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groningen**

**faculty of science
and engineering**

**Investigating the efficacy of using
carbon dioxide-water equilibration
to make $^{17}\text{O}\text{-CO}_2$ reference gases**

Isidro Fernandez Garcia



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**Investigating the efficacy of using carbon dioxide-water
 equilibration to make ^{17}O -CO₂ reference gases**

Bachelor's Thesis

To fulfill the requirements for the degree of
 Bachelor of Science in Physics
 at University of Groningen under the supervision of
 Prof. dr. H.A.J. Meijer (Centre for Isotope Research, University of Groningen)
 and
 Prof. dr. U. Dusek (Centre for Isotope Research, University of Groningen)

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Abstract

The $\delta^{17}\text{O}$ to $\delta^{18}\text{O}$ relation in atmospheric CO_2 is theorised to be a good indicator for gross primary production, suggesting it can be used to detect sources and sinks of atmospheric carbon. The problem is that the ^{17}O scale for CO_2 is not well defined, as there is no international consensus on the $\delta^{17}\text{O}$ values of primary reference materials. The aim of this thesis is to test whether CO_2 with water equilibration is a valid method for the creation of CO_2 samples of a given $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ value which can then be used as reference materials. In this investigation, it was shown that using a rudimentary extraction setup was already significantly consistent (difference of $<0.3\text{‰}$) in producing a sample with measured $\delta^{18}\text{O}$ values close to the expected equilibration $\delta^{18}\text{O}$ values, suggesting that the same can be said for $\delta^{17}\text{O}$.

1 Introduction

Global warming is one of the biggest problems that humanity as a whole will face in the coming years. To combat this, we need a better understanding of how the environment works and which factors are influenced by human behaviour. It is known that carbon dioxide (CO₂) emissions are one of the biggest anthropogenic causes of global warming, due to its high radiative forcing [1]. For this reason, it is vital to understand the potential sources and sinks for CO₂ in the carbon cycle. The feedback from climate change to the carbon cycle has the potential to change the behaviour and impact of these sources and sinks, meaning that a thorough understanding and quantification of them is fundamental for fighting climate change. The biosphere plays a major role in the capturing of CO₂, so having an insight into this system will help us predict the consequences of CO₂ emissions for our climate and could be a breakthrough in reducing the effects of global warming. Stable isotope measurements of atmospheric CO₂ are important tools for the identification and quantification of sources and sinks because samples can be taken almost anywhere and analysed, allowing for quick analyses of CO₂ sources and sinks.

1.1 Stable Isotopes

Stable isotopes are non-radioactive atoms with the same amount of protons but differing amounts of neutrons (¹⁶O, ¹⁷O and ¹⁸O are stable isotopes of oxygen). These isotopes are present in the molecules that they form (isotopologues), meaning that there can be various isotopologues of CO₂ within a given CO₂ sample. For example, the most common isotopologue of CO₂ is made up of the light isotope containing atoms (¹²C¹⁶O¹⁶O, making up 98.42% of atmospheric CO₂ [2]), but there are various other ones (¹²C¹⁷O¹⁶O, 734ppm and ¹²C¹⁸O¹⁶O, 0.39% for instance [2]) that are far less abundant. The relation of the measured amounts of these isotopologues relative to the most abundant CO₂ is given by a ratio, which is usually scaled relative to a reference and expressed as a delta value:

$$\delta^H A = \frac{(H_{AS}/L_{AS})}{(H_{AR}/L_{AR})} - 1 \quad (1.1)$$

where H is the atomic number of the heavy isotope, L is the atomic number of the light isotope, A is the element being considered¹ and S and R stand for sample and reference respectively. Since the ratio between the sample and the reference tends to be quite close to 1, the values of $\delta^H A$ are expressed in per mill (‰). Using a scale relative to a reference material allows for better precision than doing absolute measurements. The particular choice of scale is explained in more detail in Section 2.1. A key component in the global carbon cycle is the Gross Primary Production (GPP) which denotes the total amount of carbon (in the form of CO₂) taken in by vegetation during photosynthesis [3]. Since certain biological processes favour lighter isotopologues, heavier isotopologues are more likely to remain in a given air sample (because the heavier ones have both higher binding energies and move slower than the lighter isotopologues) that has interacted with the biosphere. This process is described as isotopic fractionation, in which the original mixture of isotopologues is changed by a physical, chemical or biological factor. Since there is data suggesting a potential link between the relation of $\delta^{17}\text{O}$ to $\delta^{18}\text{O}$ (also known as $\Delta^{17}\text{O}$, explained in Section 2.3) and the GPP [4], it is interesting to measure and keep track of these values over time.

¹This thesis will almost exclusively focus on the oxygen isotopes, so in most cases A → O and L → 16

1.2 Measurement Techniques

The current problem with $\Delta^{17}\text{O}$ measurements is that until recently the most widespread method of measuring these isotopologue concentrations is using the Isotope Ratio Mass Spectrometer (IRMS). This requires extensive preparation of samples for measurements to be taken, as it is designed for the measurement of pure gases and therefore cannot handle direct air samples. Another issue is that it cannot distinguish different isotopologues with the same nominal mass (e.g. $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ both have a mass of 45) as these would give the same readings when measured. Using laser absorption spectrometry (LAS), measurements can be taken much faster than with the IRMS due to easier sample preparation while approaching similar levels of precision. Using LAS also circumvents the problem of having the same mass isotopologues. The equipment used is the Stable Isotope of CO_2 Absorption Spectrometer (SICAS) manufactured by Aerodyne, which uses a dual laser system to measure CO_2 isotopologue ratios directly from an air sample (so the CO_2 doesn't need to be isolated). This is particularly useful for $\delta^{17}\text{O}$ measurements, as these are practically impossible to achieve by IRMS (only through very complicated preparations). Since this equipment is fairly new and there is no global consensus on the calibration for this equipment and ^{17}O in CO_2 in general, this thesis aims to create CO_2 samples of known $\delta^{17}\text{O}$ values to reach a good calibration of the $\delta^{17}\text{O}$ CO_2 scale.

1.3 Thesis Outline

In section two the concepts of fractionation, references and scales, excess ^{17}O , and previous experiments are introduced. The main problems with the scaling of ^{17}O are explained.

In section three the experimental set-up for the preparation of the sample is discussed. The various steps of the equilibration, extraction and measuring are explained alongside the functioning of the measuring equipment.

In section four, the results are shown and briefly described.

In section five, the results are discussed and the potential reasons for any discrepancies are considered. Finally, section 6 contains the conclusion.

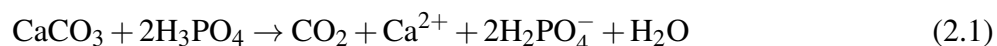
2 Background Information

2.1 References and Scales

As previously mentioned (Equation 1.1), the amount of an isotopologue in a sample is expressed by comparing the amount of the heavy isotope relative to the light isotope in a sample with a known ratio from a reference. These references have to be internationally consistent to have meaningful values that can be compared between laboratories. These reference values are based on globally recognised reference materials, which in this case mainly focus on Vienna Standard Mean Ocean Water (VSMOW). This allows for the definition of a zero point for the relative ratios of CO₂ isotopologues

The typical scale for water is the VSMOW-SLAP scale, which is based on the average heavy-to-light isotope ratio in ocean water (Standard Mean Ocean Water, SMOW) and the average heavy-to-light isotope ratio in antarctic precipitation (Standard Light Antarctic Precipitation, SLAP). A representative sample was agreed upon at an International Atomic Energy Agency (IAEA) consultant's meeting in Vienna that had an isotope composition very close to that of SMOW, resulting in the creation of VSMOW [5]. This reference, by definition, has a $\delta^{18}\text{O}$ of 0‰, and, although there is officially no international consensus about the $\delta^{17}\text{O}$ value, the unofficial consensus value is also 0‰. The second reference sample (SLAP) has a consensus value of $\delta^{18}\text{O}$ of -55.5‰ with VSMOW as a reference [5]. Although not formally established, $\delta^{17}\text{O}$ is often defined to be -29.699‰ , assuming the same $\delta^{17}\text{O}$ to $\delta^{18}\text{O}$ relation as VSMOW. This scale is linked to CO₂ via water equilibration, meaning that any CO₂ sample in equilibrium with any water will be fractionated by the same factor of $\alpha=1.0412$ [5][6].

For carbon dioxide, the most common scale is the Vienna-Pee Dee Belemnite (VPDB) scale. It is a theoretical sample agreed upon by the IAEA in Vienna, based on a calcite named Pee Dee Belemnite from South Carolina [7]. It is currently anchored by IAEA-603 with values of $\delta^{18}\text{O}$ of $-2.37 \pm 0.04\text{‰}$ and $\delta^{13}\text{C}$ of $2.46 \pm 0.01\text{‰}$ on the VPDB scale [8]. This scale is linked to CO₂ via a reaction with phosphoric acid shown in Equation 2.1.



This reaction has some issues. It is a temperature-dependent reaction, meaning that the isotope distribution over the reaction products changes with the temperature. Keeping a constant temperature throughout the reaction is important as this allows one to calculate the isotope distribution among the products. However, this reaction is exothermic and maintaining the temperature constant is not easy. The calcite (CaCO₃) has three oxygen components being distributed at a ratio of 2:1 into CO₂ and H₂O respectively, meaning it is difficult to know what fraction of which oxygen isotope is present in the CO₂ (due to it being temperature-dependent). This means that there is a doubt in regards to the actual δ values of the CO₂ representing the calcite. This inconsistency is a large component of the motivation for the research into a better definition of the $\delta^{17}\text{O}$ (and $\delta^{18}\text{O}$) scale.

This scale is linked to the VSMOW scale via Equation A.10.

2.2 Fractionation

As previously mentioned, isotopic fractionation is the change of an isotopologue composition due to a sample undergoing a certain physical, biological or chemical process. The two fundamental types are kinetic fractionation, typically due to an irreversible (physical or chemical) reaction, and equilibrium fractionation, which occurs during thermodynamic equilibrium. This thesis focuses on both of these effects in differing conditions. A large part of the fractionation occurring in the atmosphere is due

to kinetic effects, such as CO₂ becoming biological carbon in plants via photosynthesis, whereas the experiment focuses on equilibrium fractionation to establish a CO₂ sample with a well-known δ¹⁷O. The investigation of isotopologue concentrations in relation to CO₂ sources and sinks takes advantage of the fact that heavier isotopes usually have higher binding energies (see Figure 1) and the fact that they have a higher mass. This means they travel slower than lighter particles under the same conditions (see Equation 2.2), reducing the chance of collision with other molecules and leading to an overall slower rate of reaction.

$$k_B T = \frac{mv^2}{2} \implies v = \sqrt{\frac{2k_B T}{m}} \quad (2.2)$$

Where k_B is the Boltzmann constant, m is the mass of the molecule, v is the velocity of the molecule and T is the temperature.

Using this information, one can keep track of the rate at which CO₂ is being absorbed and emitted

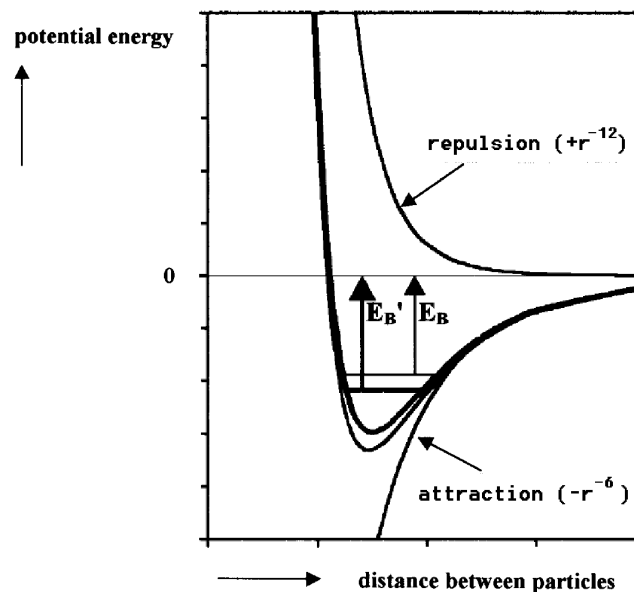


Figure 1: Graph depicting a potential well containing the binding energies of light and heavy isotopologues, written as E_B and E'_B respectively. These are expressed at the same temperature, so if it were to increase, E'_B would remain greater than E_B (although the difference between them would be smaller). The thin curves labeled attraction and repulsion represent the forces acting on the molecules and the thicker curve is the net effect of these. This figure is adapted from the figures in Chapter 3 of *Environmental isotopes in the hydrological cycle: Principles and applications* [9].

into the atmosphere as an enriched sample (the ratio of heavy isotopes relative to light isotopes is higher than the typical atmospheric ratio) could imply carbon uptake by plants and a depleted sample (ratio of heavy isotopes relative to light isotopes is lower than the typical atmospheric ratio) could imply anthropogenic CO₂ emissions (e.g. Fossil fuel burning [10]). This also relates to the changes in the excess ¹⁷O mentioned in Section 2.3.

2.3 Excess ^{17}O

Excess ^{17}O ($\Delta^{17}\text{O}$) exists as a measure of the discrepancy between the mass fractionation between ^{17}O and ^{18}O [4], meaning that a deviation from it must be due to an external factor. It is given as:

$$\Delta^{17}\text{O} = \ln(\delta^{17}\text{O} + 1) - \lambda \times \ln(\delta^{18}\text{O} + 1) \quad (2.3)$$

where $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ are the isotopologue concentrations of ^{17}O and ^{18}O respectively and λ is the reference line. Stratospheric CO_2 carries a strongly positive $\Delta^{17}\text{O}$ and thus influences this balance, which is counteracted in the troposphere due to carbon exchange between CO_2 and water, primarily in vegetation. Although the $\delta^{18}\text{O}$ content is known to be altered when exposed to the biosphere, this by itself is not a good enough measurement due to the fact that the $\delta^{18}\text{O}$ values can greatly vary, meaning that a lot more of the system's conditions need to be known to draw any conclusions from the data [11]. Atmospheric CO_2 in equilibrium with water will have a $\Delta^{17}\text{O}$ close to zero, due to the fact that the pool of oxygen molecules in the water is much higher than the oxygen molecules in the CO_2 gas. Knowing that a sample is in equilibrium allows one to calculate the desired isotope ratio, as long as the other one is known. Investigating $\Delta^{17}\text{O}$ is relevant due to the fact that it has been suggested as an indicator of GPP and anthropogenic emissions such as fossil fuel burning. Biosphere activity has been linked to bringing the excess ^{17}O closer to zero, while strong depletions have been associated with combustion emissions. This can therefore be used to detect potential carbon dioxide sources and sinks.

2.4 Similar Experiments

The method used in this thesis for the CO_2 sample preparation (see Section 3.2) is not new, as it has been a known method since it was implemented by Epstein and Mayeda in 1953 [12]. Their paper uses a CO_2 - H_2O mole ratio of about 96, whereas this thesis uses one of approximately 50 (see Section 4.1). In a perfect experiment, one would want a negligible amount of CO_2 in comparison to the water, as this allows for the water to dominate the oxygen isotope composition and therefore influence the CO_2 more effectively. Since the $\delta^{17}\text{O}$ of the CO_2 is not well known, it is ideal to minimise the uncertainty caused by its participation in the equilibration.

2.5 Measurement Systems

2.5.1 Mass Spectrometry

Mass spectrometry has been a common tool to measure isotopologue ratios since the 1950s. It uses the principle that different isotopes have different masses, meaning that the isotopologues made up of these will also have differing masses. By ionizing these masses, they can be affected by a magnetic field and therefore be separated from each other by accelerating them around a bend. The separation occurs due to the heavier isotopologues experiencing a larger centripetal force (see Equation 2.4) and therefore taking a different path.

$$F_C = \frac{mv^2}{r} \quad (2.4)$$

Where m is the mass of the molecule, v is the velocity at which it travels and r is the radius of the bend in its trajectory.

This process works very well for molecules with distinct masses, but it starts to run into issues when measuring isotopologues with very similar masses. As previously mentioned, the masses of $^{13}\text{C}^{16}\text{O}_2$

and $^{12}\text{C}^{16}\text{O}^{17}\text{O}$ are both very close to 45, meaning that mass spectrometry with limited mass resolution (such as used for isotope measurements) cannot be used to identify the amounts of each for a sample.

Isotope Ratio Mass Spectrometer (IRMS)

The IRMS is a mass spectrometer with a narrow mass range, allowing for more precise measurements [13]. It works by injecting the desired gas from a vial into a chamber in which they are bombarded with electrons and become ionised. The isotopologues are then accelerated until they enter the magnetic field, where they are forced to go around the bend and are separated by mass. The detectors produce an electrical signal proportional to the number of molecular ions arriving, which can be used to determine the ratio between the masses. A diagram showing the general functioning of the IRMS is given in Figure 2. The specific IRMS used is the PrecisION IRMS from Elementar Analysensysteme GmbH. It uses dual inlet IRMS to alternate measurements of the sample and of a reference gas to measure the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of a given CO_2 sample.

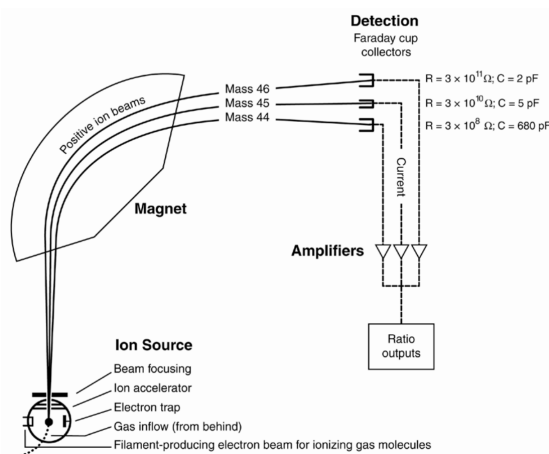


Figure 2: Diagram showing the basic functioning of the IRMS, taken from $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ determination in nitrate [14].

2.5.2 Laser Absorption Spectrometry

In the 2000s, the development of laser absorption spectrometry enabled a large development in applications in isotope measurements. Not only do they reach similar levels of accuracy as using mass spectrometry, but they also allow for direct isotopologue measurements, differentiating between isotopologues with very similar masses. These measurements can be taken in a shorter time span, cost less per measurement, and have adjustable spectra to cover a wide range of isotopologues.

The process of laser absorption spectrometry follows the principle that different isotopologues have slightly different vibration frequencies, leading to different absorption spectra. Following the simple harmonic motion equation, this is easy to explain:

$$f = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \quad (2.5)$$

Where f is the frequency of the oscillation, k is a constant dependent on the forces experienced by each atom in the molecule and m is the mass of the oscillating particle. Since k and m vary per combination of isotopes, this leads to a distinct oscillation frequency for each molecule. Assuming there

is a constant laser, this will result in an output spectrum with varying absorption lines. Since there are two LAS-based instruments used in this thesis with their own distinctions, their details are separated into the following two parts.

Triple Liquid Water Isotope Analyser (TLWIA)

The Triple Liquid Water Isotope Analyser (TLWIA) uses Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) to measure the concentration of the water isotopologues. It uses the fact that these isotopologues have frequencies relatively close (but still distinct) to each other to measure them with the same laser beam.

When the laser is shone through the gas sample being measured, there will be photons of certain frequencies that match the excitation energy of the molecules, resulting in these photons being absorbed. This means that the total amount of photons at those frequencies reaching the receptor will be lower, resulting in a lower intensity. By measuring the change in the intensity, they are capable of identifying the amount of each isotopologue [15].

$$I = I_0 \cdot e^{-\alpha \cdot L} \quad (2.6)$$

Where I_0 is the original light intensity and I is the light intensity after having passed through a gas with an attenuation coefficient α over a distance L . The value α can be found following the Lambert-Beer law:

$$\alpha \cdot L = A = L \cdot S \cdot N \implies \alpha = S \cdot N \quad (2.7)$$

Where A is the absorbance, S is the molecular absorption coefficient and N is the number density of the absorbing molecule. Therefore, the number density of the isotopologue can be expressed as:

$$N = \frac{1}{S \cdot L} \ln \left(\frac{I_0}{I} \right) \quad (2.8)$$

With this knowledge, the isotopologue ratio of a molecule can be found by comparing the number densities:

$${}^H R = {}^H A / {}^L A = {}^H N_A / {}^L N_A \quad (2.9)$$

Where H and L represent the heavy and light isotope respectively and A represents the element being measured.

TLWIA vials are filled with a few milligrams of the desired water sample, which is injected into the cavity and vapourised. This allows the laser to be shone through it and measure the isotope compositions. The specific TLWIA used is the LWIA for Liquid water isotope analyzer from LGR, ABB Group. It is used to measure the $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of the water samples.

OA-ICOS is not the only type of LAS, however. Certain Laser Absorption Spectrometers (such as the Stable Isotope of CO_2 Absorption Spectrometer) use the integration of the absorption peaks as well as the knowledge of the system's parameters to measure the number of isotopologues, the main difference lying in the optical cavities. The following explanation of the functioning is adapted from *Simultaneous measurement of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ of atmospheric CO_2 – performance assessment of a dual-laser absorption spectrometer* [16].

Stable Isotope of CO_2 Absorption Spectrometer (SICAS)

For the isotopologue measurements to be taken, the setup in Figure 3 is used. The notation for the isotopologues is the last digit of the mass number, e.g. $^{16}\text{O}^{12}\text{C}^{17}\text{O}$ is expressed by 627 and so on.

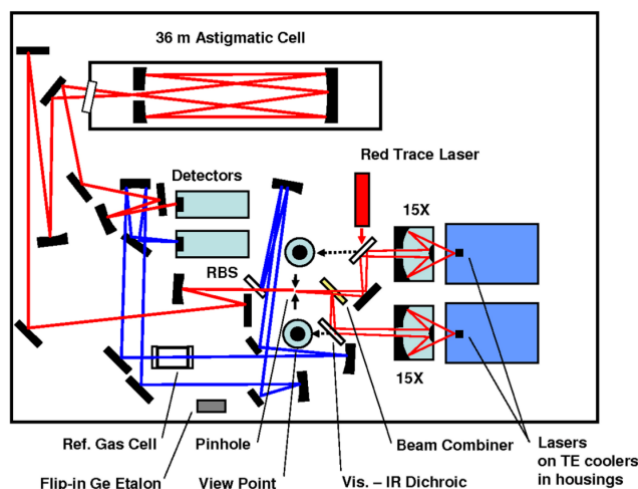


Figure 3: Diagram depicting the inside of the SICAS, taken from *Simultaneous measurement of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ of atmospheric CO_2 – performance assessment of a dual-laser absorption spectrometer* [16].

This contains two lasers; laser 1 (detects 626 and 627), operating at a wavenumber of 2350cm^{-1} at a temperature of -1.1°C and laser 2 (detects 626, 636 and 628), operating at a wavenumber of 2310cm^{-1} at a temperature of $+9.9^\circ\text{C}$. These laser beams travel into a multi-pass cell (optical length of about 36 meters) in which the sample is present at low pressure. These beams then travel to an infrared detector, which measures the signals from these lasers. To keep the measurements consistent, the housing is continuously flushed with Nitrogen, preventing CO_2 absorption outside the cell to occur, as well as kept at 20°C so as to not change the absorbance of the molecules. A software called "TDLWintel"[17] uses the integration of the peaks at specific wavelengths, pressure, temperature and optical length to calculate the isotopologue mole fractions present in the sample.

For this investigation, the SICAS takes its measurements in static mode, taking sample flasks as input. Since it is typically used for atmospheric pressures and similar CO_2 concentrations, this means that the samples it measures should have pressures ranging from 700 to 1500mbar, as well as CO_2 concentrations ranging from 375 to 424 parts per million.

3 Methods and Setup

As previously mentioned, this thesis aims to calibrate the ^{17}O scale by attempting a new method of making a CO_2 sample with a well-defined $\delta^{17}\text{O}$ value. To know the $\delta^{17}\text{O}$ of this sample, the sample must be created with care as any mistake in the process could lead to a different outcome in the isotope composition.

3.1 Water Samples

To have a meaningful calibration for the SICAS, the samples that are used to calibrate it should cover a wide enough range of $\delta^{17}\text{O}$ compositions. Since the amount of water and CO_2 being used is known, the final $\delta^{17}\text{O}$ values of the CO_2 can be calculated using the process outlined in Appendix A. The $\delta^{17}\text{O}$ values of the final water samples used are given in Table 1. These, when equilibrated with the same CO_2 sample of $\delta^{18}\text{O}_{\text{VSMOW}} = 5.52\text{‰}$, would result in a set of $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values for the equilibrated CO_2 listed in Table 5.

Table 1: The chosen water $\delta^{17}\text{O}$ values, measured by the Triple Liquid Water Isotope Analyser (TL-WIA) and their descriptions.

Sample Description	$\delta^{17}\text{O}$ values [VSMOW, ‰]
Depleted Water	-5.16 ± 0.04
Demineralised Water	-3.51 ± 0.04
Enriched Water	0.59 ± 0.04

In terms of preparation, the demineralised water was the easiest water sample, being obtained directly from the laboratory supply. It was chosen as a midpoint between the other water samples to be able to check that the measurement results are consistent with each other.

The enriched water was prepared by filling a large beaker with two litres of water and letting it evaporate over a few days until it was at approximately 1.5L. This process works due to evaporation favouring the lighter H_2O isotopologues, leaving behind a higher concentration of ^{17}O - and ^{18}O -containing water molecules. Originally, this was attempted using a flask instead of a beaker but the exposed surface area was too small and the change in $\delta^{17}\text{O}$ was barely noticeable.

Finally, the depleted water was created by mixing demineralised water and a depleted SLAP-like water sample, determining the required masses of the two water samples using the Two End Member Mixing model [9], which is given by Equation 3.1.

$$\delta_{\text{Final}} = \frac{\delta_1 m_1 [1 + R_r(1 + \delta_2)] + \delta_2 m_2 [1 + R_r(1 + \delta_1)]}{1 + R_r [(1 + \delta_2)m_1 + (1 + \delta_1)m_2]} \approx \frac{\delta_1 m_1 + \delta_2 m_2}{m_1 + m_2} \quad (3.1)$$

Where δ_{Final} is the final isotope ratio, R_r is the heavy isotope abundance ratio, δ_1 and δ_2 are the respective isotope ratios of the mixing components, and m_1 and m_2 are the respective masses of the mixing components. The approximation is quite close to the real value, meaning that it can be used as a valid estimate for the amount of each sample needed to create the desired sample of depleted water. In this investigation, the decision was made to use the samples listed in Table 2 as it would result in a sample of water depleted enough to put the $\delta^{17}\text{O}$ value of the demineralised water close to the midpoint of the three samples. This calculation was carried out using the measured ^{18}O ratios. Using the VSMOW scale, the ^{18}O to ^{16}O ratio is 0.0020052 [18]. This leaves us with an expected $\delta_{\text{Final}}^{18}$ of

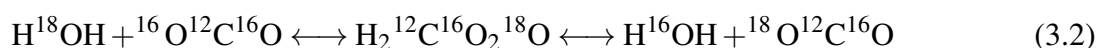
Table 2: The values used in the calculation of the mixed depleted water sample.

Sample Type	δ^{18} [‰, VSMOW]	Mass [g]
SLAPLEN Water	-56.40 ± 0.02	125.00 ± 0.05
Demineralised Water	-6.59 ± 0.04	1875.03 ± 0.05

$-9.79 \pm 0.04\text{‰}$, which was measured to be $-9.73 \pm 0.04\text{‰}$. The sample titled "SLAPLEN Water" is a SLAP-like water source with a depletion very close to that of SLAP water.

3.2 Equilibration

The equilibration of the CO_2 with the different water samples occurs via oxygen isotope exchange due to equilibrium with carbonic acid (see Equation 3.2).



The end goal of the equilibration is to have a CO_2 sample that has equilibrated with the water samples of known $\delta^{17}\text{O}$ values, as this allows us to know the $\delta^{17}\text{O}$ of the CO_2 after the equilibration. For this to be achieved, these two substances need to be isolated in a container to prevent any external influence on the isotopologue ratios. To do this, a glass sample flask with a volume of approximately 2.3 litres is used as the container. Before beginning the experiment, the flask is tested for strain by shining polarised light through the flask and viewing it through another polariser. If it passes the test, it is safe to use for the equilibration as it will survive the potential stress exerted by the freezing and thawing of the sample preparation. Once this experimental step is completed, there will be four flasks full of equilibrated air: one using depleted water, one using demineralised water and two using enriched water to test the repeatability.

To prepare the sample, the flask is filled with 200ml of the chosen water. Once this has been weighed out, it is placed in a custom-made dewar filled with dry ice and silicone oil, making sure that the long tube of the flask is not submerged in the water, as shown in Figure 4. The choice of silicone oil over ethanol is due to the molar mass of ethanol being 46 g/mol (same as ${}^{16}\text{O}^{12}\text{C}^{18}\text{O}$), meaning that any contamination would significantly skew the sample measurement.

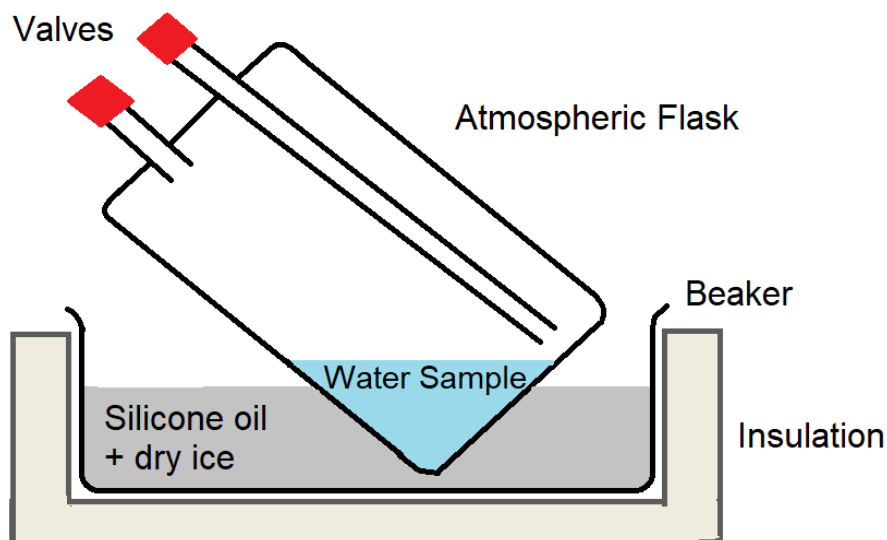


Figure 4: The setup used to freeze the water samples for evacuation inside the atmospheric flasks. The valve attached to the short tube inside the flask is used for evacuation and CO₂ filling.

After approximately one hour, the water contents of the flask will be ice, which means that the (atmospheric) air present in the flask can be evacuated (using the system shown in Figure 5) without fractionating the water sample. This results in a flask consisting of a vacuum and 200ml of water. The flask is then removed from the dewar, allowing it to thaw and thereby releasing gases that were previously dissolved in the water into the vacuum within the flask. This process is repeated once more

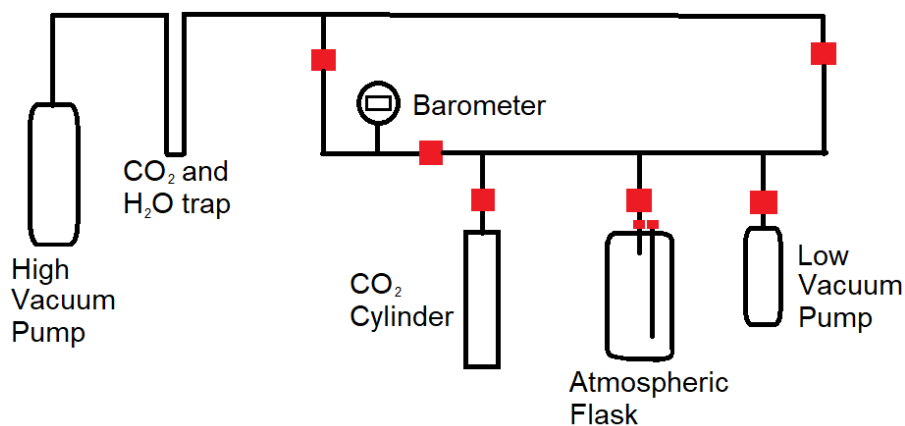


Figure 5: The system used to evacuate the air out of the atmospheric flask and fill the CO₂ from the cylinder. The red squares indicate the relevant valves in the system. The carbon dioxide and water traps are parts of the system tubing that can be submerged in liquid nitrogen to prevent moisture from reaching the strong vacuum pump. The "low vacuum" pump is a KNF N813.3ANE diaphragm pump and the "high vacuum" pump is a Drytel drag vacuum pump. To avoid over-exerting the high vacuum pump, the low vacuum pump is used to bring the system pressure to approximately 50 mbar.

before the CO₂ is added into the flask, evacuating the released gases. The CO₂ gas is pumped into the flask (once again using the system shown in Figure 5) while the water is still frozen until a pressure of approximately 1000mbar is reached. The flask can finally be removed from the dewar so the ice

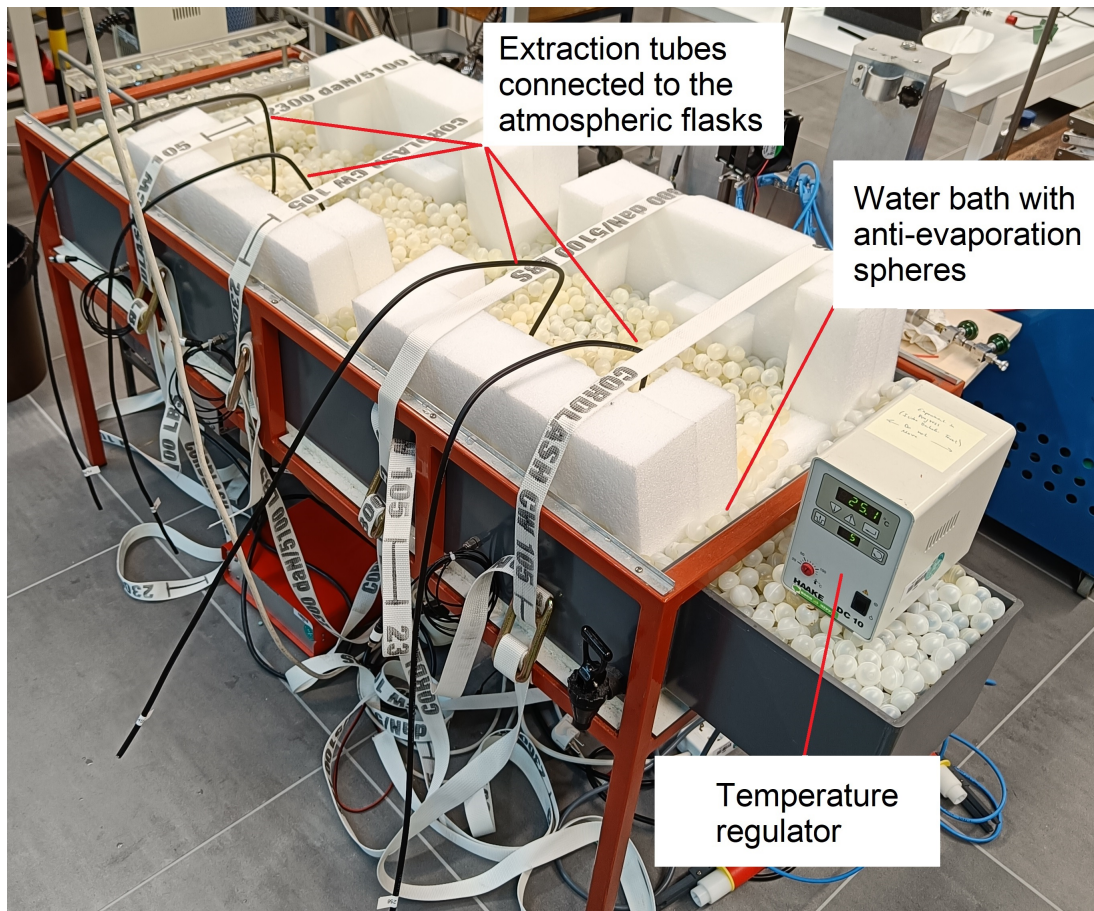


Figure 6: The submerged flasks with their extraction tubes protruding out of the water bath. Key components are labelled with straight red lines. The water bath is contained within the entirety of the orange table shown in the image, where the flasks are kept submerged by foam supports. These are secured in place with loading straps.

can melt and the CO_2 and the water can equilibrate.

As the fractionation between the water and the CO_2 oxygen isotopes due to equilibration is temperature dependent, the flasks are fully submerged in a water bath at $25.0 \pm 0.1^\circ\text{C}$ and left for at least a week. This guarantees that using the theoretically ideal values for the equilibration calculations is as accurate as possible.

3.3 CO_2 Extraction

Once the CO_2 and the water have equilibrated at a controlled temperature long enough, the CO_2 is ready to be extracted. For this, special care has to be taken to prevent fractionation and to prevent water from getting into the CO_2 storage cylinder. Since the flasks were originally placed underwater without a way of extracting the air, a tube had to be attached to the flasks (see Figure 6). After this was done, the flasks were placed underwater for a few more days to return to their $25.0 \pm 0.1^\circ\text{C}$ equilibrium.

A mobile system was custom-built to be able to extract the CO_2 from the submerged flasks (see Figure

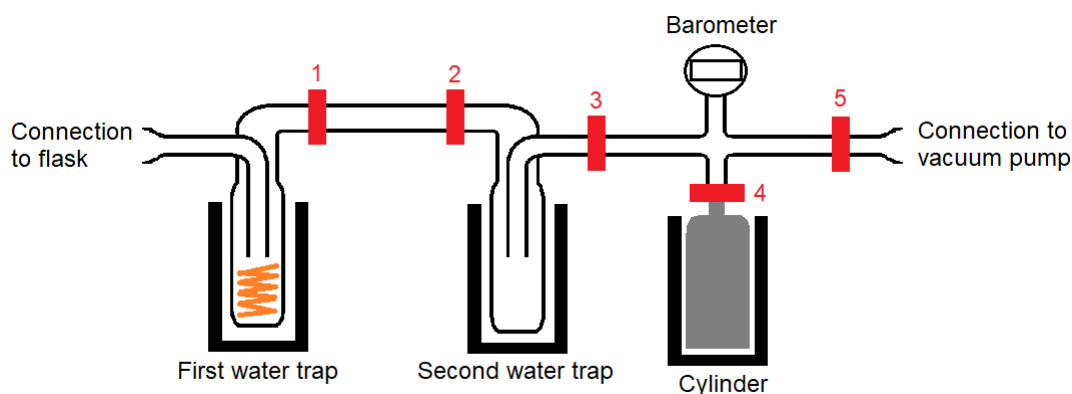


Figure 7: The system used to extract the CO₂ from the submerged flasks. The red rectangles represent valves. Two water traps are used to minimise the chances of any water making it to the cylinder, as this could ruin the sample. The first water trap contains a copper coil to help distribute the heat, preventing frozen water from clogging the tubing. Both water traps are placed inside dewars filled with silicon oil and dry ice. The dewar around the cylinder is to be filled with liquid nitrogen to collect the CO₂ into the cylinder.

7). To prevent any damage to the vacuum pump due to it drawing high quantities of water, the first water trap (with silicone oil and dry ice inside the dewar) is disconnected (closing valve 1) from the evacuation system to catch water in the event of there being a leak in which the water from the bath enters the tubing. Once this has been left for a sufficient amount of time and it is determined that there is no leak, it is reconnected to the system using valve 1. The entire system, including the CO₂ cylinder, is then evacuated for approximately 15 minutes until the pressure on the barometer reads below 1×10^{-2} Torr (the flask containing the equilibrated CO₂ and water is still closed). Following the evacuation, the following steps are taken to complete the extraction:

- The connection to the vacuum pump is closed (close valve 5) and the remaining pressure in the system is noted
- The flask containing the equilibrated CO₂ and water is opened underwater and the pressure in the system is noted
- Liquid nitrogen is poured into the dewar surrounding the CO₂ cylinder, refilling as it evaporates
- Once the pressure starts to drop due to the CO₂ solidifying inside the bottom of the cylinder, a timer is started to have an approximate idea of when the process is finished².
- When the pressure on the barometer stops decreasing (usually at the order of 10^0 Torr), this pressure is noted and the cylinder valves are sealed. The flask can be sealed underwater and the system can be taken out of its vacuum state and prepared for the next extraction.

The pressure values recorded in this part of the method can be found in Table 9 in Appendix B.3.

3.4 Measuring the amount of CO₂

Since the volume of the atmospheric flasks is not calibrated, the exact amount of CO₂ used in the equilibration is unknown. Inside the atmospheric flask, there was over 2L of CO₂ at a pressure of

²Since the flasks are beneath a layer of opaque anti-evaporation spheres, using a visual cue is not an option and therefore it is loosely based on the time passed.

approximately 1 bar. This was extracted into a cylinder with a volume of less than 200ml, meaning it became highly pressurised. Therefore, to safely measure the amount of CO₂ a large volume is required as this allows for the gas to expand and reduce the pressure. The system depicted in Figure 8 was used to expand the CO₂ into sections with known volumes and then the pressure can be measured to calculate the total amount of molecules with the ideal gas (in this case, the gas is not ideal but the deviation from it being ideal is negligible) equation:

$$n_{CO_2} = 2 \left(\frac{V_{CO_2} \cdot P}{R \cdot T} \right) \quad (3.3)$$

Where n_{CO_2} is the amount of moles of oxygen due to CO₂, V_{CO_2} is the volume [m³] of the CO₂, P is the pressure [Pa] of the CO₂, T is the temperature [K] of the CO₂ and R is the gas constant [J·mol⁻¹·K⁻¹]. The factor of two is due to the fact that there are two oxygen atoms in one carbon dioxide molecule.

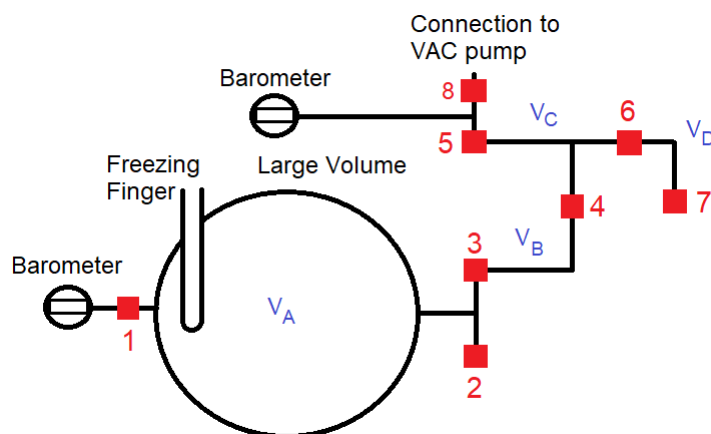


Figure 8: The setup used to both measure the quantity of CO₂ extracted into the cylinders and fill the vials for IRMS measurements. The red squares and their respective numbers indicated valves and the blue text labels the volumes with their respective letters (i.e. V_A = Volume of section A). The straight black lines indicate glass tubing. Two barometers (the one closest to the vacuum pump is a PCM301 Busy Bee barometer and the one connected to the large volume is an MKS 910 Dual Trans barometer) are used to keep track of the pressure distribution in the system, allowing one to know when all of the CO₂ is inside Volume A. Valve 7 is used to connect external components (such as a CO₂ cylinder) to the system, valve 2 is permanently closed and valve 1 is permanently open. The volumes of the sections and how they were calculated can be found in Appendix B.1.

First, the entire volume is evacuated up to the connection of the cylinder at valve 7 (all valves except 2 are open), ensuring that there is no contribution from any contaminants present. After this is done (which could take upwards of 30 minutes due to the large volume), liquid nitrogen is poured into the freezing finger, refilling when necessary until it stops boiling. Once this is done, the connection to the vacuum pump is closed (valve 8) and the connection to the cylinder is opened (valve 7). As soon as valve 7 is opened, one can see the CO₂ precipitating onto the freezing finger inside of volume A and the pressure reading at both barometers starting to drop. After another 30 minutes have passed (while making sure the freezing finger is continuously filled with liquid nitrogen), the pressures measured at the barometers are recorded. The entire system is briefly evacuated to remove any contamination that is not frozen onto the freezing finger and valve 3 is closed, isolating all of the CO₂ inside of volume

A. The CO₂ is left to thaw, and as soon as the solid mass is no longer visible (meaning the CO₂ has fully sublimated), the system is left for 30 minutes to allow for the isotopologue distribution to settle³ and for the temperature to become homogeneously room temperature. This is done to prevent fractionation from occurring during the filling of the vials in the following steps. Now, the pressure reading can be noted and the CO₂ amount is known. The results for this are shown in Table 8 in Appendix B.2.

3.5 IRMS Vial Preparation

The samples that the IRMS takes as input are small vials, that require to be filled with a pressure ranging between 150 to 200mbar due to the optimal amount of CO₂ the IRMS can measure. These use a very narrow valve so extra care must be taken to fully evacuate and fill the vials as it does take longer than when using the standard valves. Since this step was carried out using the same system shown in Figure 8 directly after the CO₂ was expanded into volume A in Section 3.4, the procedure begins with the CO₂ already inside the system.

In the first place, the (now empty) cylinder is removed and one of the vials is put in its place. The entire system except volume A is evacuated, letting it run slightly longer to allow the slower draining valve of the vial to fully evacuate. Since the pressure in volume, A is about 550 mbar, this needs to be reduced below 200 mbar to allow the IRMS to measure it properly. Reducing the pressure is achieved by expanding the CO₂ into volume B (by closing valve 4, opening valve 3 and giving the system some time to settle), followed by closing valves 3 and 5 and opening valve 4, expanding the gas into volumes C and D. Seeing as the combination of volumes B, C, and D (the volume of the vial is included in D) is approximately three times the volume of B, this expansion results in a final pressure within the ideal IRMS range in the vial. Keeping the narrow vial valve in mind, this is left to rest for a few minutes for the isotopologue distribution to become as homogeneous as possible. This step is repeated two more times, swapping the full CO₂ vial out for an empty one and evacuating the system up to valve 3 once more. The pressures for the filling of the vials in this section can be found in Table 8 in Appendix B.2.

To store the CO₂ back in the cylinder, it is connected back to valve 7 and the system is evacuated up to the point of valve 3. Then the cylinder is placed inside a dewar, filling slowly with liquid nitrogen to start cooling it down. After a few minutes, valve 8 can be closed to stop the evacuation and valve 3 can be opened, releasing the CO₂ into the system. Due to the temperature gradient, the gas will be drawn to the cold cylinder and solidify there. Using the two barometers one can then make sure all of the CO₂ is extracted and once this is done (after approximately 30 minutes) the cylinder can be sealed and removed from the system. The pressures during the extraction of the CO₂ from this system can be found in Table 9 in Appendix B.3.

³It has been shown that changes of phases have mass-dependent fractionation effects, meaning that sublimation will impact the isotopologue values significantly on a short term scale [19].

4 Results

4.1 Expected values from Equilibration

In total, four atmospheric flasks were filled. The values for the water used in this calculation are listed in Table 3: Once ready, each of these flasks was filled with CO₂ from the same cylinder. The

Table 3: The properties of all the different water samples used for the equilibration inside the atmospheric flasks. The scale used for these water measurements is the VSMOW-SLAP scale.

Atmospheric Flask	Description	$\delta^{17}\text{O}$ ($\pm 0.04\text{‰}$)	$\delta^{18}\text{O}$ ($\pm 0.04\text{‰}$)	Mass ($\pm 0.05\text{g}$)
358	Demineralised Water	-3.51	-6.68	200.02
256	Depleted Water	-5.16	-9.73	200.01
423	Enriched Water	0.59	1.14	200.02
046	Enriched Water	0.59	1.14	200.01

quantity of CO₂ per flask is given in Table 4. Unfortunately, the sample in Atmospheric Flask 358 was irreversibly lost due to human error during the extraction process and therefore has no more calculations or measurements associated with it. Following the procedure explained in Appendix A,

Table 4: The amount of O moles originating from the CO₂ per flask, calculated using Equation 3.3.

Atmospheric Flask	Cylinder	Pressure in V _A ($\pm 0.1\text{mbar}$)	O moles from CO ₂ (± 0.00045)
256	1	548.4	0.19664
423	2	561.8	0.20144
046	3	539.3	0.19337

the expected δ values are presented in Table 5.

Table 5: The theoretical δ values for the CO₂-H₂O equilibration on the VSMOW-SLAP scale.

Atmospheric Flask	Expected CO ₂ values [‰ , VSMOW-SLAP]	
	$\delta^{17}\text{O}$	$\delta^{18}\text{O}$
256	15.69 \pm 0.04	30.88 \pm 0.04
423	21.44 \pm 0.04	41.98 \pm 0.04
046	21.47 \pm 0.04	42.01 \pm 0.04

4.2 IRMS Results

Using the IRMS to measure the vials filled from the flasks, the results in Table 6 were found. The IRMS also measures the $\delta^{13}\text{C}$ values (by measuring the number of molecules for mass 45 and calculating the expected $\delta^{13}\text{C}$ based on the typical natural relation of ¹⁷O and ¹⁸O), which can be influenced by the some of the CO₂ remaining dissolved in the water as bicarbonate, but it should have a small influence.

Table 6: The δ values measured from the vials filled for the IRMS. The carbon measurements are on the VPDB scale while the oxygen measurements are shown both on the VPDB-CO₂ (native output of the IRMS) and VSMOW (for consistency with previous scales) scale.

Atmospheric Flask	Measured CO ₂ values [‰]		
	$\delta^{13}\text{C}$ [VPDB]	$\delta^{18}\text{O}$ [VPDB-CO ₂]	$\delta^{18}\text{O}$ [VSMOW]
CO ₂ Tank	-26.682±0.004	-34.52±0.01	5.52±0.01
256	-26.71±0.003	-10.385±0.002	30.651±0.002
423	-26.641±0.002	0.409±0.003	41.893±0.003
046	-26.644±0.003	0.361±0.003	41.843±0.003

As seen in the tables, the results from the IRMS have a very low uncertainty, meaning that the difference between the expected values and the measured values is due to random error in the experimental process. The largest uncertainty is due to the calibration of the IRMS, which is based on the ¹⁸O of carbonate. Furthermore, the predicted data lines up quite closely with the measured values (the largest deviation is of 0.23 ‰) but still do not fit within each other's uncertainties.

5 Discussion

5.1 Results

5.1.1 IRMS

The results shown in Table 6 are quite promising, showing a very good correlation with the expected values calculated in Table 5. The method also seems to be quite successful seeing as the repeat samples achieved such similar results. Since only the $\delta^{18}\text{O}$ measurements are comparable, it has to be assumed that the $\delta^{17}\text{O}$ measurements follow similarly. Despite none of the measured values fitting perfectly (the closest value was within 0.086‰) with the predicted values, they are good enough to confirm that this method is a valid technique of creating CO_2 samples with known δ values. The reasons for the slight differences between the results are explained in the following sections.

The IRMS also gives a measure of $\delta^{13}\text{C}$ (shown in table 6), which theoretically should remain quite similar across all samples. This could give an indication of the fractionation in the sample and is therefore interesting to supervise. The first value is the CO_2 that was used for the equilibration, meaning that if there was no change in the ^{13}C composition, all values should be equal to that one. The data shows deviations in these though, meaning that there was potentially some fractionation occurring. This could be due to the CO_2 remaining dissolved in the water as bicarbonate while removing molecules from the gas- CO_2 and fractionating the sample. Another explanation is that since the IRMS filters by mass, the different amounts of ^{17}O in each CO_2 sample could be registered under the ^{13}C measurement. This is consistent with the depleted samples having a more negative ^{13}C value and the enriched samples having a less negative ^{13}C value. This is an unlikely reason, however, as the influence of the ^{17}O on the ^{13}C measurement would mean there is a very high $\Delta^{17}\text{O}$ in the sample, which is not the case. The varying $\delta^{13}\text{C}$ problem could be addressed in the future by having the water samples be slightly acidic, preventing the loss of carbon in the sample.

5.1.2 SICAS

Unfortunately, the dilution system for the SICAS sample preparation was too inconsistent to measure the CO_2 samples in time. After several series of multiple flask measurements, the repeatability of the measurements was too low, yielding inconsistent results. It is indeed possible to take highly repeatable measurements on the SICAS (see "*Simultaneous measurement of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ of atmospheric CO_2 – performance assessment of a dual-laser absorption spectrometer*" [16]), however, this was not achieved in this thesis. The SICAS results would be highly interesting to look at, as it would give an actual value for the $\delta^{17}\text{O}$ without having to assume based on the $\delta^{18}\text{O}$ values. This would give the necessary proof for the validity of this method in creating a CO_2 reference. These measurements would also enable the comparison of the $\delta^{13}\text{C}$ to the original value without the influence of the ^{17}O .

5.2 Sources of Uncertainty

The most important source of uncertainty when comparing the measurements of the IRMS and the expected equilibration values is the calibration of the IRMS on the VPDB scale, while the water measurements are on the VSMOW scale. Although this discrepancy has the largest impact on the measurements, there are other minor sources of uncertainty that should be contemplated considering the circumstances of this investigation.

5.2.1 Equilibration

A minor source of uncertainty in this process is that the mole ratio between the water and the CO₂ is slightly lower than ideal. Since the $\delta^{17}\text{O}$ of the CO₂ is not well known and must therefore be approximated, it is ideal to have the initial values of the CO₂ influence the final values after equilibration as little as possible. In an ideal case, the amount of water massively outweighs the amount of CO₂ but in this case, it is only around 50-60 times greater. Hence, it would reduce the uncertainty if the ratios were changed in favour of the water by reducing the total CO₂ used in the equilibration.

Another problem with the equilibration was noticed when filling the IRMS vials from the CO₂ tank used to fill the atmospheric flasks. It showed that when not flushed thoroughly, the repeatability was very low (standard deviation of almost 0.1‰), suggesting that there was some type of contamination when extracting CO₂ from the tank. This contamination could have been present when filling the atmospheric flasks and therefore increase the uncertainty of the expected equilibration δ values. These gases could also have been responsible for the remaining pressures (should theoretically go to vacuum) during extraction, as this gas would partly not be captured in water traps or the CO₂ freezing.

Finally, the last potential issue when filling the atmospheric flasks for the equilibration was the use of the needle valve as a pressure regulator. When filling the flasks, the needle valve knob was turned on the tank's pressure regulator to stop the flow of CO₂ when the desired pressure was reached. This, however, has the potential to fractionate the sample, which was not taken into account during the filling process. Luckily, the CO₂ has less influence on the equilibration than the water so this is, if at all, a minor source of uncertainty.

5.2.2 Extraction

During the extraction process, there were many instances in which it was impossible to determine whether the CO₂ had been completely extracted. During the original extraction (see Section 3.3), the pressures recorded while freezing the CO₂ from the atmospheric flasks into the cylinder varied slightly. While the setup was tested, it was noticed that when too much water was entering the system, the first water trap could clog up and prevent any more gas from travelling through the system. Since the lighter isotopologues can reach the cylinder with more ease, they could have gotten through while the heavier ones stayed behind, fractionating the sample.

During the extraction, the pressure decreased at a very slow pace, meaning that it was very difficult to identify the moment at which the extraction was "complete". This could have resulted in fractionation for the same reason as listed above.

A third reason that could have led to fractionation and thus resulted in unexpected results is that the cylinder did not have a sufficiently large surface area to capture all of the CO₂ present in gas form. With all cylinders, the final pressure reached was of the order of 1×10^0 Torr. This could be an issue in the sample as it is known that when freezing CO₂, the measured pressure can reach a lot lower than that. Since the lighter isotopes would be the first to freeze, this would create a sample with a larger light-to-heavy isotope ratio than expected.

All of these problems could be remediated by scaling down the experiment. This experiment would significantly benefit from using less water and CO₂ inside of the atmospheric flasks, as the reduced CO₂ would significantly shorten the duration of the extraction process and the reduced water would make the clogging of the water traps less of a concern. These reasons would therefore make a successful extraction more probable. As long as the ratios of the oxygen moles due to water and CO₂ remain the same, the equilibration should not be affected by the absolute change in moles.

One final change that could have led to less fractionation is that in similar experiments, the CO₂ was not fully extracted (via freezing it into a vessel using liquid nitrogen), but rather expanded into a vol-

ume. This would have a smaller impact as this does not rely on the entire CO₂ volume making it out of the atmospheric flask to prevent fractionation.

5.2.3 Measuring the Amount of CO₂

A similar issue arose when freezing from CO₂ the cylinders into the known volumes to measure the total quantity. Since the surface area was limited, there was no guarantee that all of the CO₂ was frozen onto the freezing finger. This doubt is heightened by the fact that the pressure recorded in volume A (Figure 8) did not go below 1×10^{-1} Torr during freezing, despite the fact that it theoretically could go lower. If all of the CO₂ was not successfully frozen into this volume, this would certainly have led to fractionation and therefore changed the isotopologue ratios of the sample.

5.2.4 IRMS Vial Filling

The filling of the IRMS vials with the CO₂ sample proved to be highly effective, resulting in extremely low standard deviations (the highest standard deviation of the samples was 0.009 ‰) and therefore very high repeatabilities. This is therefore a very effective method of vial filling.

6 Conclusion

6.1 Summary of Main Contributions

This thesis set out to show whether the equilibration of CO₂ with water was a valid method of creating CO₂ samples with well-known $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values. Despite the plethora of roadblocks such as the lack of an established extraction setup and the high amount of CO₂ in need of extraction, the measured results had a minimal discrepancy in regards to the expected values. Therefore, it is easy to conclude that this equilibration method was a success, as this was a pilot study for this laboratory. Although there were no measurements taken on the SICAS to confirm the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values as well as measuring the $\delta^{17}\text{O}$ values, the accuracy of the $\delta^{18}\text{O}$ measurements on the IRMS is enough to assume the process is good enough to make samples with a consistent $\delta^{17}\text{O}$.

The reliability of this method could mean that the calibration of the ¹⁷O scale becomes more consistent internationally.

6.2 Future Work

Although the experiment can be seen as a success, many improvements can be made to further improve the precision.

One improvement to potentially reduce the variation in the $\delta^{13}\text{C}$ is to partially acidify the water, reducing the amount of CO₂ remaining in the form of carbonates. The measurement of $\delta^{13}\text{C}$ would be useful as it could be an indicator of fractionation in the sample. This has the drawback of prolonging the equilibration time, which would not be a significant downside.

Another improvement would be to have a pre-built and more rigorously tested extraction system to prevent the inconsistencies associated with the improvised system. This would help reduce the risk of both contamination and fractionation in the sample. Another great problem associated with this thesis was the high amount of CO₂ and water used, leading to uncertainties during the extraction as to whether the full sample was extracted. The solution to this would be to use a smaller amount of both samples as this would guarantee a faster and fractionation-free extraction. The ratio of water to CO₂ was also relatively small (50 moles of water per mole of carbon dioxide), meaning that this could be increased in favour of the water to have a smaller uncertainty when predicting the outcome of the equilibration.

During the measurement of the Tank-CO₂, it was noticed that the CO₂ was contaminated when the pressure regulator volume was not flushed for long enough. The solution to this would be to flush the system for a few extra minutes to ensure that the only gas being pumped in is the desired CO₂. The use of a needle valve for regulating the pressure was also a potential source of error when filling the atmospheric flasks with CO₂, which could be remediated by setting the right back pressure on the regulator. This could be mediated by using an expansion system instead.

An obvious component of future work would be to measure the samples on the SICAS, giving an insight into the δ values that the IRMS cannot measure.

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Appendices

A Calculating the outcome of the equilibration process

A.1 Mole Quantities

To calculate the number of moles of water present in the equilibration, the following equation is used:

$$n_{H_2O} = \frac{m_{H_2O}}{M_{H_2O}} \quad (\text{A.1})$$

Where n_{H_2O} is the number of moles of water, m_{H_2O} is the mass [g] of the water and M_{H_2O} is the molar mass (18.02 g/mol) of water. Since there is one atom of oxygen per water molecule, this is equal to the number of moles of oxygen in the water.

To calculate the number of moles of oxygen due to carbon dioxide present in the equilibration, equation 3.3 is used.

A.2 Ratio Calculations

In this section, the process for the equilibration for ^{17}O is shown. This, of course, can be replaced with the appropriate ratios for ^{18}O to calculate the equilibrium.

Calculating the ratio of ^{17}O isotopologues in the water (pre-equilibration):

$$^{17}R_{H_2O-pre} = (1 + \delta^{17}\text{O}_{Sample(H_2O)}) \times ^{17}R_{VSMOW} \quad (\text{A.2})$$

Where $\delta^{17}\text{O}_{Sample(H_2O)}$ is the isotopologue ratio of the water molecules on the VSMOW scale [‰] and $^{17}R_{VSMOW}$ is the ratio of ^{17}O isotopologues in VSMOW (0.0003799 [18]).

Assuming that the $\Delta^{17}\text{O}$ is approximately 0‰ (following from Equation 2.3), an estimate for the $\delta^{17}\text{O}$ of the CO_2 can be found:

$$\delta^{17}\text{O} = (\delta^{18}\text{O} + 1)^\lambda - 1 \quad (\text{A.3})$$

The value of the reference line (λ) here is taken to be 0.5229 [4]. Calculating the ratio of ^{17}O isotopologues in the carbon dioxide (pre-equilibration) is now achieved using:

$$^{17}R_{CO_2-pre} = (1 + \delta^{17}\text{O}_{Sample(CO_2)}) \times ^{17}R_{VPDB} \quad (\text{A.4})$$

Where $\delta^{17}\text{O}_{Sample(CO_2)}$ is the isotopologue ratio of the carbon dioxide molecules on the VPDB- CO_2 scale [‰] and $^{17}R_{VPDB-CO_2}$ is the ratio of ^{17}O isotopologues in VPDB- CO_2 (0.0003808 [20]).

Therefore, the total ratio is given by:

$$^{17}R_{Total} = n_{H_2O} \cdot ^{17}R_{H_2O-pre} + n_{CO_2} \cdot ^{17}R_{CO_2-pre} = n_{H_2O} \cdot ^{17}R_{H_2O-post} + n_{CO_2} \cdot ^{17}R_{CO_2-post} \quad (\text{A.5})$$

Here, n_{H_2O} is the number of moles of water and n_{CO_2} is the number of moles of carbon dioxide. Assuming that the amount of moles of water and carbon dioxide is constant (i.e. the amount of carbonic acid remaining in the solution is negligible), the total ratio is the same before and after the equilibration. This is an approximation which holds due to the left-hand side and the right-hand side of

Equation A.5 being almost equal.

Calculating the ratio of ^{17}O isotopologues in the water (post-equilibration):

$$^{17}R_{H_2O-post} = \frac{^{17}R_{Total}}{n_{H_2O} + n_{CO_2} \cdot \alpha_{CO_2/H_2O}} \quad (\text{A.6})$$

Here α_{CO_2/H_2O} is the ratio of VSMOW- CO_2 to VSMOW for ^{17}O (1.021254 ± 0.00004 [21][6]).

Calculating the ratio of ^{17}O isotopologues in the carbon dioxide (post-equilibration):

$$^{17}R_{CO_2-post} = ^{17}R_{H_2O-post} \times \alpha_{CO_2/H_2O} \quad (\text{A.7})$$

A.3 Final $\delta^{17}\text{O}$ Values

Finally, the $\delta^{17}\text{O}$ of the water is calculated using:

$$\delta^{17}\text{O}_{Sample(H_2O)} = \frac{^{17}R_{H_2O-post}}{^{17}R_{VSMOW}} - 1 \quad (\text{A.8})$$

Similarly, the $\delta^{17}\text{O}$ of the carbon dioxide is calculated using:

$$\delta^{17}\text{O}_{Sample(CO_2)} = \frac{^{17}R_{CO_2-post}}{^{17}R_{VPDB-CO_2}} - 1 \quad (\text{A.9})$$

To express the values of the VPDB- CO_2 scale in the VSMOW scale, this can be done following:

$$\delta^{17}\text{O}_{Sample(VSMOW)} = \frac{^{17}R_{VPDB-CO_2}}{^{17}R_{VSMOW}} (\delta^{17}\text{O}_{Sample(VPDB-CO_2)} + 1) - 1 \quad (\text{A.10})$$

This can also be rearranged to change the scale from VSMOW to VPDB- CO_2 .

B CO₂ Sample Preparation

B.1 Volume Calculations

A key part of measuring the quantity and extracting CO₂ from inside of the cylinders is knowing the volumes of each section in the expansion system shown in Figure 8. These were calculated using the principle that at constant temperature and number of molecules, the pressure relation between two volumes is given by:

$$P_a V_a = P_b V_b \quad (\text{B.1})$$

Where P and V are the pressure and the volume respectively, and the subtext indicates which part of the system it belongs to. In the following equations, the following notation is used for simplicity: $V_{ABCD} = V_A + V_B + V_C + V_D$, $V_{AB} = V_A + V_B$, etc.

To begin, there has to be a well-defined volume and pressure which can be expanded into the system. This was achieved by taking a large glass flask and completely filling it up several times with water and weighing it out. Knowing the density of the water, this enables the calculation of the volume of the flask. This resulted in the calibrated volume of the flask being $V_E = 1140.53 \pm 0.04\text{ml}$.

The system is fully evacuated up to the point of valve 7 (Figure 8). By filling the control volume flask with atmospheric air ($P_E \approx 1022\text{mbar}$) and attaching it to valve 7, the process can begin. Firstly, valve 5 is closed and then the gas from valve 7 is slowly released into the system to prevent condensation of the water vapour in the air⁴. The volume of the system (ABCD) can be found using the following equations:

$$V_E P_E = V_{ABCDE} P_1 \implies V_{ABCDE} = \frac{V_E P_E}{P_1}$$

$$V_{ABCD} = V_{ABCDE} - V_E$$

After the volume ABCD is found, valves 4 and 7 are closed and the system is evacuated by opening valve 5. Once fully evacuated, valve 5 is closed and the gas in volume AB (still at pressure P_1) is expanded into the rest of the system, resulting in a pressure of P_2 and allowing us to determine the volume of both AB and CD.

$$V_{AB} P_1 = V_{ABCD} P_2 \implies V_{AB} = \frac{V_{ABCD} P_2}{P_1}$$

$$V_{CD} = V_{ABCD} - V_{AB}$$

To calculate C (and therefore D), valves 4 and 6 are closed and the system is evacuated by opening valve 5. When evacuated, valve 5 is closed and valve 4 is opened, expanding the air in volume AB at pressure P_2 into volume C. This will result in a new pressure measured in volume ABC of P_3 .

$$V_{AB} P_2 = V_{ABC} P_3 \implies V_{ABC} = \frac{V_{AB} P_2}{P_3}$$

$$V_{ABC} - V_{AB} = V_C$$

$$V_{CD} - V_C = V_D$$

Finally, to calculate the volumes of A and B, the system is evacuated up to valve 3. Once evacuated, valve 4 is closed and valve 3 is opened to expand the gas from volume A (at P_3) into volume B (resulting in a pressure of P_4 in volume AB).

$$V_A P_3 = V_{AB} P_4 \implies V_A = \frac{V_{AB} P_4}{P_3}$$

⁴It was observed that when the gas was released too quickly, the rapid cooling of the air would cause condensation to appear, reducing the number of molecules in gas form and potentially giving an inaccurate pressure reading.

$$V_{AB} - V_A = V_B$$

This entire volume calculation process was carried out a total of 6 times. The volumes calculated can be found in Table 7.

Table 7: Table containing the values of the volumes depicted in Figure 8.

Name	Volume (ml)	Standard Error (ml)
V_{ABCD}	4440	8
V_A	4399	8
V_B	14.93	0.05
V_C	11.7	0.5
V_D	14.2	0.7

B.2 IRMS Vials

The following table shows the pressures of parts of the system as the vials were filled. The first measurement of each cylinder (the highest pressure) is used to calculate the amount of moles used for equilibration.

Table 8: The pressures recorded during the filling of each vial. The cylinder from which the CO₂ came is indicated at the top of the table. The number in the brackets indicates which atmospheric flask the cylinder was filled.

	Pressure [± 0.1 mbar]								
	Cylinder 1 (256)			Cylinder 2 (423)			Cylinder 3 (046)		
Volume	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	Vial 9
V_A	548.8	547.1	545.5	561.8	560.2	558.6	539.3	537.8	528.5
V_B	546.6	545.2	543.6	559.9	558.2	556.6	537.4	535.9	526.6

B.3 Extraction Pressures

The following table (Table 9) depicts the pressures recorded during the extraction of CO₂ (the Atmospheric Flask Extraction is explained in Section 3.3 and the IRMS Vial System Extraction is explained in the last paragraph of Section 3.5), measured on the Busy Bee PCM301. Note that Cylinder 1 recorded a much lower pressure when extracting, suggesting that the second extraction captured more of the CO₂ better or could have been of higher purity. Cylinder 3 does not have a secondary extraction pressure as the sample was contaminated with atmospheric air and had to be discarded.

Table 9: The pressures recorded during the extraction of the CO₂ from the atmospheric flasks and from the IRMS vial filling

	Pressures [± 0.0001 mbar]	
Cylinder	Atmospheric Flask Extraction	IRMS Vial System Extraction
1	6.91	0.0607
2	3.13	2.40
3	4.53	-