**Appendix**

The equations used in the mixing model (S.1) and box model (S.2) are detailed here. The models with the parameters used are available on the Github site for this manuscript: https://github.com/Tripati-Lab/ Lucarelli-et-al-D48exp2022.

**S.1 Mixing model equations**

The mixing model calculates *δ*45, *δ*46, *δ*47, *δ*48, *δ*18O, *δ*13C, *Δ*47, and *Δ*48 values of CO2 gas produced by phosphoric acid digestion of carbonate relative to a working gas (WG) for two endmembers and the mix of the endmembers. The equations used here for calculation of *δ*45, *δ*46, *δ*47, *δ*48, *δ*18O, *δ*13C, *Δ*47 are based on equations from Defliese and Lohmann (2015). Here, we add the calculation of *Δ*48 to the model. We have updated the model to use the IUPAC absolute abundance ratios from Brand et al. (2014) to calculate the endmember *R*13 and *R*18 in equations S1 and S2.

*R*13 = (*δ*13C/1000 + 1) × 0.01118      (S1)

*R*18 = (*δ*18O/1000 + 1) × 0.0020052             (S2)

*R*17 = (*R*18/0.0020052)0.528 × 0.00038475

*R* values were then used to calculate the absolute abundance of each isotope in equations S3-S7.

[12C] = 1/(1 + *R*13)           (S3)

[13C] = *R*13/(1 + *R*13)       (S4)

[16O] = 1/(1 + *R*17 + *R*18)       (S5)

[17O] = *R*17/(1 + *R*17 + *R*18)       (S6)

[18O] = *R*18/(1 + *R*17 = *R*18)       (S7)

The C and O isotopic abundances were used to calculate the stochastic distribution of model sample and working gas CO2 isotopes, *R\** and *R\**WG, by equations S8-S11.

*R*45\* =( [13C] [16O]2 + 2[12C] [16O] [17O]) / [12C] [16O]2       (S8)

*R*46\* = (2[13C] [16O] [18O] + [12C] [17O]2 + 2[13C] [16O] [17O]) / [12C] [16O]2       (S9)

*R*47\* = (2[13C] [16O] [18O] + [12C] [17O]2 + 2[12C] [18O] [17O]) / [12C] [16O]2 (S10)

*R*48\* = ([12C] [18O]2 + 2[C13] [17O] [18O]) / [12C] [16O]2     (S11)

The endmember *δ*45 and *δ*46 were calculated by equations S12 and S13.

*δ*45 = (*R*45\*/*R*45\*WG - 1) × 1000     (S12)

*δ*46 = (*R*46\*/*R*46\*WG - 1) × 1000     (S13)

The *δ*47 values were calculated using equations S14 and S15.

*δ*47 = [*Δ*47 SC + 1000) × *R*47\* - 1000 × *R*47\*WG] / (*R*47\*WG - *SC* × *R*47\*)    (S14)

*Δ*47 SC = (*Δ*47 CDES90 - ETFIntercept) / ETFSlope (S15)

where *Δ*47 SC is the non-linearity slope corrected *Δ*47 value, *Δ*47 CDES90 is the *Δ*47 value projected into the absolute CDES90 (Bernasconi et al., 2021) reference frame, *SC* is non-linearity slope correction, and ETFslope and ETFintercept are the empirical transfer function slope and intercept (Dennis et al., 2011; Defliese and Lohmann, 2015).

*δ*48 and *Δ*48 were calculated similarly using equations S16 and S17.

*δ*48 = [*Δ*48 SC + 1000) × *R*48\* - 1000 × *R*48\*WG] / (*R*48\*WG - *SC* × *R*48\*) (S16)

*Δ*48 SC = (*Δ*48 CDES 90 - ETFIntercept) / ETFSlope              (S17)

*Δ*48 SC is the non-linearity corrected *Δ*48 value, *Δ*48 CDES 90 is the *Δ*48 value projected into the CDES 90 absolute reference frame, and ETFslope and ETFintercept are the slope and intercept of the empirical transfer function.

*δ*13C, *δ*18O, *δ*45, *δ*46, *δ*47, and *δ*48 were assumed to have linear mixing and are calculated by equation S18.

*δ*imix = *x1δi1* + *x2δi2* … + *xnδin*      (S18)

where *δ*i is a linearly mixed component, *x* is the fractional contribution of each endmember (*Σx* = 1), n represents the endmember composition. The stochastic distribution of isotopes in the endmember mix, *R*45\*mix, *R*46\*mix, *R*47\*mix, and *R*48\*mix, were calculated using *δ*13Cmix and *δ*18Omix in equation S19. The isotopic ratios of the mix were calculated using:

*R*imix = [(*δi*mix / 1000) + 1] × *R*i\*WG     (S19)

where *i* represents CO2 with *m/z* of 45, 46, 47, and 48. *Δ*47 CDES90 mix and *Δ*48 CDES90 mix were calculated using equations S20-S25.

*Δ*47[SGvsWG] mix = [(*R*47 mix/*R*47\*mix - 1) - (*R*46 mix/*R*46\*mix - 1) - (*R*45 mix/*R*45\*mix - 1)] × 1000 (S20)

*Δ*47 SC = *Δ*47[SGvsWG]mix - *δ*47mix × *SC*    (S21)

*Δ*47 CDES 90 mix = *Δ*47 SC x ETFslope + ETFintercept     (S22)

*Δ*48[SGvsWG] mix = [(*R*48 mix/*R*48\*mix - 1) - 2(*R*46 mix/*R*46\*mix - 1)] × 1000     (S23)

*Δ*48 SC = *Δ*48[SGvsWG]mix - *δ*48mix × *SC*             (S24)

*Δ*48 CDES 90 mix = *Δ*48 SC × ETFslope + ETFintercept                  (S25)

**S2. Box model**

This box model tracks the isotopic composition of DIC species in a homogeneous (well-stirred)\_ solution into and out of which there are two fluxes: (1) a CO2 influx, and (2) a CaCO3 outflux. The incoming CO2 has a fixed isotopic composition that is assumed to be in equilibrium with water. The CaCO3 flux depends on the degree of supersaturation as well as the Ca2+:CO32- ratio according to the ion-by-ion model (Wolthers et al., 2012). The box model used here is based on the framework of Chen et al. (2018) for *δ*18O and *δ*13C. The oxygen isotope mass balance was updated by Christensen et al. (2021). The calculation of *Δ*47 was added to the model by Uchikawa et al. (2021) in the ExClump38 model. Kinetic effects associated with growth rate are calculated with equations from Watkins and Hunt (2015) and Watkins and Devriendt (2021). The calculation of *Δ*48 was added by Watkins and Devriendt (2021).

As CO2 enters solution, it gets converted to HCO3- and CO32-. The carbon and oxygen isotope exchange reactions for (de)hydration and (de)hydroxylation of CO2 involve six isotopically distinct DIC species (note that 12C = 2, 13C = 3, 16O = 6, 18O = 8, and superscripts on charged ionic species have been dropped):

266 + H26 H2666 + H (S26)

266 + 6H H2666 (S27)

366 +H26 H3666 + H (S28)

366 + 6H H3666 (S29)

266 + H28 H2866 + H (S30)

286 + H26 H2866 + H (S31)

266 + 8H H2866 (S32)

286 + 6H H2866 (S33)

These reactions are tracked through a system of ordinary differential equations (ODEs) where and are written together as equilibrated inorganic carbon, EIC (Chen et al., 2018). For example, H2666 + 2666 = E2666. It is assumed the [266] [CO2], [H2666] ≈ [HCO3−], [6H] ≈ [OH−], and [H26] ≈ [H2O] = 1. It is also assumed that and achieve instantaneous isotopic equilibrium, as the protonation and deprotonation reactions proceed at much faster rates than hydration and hydroxylation and their reverse reactions.

(S34)

(S35)

(S36)

(S37)

(S38)

(S39)

The term is the fraction of HCO3- in EIC (Chen et al., 2018).

(S40)

(S41)

(S42)

The term is the equilibrium fractionation factor between and . K2 is the equilibrium constant for the dissociation of to and H+, which are related to the kinetic rate constants, and .

(S43)

(S44)

Other equilibrium constants are as follows:

K1  (S45)

Kw . (S46)

The kinetic fractionation factors (KFFs) for carbon and oxygen isotope fractionation are directly related to the forward rate constants, with the numerator of the left hand side of the equation being the singly heavy isotope substituted isotopologue and the denominator being the most abundant isotopologue. For the hydration reactions, the KFFs are:

(S47)

(S48)

. (S49)

For hydroxylation reactions, the KFFs are:

(S50)

(S51)

. (S52)

After combining the equilibrium constant and rearranging, forward and backward kinetic rate constants satisfy equilibrium, which results in the following relationships:

*k*+1[266][H26] = *k*-1[H2666][H] (S53)

*c*+1[366][H26] = *c*-1[H3666][H] (S54)

*a*+1[266][H28] = *a*-1[H2866][H] (S55)

*b*+1[286][H26] = *b*-1[H2866][H] (S56)

*k*+4[266][6H] = *k*-4[H2666] (S57)

*c*+4[366][6H] = *c*-4[H3666] (S58)

*a*+4[266][8H] = *a*-4[H2866] (S59)

*b*+4[286][6H] = *b*-4[H2866] . (S60)

To add carbonate clumped isotopes, *Δ*47  and *Δ*48, to the model, equations from Uchikawa et al. (2021) were used for *Δ*47 and new equations from this study were used for *Δ*48. Clumping in CO2 is based on the following reactions and equilibrium constants for the internal equilbium of 13C–18O–16O and 12C–18O–18O:

(S61)

(S62)

47  (S63)

48 . (S64)

The abundance of m/z 47 and m/z 48 CO2 isotopologues are defined by equations S65 and S66, respectively,

47 (S65)

48 (S66)

47R (S67)

48R (S68)

where R is the ratio of heavy isotope substituted isotopologue to the most abundant isotopologue, and R\* is the stochastic ratio. It is customary to present *Δ*47 and *Δ*48 in part per thousand (‰), and a factor of 1000 is multiplied by equations S65 and S66. The stochastic ratios are related to the equilibrium constants by the following equations (Watkins and Hunt, 2015; Uchikawa et al., 2021; Watkins and Devriendt, 2021):

47  (S69)

48 . (S70)

Similarly, expressions relating the equilibrium constants and isotope ratios can be written for and :

63  (S71)

64  (S72)

63 (S73)

64  (S74)

The model includes the following reactions that result in the formation 13C–18O–16O and 12C–18O–18O species in and , where reactions that form a clumped isotopologue from singly substituted reactants is “primary”, denoted *p*, and the others are secondary, denoted *s* (Guo, 2020):

366 + H28 H3866 + H (S75)

386 + H26 H3866 + H (S76)

366 + 8H H3866 (S77)

386 + 6H H3866 (S78)

286 + H28 H2886 + H (S79)

288 + H26 H2886 + H (S80)

286 + 8H H2886 (S81)

288 + 6H H2886 (S82)

From these reactions, the following ODEs are obtained (Uchikawa et al., 2021; Watkins and Devriendt, 2021)):

(S83)

(S84)

(S85)

(S86)

The terms and take the same form and and because the fraction of EIC that is has the same pH dependence for clumped, oxygen, and carbon isotopes (Hill et al., 2014).

(S87)

(S88)

(S89)

(S90)

The model assumes that the kinetic clumped isotope effects (47KIE and 48KIE) for hydration and hydroxylation are a combination of kinetic effects in carbon and oxygen isotopes, 13αKIF, 18αKIF, with an additional fractionation specific to clumped isotope species, (47αKIF and 48αKIF) (Watkins and Hunt, 2015; Guo, 2020). For the forward reactions, the relationships are as follows:

(S91)

(S92)

(S93)

(S94)

(S95)

(S96)

(S97)

. (S98)

The reverse reaction rate constants can be calculated via the following relationships (Uchikawa et al., 2021):

(S99)

(S100)

(S101)

(S102)

(S103)

(S104)

(S105)

. (S106)

To account for kinetic effects from carbonate precipitation, we use the ion-by-ion model from Watkins and Hunt (2015). The model describes crystal growth ( > ) through the attachment and detachment rates of and isotopologues to a calcite crystal, which affects the isotopic composition of residual EIC. The CO2 flux in the modelled solution reacts via hydration and hydroxylation reactions to form and , and the calcite growth rate depends on the [Ca2+] and [].

Ca2+ + CaCO3 + H+ (S107)

Ca2+ + CaCO3 (S108)

The rate constants in equations S107 and S108 are mass dependent and using notation from Wolthers et al. (2012). The effect of CaCO3 precipitation on the clumped isotope composition of residual EIC is calculated by equation S109:

(S109)

The equations solved in the ion-by-ion model are:

(S110)

(S111)

(S112)

(S113)

(S114)

(S115)

(S116)

(S117)

(S118)

In the model, we begin with [Ca2+] = 10 mM and enough DIC to bring the degree of supersaturation to unity. Hence, the amount of initial DIC depends on the specified pH. Since  = 1, the precipitation flux of CaCO3 = 0 initially but then monitonically increases until it exactly balances with the incoming constant CO2 flux. Depending on the rate of CO2 flux, the system will eventually reach of steady state that is not in equilibrium. The CO2 flux rate used was X and achieved steady state within 12 hours. The flux of CaCO3 was calculated by multiplying the growth rate by an average reactive surface area of 0.1 m2, as estimated to be representative of the experiments.

The addition of carbonic anhydrase increases the hydration rate constant according to (Uchikawa and Zeebe, 2012):

, (S119)

Where [CA] is in moles/L, *k*cat is the turnover number and *K*M is the Michaelis-Menten constant. Uchikawa and Zeebe (2012) determined *k*cat/*K*M = 2.7 x 107 M-1 s-1 for the same type of CA that we used in our experiments.

|  |  |  |
| --- | --- | --- |
| Symbol | Expression or value at 25oC | Reference |
| K1 |  | - |
| K2 |  | - |
| Kw | [6H][H] | - |
| k+1 |  | Uchikawa and Zeebe (2012) |
| k-1 | k-1 = k+1/K1 | - |
| k+4 |  | Uchikawa and Zeebe (2012) |
| k-4 | k-4 = k+4 | - |
|  |  | - |
| **Carbon isotope parameters** |  |  |
|  | -9.866*T*-1 + 1.02412 | Zhang et al. (1995) |
|  | -0.867 *T*-1 + 1.00252 | Zhang et al. (1995) |
|  | 0.9872 | Yumol et al. (2020) |
|  | 0.9814 | Christensen et al. (2021) |
| c+1 | c+1 | - |
| c-1 | c-1 | - |
| c+4 | c+4 | - |
| c-4 | c-4 | - |
|  |  | - |
| **Oxygen isotope parameters** |  |  |
|  | exp(2520 | Beck et al. (2005) |
|  | exp(2590 | Beck et al. (2005) |
|  | exp(2390 | Beck et al. (2005) |
|  | 5.6676 | Zeebe (2020) |
|  | 1.0000 | Yumol et al. (2020) |
|  | 0.9812 | Yumol et al. (2020) |
|  | 0.9988 | Christensen et al. (2021) |
|  | 1.0000 | Christensen et al. (2021) |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
| ***Δ*47 parameters** |  |  |
|  |  | Hill et al. (2020) |
|  |  | Hill et al. (2020); Tripati et al. (2015) |
|  |  | Hill et al. (2020); Tripati et al. (2015) |
|  |  | - |
|  |  | - |
|  |  | - |
|  | 1-0.146/1000 | Guo (2020) |
|  | 1-0.219/1000 | Guo (2020) |
|  | 1-0.016/1000 | Guo (2020) |
|  | 1-0.175/1000 | Guo (2020) |
|  |  | Uchikawa et al. (2021) |
|  |  | Uchikawa et al. (2021) |
|  |  | - |
|  |  | - |
|  |  | Uchikawa et al. (2021) |
|  |  | Uchikawa et al. (2021) |
|  |  | - |
|  |  | - |
| ***Δ*48 parameters** |  |  |
|  |  | Hill et al. (2020) |
|  |  | Hill et al. (2020) |
|  |  | Hill et al. (2020) |
|  |  | - |
|  |  | - |
|  |  | - |
|  | 1+0.049/1000 | Guo (2020) |
|  | 1-0.146/1000 | Guo (2020) |
|  | 1-0.144/1000 | Guo (2020) |
|  | 1-0.086/1000 | Guo (2020) |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |
|  |  | - |



**Figure S.1.** Example showing the time evolution to steady-state for bulk and clumped isotopes.The time to reach steady state depends on *F*CO2, with lower *F*CO2 taking longer to reach steady state.