

Centre for Isotope Research
(CIO)

**“Developing methods
to measure the $\delta^{13}\text{C}$ and
 $\delta^{18}\text{O}$ compositions of
extracted CO_2 -samples
from the Amazon
region.”**

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Abstract:

The research project aims at developing methods allowing measurement of the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ compositions of extracted CO_2 -samples from the Airbone Stable Isotopes of Carbon from the Amazon (ASICA) project. Methods were developed for two different instruments: an optical spectrometer, the Stable Isotope of CO_2 Absorption Spectrometer (SICAS), and the Dual-Optima, an isotope ratio mass spectrometer (IRMS). Since the SICAS only measures CO_2 isotopes in ambient air, the extracted CO_2 has to be diluted to the atmospheric concentration of ~ 400 ppm which is done on glass line specifically designed for this purpose. The IRMS can measure CO_2 isotopes from the extracted CO_2 -samples of ASICA. Since the IRMS is water sensitive, and many of the ASICA samples still contain water, a drying step is required. The drying step was developed containing magnesium perchlorate. Results from SICAS and IRMS were analyzed and were compared with each other. It turned out that the SICAS had a satisfactory reproducibility with high precision, and probably high accuracy. The IRMS had large uncertainties in its calibrations, so the results could not be corrected and therefore further analyzed. Without this drying step, the IRMS also gave a good result with acceptable precision and probably good accuracy. Finally, there still were large uncertainties in the drying step for the IRMS.

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1. Introduction:

1.1 Carbon cycle and isotopes of CO₂

The increase of atmospheric CO₂ is the main reason for the already observed and predicted global warming. Nowadays global climate is 1.0°C warmer than pre-industrial levels with a likely range of 0.8°C to 1.2°C (IPCC, 2018). Since the industrial revolution, the atmospheric carbon dioxide (CO₂) has been increasing rapidly from 280 ppm to 413 ppm at present (Lindsey, 2020; CO₂.Earth, 2020). The biggest part of the increase of CO₂ is caused by humans known as anthropogenic emissions. There are two primary sources of these anthropogenic emissions. The first is from the combustion of fossil fuels and CO₂ emissions from industries, which contributes 88% of the total carbon emissions (Le Quéré et al., 2018). Fossil fuels originate from organic substances from millions of years ago. The combustion of these fossil fuels leads to the production CO₂ (and also water). Since the industrial revolution, humans have increased their fossil fuel consumption gradually, from 3.0 Peta-grams of Carbon (PgC) annual from 1960 to 1969, till 10 PgC in 2018 (Friedlingstein et al., 2019).

The other primary source of anthropogenic emissions is land-use change. The land-use change contributes 12% of the total carbon emissions (Le Quéré et al., 2018). During pre-industrial times, anthropogenic land-use resulted in an accumulated carbon release of 114 PgC, while 148 PgC was emitted during the industrial time from 1850 until 1990 (Olofsson & Hickler, 2007). Nowadays land-use change remains a net source, emitting around 1.5 PgC annually (Friedlingstein et al., 2019) and contributes to the increase of CO₂ concentration in the atmosphere.

Three main sinks for CO₂ from the combustion of fossil fuels, land-use change and all other natural CO₂ sources are the biosphere/land, the ocean and the atmosphere. Out of the 100%, the ocean captures 22% of emitted CO₂ in compare to the biosphere/land part with 28%. The rest of the emitted CO₂ stays in the atmosphere which is 44% (Le Quéré et al., 2018). There is a remaining unattributed budget imbalance of 5%, therefore the uncertainties in the sinks are considerable. The storage in the biosphere/land is a considerably high part of the total carbon sink. The Amazon rainforest is one of the foremost critical ecosystems of the Earth and occurs 45% of the world's tropical forest. Globally, around 20% of carbon residing in terrestrial

vegetation occurs in the Amazon rainforest. The Amazon rainforest processes about three times as much carbon through photosynthesis and respiration as anthropogenic released by fossil fuel combustion into the atmosphere (Malhi et al., 2002).

Vegetation dynamics in the Amazon rainforest can be described by the slight imbalance of the net ecosystem exchange (NEE) of CO₂, which is equal to the gross primary production (GPP) whereby CO₂ has assimilated from the atmosphere, minus the terrestrial ecosystem respiration (TER) whereby CO₂ has released into the atmosphere. In other words, the equation is $NEE = GPP - TER$. When GPP is known, rather estimating or predicting, then predictions of atmospheric CO₂ concentrations in a changing climate with more severe droughts will be improved (Ciais et al., 2005; Gatti et al., 2014). In the next paragraph, it will be described how plants, also in the Amazon regions, adapt their water-use efficiency under their increasingly drier environment. This leads to a massive decrease of GPP and a TER reduction from plant respiration and the microbial soil respiration which results in a reduced net carbon uptake (NEE). GPP is very hard to determine, because the change that is observed in the atmosphere, is atmospheric NEP. This atmospheric NEP is just a part of the sink atmosphere for CO₂, and the development of NEP with changing, increasingly drier, the climate is an important feedback mechanism to GPP.

Isotopes

Isotopes are atoms with the same number of protons and electrons, but these atoms contain different numbers of neutrons. The different numbers of neutrons cause different masses of isotopes. There are no different chemical properties of the isotopes since the isotopes contain the same numbers of electrons and protons. Isotopes, therefore, undergo the same chemical and physical reactions (Hoefs, 2008). Differences in mass, however, lead to slightly different behaviour in many processes. Examples are the rate of chemical, biological, or physical reactions, and this can lead to the partitioning of isotopes differentially among phases. Isotopic distribution during these different processes is called isotopic fractionation (Hoefs, 2008). The isotopic fractionation also takes place during the uptake of CO₂ by photosynthesis in C₃-, C₄- and CAM-plants (O'Leary, et al., 1992). There is a ratio of the rare isotope and the abundant isotope and is called an isotope ratio. Since natural variations in isotope ratios are small-scale, the delta (δ) notation is used. The δ notation expresses the variation of an isotopic

ratio of an element (in R_{sample}), relative to the isotopic ratio of a standard/reference (in $R_{\text{reference}}$). An element R stands for the heavy/light isotope ratio for element R. The δ notation is expressed in permille, in ‰ (Slater, 2001). The formula for the delta notation of ^{13}C is:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} \right) * 1000 = \left(\left(\frac{R_{\text{sample}}}{R_{\text{reference}}} \right) - 1 \right) * 1000$$

This delta notation was invented to have a convenient and readable way to understand the variability of isotopic ratio in many natural systems in which the range is in the third to the fifth decimal place. In this research, the main focus will be on isotopic ratio carbon ($^{13}\text{C} / ^{12}\text{C}$).

Each of C3-, C4 and CAM-plants also have their characteristic isotope ratio of carbon. The C3 plants have 3-phosphoglycerate (3PG), which contains 3 carbon atoms, as the first stable product of C3 cycle or Calvin cycle for the dark reaction of photosynthesis. These plants can assimilate CO_2 through photosynthesis (Liang, Wang & Li, 2012). The C3 plants are the majority of plants that occur on earth (~95% of total green plants). These C3 plants are cool-season plants and commonly seen in cool and wet areas. They are because of this reason abundant in temperate conditions. There are also C4 plants which have oxaloacetic acid (OAA) as the first stable product instead of 3PG at C3 plants. These C4 plants have a different C4 cycle or Hatch-Slack Pathway for the dark reaction of photosynthesis. These plants are warm-season plants, and therefore commonly seen in tropical conditions.

Finally, there are CAM plants too. These CAM plants utilize both carbon fixation pathways in C3 and C4 plants. They are adapted to dry environments and can be therefore found in very hot, dry areas like deserts (Lüttge, 2004). In this report, it is especially focussed on C3 and C4 plants which are found in the Amazon regions.

Both C3- and C4-plants have a preference for $^{12}\text{CO}_2$ isotopologues instead of $^{13}\text{CO}_2$ isotopologues. C3-plants have a relatively stronger preference than C4-plants. The reason for this preference from both plants is that $^{12}\text{CO}_2$ isotopologues are slightly lighter than $^{13}\text{CO}_2$ isotopologues. This makes the C3- and C4 plants absorb $^{12}\text{CO}_2$ isotopologues easier than $^{13}\text{CO}_2$ isotopologues (Keeling et al., 2017). The preference of these plants causes a seasonal change of ratio $^{13}\text{CO}_2$ isotopologue over $^{12}\text{CO}_2$ isotopologue in the atmosphere above the terrestrial

area. When there is a drought, then the plants will increase their water-use efficiency and adjust to their increasingly drier environment. These plants are stressed and therefore cannot absorb as much CO₂ to perform photosynthesis as they usually do (Green, J.K. et al, 2019). This will cause a shift in the isotopic preferential pathway in the plants as well (Farquhar & Richards, 1984).

The change in the isotope ratio in the atmosphere can also be caused by different CO₂ resources that add CO₂ additionally into the atmosphere. Combustion of fossil fuel is an example of this input. This will cause a change in the isotope ratio in the atmosphere. All sources and sinks have characteristic values for the isotopic ratio. The signal of the isotope ratio of ¹³C/¹²C is, therefore, a useful tracer for the sources and sinks of the atmospheric carbon cycle.

1.2 ASICA-project

The EC-funded project "Airbone Stable Isotopes of Carbon from the Amazon" (ASICA, granted to Prof. Wouter Peters) aims to create the first estimate of pan-Amazonian GPP by the collection of more than 5,000 air samples at four different locations above the Amazon regions. These samples were collected, in an early stage of the project, from unpressurized aircrafts that fly up to 6 km altitude.

After capturing air, the air was analysed and prepared at the laboratory of Dr. Luciana Gatti, at the National Institute for Space Research (INPE), São José dos Campos, Brazil for its CO₂, CH₄, N₂O, SF₆ and CO mole fractions. After the analysis, CO₂ was extracted from samples and conserved as highly concentrated CO₂ in flame-sealed tubes and were sent to the CIO. At CIO, these 5,000 samples should be measured for their isotopic composition. In this research, there are two different measuring instruments which can measure the δ¹³C and δ¹⁸O ideally also δ¹⁷O values of these ASICA samples.

These flame-sealed tubes contain highly concentrated CO₂. However, many of them, unfortunately, were not properly dried, and thus contain traces of H₂O. All of them also contain N₂O. N₂O has identical behaviour under cryogenic extraction as CO₂ and is therefore inevitably co-extracted from the air.

In this research atmospheric NEP is measured and calculated. This will help to determine NEP of the Amazon region and therefore the determination of final GPP can be improved hereby. When GPP is known then predictions of atmospheric CO₂ concentrations in a changing climate with severe droughts will be improved.

1.3. Aims of this research

In this research project, the main goal is to develop methods to be able to measure the isotopic composition of small highly concentrated Amazonian CO₂ samples at high precision and accuracy. The instruments that measure the isotopic compositions of the ASICA samples are the Stable Isotope of CO₂ Absorption Spectrometer (SICAS), and the Isotope Ratio Mass Spectrometer (IRMS). The SICAS measures the isotopic composition by dual laser absorption spectroscopy and IRMS measures the isotopic composition by using the difference in mass of the isotopologues of CO₂. For both instruments, methods must be developed to be able to measure the isotope composition of the ASICA samples.

The SICAS can only measure samples at atmospheric concentrations. The atmospheric CO₂ concentration nowadays is around 400 ppm (Showstack, 2013). That is why the sample firstly needs to be diluted to this concentration. The aim is having samples of around 400 ppm with max. 5% uncertainty which means max. ± 20 ppm standard deviation. For the SICAS, this research targets to have a well-designed preparation system to be able to dilute the CO₂ ASICA-samples to the atmospheric concentration and then measure on the SICAS afterwards.

For the IRMS, the goal is designing a method to be able to transfer the extracted CO₂-sample from a flame-sealed tube into the IRMS. The IRMS can measure the isotope compositions from pure CO₂. To release the sample from the flame-sealed tube, a breaker is required. Moreover, the objects that are created, need to be as small as possible due to the amount of sample that is needed for IRMS measurements. The sample size of the ASICA samples is barely enough for measuring it on the IRMS, and that is why the size of objects like the breaker should be as small as possible too. Since many of the ASICA samples still contain water and the IRMS is water sensitive, a drying step is required to remove water from these samples. In this research, a drying step is developed containing magnesium perchlorate. Another goal for the IRMS is

measuring the ASICA-samples from one of the four collection sites above the Amazon region on the IRMS.

When all varieties of methods for these two instruments are standardized and uncertainties are reduced, then the test-samples can be prepared, measured and analysed on both instruments. These test-samples are flame-sealed tubes filled with local reference CO₂ that is known for its isotope compositions at the Centre for Isotope Research (CIO) of the University of Groningen. The results of test-samples from both instruments are compared with the data of the certain CO₂ that is known at the CIO. Finally, these results from both instruments are compared with each other as well.

2. Methods

2.1 Stable Isotope Measurements Devices

In this research there are two different instruments to measure isotopes compositions in the samples; Stable Isotope of CO₂ Absorption Spectrometer (SICAS) and Isotope Ratio Mass Spectrometer (IRMS). The extracted CO₂ samples prepared at the laboratory of Dr. Luciana Gatti can be measured on these instruments.

The first instrument that will be described is SICAS. This is designed for direct CO₂ isotopes in ambient air, using optical spectroscopy. That is why the sample of the extracted CO₂ needs to be diluted to the atmospheric concentration to be able to use the instrument.

Then another instrument, IRMS, will be described. In this research, IRMS is used as a Dual-Optima. This instrument can measure isotopic compositions directly from pure CO₂ sample and that is why the ASICA sample firstly needs to be dried before measuring it for its isotopic compositions.

2.1.1. Stable Isotope of CO₂ Absorption Spectrometer (SICAS)

The first instrument for measuring stable isotopes of the CO₂ sample is the Stable Isotope of CO₂ Absorption Spectrometer (SICAS). The SICAS, which is called as CIO-DL-SICAS at the CIO, measures air samples with CO₂ concentrations in the atmospheric range for its isotope compositions. The CIO-DL-SICAS is a dual laser absorption spectrometer from the company

Aerodyne Research Inc. In figure 1, the optical board of the SICAS is shown. The instrument consists of two tunable quantum cascade lasers (QCL) (Nanoplus) (nr. 1 in figure 1) operating in the mid-infrared region (MIR). Moreover, there are also optical cell, two detectors and several mirrors in the instrument to combine and deflect the laser beams. The isotopologues that are measured on this SICAS are $^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{C}^{16}\text{O}_2$, $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ and $^{12}\text{C}^{16}\text{O}^{17}\text{O}$. These isotopologues will be called as 626, 636, 628 and 627, following the HITRAN database notation (Rothman et al., 2013). Laser 1 operates in the spectral range of 2350 CM^{-1} ($4.25\text{ }\mu\text{M}$) for the measurement of 627 (and 626) and laser 2 operates in the spectral range of 2310 CM^{-1} ($4.33\text{ }\mu\text{M}$) for the measurement of three isotopologues 626, 636 and 628. These two lasers are sending beams into the optical cell and the laser beams go multiple times in the optical cell by reflection on two mirrors installed on both ends of the cell reaching a total path length of 36 meters. When the beams exit the optical cell then this will be detected by two TEC-cooled infrared detectors (nr. 2 in figure 1) where the signal is measured from the lasers in the spectral range as it shows in figure 2.

These detectors, the optical cell and the lasers are all in housing where it is continuously flushed with nitrogen gas (N_2). This is to avoid that any other absorption of CO_2 than from gas in the optical cell is made. Then the software TDLWintel (McManus et al., 2005) derives the absorption spectra whereby the measured signal is fitted based on known molecular absorption profiles from the HITRAN database (Rothman et al., 2013) as it shows in figure 2. The mole fractions of these four isotopologues can be calculated by the TDLWintel software on basis of the integration of the peaks at the specific wavelengths, measured pressure and temperature in the optical cell and the constant path length.

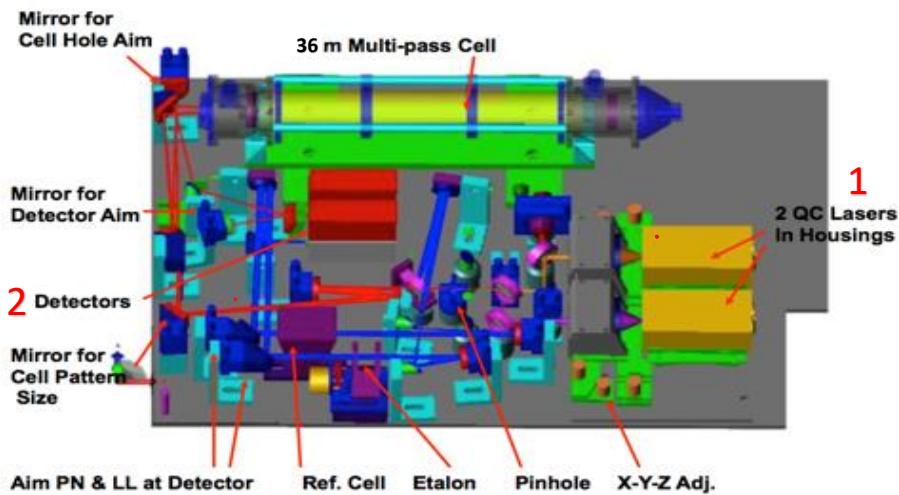


Figure 1; Optical board of the instrumental set-up of the CIO-DL-SICAS (figure adapted from Aerodyne Research, Inc).

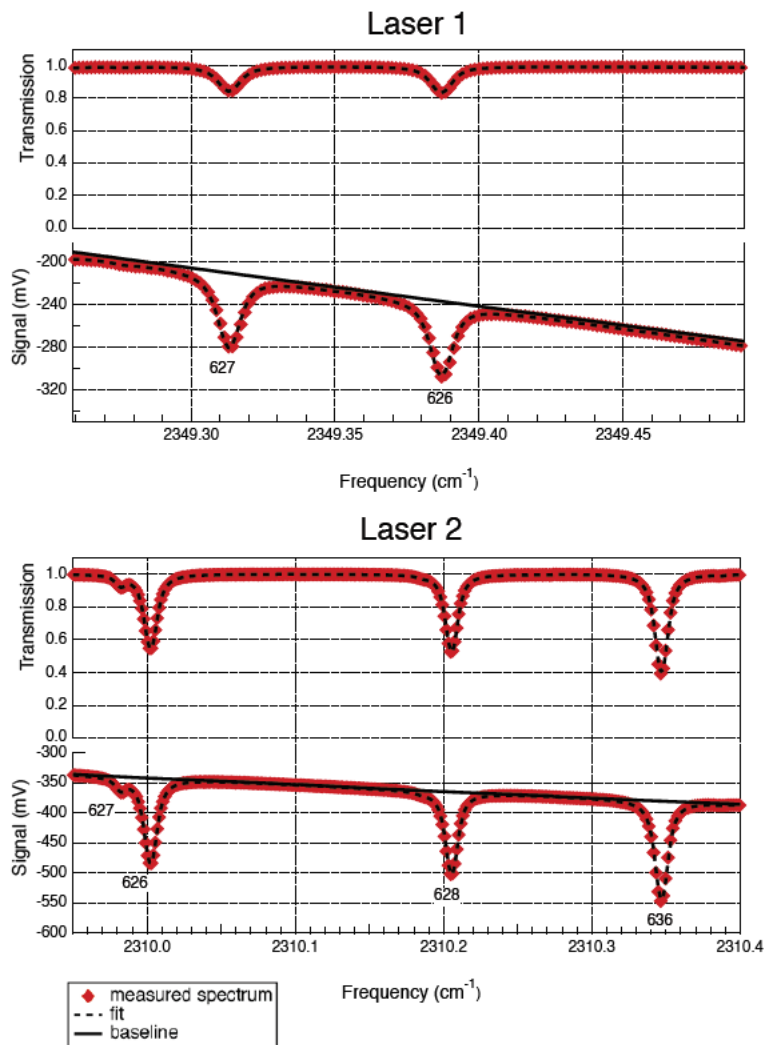


Figure 2; Absorption spectrum of laser 1 (top panel) for measurement of 627 and 626, and laser 2 (lower panel) for measurement of 626, 628 and 636.

The gas inlet system of the SICAS is shown in figure 3, and it was designed, whereby different discrete air samples can be measured by switching quickly between these measurements. The closing and opening of the valves are controlled by the TDLWintel software. There are three different inlet ports (nr. 11, 14 and 18 in figure 3) in this system and these ports are connected to the inlet volume. One of these inlet ports (nr. 11 in figure 3) is connected to a 1/8" VICI multivalve (Valco 90 Instruments) with 15 potential positions for flask samples and/or cylinders. To avoid cross-contamination in the inlet volume between different samples, valves 9 and 10 are used for a flushing procedure whereby it is pumped out by a vacuum pump. Furthermore, also valves 22 and 23 are used to evacuate the optical cell. Afterwards, the gas from the inlet volume can be immediately brought into the optical cell by opening valves 19 and 23 (and valve 22 must be closed now). The gas is measured for a period of around 60 seconds in the optical cell. The pressure in the optical cell can affect peak shapes and intensities of the isotope ratio results, and has, therefore, to be kept as stable as possible, and is regulated by the software. Instabilities in the optical cell pressure can cause by too high pressure of sample and by the flow into the optical cell is too fast and/or timing of the sealing of the cell is off. Based on previous experiments the sample pressure should therefore be no more than around 1 bar to have a stable pressure in the optical cell stable, as it shows in figure 4. The sample pressure cannot be lower than 0.22 bar, because then the pressure is too low to fill the optical cell, this is also shown in figure 4.

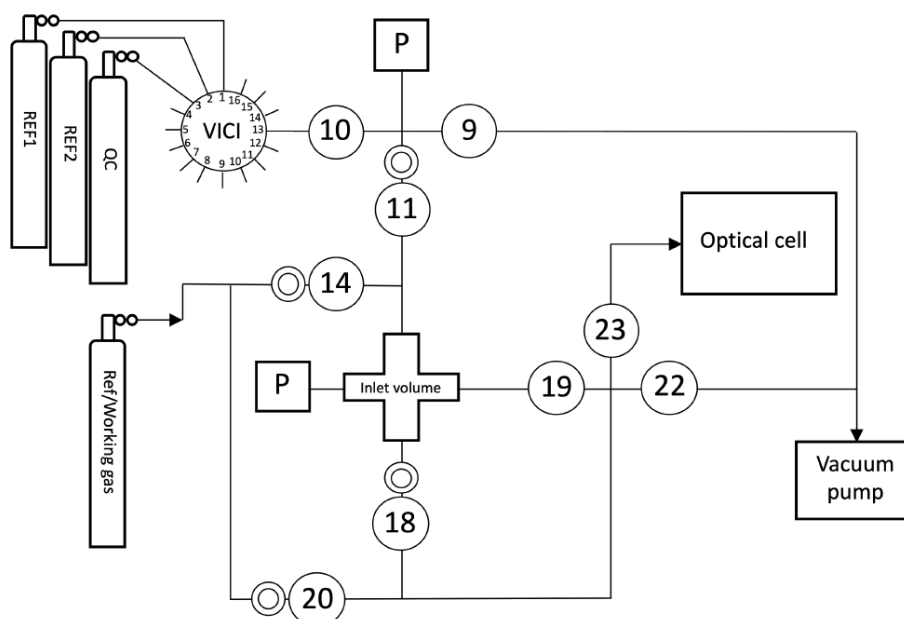


Figure 3, gas inlet system of the SICAS. In this figure valves are visualized as numbered circle.



Figure 4, pressure stability in the optical cell of the SICAS and sample pressure. This chart shows the results of pressure in the optical cell versus the sample pressure. Moreover, this chart shows that the pressure of the sample has to be around 1 bar to have a stable pressure in the optical cell. Finally, the red highlighted range of the chart shows the most stable pressure of the optical cell.

2.1.2. Isotope Ratio Mass Spectrometry (IRMS)

This is a conventional way to measure stable isotopes of CO₂ using the difference in mass of the isotopologues of CO₂ to separate them and detect the abundances. The abundances of molecules with molecular masses of 44, 45 and 46 Da in a pure CO₂ gas sample can be measured by Standard Mass spectrometers. At the CIO, there is a Dual-Inlet (DI) Mass Spectrometer that measures the stable isotopes of CO₂ from pure CO₂ gas sample. This spectrometer measures alternately a reference gas and a sample which are stored in two separate bellows as shown in figure 5. Dual inlet mass spectrometers enable rapid switching between reference measurement and sample measurement. As the delta values are calculated by $(R_{\text{sample}}/R_{\text{ref}})-1$, instrumental error will be cancelled out as both the sample and the reference measurements are drifting in the same way. Furthermore, this reference gas entered the IRMS in the same way as the sample, as it shows in figure 5. The two bellows with the reference gas and sample are connected by two capillaries to a switching block, also called as 'change-over valve'. This enables the rapid switching between sample and reference measurements. To have continuous flow through both capillaries, the switching block leads

one gas flow to the ion source, while the other capillary is led to a 'waste line', that is pumped down. Electrons are produced at the ion source and are in interaction with the CO₂ molecules. This causes ionization of the molecules and then these ionized molecules are accelerated to a few keV. Then these molecules are separated by a magnetic field: the mass differences lead to different bending of the molecules' paths. The currents of ionized molecules are detected in faraday cups in the ion collector assembly. For a sample measurement, several iterations of sample and reference measurements are conducted, and for every successive measurement of sample and reference, a delta value is calculated of which the mean value is calculated. Then the delta value is calibrated against a primary reference for expression of the isotope composition on the VPDB scale (Sharp, 2007; Werner & Brand, 2001). In this research in-house working standards GS-19, GS-20 and GS-25 are used as a primary reference, which is thus expressed the isotope composition on the VPDB scale.

To derive the ¹³C and ¹⁸O content of CO₂, some assumptions have to be made about the abundances of molecules with mass 44, 45 and 46 Da. The ion beam at mass 44 Da represents ¹²C¹⁶O₂ isotopologue only. Two isotopologues, ¹³C¹⁶O₂ and ¹²C¹⁶O¹⁷O, have the same molecular mass of 45 Da. That is why the ¹³C/¹²C ratio that is derived from the mass 45/44 current ratio should be corrected for ¹²C¹⁶O¹⁷O. In natural samples, ¹²C¹⁶O¹⁷O occupies around 6.5% of the isotopologues with mass 45 Da (Werner & Brand, 2001). Finally, the mass for three isotopologues ¹²C¹⁸O¹⁶O, ¹³C¹⁷O¹⁶O and ¹²C¹⁷O₂ is the same and that is 46 Da. To derive these three isotopologues for ¹³C / ¹²C isotope ratios, it can only be calculated by correcting the raw isotope ratio 45/44 Da with the participation of ¹⁷O-bearing molecules. This participation of ¹⁷O-bearing molecules of 45 Da can be calculated from an assumed relationship between ¹⁷O and ¹⁸O isotopic abundances and the ¹⁸O abundance as determined from the isotopic ratio 46/44. This ¹⁷O correction algorithm on the basis of the current knowledge of the three-isotope relationship for oxygen atoms is also called as 'mass dependent fractionation path' (Assonov & Brenninkmeijer, 2003).

Moreover, CO₂ is extracted cryogenically from the air when measuring atmospheric samples with CO₂. N₂O is extracted at the same time as CO₂ because it has the same molecular mass and very similar physical properties. That is why the CO₂ sample contains N₂O as well. Since this has the same molecular masses as CO₂, it will also be detected by the IRMS. This will

therefore influence the measured ion intensities as well (Sirignano, et al., 2004; Werner & Brand, 2001). This is why N₂O corrections should be taken into account to the N₂O/CO₂ ratio in the sample and the ionization efficiency ratio of N₂O over CO₂ which differs from instrument to instrument. This might also be changing over time (Ghosh & Brand, 2004; Sirignano et al., 2004). Typically, the influence of N₂O on the isotopic values for CO₂ is from 0.20 to 0.22‰ for d¹³C and from 0.29 to 0.32‰ for d¹⁸O (Sirignano et al., 2004).

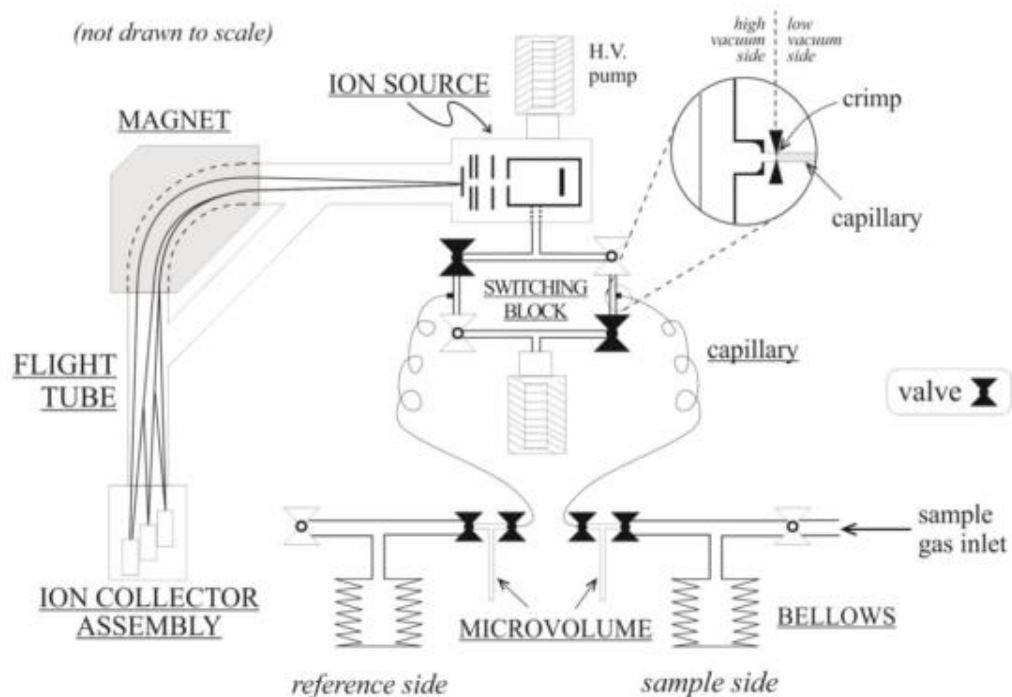


Figure 5, Dual-Inlet Mass Spectrometer, DI-Optima (Sharp, 2007).

2.2. ASICA Samples

The CO₂ sample was extracted and captured in a flame-sealed tube. At CIO this extracted sample needs to be released from the flame-sealed tube, so both instruments can measure the isotopic compositions of the sample. For each of the instruments, there are different methods developed to release the sample. For the SICAS, a cracker is developed and for the IRMS a breaker and drying step are developed.

At the SICAS, a cracker is developed where it is connected to the preparation system through glass tubes. Moreover, this cracker is also connected to a home-built pressure sensor. This

sensor can measure the pressure in the cracker. The flame-sealed tube firstly needs to be carved, then it is plugged just over the notch of the cracker. The notch is especially meant to hold the tube in balance in the cracker and makes it easier to crack the carved tube, as it shows in figure 8.

At the IRMS, a breaker and drying step are developed. As described earlier in this report, all objects that are developed, have to as small as possible because of the sample size of the ASICA samples. A tiny breaker is developed that connects to a glass tube with magnesium perchlorate to remove water from the ASICA sample. This tube with magnesium perchlorate also connects with the IRMS, so the dried sample can flow into the instrument directly after the drying step.

2.3. Sample Preparation

After releasing the ASICA sample from a flame-sealed tube, there are different methods developed to get these samples prepared for measuring it on both instruments. For each of the instruments, the sample needs to be prepared at different methods.

At the SICAS, the ASICA-samples need to be diluted first, so it will be measurable on the SICAS.

For the IRMS there is less preparation needed for the sample. It only requires developing a method to translocate the sample from the flame-sealed tubes into the IRMS without contamination during translocating. Furthermore, this method also requires a drying step to remove water from the sample.

2.3.1. Stable Isotope of CO₂ Absorption Spectrometer (SICAS)

The SICAS was developed for measuring whole air samples with an atmospheric CO₂ concentration of around 400 ppm. The CO₂ concentration of the sample cannot deviate too much from 400 ppm, because the concentration of calibrations in the SICAS is around 343 ppm, 416 ppm and 424 ppm. That is why the aim is set up to produce samples of around 400 ppm with max. 5% standard deviation, thus max. 20 ppm.

This is the reason that ASICA-samples need to be translocated in another housing. Then the sample in that new housing can be diluted with a gas mixture similar or CO₂ free natural air till it reaches the atmospheric CO₂ concentration of around 400 ppm. In this research, a 0.8-liter-flask is used as new housing for the sample. Afterwards, the diluted sample in the 0.8-liter-flask is measured for their $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (and ideally also $\delta^{17}\text{O}$) values on the SICAS.

Before translocating the sample into the 0.8-liter-flask, the sample firstly needs to be dried. There are two different drying methods which were used to remove water from the wet samples in this research project. The first method has been developed already; glass vacuum line called as freeze finger. Moreover, a dewar (nr. blue 10 in figure 6) mixed with dry-ice of around -80°C and 96% ethanol is placed around the freeze finger to trap the water from the sample.

Another, a new, method was developed during this research using magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$) to check whether it would also work as a water trap for the ASICA-samples. There was an experiment which contained eight test-samples. Four of these samples were prepared with the freeze finger and the rest was prepared with the new drying method. For this new drying method, a glass tube with magnesium perchlorate was developed.

After this experiment, it was decided that all samples would be prepared with the freeze finger as the drying method. It was more convenient and faster to prepare samples with the freeze finger than the drying method containing magnesium perchlorate. When the freeze finger is used as the drying method, then the system will evacuate considerably faster. These tests with a magnesium perchlorate drier were less successful and therefore there was chosen to use the freeze finger.

Due to this requirement of dilution of the CO₂ sample to around 400 ppm, it needs to check if this dilution process is going well. This can be done with two different instruments where the CO₂ concentration can be measured; Gas Chromatography and Picarro. If the 0.8-liter-flask does contain atmospheric concentration of CO₂, then it is ready to measure on the SICAS for their $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ values.

The first instrument where the CO₂ concentration can be measured is Gas chromatography (GC). This is an Agilent HP 6890N GC (HPGC) which is commercially available. This HPGC-FID system has a set-up similar to the GC-system described by van der Laan et al. (Van der Laan, Neubert, & Meijer, 2009). The GC has been modified to be able to measure with higher precision and long-term stability of the atmospheric mixing ratio of greenhouse gases CO₂, CH₄ and CO from the same sample at the same time.

The precision is <0.1 ppm for CO₂ and other greenhouse gases: <1 ppb for CH₄ and <2 ppb for CO and calibration is done using three working standard cylinders that were linked to the internationally recognized scale provided by the WMO.

There is another instrument to measure atmospheric CO₂ mixing ratios. It is a Picarro of Inc., CA, USA, model G2401-m using the cavity ring-down spectroscopy (CRDS) technique (Chen et al., 2010). The precision is <0.1 ppm for CO₂, for CH₄ <0.6 ppb and for CO <2 ppb.

Picarro takes less time to measure the CO₂ concentration of the samples, compared to GC. Samples do not need to be overnight at Picarro to be measured. At Picarro, it only takes around 5 minutes for measuring one sample. Picarro cannot measure multiple samples at the same time. Every five minutes, the sample has to be replaced with another sample. Compared to GC, Picarro would take around 45 minutes to measure eight samples. At GC, multiple samples, with a maximum of eight samples, can be overnight to be measured all of the samples which takes around 8 hours.

2.3.1.1. Sample Preparation System of SICAS

As described earlier in this report, the ASICA-sample in the flame-sealed tube needs to be diluted and the water vapour needs to be removed from the sample. The highly concentrated CO₂ of ASICA-sample needs to be diluted with CO₂ free natural air to the atmospheric CO₂ concentration close to 400 ppm. First, the ASICA-sample is transferred from the flame-sealed tube into a 0.8-liter-flasks, where the sample can be diluted. The diluted sample can be measured on the SICAS afterwards. This requires a well-designed preparation system to produce 0.8-liter-flask with diluted ASICA-sample at atmospheric values. This preparation system is shown in figure 6.

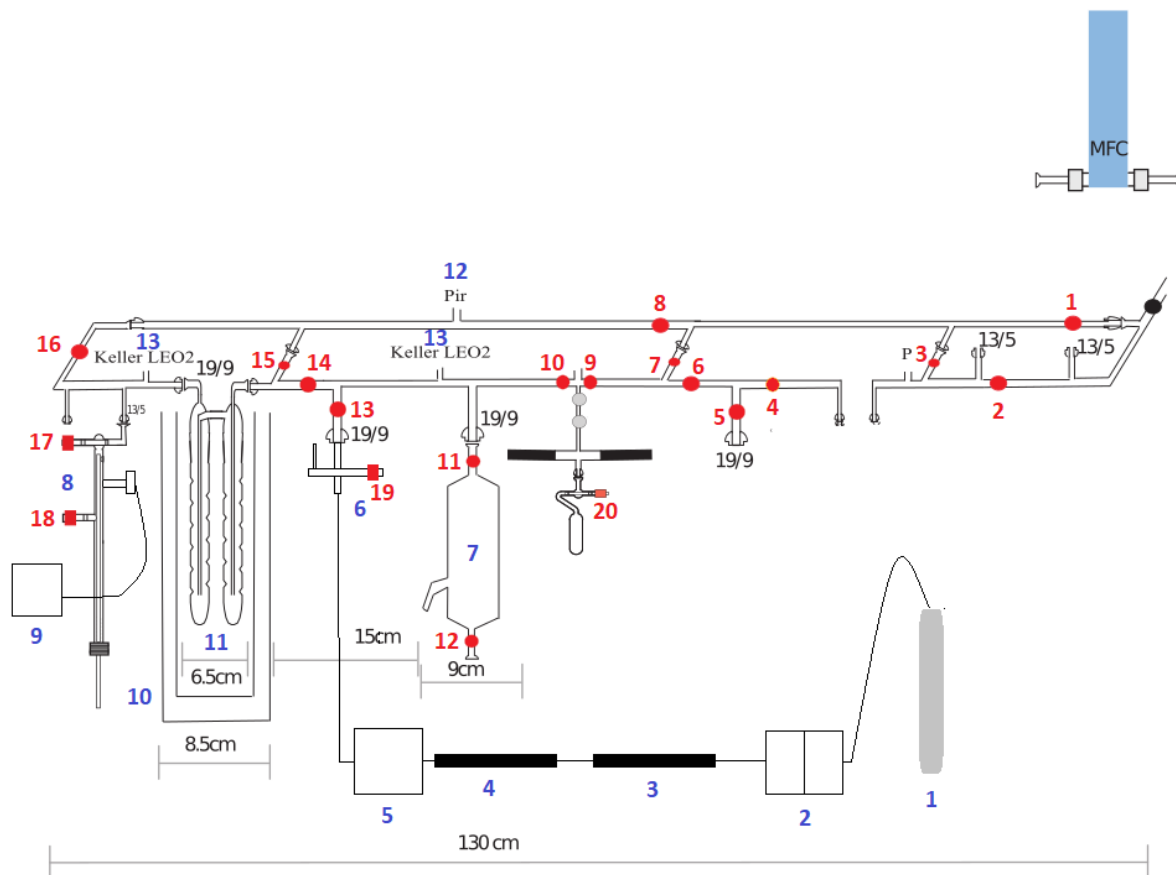


Figure 6, the preparation system where the test- and ASICA-samples are prepared. The sample can be transferred from the flame-sealed tube in the cracker (nr. blue 8) to another housing (nr. blue 7) and be diluted with the CO₂ and H₂O free air till a certain pressure is reached in the housing. All red dots (nr. red 1-16) and red squares (nr. red 17-20) stand for valves that can be closed and opened.

A cylinder (nr. blue 1 in figure 6) containing natural air connects to the preparation system. To be able to flow natural air into the system, the flow controller was installed as it shows nr. blue 2 in the figure. When the natural air is flowed into the system, it first goes through a tube with ascarite (nr. blue 3 in figure 6) to remove all CO₂. After the ascarite trap, the CO₂ free natural air goes through the water trap (nr. blue 4 in figure 6), which is a tube contains Sicapent® with indicator (phosphorous pentoxide drying agent for desiccators) to remove all water from the ambient air. Now, this air contains virtually no CO₂ and H₂O anymore and is ready to fill the 0.8-liter-flask with extracted CO₂-sample of ASICA. The mass flow controller (nr. blue 5 in figure 6) can be installed, so the air can be flowed at a certain flow velocity (in liter/min) into the preparation system (which takes place at nr. blue 6 in figure 6). For this research, it was investigated whether the natural air would still contain CO₂. It did, but very limited and the average CO₂ concentration was around 0.13 ppm with standard deviation of 0.11 ppm.

It is important that there is no contamination in the preparation system. The contamination of undesirable gasses during the preparation of samples can cause a disturbance of measuring the sample. The system is evacuated using a turbo-molecular pump (Drytel 1025, Alcatel), which is shown in figure 6 as 'MFC'. This can pump directly from atmospheric pressures all the way down to around $1.33 \cdot 10^{-6}$ millibar (Ideal Vacuum Products LLC, 2020). Between the preparation system and the Alcatel pump there is a vacuum pressure sensor (MKS series 910) that helps to control the evacuation process (shows as nr. blue 12 in figure 6).

In figure 8, a cracker is shown and this is also shown in figure 6 as nr. blue 8. This is where the small flame-sealed tube with ASICA-sample is cracked. First, the tube with ASICA-sample needs to be carved at the place where the glass pin touches the tube in the cracker. Then the tube can be plugged into the cracker. The ultra-torr connection must be very tight, to make sure that there is no leak from the preparation system. Now the tube with the ASICA sample can be cracked and the ASICA-sample will be released into the cracker from the tube. A home-built pressure sensor in the cracker (nr. blue 9 in figure 6) registers the pressure with a resolution of 1 mbar.

Before translocating the ASICA sample into a 0.8-liter flask (nr. blue 7 in figure 6), the sample first needs to be dried. A freeze finger (nr. blue 11 in figure 6) is installed between the cracker and the 0.8-liter-flask. In figure 7, the 0.8-liter-flask is shown. Due to the production process of the 0.8-liter-flasks, their volumes vary around 0.8 liters by ± 25.8 ml.

There is a CO₂ trap in the 0.8-liter-flask and this happens in the side arm of the 0.8-liter-flask. In figure 7, the side arm of the 0.8-liter-flask is shown. The side-arm is put in the dewar with liquid N₂ (LN₂), so the temperature will drop to around $\sim -200^\circ\text{C}$. Since the freezing point of CO₂ is -78.46°C , CO₂ from the cracker will be trapped in the side arm where it is around $\sim -200^\circ\text{C}$. After around 5 minutes all CO₂ from the ASICA-sample should be trapped in the side arm of the flask, then the dewar with LN₂ is removed. Now the CO₂ and H₂O free air (from nr. blue 6 in figure 6) can be flowed into the 0.8-liter-flask while the CO₂ remains trapped in the side arm of the flask. Inflow of CO₂ free air is controlled with a mass flow controller that is installed at flow of 0.450 L/min, so there is enough time to close the sample flask to reach the preferred pressure. Furthermore, it is undesired to have a high flow as all CO₂ has to be

removed from the ambient air. If there is a considerably high flow, then the chance exists that not all CO₂ is removed by the ascarite. In the right part of the preparation system, just before the 0.8-liter-flask, there is a pressure sensor Keller LEO2 (nr. blue 13 in figure 6) that can measure the pressure when filling the 0.8-liter-flask with the CO₂ and H₂O free air. The Keller LEO2 sensor measures the pressure twice per second and its accuracy is ±0.1% over the temperature range of 0 to 50°C. It has a resolution of 1 millibar (Keller AG für Druckmesstechnik, 2018). This pressure sensor is also present in the left part of the preparation system and was placed between the cracker and the freeze finger.

After the filling process is ready, the flask is closed and the trapped CO₂ becomes gaseous again. To make sure that CO₂ and the CO₂ free air are mixed thoroughly, the flask is allowed mixing for at least 12 hours before measuring on the SICAS.

When the ASICA (test-)samples are prepared, then these samples will be measured on the SICAS. In addition, in-house air working standards will also be measured, which are coupled to the VPDB-CO₂ scale since these are measured in the central calibration lab in Jena (Wendeberg et al., 2013). After the measurement, the isotopes compositions of the samples can be corrected by data of in-house air working standards.

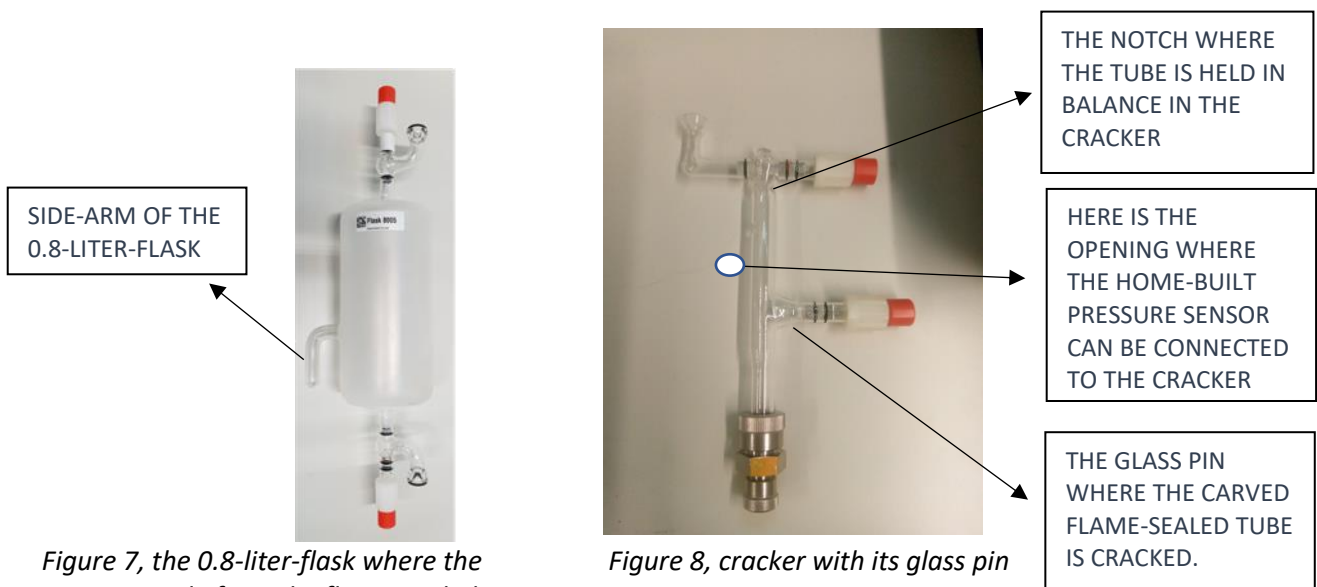


Figure 7, the 0.8-liter-flask where the ASICA-sample from the flame-sealed tube is translocated into. Then this flask needs to be filled with the CO₂ and H₂O free air till a certain pressure in the flask.

Figure 8, cracker with its glass pin

Now all parts of the preparation system have been described. The next step is filling the 0.8-liter-flask contained ASICA sample with the CO₂ free air till it reaches the atmospheric CO₂ concentration of around 400 ppm. The Boyle's law is used to figure out how much the pressure should be in the 0.8-liter-flask to reach the CO₂ concentration of around 400 ppm. This experimental gas law describes how the pressure of a certain gas tends to increase as the volume of the mentioned gas decreases. The Boyle's law can be used when the pressure and volume are known of a certain and trapped gas and this holds as long as the temperature is constant and amount of the gas remains unchanged within a closed system. Then this certain gas can be compared under two different sets of conditions in the preparation system, and these are cracker and 0.8-liter-flask. For this preparation system, the Boyle's law can be expressed as:

$$\frac{P_{cracker} * V_{cracker}}{T_{cracker}} = \frac{P_{flask} * V_{flask}}{T_{flask}} \rightarrow P_{cracker} * V_{cracker} = P_{flask} * V_{flask}$$

when $T_{cracker} \approx T_{flask}$

In this preparation system, the temperature is overall constant and the gas (CO₂) remains unchanged within a closed preparation system. When CO₂ is translocated from the cracker into the 0.8-liter-flask, the whole intern preparation system is opened. From the cracker till the 0.8-liter-flask, so the highly concentrated CO₂ sample is diffused over the whole system. Then the small box with liquid nitrogen (LN₂) is brought under the side-arm of the 0.8-liter-flask to change the temperature from room-temperature to around ~-200°C to trap the highly concentrated CO₂ sample in that side-arm of the flask. After 5 minutes all of CO₂ should be trapped and then the part with the cracker is excluded from the preparation system. Then the dewar with liquid nitrogen will be removed from the side-arm of the 0.8-liter-flask, so temperature of the side-arm of the flask will increase back to room-temperature. That is how the temperature remains similar/constant under two different sets of conditions.

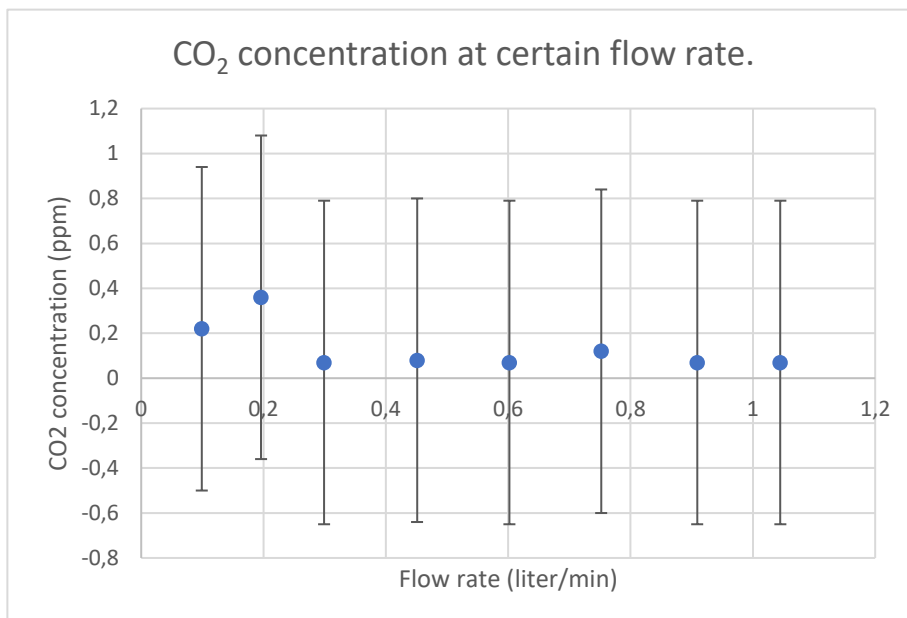
In this preparation system, P_{cracker}, V_{cracker} and V_{flask} can be calculated and measured easily. Only P_{flask}, the pressure in the 0.8-liter-flask, cannot be measured and needs to be calculated based on the Boyle's law. It is necessary to know how much the pressure P_{flask} is, then it can be filled with the CO₂ free air till the highly concentrated CO₂ reaches the 400th part of the million (400

ppm). This means that the pressure of the CO₂ free air will be 1,000,000/400 = 2,500 times higher than the pressure of the highly concentrated CO₂ sample in the flask. To test this, flame-sealed tubes are used that are filled (with a similar amount of highly concentrated CO₂ as the real ASICA samples) with a local reference CO₂ with known isotope values.

2.3.1.2. Flow velocity of the CO₂-free air

Before filling the 0.8-liter-flask with CO₂ and H₂O free air, it is investigated whether different flow rates of CO₂ and H₂O free air would affect the CO₂ concentration in the 0.8-liter-flask. First empty 0.8-liter-flasks are filled with the CO₂ and H₂O free air till it reaches the atmospheric pressure of 1.000 Bar. This will be done at different flow rates.

Eight till 1.000 Bar filled flasks were measured at GC. All of these measurements had a measurement error of 0.72 ppm. In graph 1, it shows the result of these measurements at different flow rates. As the graph shows that at lower rates, especially around 0.100 and 0.200 liter/min, it shows a little higher CO₂-concentration in the 0.8-liter-flask, however these differences were smaller than the measurement error, so the flow rate does not influence the remaining CO₂ concentration in the 0.8-liter-flask.



Graph 1; Results of CO₂ concentration at a certain flow velocity (liter/min) with the CO₂ and H₂O free air to fill the 0.8-liter-flask till 1.000 Bar. Including the measurement error of 0.72 ppm.

This result gives an average CO₂ concentration of 0.13 ppm with standard deviation of 0.11 ppm. Given the constraints: short filling time, least possible remaining CO₂ and accurate control of filling pressure led to the choice of 0.450 l/min. This is a quite high velocity and is still easy to stop the flow when it reaches 1.000 bar in the 0.8-liter-flask. Moreover, 0.450 l/min is a pleasant flow rate, so the chance would be considerably high that all CO₂ is removed by the ascarite.

2.3.1.3. Volumes and uncertainties of the sample preparation system

Now the next step is determining values for the Boyle's formula, hereby the theoretical pressure P_{flask} can be calculated. Then the 0.8-liter-flask can be filled with CO₂ and H₂O free air at flow rate of 0.450 l/min till it reaches the theoretical pressure P_{flask} . This will help to achieve the CO₂ concentration of around 400 ppm for the sample with max. 5% uncertainty, so ± 20 ppm standard deviation.

To get a value for V_{flask} , the exact volume of the 0.8-liter-flask is determined. These flasks were handmade by a glassblower, so they might be varied. There were three 0.8-liter-flask which were measured for its volume. The average volume of these three 0.8-liter-flask is 828.44 ± 0.03 mL. The value for the V_{flask} is therefore 0.82844 L.

To get a value for V_{cracker} , the volume of the cracker is determined including the flame-sealed tube. Moreover, it was decided to plug all the flame-sealed tubes into the cracker at the very same place. This has decreased uncertainty of the cracker's volume. This can be done by marking flame-sealed tubes exactly on the place until where it will be plugged into the cracker. The average volume of the cracker including the volume of the flame-sealed tube is 21.36 ± 0.09 mL. The value for the V_{cracker} is therefore 0.02136 L.

2.3.1.4. Theoretical pressure of the 0.8-liter-flask

Now the values for V_{cracker} and V_{flask} have determined, the theoretical pressure of the 0.8-liter-flask can be calculated. As described earlier in this report, the Boyle's formula is used to calculate the theoretical pressure. Theoretical P_{flask} has to be 400th part of million, makes it 400 ppm CO₂ as the final concentration in the sample, which leads to this formula.

$$P_{tot} = \left(\frac{P_{cracker} * V_{cracker}}{V_{flask}} \right) * \left(\frac{1,000,000}{400} \right) = \left(\frac{P_{cracker} * 0.02136 \text{ liter}}{0.82844 \text{ liter}} \right) * \left(\frac{1,000,000}{400} \right)$$

P_{tot} = total pressure needed in the flask to have 400 ppm CO₂ in the 0.8-liter-flask.

$P_{cracker}$ = pressure in the cracker, which can be read from the home-built sensor.

$V_{cracker}$ = volume of the cracker, which is 21.36±0.09 mL = 0.02136 liter.

P_{flask} = pressure in the 0.8-liter-flask after the sample is translocated into the 0.8-liter-flask.

V_{flask} = volume of the 0.8-liter-flask, which is 828.4±0.3 mL = 0.82844 liter.

When the flame-sealed tube is broken in the cracker, then the home-built pressure sensor will indicate the $P_{cracker}$. With this pressure value, the Boyle's formula will give a theoretical P_{tot} pressure of the 0.8-liter-flask as outcome. Now the 0.8-liter-flask would theoretically have an atmospheric CO₂ concentration of 400 ppm which will be measurable on the SICAS.

2.3.2. Isotope Ratio Mass Spectrometry (IRMS)

For IRMS it is important that these samples are dried. Wet samples can affect measuring the isotope compositions because the amount of water vapour can influence the pressure/current balancing of the IRMS, and possibly the flow characteristics in the machine.

Hence, a dryer is developed to remove all water from the ASICA samples. In this research, magnesium perchlorate (Mg(ClO₄)₂) is used as a regenerable drying agent. Magnesium perchlorate strongly absorbs water from air and is crystallized with 6 H₂O and will decompose above 250 °C.

Moreover, it is critical that there is less space as possible in an object, including the cracker, for translocating the sample from the flame-sealed tube into the IRMS. This is because of small amount of the ASICA samples, that IRMS barely can measure. To prevent that the sample is lost, the dead space is reduced as much as possible. That will improve measuring the isotope compositions. This is why the tube with magnesium perchlorate in it, should also be as small as possible and at the same time it should absorb all water from the sample. Moreover, this is the reason that a smaller cracker will be developed to reduce even more dead space in the cracker.

The glass tube is created and is filled with grains of magnesium perchlorate. Rock wool is placed at the ends of the tube to avoid contamination of the IRMS with Magnesium Perchlorate. This dryer is tested in this research for its efficiency.

The ASICA samples are measured together with in-house working standards such as GS-19, GS-20 and GS-25. These are the reference of the IRMS. The isotopes compositions of these in-house working standards are known, so the calibrations can be made from these in-house working standards. Based on these known isotopes compositions of GS-19, GS-20 and GS-25, the measured isotopes compositions of samples can be corrected.

3. Results

In this part, all results of this research will be shown. At first, the results from the method development for the SICAS samples and then the method development for the IRMS. Moreover, a comparison will be made between these two different methods.

3.1 Method development for the SICAS samples

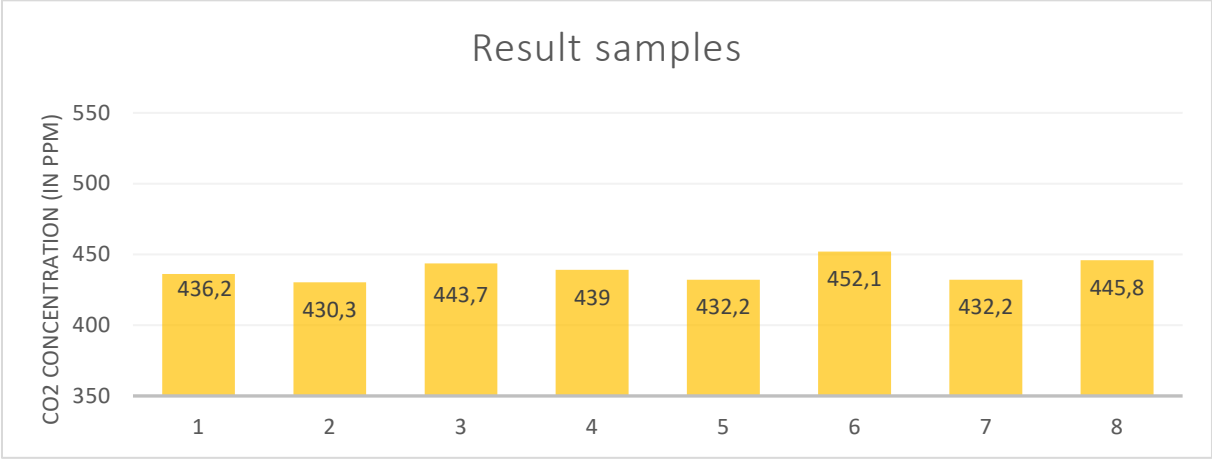
In this part, it will be focussed on the method developments for the SICAS only. For the SICAS, the proper sample dilution process is crucial. Here, 'proper' means both reliable, accurate and reproducible dilution of CO₂ samples into CO₂-free air, resulting in a mixing ratio as close to 400 ppm as possible. In practice it meant calibration of the various volumes, controlling the filling process of the flask with CO₂-free air up to the desired pressure, and both predict and test the precision and accuracy by which this can be done. Below the experiments are described in detail.

3.1.1 Whole air sample preparation results

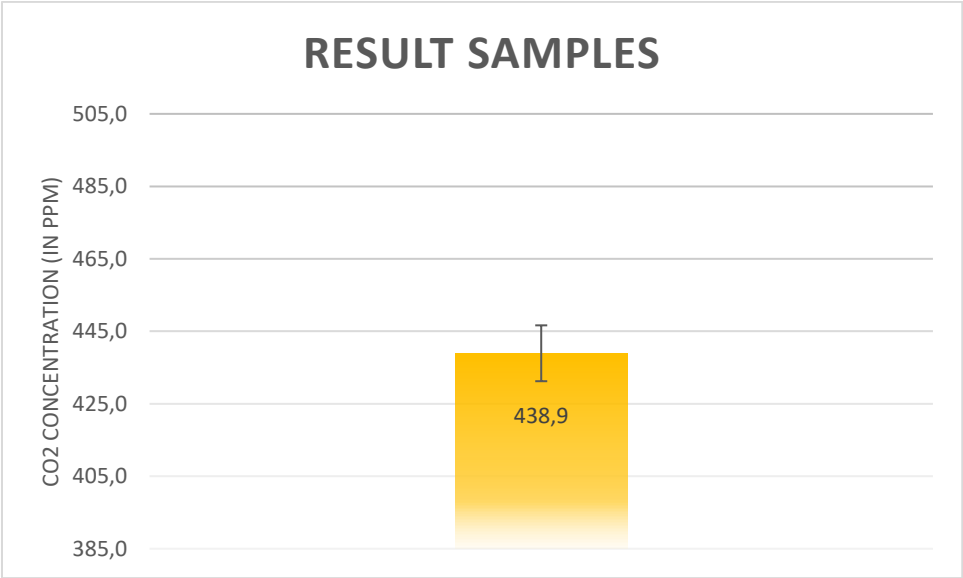
For the final result, there were in total eight 0.8-liter-flasks that were prepared with test-samples which contained in-house working reference CO₂ gas. These test-samples were also captured in flame-sealed tube that was similar to the original ASICA flame-sealed tube. These test-samples were prepared based on the Boyle's formula for the theoretical pressure of P_{flask}. In the appendix on page 41, there is an instruction how to translocate the sample from the

flame-sealed tube into the 0.8-liter-flask using the preparation system and then diluting the sample till it reaches the theoretical P_{flask} pressure in the flask.

Afterwards the prepared test-sample in the 0.8-liter-flasks were measured for their CO_2 concentration on the Picarro. The result of these eight samples is shown in graph 2 and in graph 3 below.



Graph 2, eight prepared test-samples in the 0.8-liter-flask measured on the Picarro.



Graph 3, the average result of whole air sample preparation with the standard deviation of 7.7 ppm.

3.1.2 Precision and accuracy of the results

Uncertainties have been reduced considerably. The goal is having samples of around 400 ppm with max. 5%, so max. 20 ppm. The precision of the final results is very good with 7.7 ppm standard deviation. The average of these samples is 438.9 ppm, higher than it supposed to be, which is around 400 ppm. Accuracy of these result is therefore quite low and needs to be improved.

3.2. Method development for the IRMS samples

This part is focussed on method developments for the IRMS. Since the IRMS is sensitive to wet samples, it is necessary to dry these samples before measuring for their isotope compositions on the IRMS. For method developments, in-house working standard (GS-25) is used as test sample. The isotope compositions are known for this in-house working standard. Furthermore, this in-house working standard was dried already. The measurements are made, with and without the drying step. Also, two other reference gas (GS-19 and GS-20) are measured to correct the isotope compositions of the measured samples. The results of samples with drying method and without drying method will be compared with each other, to examine whether the drying method is well developed.

Breakseals were used for the method with the drying step, after breaking the breakseal the test-sample goes through the drying step which is a tube with grains of magnesium perchlorate in it. There were two breakseals for the experiment.

For the method without the drying step, the in-house working standard GS-25 was captured in a smaller cylinder from the reference GS-25 cylinder. This in-house working standard in smaller cylinder was measured immediately after releasing into the IRMS. There were three samples measured without the drying step. Also, two reference gasses (GS-19 and GS-20) were measured for the calibrations. The results are shown in table 1.

Table 1, result of CO₂ isotope compositions from test-samples that were measured on the DI-Optima. Test samples were prepared on two different methods, one with the drying step (breakseals) and another without the drying step.

Average:	d¹³C	St. Dev.	d¹⁸O	St. Dev.
GS-25 breakseals:	-3.58‰	0.05‰	-11.68‰	0.13‰
GS-25:	-3.73‰	0.02‰	-11.80‰	0.04‰
Assigned value:	-3.50‰		-11.15‰	

This would be the reason to distrust this measurement session altogether.

The reference gas GS-19 deviated quite a lot from the assigned value for the d¹³C and d¹⁸O. The assigned value of d¹³C for GS-19 is -7.5‰ and it was measured -7.06‰. For the d¹⁸O the assigned value is -0.19‰ and it was measured 1.793‰. Another in-house reference gas GS-20 had better values, for d¹³C it was measured -8.57‰ while the assigned value is -8.62‰. For the d¹⁸O it was measured 0.092‰ while the assigned value is -0.99‰.

Although the remarkable values of GS-19, the GS-20 seems to have an acceptable correction value. That is why GS-19 reference values were excluded for correcting the sample values, with keep in mind that this whole session remains unreliable. These measured values of test-samples were corrected with the gas reference of GS-20 only.

The result shows that a method with the drying step will especially lead to a change in the d¹³C isotope composition. In table 1, it shows that the difference between these two different methods is 0.15‰ for d¹³C and 0.12‰ for d¹⁸O.

3.2.1 ASICA-samples measured at IRMS

Then the ASICA samples of the whole flight were prepared and were measured on the IRMS, DI Optima. The drying step was still used as the last experiment, and it contains a tube with grains of magnesium perchlorate. These 25 ASICA samples were measured together with reference gases of GS-19 and GS-20. These reference gasses were used for calibration. In the appendix on page 41, there is a result of the whole measurement.

These ASICA samples have the average $d^{13}\text{C}$ of $-5.56 \pm 5.05\text{‰}$ and for $d^{18}\text{O}$ $-0.03 \pm 3.72\text{‰}$. Before doing a further data analysis on this result, these values firstly need to be corrected by calibrations. In table 2, below it shows the measurements of reference gasses GS-19 and GS-20. The reference gas GS-19 is assigned with -7.5‰ for $d^{13}\text{C}$ and -0.19‰ for $d^{18}\text{O}$, while another reference gas GS-20 is assigned with -8.62‰ for $d^{13}\text{C}$ and -0.99‰ for $d^{18}\text{O}$.

Table 2, the reference gasses GS-19, and GS-20 were measured as well as the ASICA-samples for the calibrations.

Ref. gas	Date	Peak height	$d^{13}\text{C}$	Assigned $d^{13}\text{C}$	m-a	$d^{18}\text{O}$	Assigned $d^{18}\text{O}$	m-a
GS 19 CIL	6-1-2020	2.239	-7.593	-7.500	-0.093	1.051	-0.190	1.241
GS 19 CIL	6-1-2020	2.234	-7.684	-7.500	-0.184	1.026	-0.190	1.216
GS 19 CIL	7-1-2020	2.360	-7.257	-7.500	0.243	1.671	-0.190	1.861
Ref. gas	Date	Peak height	$d^{13}\text{C}$	Assigned $d^{13}\text{C}$	m-a	$d^{18}\text{O}$	Assigned $d^{18}\text{O}$	m-a
GS 20 CIL	6-1-2020	2.309	-8.590	-8.620	0.030	0.180	-0.990	1.170
GS 20 CIL	7-1-2020	2.918	-8.533	-8.620	0.087	0.347	-0.990	1.337
Avg. Sample	6-1-2020	3.86	-5.267			1.444		
Avg. Sample	7-1-2020	3.93	-6.125			-2.653		

The peak height values for CS-19 and CS-20 are, unfortunately, much lower than they should have been. These were around 2.3-2.9 nA and should be around 4 nA. It is deviated quite a lot. Therefore these measurements of GS-19 and GS-20 cannot be used as calibration, as the $d^{13}\text{C}$ and $d^{18}\text{O}$ values measured by the IRMS depend on these major beam currents.

This is the reason why it is recommended to avoid doing a further data analysis on these results of the ASICA-samples of the whole flight. These measured values of all those ASICA-samples cannot be corrected since there is no calibration. In the following part of the discussion and recommendation, there will be some explanations why these calibrations were not around 4 nA, but around 2.2 nA.

3.3. Comparison between two methods at DI-Optima IRMS and the SICAS

Both measurements were made on the DI-Optima IRMS and on the SICAS. Now the next step is comparing these instruments with each other.

At the SICAS, GS-25 is also used as test-sample for the dilution in CO₂-free air, and subsequent measurement. In that way, the SICAS results can be compared to the IRMS results from the previous chapter. Unlike the IRMS, the freeze finger is used for the drying method at the SICAS.

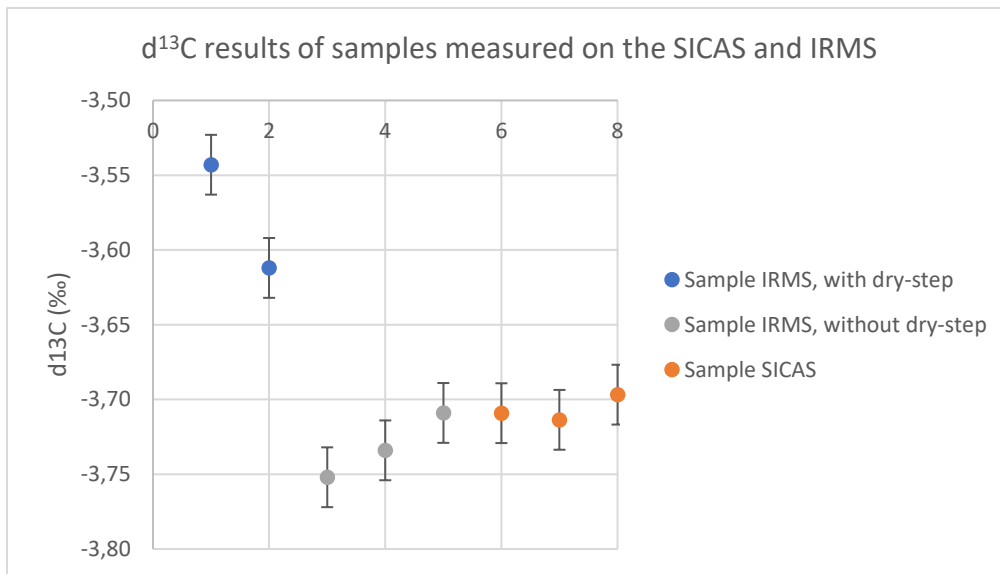
The calibration from the quality control tank seemed to be going well for the measurement on the SICAS. The SICAS produced calibrations of acceptably quality. The residual of d¹⁸O was 0.05‰ and that of d¹³C was 0.04‰.

The average CO₂ concentration of three samples is 426.1±7.2 ppm and the average d¹³C after the correction was -3.71±0.01‰. For d¹⁸O the average value was -11.35±0.01‰, as it shows in table 3. Moreover, the results are also shown in graph 4 and in graph 5.

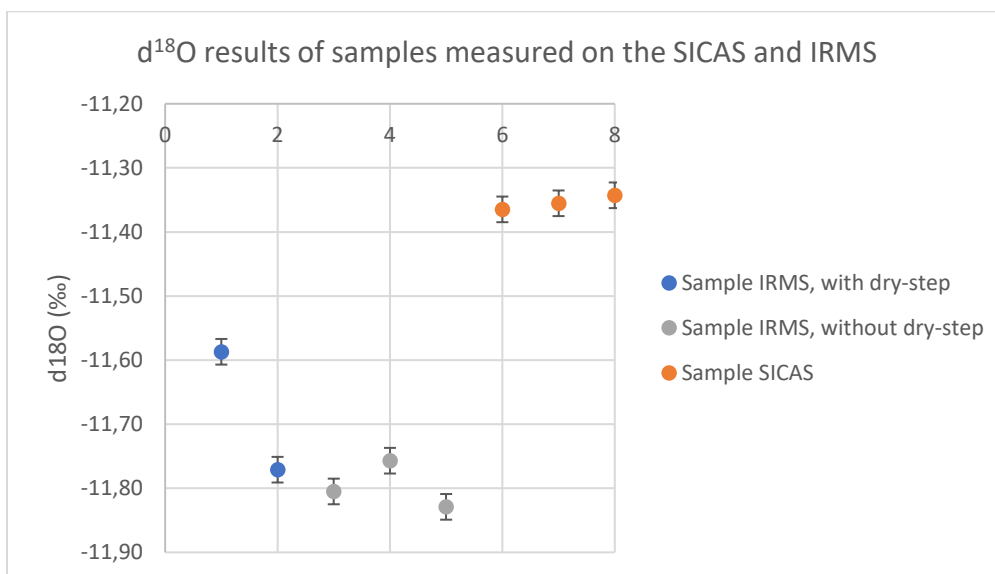
The next step is comparing these results with those from the IRMS.

Table 3, results of measurements on the IRMS and on the SICAS with samples of an in-house working standard which its isotope compositions are known. The assigned value of d¹³C for the gas sample is -3.50‰ and d¹⁸O -11.15‰.

Sample IRMS	d¹³C cor	d¹⁸O cor
GS-25 BREAK SEAL 1	-3.54‰	-11.59‰
GS-25 BREAKSEAL 2	-3.61‰	-11.77‰
GS-25 1	-3.75‰	-11.81‰
GS-25 2	-3.73‰	-11.78‰
GS-25 3	-3.71‰	-11.83‰
Average sample IRMS	-3.67‰	-11.75‰
Sample SICAS	d¹³C cor	d¹⁸O cor
Sample 1 SICAS	-3.71‰	-11.37‰
Sample 2 SICAS	-3.71‰	-11.36‰
Sample 3 SICAS	-3.70‰	-11.34‰
Average sample SICAS	-3.71‰	-11.35‰



Graph 4, $d^{13}C$ of both instruments SICAS and IRMS, with the standard deviation of an estimated and desired value of 0.02‰.



Graph 5, $d^{18}O$ of both instruments SICAS and IRMS, with the standard deviation of an estimated and desired value of 0.02‰.

The average $d^{13}C$ for measured on the IRMS was -3.67 ± 0.09 ‰. When these measurements with the drying method are excluded (so only the measurements without the drying step), then the average would be -3.73 ± 0.02 ‰. Surprisingly well, given the unreliable IRMS calibration because of the peak height values as described earlier in this report. This also points to the fact that the assigned value of -3.50 ‰ might be outdated. On the SICAS, the

average was $-3.71 \pm 0.01\%$. The values measured on the SICAS are more precise than those measured on the IRMS.

What about $d^{18}O$ measured on the IRMS, the method without the drying step also gave a better result of $-11.80 \pm 0.04\%$ which means higher precision than result including the measurements with the drying step which was $-11.75 \pm 0.04\%$. See table 3, for the results measured on the IRMS. The average value at the SICAS is $-11.35 \pm 0.01\%$.

4. Discussion and recommendations

First, discussion refers to the IRMS instrument, which improvements can be made with this instrument. Also, which recommendations are there for this instrument. Afterwards, the SICAS will be discussed with some recommendations.

Unfortunately, the calibrations of the IRMS were not satisfactory. The low peak height values caused distrusting calibrations. Ideally, the instruments would produce reliable, calibrated values that were in agreement with each other and with the assigned value. That is obviously not the case here. However, we can learn a lot from these measurements to make improvements for the research in the future.

There are explanations why those values of height peak did not reach 4 nA on the IRMS instrument which should have happened. This caused calibration with the GS-19 and GS-20 unreliable, or in fact useless.

There are two inlets in the DI-optima IRMS. One inlet is for samples only and another one is for the reference gasses. When a sample gas is being let into the sample-inlet, then the pressure (with the bellow) will be adjusted until there is a peak height of 4 nA. In other words, the pressure in the bellow is responsible for that peak height of 4 nA. When there is too little sample, then it will not reach the peak height of 4 nA, because the bellow will be closed completely then. Then the value will be close to 4 nA as possible.

The measurements of sample and reference gasses showed the sample side had higher peak height values than on the reference side in the process. This might cause by very little sample

on the sample side, so the pressure has decreased faster at the reference side. Moreover, one measurement consists of 8 iterations of sample-ref measurements, and because there is more reference gas than sample gas, the pressure difference between the first and the last iteration will be much higher at the sample side.

In short, the reason that these working standards did not reach the desirable peak height of 4 nA, might cause by the pressure. The pressure was too low because the sample was probably too little. There should be more sample added than it has done. This might affect the measurement, although it is unknown how much this might affect.

After the analysis of the measurements, it is concluded that these ASICA samples cannot be further researched. The peak height of 4 nA is almost never reached in these measurements. The recommendations for this could be, the cracker should be even smaller to have even less volume. Another recommendation for this situation could be, it should be measured at even lower peak value. So all measurements should have been done at the same and lower major beam current.

Fortunately, the direct results from the IRMS are already reasonably well calibrated by the IRMS machine reference gas. Only these direct results cannot be calibrated against a primary reference for expression of the isotope composition on the VPDB scale. This should have happened in this research project.

Also, there are some recommendations about the drying step containing magnesium perchlorate at IRMS. The results show that the drying step gave remarkable values. Magnesium perchlorate might absorb a part of CO₂ as well as water from samples. Magnesium perchlorate should not affect the isotopic compositions of the CO₂ sample. It is hard to investigate whether this has caused by the drying step, or by the machine. Therefore, further research is recommended to check this under better conditions.

Finally, for further research, it is recommended to check mass 17 (d¹⁷O) in the IRMS for remaining water in the machine. This can prove whether the drying step would absorb all water from the ASICA samples.

Now the SICAS instrument is discussed with some recommendations. Also, some improvements are provided to explain what is needed to produce even more reliable results.

During the dilution experiments for the SICAS, several flaws have been repaired, and the process has been improved considerably. Now, the system can produce CO₂ mixing ratios in the flasks with a reproducibility of 438.9±7.7 ppm. For the last sets in this work, the mixing ratio itself was still higher than aimed for, but that can easily be fine-tuned in future use. The precision is great, it is considerably lower than the goal of max. 5% uncertainty, or max. ±20 ppm standard deviation. Only the accuracy can be improved, from 438.9 ppm to around 400 ppm.

There is a reason that could explain why the accuracy was not 400 ppm but 438.9 ppm. After the experiment, it was discovered that the volume into the tube was not counted into the theoretical P_{flask} pressure calculations. The volume of the cracker included the flame-sealed tube was calibrated while the tube still was unbroken. This should be broken because the volume into the tube should also be taken into account to calculations to achieve proper flask fillings. This will probably lead to higher accuracy of the samples in the future experiment.

Second, the home-built pressure meter's resolution is rather limited, causing uncertainty and spread in the final mixing ratio of 1 mBar. P_{cracker} of 1 mBar equals to P_{flask} of 64.5 mBar. P_{flask} of 64.5 mBar equals to around ~22 ppm uncertainty in the final CO₂ concentration which is around 5.5% uncertainty. Although the great reproductibility, a pressure meter with higher resolution in the range used here (around 20 mBar) would reduce uncertainty even more.

Agreement between IRMS and SICAS results for ¹³C is surprisingly good, given the various calibration problems for IRMS. The average d¹³C from SICAS is -3.71±0.01‰ and from the IRMS, the average without the drying step is -3.73±0.02‰. The average d¹⁸O from SICAS is -11.35±0.01‰ and from the IRMS the average without the drying step is -11.80±0.04‰. For ¹⁸O the difference between the average SICAS value and the average IRMS value without the drying step is 0.45‰. This must be improved in the future.

Although those results, especially from the SICAS, showed a great reproductibility, it did not match with the assigned value of the reference gas. It might be that the assigned value of the reference gas is outdated. This should be updated and therefore it is recommended to do further research on the assigned value, so that the accuracy will probably improve too. This can be done easily, due to lack of time this could not be redone in this research.

5. Conclusions

The results from SICAS show that the dilution process is well in place, precisions, and reproducibility are accurate. The last experiment showed the average CO₂ concentration of the diluted sample is 438.9 ± 7.7 ppm. Hereby the goal has achieved to have max. 5%, or 20 ppm, standard deviation. However, this last experiment needs to be repeated, to check whether the accuracy has improved now. In the last experiment, the V_{cracker} volume of the cracker was used when the volume inside the flame-sealed tube was excluded for the Boyle's formula. This should be included and would bring the CO₂ concentration of the sample in 0.8-liter-flask even closer to 400 ppm. The results from the SICAS also show great reproducibility; $-3.71 \pm 0.01\text{‰}$ for d¹³C and $-11.35 \pm 0.01\text{‰}$ for d¹⁸O. The residual of d¹³C was 0.04‰ and that of d¹⁸O was 0.05‰.

At the IRMS, there were some issues with calibrations. The low peak height values caused distrust the calibrations, as well as measured values. These measured values could not be corrected with these unreliable calibrations. Regardless, the measured values were still used and corrected with the calibrations, with in mind that they still are untrustful. Surprisingly, the average value of the method without the drying step was comparable with the average value from the SICAS. At the IRMS, the average d¹³C value of the method without the drying step is $-3.73 \pm 0.02\text{‰}$. and from SICAS it is $-3.71 \pm 0.01\text{‰}$. The average d¹⁸O value of the method without the drying step is $-11.80 \pm 0.04\text{‰}$, and from the SICAS $-11.35 \pm 0.01\text{‰}$. This difference of d¹⁸O between these two instruments could be even more improved in future research. Including the method with the drying step, the average value was $-3.67 \pm 0.09\text{‰}$ for d¹³C and $-11.75 \pm 0.04\text{‰}$ for d¹⁸O. These differences between these two methods, with the drying step and without the drying step, are considerably large. This might be caused by the drying agent magnesium perchlorate. That is why there is more research needed about this drying agent.

It might be that this drying agent absorbs a part of CO₂, and thus might affect the isotopic compositions at the end. This should not have happened.

An advantage from IRMS, it requires less work to produce results of the samples compared to SICAS. After the measurement of ASICA samples on the IRMS, it turned out that the sample size of ASICA samples was not enough to reach the peak height of 4 nA. There was too little sample to measure for its isotopic compositions.

Moreover, it is recommended to check the results under better conditions, especially from the IRMS. Furthermore, the scales of the IRMS system and the SICAS differ. This is the reason why it will be very interesting to do more test with primary reference materials to check the scale difference between these two systems.

Finally, the deviation from the assigned value of the SICAS GS-25 measurements is much higher than the deviation of the measurement of the quality control tank. The conclusion can be taken that the strong deviation is not caused by the calibration of the SICAS, but by another unknown cause. These assigned values of the SICAS GS-25 might be outdated. This can be done easily to check whether these results also had high accuracy. Due to the time limitation of this particular research project, this is the matter of further exploration.

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7. Appendix

7.1. Instructions for future users

Preparing samples from the flame-sealed tube in the preparation system for the SICAS

- Let valves (nr. red 1 – 11, 13 – 16 and 20 in figure 6) in the system open, apart from the valve for the flow (nr. red 19) which has still to be closed.
- Let the system evacuate until everything is evacuated, when the vacuum pressure sensor (nr. blue 12 in figure 6) shows the value of $-1.33 \cdot 10^{-5}$.
- Now valves (nr. red 2 – 11, 13, 15, 16 and 20) have to be closed, so the preparation system will be a closed system now.
- Put the freeze finger into the system (nr. blue 11).

- Fill ethanol of 96% and dry ice into a dewar (nr. blue 10) and put this dewar under the freeze finger, to trap water from the samples.
- Plug the carved and marked flame-sealed tube into the cracker (nr. blue 8), until the tube reaches the notch. So all tubes will be placed on the exact same place. Make sure that the ultra-torr connection is very tight.
- *****
- Put the 0.8-liter-flask (nr. blue 7) into the system.
- Let valves (nr. red 8, 11, 13, 15 and 16) open.
- Let the valve of the cracker open (nr. red 17)
- Let the system evacuate until everything is evacuated.
- Close the valve of the cracker (nr. red 17).
- Break the carved flame-sealed tube in the cracker, by turning the glass pin into the cracker (nr. red 18).
- Reading the home-built pressure sensor (nr. blue 9) for the P_{cracker} pressure in the cracker and write it down for the Boyle's formula.
- Close the valves (nr. red 15 and 16).
- Put liquid nitrogen, LN₂, in a small dewar and put this under the side arm of the 0.8-liter-flask to trap all CO₂ from the sample into the side arm.
- Open the valve of the cracker (nr. red 17) and at the same time start running 5 minutes. Refilling the box with LN₂ when it becomes empty. Slowly raise the box containing LN₂ in small steps to make sure that all CO₂ is trapped in the side-arm of the 0.8-liter-flask.
- Close the valve (nr. red 14), so a smaller closed preparation system is created now.
- Put the box with LN₂ away from the side arm of the 0.8-liter-flask.
- Open the valve for the flow (nr. red 19), which is installed at 0.450 liter/min.
- Read from the pressure sensor (nr. blue 13) for the pressure in that closed preparation system and close rapidly the valves for the flow (first nr. red 13 and immediately afterwards nr. red 19) when it is reached the theoretical P_{flask} pressure in the 0.8-liter-flask.
- Close the valve of the 0.8-liter-flask (nr. red 11) rapidly and take the flask away from the system.
- Close the valve of the cracker (nr. red 17).
- Replace the broken tube by a new and carved flame-sealed tube in the cracker.
- Start all over again at the process step *****

7.2. Result measurements dried ASICA-samples on the DI-Optima

ASICA-sample	d¹³C	d¹³C Error	d¹⁸O	d¹⁸O Error	Peak height
AS IC 102153	-8.676	0.002	0.904	0.019	4.002
AS IC 102213	-8.611	0.005	-3.529	0.017	3.978
AS IC 102163	7.044	0.805	2.326	0.064	4.001
AS IC 102163	0.867	0.280	1.937	0.009	3.930
AS IC 102125	-8.552	0.008	-4.937	0.019	3.980
AS IC 102156	-8.684	0.004	-1.527	0.014	3.973
AS IC 102130	-8.712	0.008	-3.308	0.005	3.971
AS IC 102223	-8.540	0.008	-0.066	0.014	3.960
AS IC 102119	-8.285	0.006	-0.372	0.015	3.970
AS IC 102160	-3.566	0.065	7.267	0.045	3.949
AS IC 102160	-4.200	0.033	6.877	0.014	3.884
AS IC 102120	-7.385	0.008	-0.696	0.021	4.000
AS IC 102161	-4.114	0.099	3.723	0.054	3.972
AS IC 102161	-5.186	0.053	3.007	0.024	3.894
AS IC 102158	-2.990	0.138	6.475	0.085	3.979
AS IC 102158	-4.678	0.107	5.019	0.094	3.942
AS IC 102127	4.664	0.078	-3.384	0.155	3.901
AS IC 102127	5.445	0.019	-1.393	0.160	3.823
AS IC 102234	-9.172	0.036	-1.380	0.010	4.008
AS IC 102234	-10.220	0.045	-1.272	0.011	4.033
AS IC 102233	-9.041	0.185	-3.439	0.008	3.983
AS IC 102233	-10.786	0.106	-3.384	0.007	3.980
AS IC 102121	-8.600	0.011	-1.855	0.013	3.979
AS IC T964	-8.690	0.009	-5.850	0.006	3.661
AS IC 102131	-8.723	0.006	-1.916	0.011	3.993