms  $^pA$  and  $^qA$  of the element  $A$  in each of its positions in diisotopomer. Therefrom it follows that the quantity of isotopically distinct molecules in the isotopomer pool is determined by the ratios of abundances of isotopes  $^pA$  and  $^qA$  of the element  $A$  by its individual sites in the molecule. One should remember that the data obtained from solution of the corresponding mathematical Eqs. (5) and (7) may evidence only the existence of sites of the element  $A$  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.

The proposed approach to the assessment of the isotope composition of the element  $A$  in polyisotopomers allows their classification by distribution of isotopes of the element  $A$  within the molecules. If the probability of finding an isotope atom of the element  $A$  by all its sites in the molecule is the same (i.e.,  $R_1 = R_2 = R_3 = \dots = R_n = R_0$ ), then such a distribution of isotopes of the element  $A$  in the polyisotopomer pool is called *homogeneous*. The equation presenting the homogeneous distribution of the element  $A$  isotopes will be as

$$(R - R_0)^n = 0 \quad (9)$$

where  $R = R_0$  is the solution of this equation.

*Non-homogeneous* distribution of isotopes of the element  $A$  in the pool of polyisotopomer is distribution of isotope atoms that can be found by each of  $n$ -sites of the element  $A$  with different probabilities (i.e.,  $R_1 \neq R_2 \neq R_3 \neq \dots \neq R_n$ ). The equation reflecting the non-homogeneous distribution of isotopes of the element  $A$  in the polyisotopomer pool will be written as

$$(R - R_1) (R - R_2) \dots (R - R_n) = 0 \quad (10)$$

where  $R_1, R_2, \dots, R_n$  are the solutions of Eq.(10).

It should be noted that the values  $R_i$  ( $i = 1, 2, \dots, n$ ) may be both real and complex numbers. If polyisotopic element  $A$  contains a mixture of isotope atoms, one of which is predominant in abundance while the rest are in minor quantities, then the isotope composition of the element  $A$  in the polyisotopomer pool can be determined by the following three known methods:

1. The *conventional practice* determination of the isotope atom abundance ratios of the element  $A$  represented as a measurement of quantities of two isotopically distinct molecule species, one of which contains atoms of only predominant isotope and the other has one atom of the minor isotope along with the major isotope of the element  $A$ .
2. The *atomic isotope abundance ratios* of the element  $A$  in the pool of the analyzed polyisotopomer calculated as a ratio of the quantity of sums of molecules bearing the light ( $^pA$ ) and heavy ( $^qA$ ) isotopes, respectively.
3. The *average value* of isotope abundance ratios calculated as an average of values  $R_i$  ( $i=1,2, \dots n$ ) for all sites of the element  $A$  in the polyisotopomer.

Let us compare the data on the isotope composition of the element  $A$  in the polyisotopomer pool obtained by the above approaches by the example of a diisotopomer using the Eq. (8). In case of *homogeneous* distribution of isotopes of the element  $A$  in diisotopomer, its isotope composition is characterized by values  $R_1=R_2=R_{av}$ , where  $R_1$  and  $R_2$  are isotope abundance ratios of the element  $A$  for site 1 and site 2 in the diisotopomer, respectively;  $R_{av}$  is an average of values  $R_1$  and  $R_2$ . The equation describing the isotope distribution of the homogeneous two-site molecule pool can be written as

$$R^2 - 2R_{av}R + (R_{av})^2 = 0 \quad (11)$$

Peak intensities  $I_2$ ,  $I_1$ , and  $I_0$  in the mass spectrum of the diisotopomer are proportional to the values 1,  $2R_{av}$ , and  $(R_{av})^2$  (see Eq. 11). The *conventional* practice of the  $^pA$  and  $^qA$  isotopic analysis of the element  $A$  in diisotopomer relies on the two most intensive peaks of the diisotopomer mass spectrum as:

$$R_c = I_0/I_1 \quad (12)$$

or

$$R_c = (R_{av})^2 / (2R_{av}) = R_{av}/2 \quad (13)$$

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

$$R_a = (2I_0 + I_1) / (I_1 + 2I_2) \quad (14)$$

or

$$R_a = (2(R_{av})^2 + 2R_{av}) / (2R_{av} + 2) = R_{av} \quad (15)$$

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

In case of *non-homogeneous* isotope distribution of the element  $A$ , the two-site diisotopomer pool has two values of isotopic ratios  $R_1 \neq R_2$  for the element  $A$  in site 1 and site 2, respectively. The equation describing the isotope distribution of the non-homogeneous two-sited diisotopomer pool would be presented as

$$(R - R_1)(R - R_2) = 0 \quad (16)$$

or

$$R^2 - (R_1 + R_2)R + R_1R_2 = 0 \quad (17)$$

where  $R_1$  and  $R_2$  are the solutions of Eq. (17).

Peak intensities  $I_2$ ,  $I_1$ , and  $I_0$  in the mass spectrum of the diisotopomer are proportional to the values  $1$ ,  $(R_1 + R_2)$  and  $R_1R_2$  (see Eq. 17), respectively. If  $R_{av} = (R_1 + R_2)/2$ , then  $R_1 = R_{av} + \Delta R$  and  $R_2 = R_{av} - \Delta R$ . Eq. (17) may be rearranged to

$$R^2 - 2R_{av}R + (R_{av})^2 - (\Delta R)^2 = 0 \quad (18)$$

Determination of the  $^pA^qA$  isotopic ratios for diisotopomer pool with non-homogeneous isotope distribution by *conventional* practice would be calculated as

$$R_c = [(R_{av})^2 - (\Delta R)^2] / 2R_{av} = [R_{av} - (\Delta R)^2 / R_{av}] / 2 \quad (19)$$

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

$$R_a = [2((R_{av})^2 - (\Delta R)^2) + 2R_{av}] / (2R_{av} + 2) = R_{av} - (\Delta R)^2 / (R_{av} + 1) \quad (20)$$

The value  $\Delta R$  reflects deviations of the abundances of isotope atoms of the element  $A$  by its individual sites in the diisotopomer molecule from  $R_{av}$ . From Exps. (19) and (20) it follows that if the value  $\Delta R$  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  $\Delta R$  will be presented by imaginary numbers then the  $R_c$  and  $R_a$  values will be higher than  $R_{av}$  (i.e.,  $2R_c > R_a > R_{av}$ ).

### 3. FACTORS CAUSING THE DISTURBANCE OF HOMOGENEOUS DISTRIBUTION OF THE ELEMENT ISOTOPES IN DIISOTOPOMER POOL

#### 3.1 Different sources of element A in diisotopomer

One of the factors, which may be the reason of disturbed homogeneity of isotope distribution in the polyisotopomer pool, is different sources of the element  $A$  for its different sites in the molecule. An example may be carbon dioxide obtained at low-temperature oxidation of  $CO$  using  $I_2O_5$  in the reaction [15]:



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  $R_1 \neq R_2$ , then the  $CO_2$  pool will be non-homogeneous by oxygen isotopes. It is essential to note that  $R_1$  and  $R_2$  have to be real numbers and  $R_a < 2R_c < R_{av}$ .

#### 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, etc.) With

incomplete utilization of the molecule pool in reactions or processes, its remaining part will show a change in relations between isotopically distinct molecules relative to their initial quantity. This will result in a change of the values in Eq. 18 relative to their initial values.

Let us consider the changes in the isotope abundance ratios of the element  $A$  in the diisotopomer pool. In this case, the kinetics of the element  $A$  isotope fractionation should deal with two sets of the  ${}^pA$  and  ${}^qA$  isotopically different molecules, i.e. a)  ${}^pA_2M$  and  ${}^pA^qAM$ ; b)  ${}^pA_2M$  and  ${}^qA_2M$ . A mathematical expression describing the fractionation of the element  $A$  isotopes during the efflux, which involves the molecules  $A_2M$  isotopically distinct by the element  $A$  (variants  $a$ , and  $b$ ), may be presented as:

$$r_{f(1,2)} = r_{0(1,2)}(1-F)^{(1-\alpha_1)\alpha_1,2} \quad (21)$$

where index 1 or 2 shows the presence of one or two  ${}^qA$  atoms, respectively;  $\alpha_1$  and  $\alpha_2$  are the fractionation coefficients of  ${}^pA^qAM$  and  ${}^qA_2M$  molecules relative to  ${}^pA_2M$ .

A change in the quantities of  ${}^pA^qAM$  and  ${}^qA_2M$  isotopically different molecules relative to  ${}^pA_2M$ , depending on the share ( $F$ ) of  $A_2M$  consumed, may be expressed in  $\delta$  units as

$$\delta({}^pA^qAM) = [(1-F)^{(1-\alpha_1)\alpha_1} - 1] \cdot 1000 \text{ ‰} \quad (22)$$

and

$$\delta({}^qA_2M) = [(1-F)^{(1-\alpha_2)\alpha_2} - 1] \cdot 1000 \text{ ‰} \quad (23)$$

In case of mass-dependent fractionation of the molecules of  $A_2M$ , the isotopically different by the element  $A$ , the coefficients  $\alpha_1$  and  $\alpha_2$  are known to be proportional to the square root of a ratio of masses of the respective molecules, i.e.

$$\alpha_1 \sim ([{}^pA^qAM]/[{}^pA_2M])^{1,2} \quad (24)$$

and

$$\alpha_2 \sim ([{}^qA_2M]/[{}^pA_2M])^{1,2} \quad (25)$$

By dividing Exp. (22) by (23), we find that the mass-dependent fractionation of  $A_2M$  isotopically different by the element  $A$  can be expressed as:

$$k = \delta({}^pA^qAM)/\delta({}^qA_2M) \approx \alpha_2(1-\alpha_1)/\alpha_1(1-\alpha_2) \quad (26)$$

Let a certain reservoir contains a pool of diisotopomer with homogeneous distribution of isotopes of element  $A$  ( $R_0$ ). In the course of a certain reaction, diisotopomer molecules bearing predominantly an isotopically light element  $A$  are released from the reservoir. As a result, the share of isotopically heavy molecules will be increasing in the portion of diisotopomer remaining in the reservoir as diisotopomer will efflux, while the other reservoir where some part of molecules enters will contain, respectively, isotopically light molecules of diisotopomer as compared with the initial reservoir. In both cases (efflux and influx), the isotopic composition of the element  $A$  in diisotopomer molecules will be characterized by the equation (18).

If homogeneity in distribution of the isotopes of element A is not disturbed in the remaining pool of diisotopomer under its efflux, then  $\Delta R = 0$  and  $R_{av} > R_0$ . We will name such fractionation of the isotopes of the element A as a *collinear* (proportional) fractionation. If each of the isotopic forms of diisotopomer under efflux and influx is characterized by different constants of reaction rates, then the value  $\Delta R \neq 0$  (*non-collinear* fractionation of isotopes). It can be easily seen that in case of efflux the free member in the equation (18) will be less than that observed under homogeneous distribution of the isotopes of the element A in the diisotopomer pool. This implies that  $(R_{av})^2 - (\Delta R)^2 < (R_{av})^2$ , or  $(\Delta R)^2 > 0$ . In case of influx of isotopically different molecules into the reservoir, the quantity of isotopically light molecules of diisotopomer will be higher than under homogeneous distribution of the element A isotopes in this reservoir, i.e.,  $(R_{av})^2 - (\Delta R)^2 > (R_{av})^2$ , or  $(\Delta R)^2 < 0$ . From the latter inequality it follows that the value  $\Delta R$  will be an imaginary number, while  $R_1$  and  $R_2$  are represented by complex numbers. It is obvious that at non-collinear fractionation of isotopes the value  $\Delta R$  may depend both on the weight of isotopically different molecules of diisotopomer (mass-dependent fractionation) and on other factors (e.g., the secondary isotopic effects determined by the influence of neighboring isotopic atoms during the dissociation or formation of bonds in a molecule). Thus, the above analysis has offered the main parameters determining the isotopic composition of polyisotopomer. Table I shows isotopic characteristics of polyisotopic element A, which follow from the analysis of the polyisotopomer pool.

Table I. Isotopic characteristics of the diisotopomer pool bearing polyisotopic element A

Isotopic characteristics	Remarks
1. <i>Symmetrical</i> distribution of A 2. <i>Asymmetrical</i> distribution of A	In addition to element A, molecule contains other elements
3. <i>Monocluster</i> isotope pool of A 4. <i>Polycluster</i> isotope pool of A	Molecule has monoisotopic B Molecule has polyisotopic B
5. <i>Homogenous</i> distribution of isotopes 6. <i>Non-homogenous</i> distribution of isotopes	$R_1 = R_2 = R_{av}; \Delta R = 0$ $R_1 = R_{av} + \Delta R; R_2 = R_{av} - \Delta R; \Delta R \neq 0$
7. Fractionation of isotopes: <i>Efflux</i> of isotopically different molecules <i>Influx</i> of isotopically different molecules	$R_1 \neq R_2; (\Delta R)^2 > 0$ $R_1 \neq R_2; (\Delta R)^2 < 0$
8. <i>Collinear</i> efflux/influx 9. <i>Non-collinear</i> efflux/influx	$R_1 = R_2; R_1 \neq R_2; \Delta R = 0$ $R_1 \neq R_2; \Delta R \neq 0$
10. <i>Mass-dependent</i> fractionation 11. <i>Mass-independent</i> fractionation	$k \approx \alpha_2(1 - \alpha_1) / \alpha_1(1 - \alpha_2)$ $k \neq \alpha_2(1 - \alpha_1) / \alpha_1(1 - \alpha_2)$

#### 4. APPLICATIONS

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  $^{12}\text{C}$ -cluster of  $\text{CO}_2$  molecules isotopically different by  $^{16}\text{O}$  and  $^{18}\text{O}$ , i.e.,  $\text{C}^{16}\text{O}_2$ ,  $\text{C}^{16}\text{O}^{18}\text{O}$ ,



and  $C^{18}O_2$ . An equation reflecting the distribution of oxygen atoms in this case can be written as

$${}^{48}I R^2 - {}^{46}I R + {}^{44}I = 0 \quad (27)$$

where  ${}^{44}I$ ,  ${}^{46}I$  and  ${}^{48}I$  are quantitative characteristics of isotope molecules in the  $CO_2$  pool, respectively.

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  $C^{16}O^{18}O$  and  $C^{18}O_2$  relative to  $C^{16}O_2$  (i.e.  ${}^{44}I_{fe}=1.0$ ,  ${}^{46}I_{fe}$  and  ${}^{48}I_{fe}$ ) will be found by expressions

$${}^{46}I_{fe} = {}^{46}I_0(1-f)^{1-\alpha_1-\alpha_2} \quad (28)$$

$${}^{48}I_{fe} = {}^{48}I_0(1-f)^{1-\alpha_2-\alpha_2} \quad (29)$$

In case of  $CO_2$  influx into the reservoir, the quantities of  $C^{16}O^{18}O$  and  $C^{18}O_2$  relative to  $C^{16}O_2$  (i.e.  ${}^{44}I_{fi}=1.0$ ,  ${}^{46}I_{fi}$  and  ${}^{48}I_{fi}$ ) will be calculated as

$${}^{46}I_{fi} = {}^{46}I_0(1-(1-f)^{1-\alpha_1})/f \quad (30)$$

$${}^{48}I_{fi} = {}^{48}I_0(1-(1-f)^{1-\alpha_2})/f \quad (31)$$

where  $\alpha_1 = ([C^{16}O^{18}O]/[C^{16}O_2])^{1/2}$  and  $\alpha_2 = ([C^{18}O_2]/[C^{16}O_2])^{1/2}$  are coefficients of fractionation of isotopically different molecules of carbon dioxide.

The average value of oxygen isotope composition by two sites in  $CO_2$  molecule ( $R_{av}$ ) and the value  $\Delta R$  during the  $CO_2$  efflux from the reservoir (index  $e$ ) and the  $CO_2$  influx into the other reservoir (index  $i$ ) will be determined using expressions

$$R_{av(e,i)} = ({}^{46}I_{f(e,i)} / 2 + {}^{48}I_{f(e,i)}) \quad (32)$$

$$\Delta R_{(e,i)} = [({}^{46}I_{f(e,i)})^2 - 4 {}^{48}I_{f(e,i)} {}^{44}I_{f(e,i)}]^{1/2} / 2 {}^{48}I_{f(e,i)} \quad (33)$$

From (32, 33) it follows that with  $CO_2$  efflux/influx from/into reservoir the value  $R_{av}$  will decrease, while the absolute value  $\Delta R$ , on the contrary, will increase.

To be specific, let us consider the change in the isotope composition of carbon dioxide characterized by  $\delta^{18}O = -0.73$  ‰ relative to PDB standard, under its efflux from one reservoir and influx into another. In case of homogeneous distribution of  ${}^{18}O$  in the initial pool of  $CO_2$ , the average value of isotope contents by two sites of oxygen will be  $R_{av} = 471.77$ , while the corresponding equation will be presented as

$$4.4930 R^2 - 4239.34 R + 10^6 = 0 \quad (34)$$

As mentioned above, the quantitative parameters in the equation (34) will change at the efflux of this  $CO_2$  from one reservoir and its influx into another reservoir. Values  $R_{av}$  and  $\Delta R$  characterizing distribution of oxygen isotopes in  $CO_2$  in the first and second reservoirs will also change.

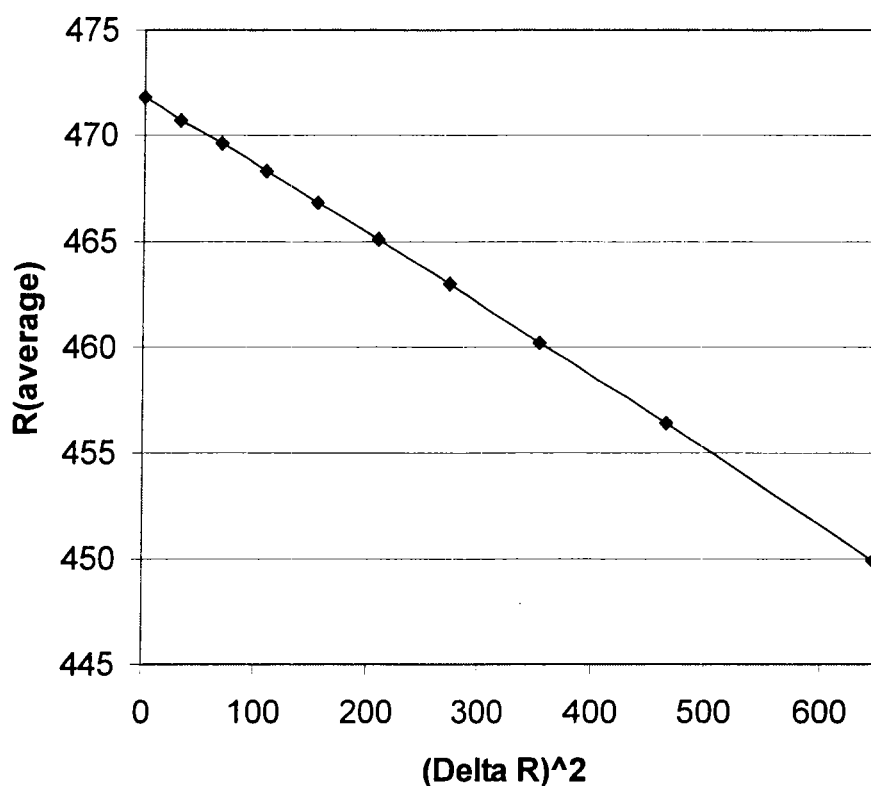


FIG. 1. Relationship between values  $R_{av}$  and  $(\Delta R)^2$  of carbon dioxide model reservoir for different values  $f$  reflecting the increasing portions of reservoir depletion from 0 to 0.9 is represented by  $R_{av} = -(0.03368 \pm 0.00014) (\Delta R)^2 + (472.0 \pm 0.08)$ . The variation coefficient for the last term of this  $R_{av}$  expression is 0.02%. The discrimination coefficients for the functions  $^{46}I_{fe}$  (Expr. 28) and  $^{48}I_{fe}$  (Expr. 29) are  $\alpha_1 = 1.022474$  and  $\alpha_2 = 1.0444659$ . Values of  $^{46}I_0$  and  $^{48}I_0$  are borrowed from Expr. 34.

Figure 1 shows a linear dependence between  $R_{av}$  and  $(\Delta R)^2$  for different values  $f$  reflecting the portion of  $\text{CO}_2$  efflux from one reservoir:

$$R_{av} = k(\Delta R)^2 + R_0 \quad (35)$$

where  $R_0$  is the characteristic of distribution of isotopes  $^{16}\text{O}$  and  $^{18}\text{O}$  in the initial reservoir;  $k$  is the coefficient of proportionality. From expression (35) it follows that determination of no less than two values of the pair of parameters  $R_{av}$  and  $(\Delta R)$  in the course of efflux and/or influx of  $\text{CO}_2$  will allow the calculation of value  $R_0$  characterizing oxygen isotope composition in the initial reservoir.

## 5. DETERMINATION OF ISOTOPE COMPOSITION OF OXYGEN IN CARBONATE

The basic postulate of determination of isotope distribution was an assumption that the distribution of  $^{18}\text{O}$  in carbonate is homogeneous. An object of the analysis was barium carbonate enriched with  $^{18}\text{O}$  isotope, whose oxygen isotope composition was strongly different from natural water and orthophosphate acid used as a reagent. Carbonate dissociation by acid results in the formation of  $\text{CO}_2$ , whose oxygen isotope composition may be non-homogeneous due to incompleteness of the reaction, its partial exchange with the water present in the acid, etc. Temperature and time of the reaction, as well as the amount of

carbonate participating in the reaction, were used as factors affecting the degree of carbonate dissociation and homogeneity of  $^{18}\text{O}$  distribution in  $\text{CO}_2$ .

Table II gives the results of analyzing the content of  $\text{CO}_2$  molecules, isotopically different by oxygen, which were obtained under acid dissociation of carbonate, and the values  $R_{av}$  and ( $i\Delta R$ ) calculated by expressions (32) and (33).

Table II. Isotope characteristics of  $\text{CO}_2$  obtained at barium carbonate dissociation by orthophosphoric acid

N	$^{44}\text{I}$	$^{46}\text{I}$	$^{48}\text{I}$	$R_{av}$	$\pm i\Delta R$	Note
1.	1.000	1.20207	0.53556	1.1223	0.7796	28°, 30 min, 30 mg
2.	1.000	1.17495	0.50846	1.1554	0.7948	25°, 30 min, 30 mg
3.	1.000	1.25270	0.47268	1.1903	0.8359	20°, 30 min, 30 mg
4.	1.000	1.06270	0.43970	1.2084	0.9022	20°, 30 min, 16 mg
5.	1.000	0.95730	0.37670	1.2706	1.0200	100°, 60 min, 30 mg

As follows from Table II, the characteristics of oxygen isotope composition in  $\text{CO}_2$  formed indicate a non-homogeneous distribution of  $^{18}\text{O}$  in  $\text{CO}_2$ , depending on the conditions of carbonate dissociation (temperature and time of reaction, amount of carbonate). At the same time, the values  $R_{1,2} = R_{av} \pm i\Delta R$  represented by complex numbers evidence the influx of  $\text{CO}_2$  to the analyzed reservoir. Using the values  $R_{av}$  and  $i\Delta R$  for  $\text{CO}_2$  samples obtained at acid dissociation of barium carbonate (Table II), an equation (36) has been defined by the method of least squares, which reflects the dependence between  $R_{av}$  and  $(i\Delta R)^2$ .

$$R_{av} = -(0.2854 \pm 0.0145)(i\Delta R)^2 + (0.9765 \pm 0.0126) \quad (36)$$

The coefficient of variations for the free member in the equation (36) was 2.3%. At  $(\Delta R)^2 = 0$  we determine the value  $R_0$  characterizing the oxygen isotope composition in the analyzed barium carbonate. In this case,  $R_0 = 0.9765 \pm 0.0126$ , which corresponded to the content of  $^{18}\text{O}$  in carbonate of  $50.6 \pm 0.3\%$ .

Thus, the above example demonstrates the possibility to determine the true ratio of oxygen isotopes in carbonate, using several  $\text{CO}_2$  samples obtained at partial dissociation of this carbonate.

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