

## A new inlet system for microscale carbon and oxygen stable isotope analysis using dual inlet isotope ratio mass spectrometer at Niigata University, Japan

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### Abstract

A new Thermo Scientific™ MAT-253 isotope ratio mass spectrometer was installed at Department of Geology, Niigata University, Japan under the MEXT Grant-in-Aid for Scientific Research on Innovative Areas. The mass spectrometer was set up for carbon and oxygen isotope measurement of small volume CO<sub>2</sub> samples. A new inlet vacuum line was constructed for transferring standard gas, to inlet externally prepared CO<sub>2</sub> gas samples and transfer CO<sub>2</sub> gas prepared in the online carbonate reaction system. The line is evacuated using a turbo molecular pump and pneumatically activated valves controlled by an electrical control board. The vacuum line also includes a reference CO<sub>2</sub> gas (Working Standard) reservoir and a laboratory standard CO<sub>2</sub> gas (Machine Standard) reservoir for routine analyses, as well as SF<sub>6</sub> reference gas reservoirs. The preparation line can hold high vacuum conditions without appreciable leaks for several hours, required for the completion of a single session of sample measurements. Long-term laboratory standard measurement results are reported here, which shows an average of  $\delta^{13}\text{C}_{(\text{V-PDB})}$  value of  $-29.796 \pm 0.041\%$  and  $\delta^{18}\text{O}_{(\text{V-SMOW})}$  value of  $3.971 \pm 0.062\%$  ( $n = 44$ ;  $1\sigma$ ). During a period of one year of analysis, a drift of 0.04‰ for both carbon and oxygen is observed for the reference CO<sub>2</sub> gas. NIST standards (NBS-20 limestone and NBS-21 graphite) and a laboratory diamond standard were analyzed to characterize the minimum volume of sample required to obtain carbon and oxygen isotopic composition with accuracy and precision <0.1‰. We also report here the routine analytical procedure for carbonate samples (calcite/aragonite and dolomite/siderite/magnesite) and elemental carbon (graphite and diamond).

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*Key words:* carbon and oxygen isotopes, MAT-253 inlet system, NIST standard measurements.

### Introduction

A new Thermo Scientific™ MAT-253 isotope ratio mass spectrometer (IRMS) was installed at Department of Geology, Niigata University, Japan under the MEXT Grant-in-Aid for Scientific Research on Innovative Areas. Here we report the analytical set up for carbon and oxygen isotope measurements for small volume samples. Conventionally, stable isotope measurements of carbon and oxygen isotopes are carried out using CO<sub>2</sub> gas in a dual inlet gas source mass spectrometer (Nier et al., 1947; Hoefs, 1997). In order to measure a sample gas using a dual inlet gas source mass spectrometer, it is essential to have a reference CO<sub>2</sub> gas reservoir. Carbon and oxygen isotopes are measured by simultaneously acquiring the CO<sub>2</sub><sup>+</sup> ions in the Faraday cup at m/z 44 (<sup>12</sup>C<sup>16</sup>O<sup>16</sup>O), 45 (<sup>13</sup>C<sup>16</sup>O<sup>16</sup>O) and 46 (<sup>12</sup>C<sup>18</sup>O<sup>16</sup>O).

Carbon isotopes are conventionally expressed as  $\delta^{13}\text{C}$  values (in ‰) to the Pee Dee Belemnite (PDB) scale (Craig, 1957), which was later on revised to the V-PDB scale (Coplen, 1988, 1994, 1996). The primary standards that are available include NBS-19 and NBS-20, and both have been assigned carbon isotopic values on the V-PDB scale. Oxygen isotopes, commonly expressed as  $\delta^{18}\text{O}$  values (in ‰) to the standard mean ocean water (SMOW) scale (Craig, 1957), which was later on revised to the international V-SMOW scale (Coplen, 1988). The V-SMOW scale is fixed by the standards V-SMOW and standard light Antarctic precipitation (SLAP), having assigned isotopic values. Conversion equations for oxygen isotope values for carbonate samples, from one international scale to the other, V-PDB to V-SMOW and vice versa are given below (after Coplen, 1988)

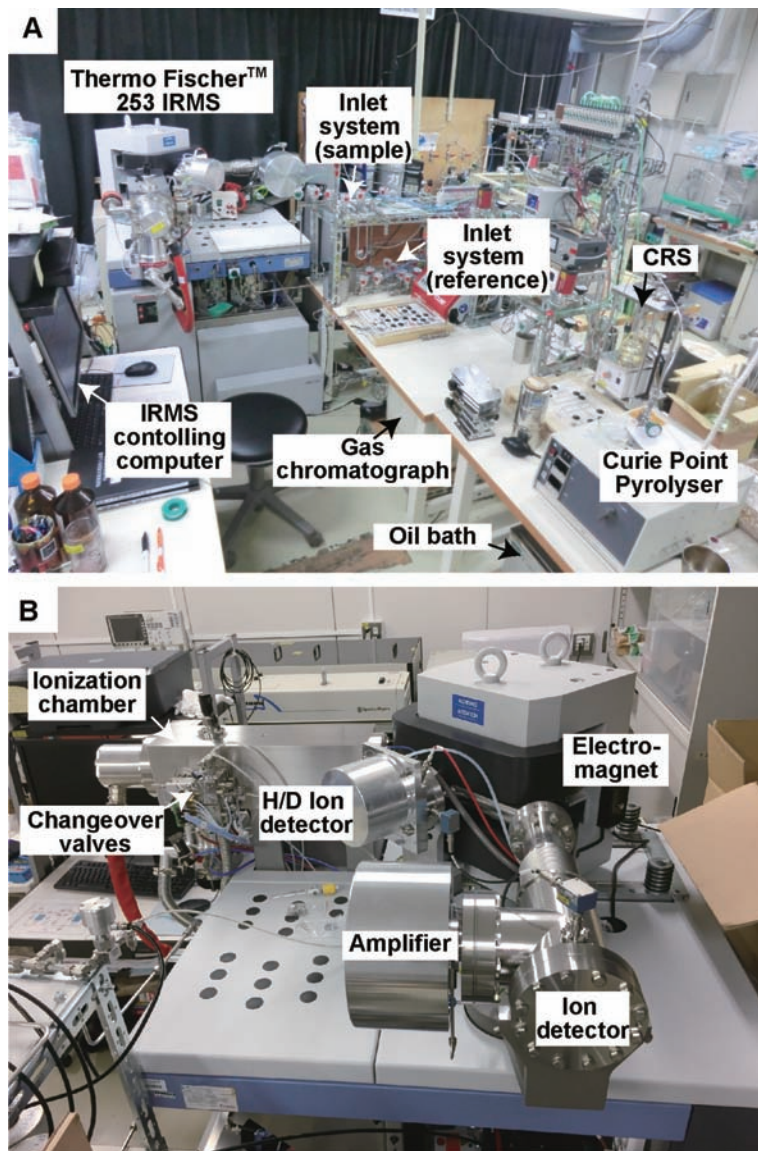
$$\delta^{18}\text{O}_{\text{V-PDB}} = 0.97002 \delta^{18}\text{O}_{\text{V-SMOW}} - 29.98$$

$$\delta^{18}\text{O}_{\text{V-SMOW}} = 1.03091 \delta^{18}\text{O}_{\text{V-PDB}} + 30.91$$

A new inlet vacuum line for transferring reference gas and sample gas to the built-in reservoirs in the mass spectrometer, to inlet offline CO<sub>2</sub> gas samples and CO<sub>2</sub> gas from online carbonate reaction system were built. In the following sections, we describe the basic set up of IRMS, the components of the inlet vacuum line and results of international standards in detail. A companion paper will describe the instrumental set up for multiple sulfur isotope measurements and sample preparation for SF<sub>6</sub> gas from rock samples with sulfide minerals.

### Basic set up of IRMS

The dual inlet gas source IRMS at Niigata University (Fig. 1A) is designed to measure carbon (as CO<sub>2</sub> or CO gas samples), oxygen (as O<sub>2</sub>, CO<sub>2</sub> or CO), sulfur (as SF<sub>6</sub>), nitrogen (as N<sub>2</sub>)

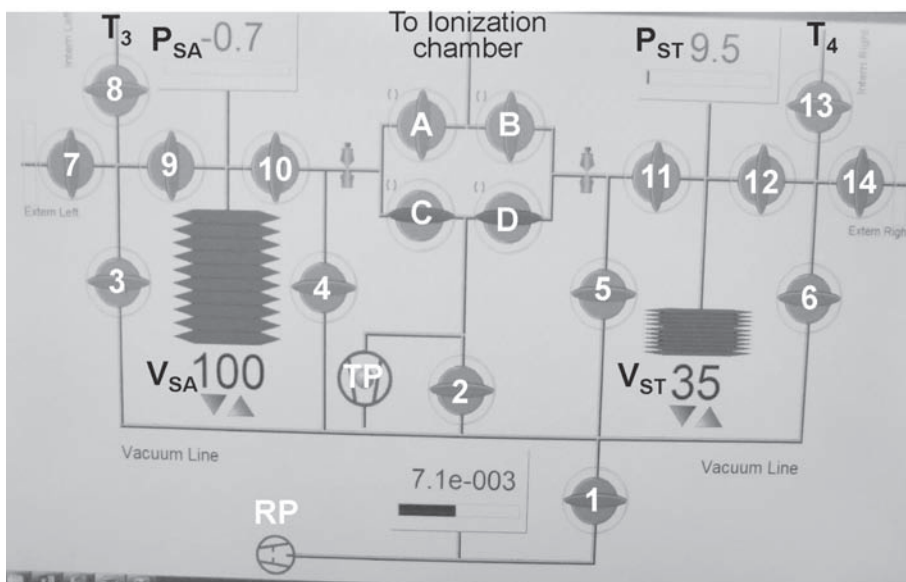


**Fig. 1.** A) Generalized view of the Thermo Scientific™ MAT-253 isotope ratio mass spectrometer and associated gas preparation vacuum lines. B) Enlarged view of ionization chamber, changeover valves, the detection chamber and amplifying unit.

and hydrogen (as  $H_2$ ) isotopes. The IRMS has a customized collector assembly of 8 Faraday cups, in addition to the H/D collector with 2 Faraday cups (Fig. 1B). The Faraday cups are aligned for the simultaneous measurement of multiple sulfur isotopes of  $^{32}S$ ,  $^{33}S$ ,  $^{34}S$  and  $^{36}S$ . The alignment of Faraday cups and their respective resistors for amplifiers are shown in Fig. 2. Faraday cups 1, 2 and 4 are used for  $CO_2$  measurements corresponding to resistors of

Slit Width (mm)		3.5	1.5	1.5	1.5	3.5	1.5	1.5	1.5
<b>Cup No.</b>		1	2	3	4	5	6	7	8
<b>Ampl. Channel</b>		1	2	3	4	5	6	7	8
<b>Gases/ Masses</b>	44,45,46	CO2	44	45	46				
	28,29,30	N2/CO	28		29	30			
	32,33,34	O2	32		33	34			
	127,128,129,131	SF6				127	128	129	131
<b>Resistors (Ohm)</b>	CO2	3E+10	3E+8		1E+11				
	N2/CO	3E+8		3E+10		1E+11			
	O2	3E+8		3E+11		1E+11			
	SF6					3E+8	3E+10	1E+10	1E+12

**Fig. 2.** Schematic sketch showing the alignment of Faraday cups and their respective resistors for amplifier in the mass spectrometer.



**Fig. 3.** Schematic layout of the built-in inlet system of the IRMS. The line is evacuated using a turbomolecular pump (TP) or a rotary pump (RP). Sample and reference gas reservoirs ( $V_{SA}$  and  $V_{ST}$ ) are directly connected to two pressure gauges. There are 14 pneumatic computer-controlled valves (Nos. 1 to 14) and a changeover valve (A-D), which switches between sample side and reference side flow of gas to the ionization chamber or to the ion pump.

$3 \times 10^{10}$ ,  $3 \times 10^8$  and  $1 \times 10^{11}$  ohms, respectively. The IRMS was set up for routine measurement of carbon and oxygen isotopes using carbon dioxide as a gas medium.

The built-in inlet system of the IRMS comprises of two gas reservoirs and a series of pneumatic valves as shown in Fig. 3. The line is connected to the external inlet system through 1/4" connectors; the sample side is being connected through the valve No. 7 and

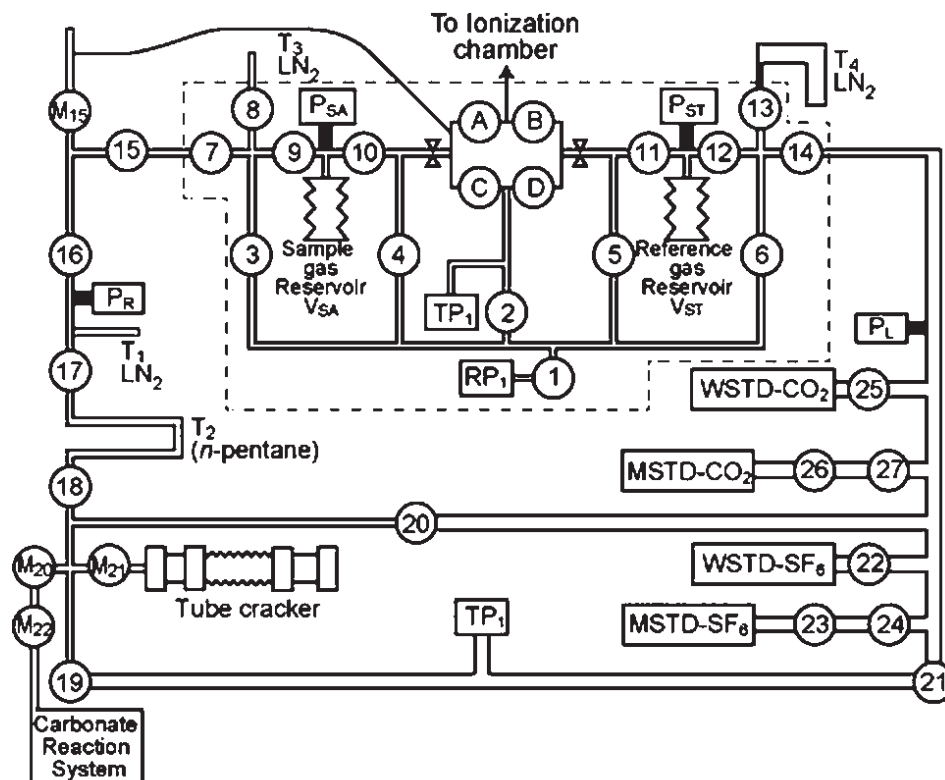


Fig. 4. Schematic layout of the vacuum line reference and laboratory standard gas reservoirs connected to the IRMS. The section demarcated using a dashed line is the built-in-inlet system of the IRMS. Thickness of tubes corresponds to 3/8" or 1/4" diameter tubes and connectors. Abbreviations are; CRS: Carbonate reaction system; M<sub>1</sub>, M<sub>2</sub>: Manual valves; MASS SA: Capillary connection of sample gas to ionization chamber; MASS ST: Capillary connection of standard gas to ionization chamber; MSTD-CO<sub>2</sub>: Machine standard CO<sub>2</sub> gas reservoir; WSTD-CO<sub>2</sub>: Working standard CO<sub>2</sub> gas reservoir; MSTD-SF<sub>6</sub>: Machine standard SF<sub>6</sub> gas reservoir; WSTD-SF<sub>6</sub>: Working standard SF<sub>6</sub> gas reservoir; P<sub>1</sub>-P<sub>2</sub>: Pirani gauge sensors; T<sub>1</sub>, T<sub>3</sub>-T<sub>4</sub>: Liquid nitrogen traps; T<sub>2</sub>: Pentane slush; TP: Turbo pump; Numerals 14 to 28 represent pneumatic bellows valves.

standard side through valve No. 14 (Fig. 3). The volume of the sample and standard reservoirs ( $V_{SA}$  and  $V_{ST}$ ) can be adjusted from c. 3 cc to c. 40 cc and both the reservoirs are directly connected to pressure gauges ( $P_{SA}$  and  $P_{ST}$ ) for monitoring the pressure of the gas inside the reservoirs (Fig. 3). The valves were numbered from 1 to 14 and the changeover valves as A, B, C and D (Fig. 3). The vacuum line can be evacuated either by a rotary pump (RP) or a turbo molecular pump (TP) (Fig. 3).

### Gas inlet system

The structure of the newly built inlet vacuum line follows the design described in detail in Wada et al. (1982, 1984a, b, 2008) and is schematically illustrated in Fig. 4. The vacuum

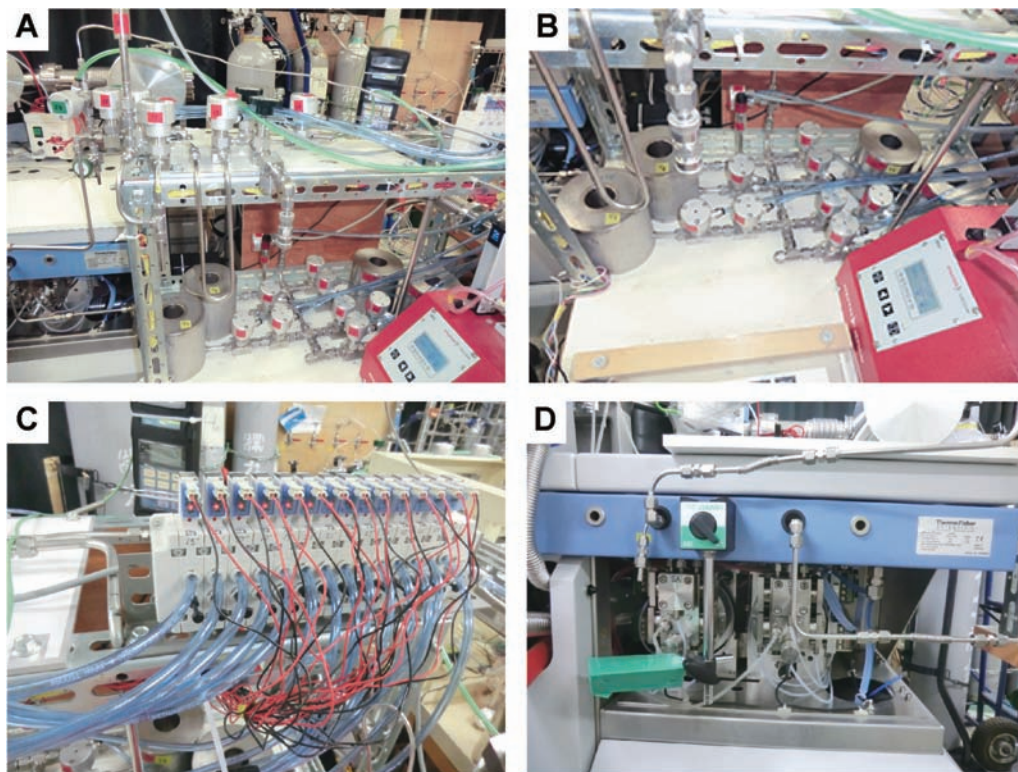




**Fig. 5.** A broad view of the inlet system for  $\text{CO}_2$  and  $\text{SF}_6$  standard gas and sample gas for carbon, oxygen and sulfur isotope analysis.

line for reference and standard gases was constructed using a combination of 3/8" and 1/4" stainless steel pipes and connectors (Figs. 5, 6), in order to minimize the dead volume inside the line and for quick transfer of gas to reservoirs. The pipes are connected using Swagelok® joints and Cajon® pneumatic bellows valves (Fig. 6A). The line is evacuated using a turbo molecular pump (PFEIFFER® HiCUBE80 Classic). There are two pressure gauges ( $P_L$  and  $P_R$ ; Pirani gauges, WAKAIDA SCIENCE CORPORATION, PG-2B02),  $P_L$  is located at the standard segment in the line and  $P_R$  is located at the sample segment (Fig. 4). Altogether, the vacuum line is composed of 14 Cajon® pneumatic bellows valves (Fig. 6B) operated by electromagnetic switches (Fig. 6C), which are controlled by electrical switches in a control board (Fig. 7). In addition, there are 4 manual valves. Manual valve  $M_{15}$  is connected to the microvolume gas inlet to the IRMS through the capillary tube (Fig. 4). Manual valves  $M_{20}$  and  $M_{22}$  connects the carbonate reaction system to the inlet line and the manual valve  $M_{21}$  is connected to a flexible tube cracker for breaking 6 mm diameter glass tubes containing  $\text{CO}_2$  gas samples prepared offline.

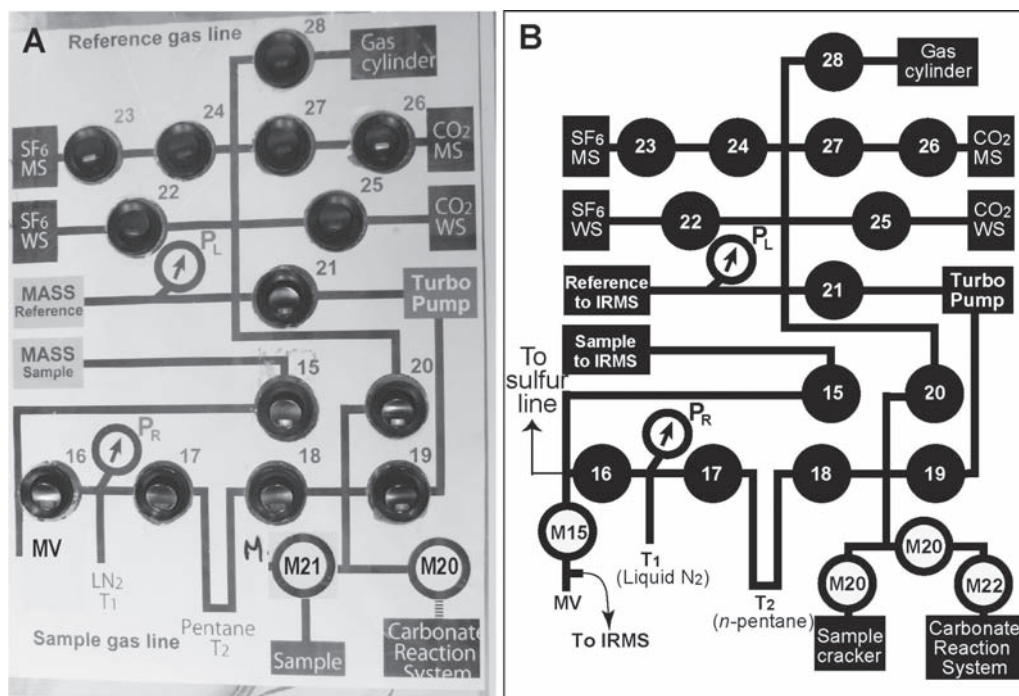
There are 3 cold traps ( $T_1$ - $T_3$ ) in the  $\text{CO}_2$  gas inlet line (Fig. 4). These cold traps are used for cleaning a mixed sample gas containing  $\text{CO}_2$  and impurity gases, and separate



**Fig. 6.** Photographs showing important segments of the inlet system. A) A general view of the vacuum line. B) Working standard (WSTD) and machine standard (MSTD) gas reservoirs containing  $\text{CO}_2$  gas. C) Electromagnetic valves used for opening and closing pneumatic valves in the vacuum line. D) View of the built-in vacuum line and  $T_3$  trap for collecting sample gas.

condensable and non-condensable gases cryogenically.  $T_1$  is 5 cm long stainless-steel tube with a welded cap and  $T_2$  is a U-shaped 3/8" stainless pipe cold trap, (Fig. 6A). These two cold traps are designed to cryogenically separate  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and other non-condensable gases, using liquid  $\text{N}_2$  ( $-196^\circ\text{C}$ ) and *n*-pentane slush (c.  $-125^\circ\text{C}$ ), respectively. The *n*-pentane slush is maintained at a temperature around  $-125$  to  $-130^\circ\text{C}$  (Wada et al., 1984a) by constantly cooling it using liquid  $\text{N}_2$  and monitored using a thermometer (Fig. 4).

The gas inlet system is connected to the inlet reservoirs and vacuum system of the IRMS by 1/4" stainless steel pipe and flexible tubes. A small volume trap  $T_3$  was set up in the IRMS inlet line using a 3 cm long 1/4" stainless steel tube with a welded cap (Fig. 6D) and filled with stainless steel balls. This cold trap is connected to the inlet line with a 3 mm diameter stainless steel pipe in order to reduce the inner dead volume. A similar assembly system is used in the microvolume trap (MV trap) as well (Fig. 4). The sample gas is transported from  $T_2$  to  $T_3$  cryogenically using liquid nitrogen and then expanded to the sample gas reservoir ( $V_{sA}$ ) in the IRMS, which is then flowed to the ionization chamber through a capillary tube (Fig. 3).



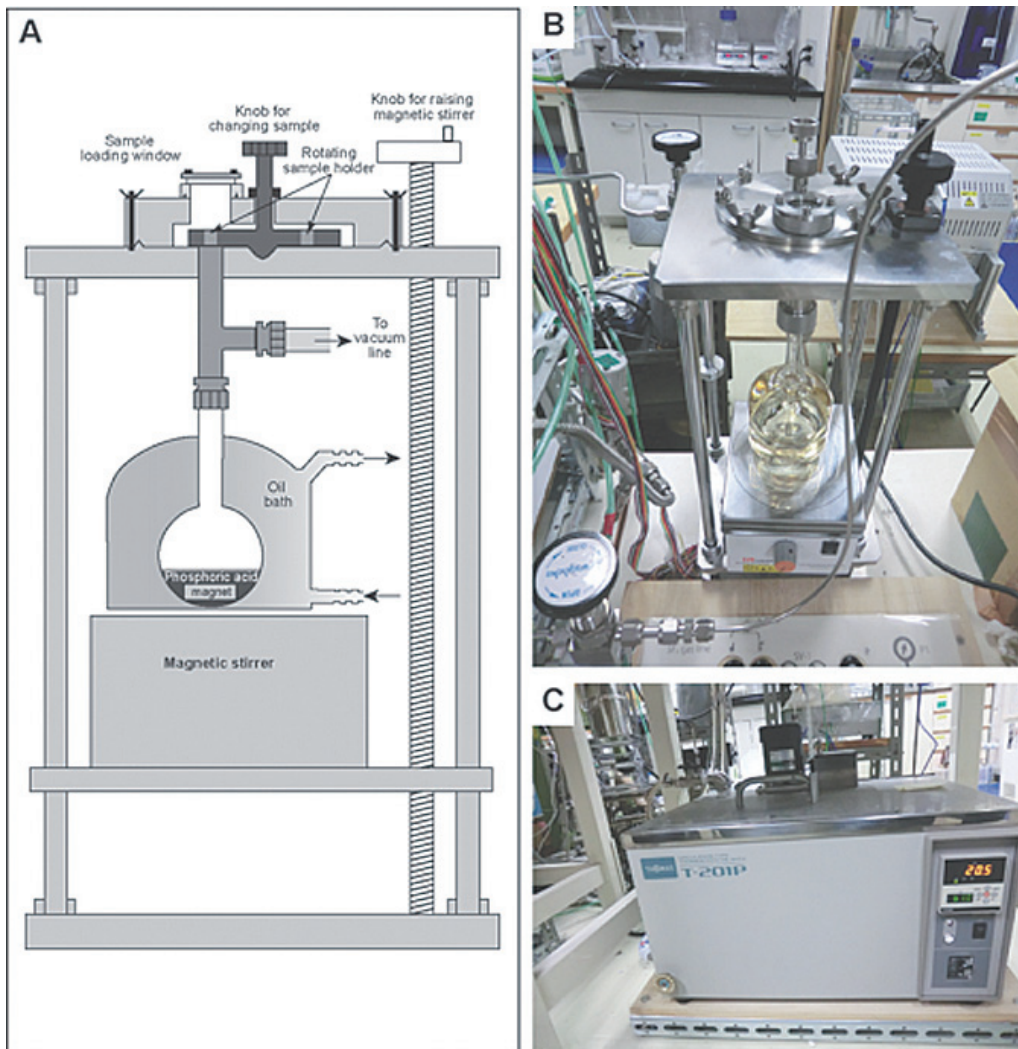
**Fig. 7.** Electrical switchboard for controlling the pneumatic valves of the inlet system. A) photograph of the control board. B) Schematic figure showing the inlet system.

### Carbonate reaction system

The carbonate reaction system (CRS) was designed based on the specifications given in Wada et al. (1982, 1984a) (Fig. 8A). It consists of a specially designed double chamber reaction vessel made of glass, the inner chamber is connected to the vacuum line and contain phosphoric acid and the outer chamber is used to circulate heated oil from a temperature-controlled oil bath (Fig. 8A). A magnetic stirrer is placed below the reaction vessel and a glass covered magnetic rod is used to stir the sample cups and to make sure that the reaction between carbonate powder and acid is completed (Fig. 8B). The rotating sample holder, made of stainless steel, can hold up to 24 sample cups at a time (Fig. 8B). The temperature-controlled oil bath is used for heating the phosphoric acid to ambient temperature for carbonate digestion (Fig. 8C). The merit of using an oil bath is that the temperatures in the range of 25 to 180 °C can be achieved.

Reaction rate of carbonate samples is dependent on the composition of the carbonate mineral, the surface area of the grains, the reaction temperature and the viscosity of the phosphoric acid. At Niigata University, calcite and aragonite are digested at 60 °C and other carbonates including dolomite and magnesite are digested at 100 °C. Appropriate oxygen isotope fractionation factors from the literature is used to for correcting the data (e. g.





**Fig. 8.** A) Sketch of the carbonate reaction system (after Wada et al.,1982). B) Photograph of the carbonate reaction system. C) Oil tank with heating unit used for circulating the oil to reaction vessel.

Sharma and Clayton, 1965; Northrop and Clayton, 1966; Rosenbaum and Sheppard, 1986; Cornides and Kusakabe, 1977).

### Vacuum condition

Initial vacuum tests of the system were carried out in order to check the high-vacuum condition for intervals of time required for the preparation and analysis of samples during a session of measurement, beginning and ending with laboratory standard (MSTD). A single session usually consists of more than 10 hours of measurements and each sample

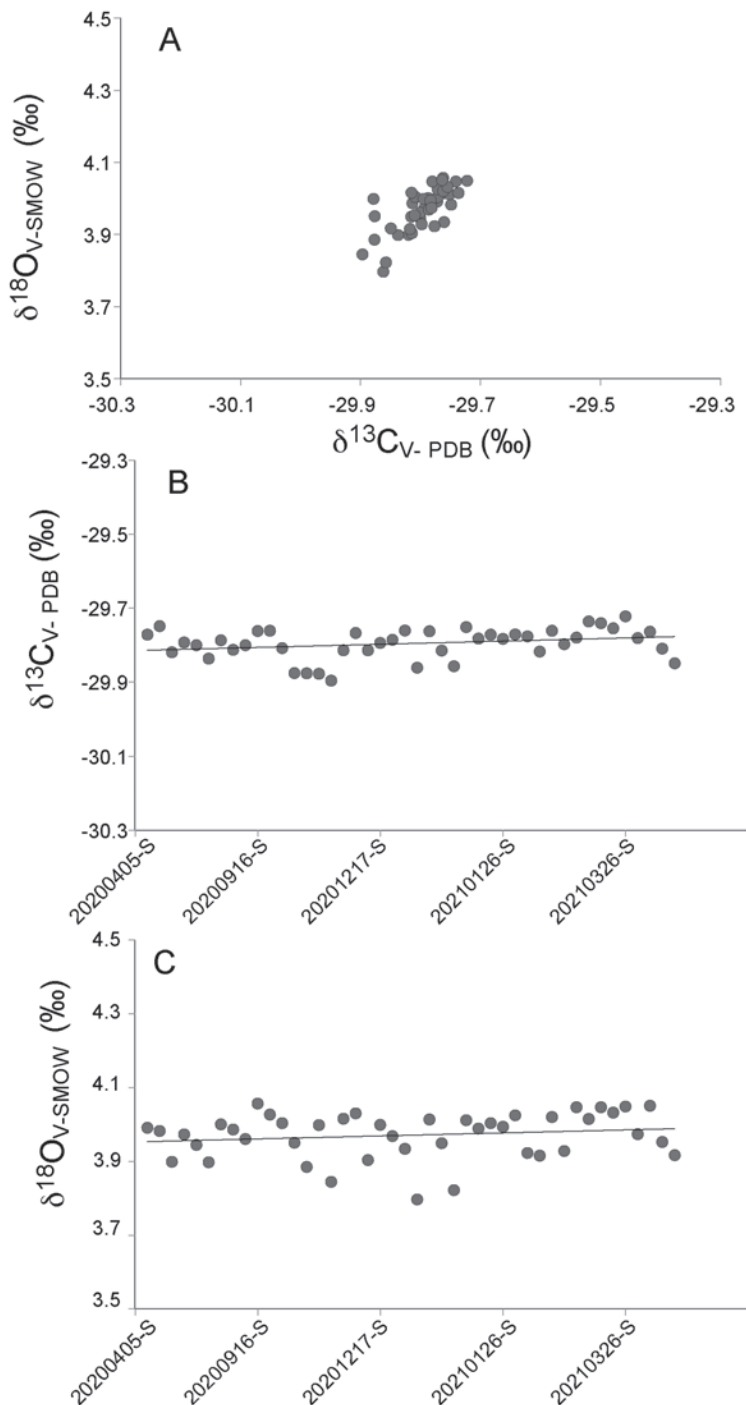
measurement can be accomplished within an hour. The system was evacuated overnight to the minimum pressure measured at the pirani gauges ( $P_L$  and  $P_R$ ) and vacuum check was conducted based on the following procedure. Initially, all the valves were kept closed for a specific time (e.g., 12 hours) and then, starting from the valve nearer to the gauge, and valves were opened one by one, while reading the shift in  $P_L$  and  $P_R$  values. For example, in the case of sample gas inlet segment's vacuum check,  $P_R$  is monitored initially with all valves closed. After keeping it for 12 hour the valve Nos. 17, 18, 16 and 15 (Fig. 4) were opened one by one and the shift in  $P_R$  is recorded. Similarly, the vacuum condition of the standard gas segment was also tested by monitoring the shifts in  $P_L$  values from the minimum value (Fig. 4). The results indicate that all segments in the vacuum line can essentially keep high vacuum condition for more than 12 hours, with limited increase of 0.2 and 0.4 Pa, for  $P_L$  and  $P_R$  values, respectively. Vacuum condition tests were also performed for the built-in vacuum line of the IRMS, which also indicated that the line could withstand high-vacuum condition for more than 12 hours duration (Fig. 3). These vacuum tests assure that for a single session of measurements there is basically no leaks and thereby confirming that no external contamination of gases to the vacuum system.

### Measurement of laboratory standard CO<sub>2</sub> gas

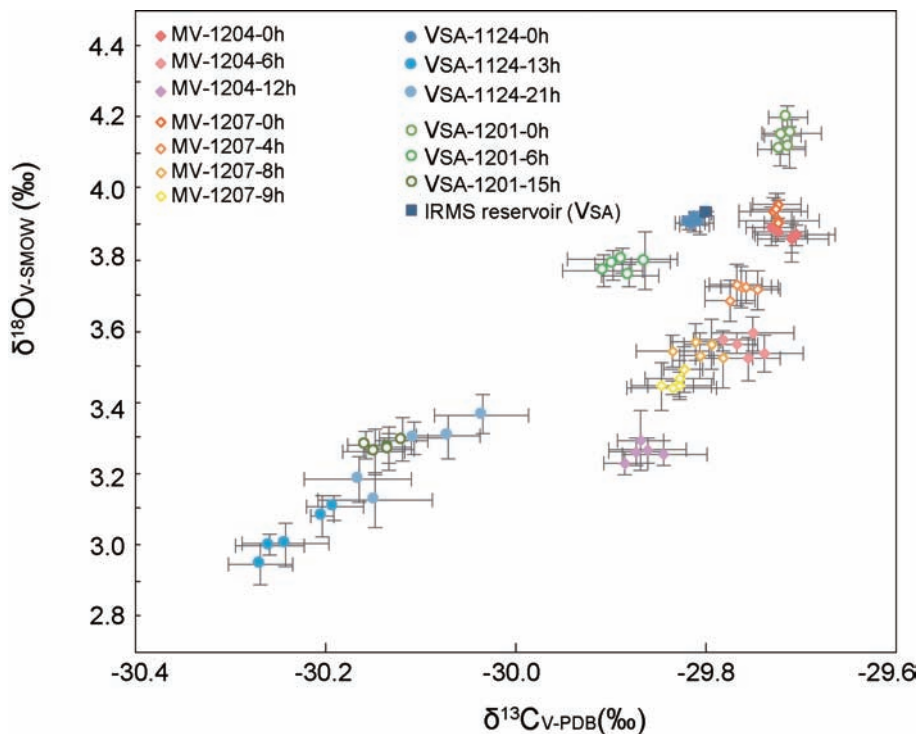
Initial tests were carried out using laboratory machine standard (MSTD) CO<sub>2</sub> gas for determining the precision of carbon and oxygen isotope measurement. The procedure for transferring the reference working standard gas and the measurement of MSTD are given in Appendix 1. The results are shown in Fig. 9. The average values of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  for MSTD are  $-29.796 \pm 0.041\%$  and  $3.971 \pm 0.062\%$  ( $n = 44$ ;  $1\sigma$ ), respectively, which shows a very good accuracy and precision (Fig. 9A). The MSTD values were also monitored for long term variations in the reference working standard and the results are presented in Figs. 9B and 9C. During a period of one year of analysis, a drift of 0.04‰ is observed for  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values for the reference CO<sub>2</sub> gas.

### Capillary flow and pressure effect on carbon and oxygen isotopic ratios

The dual inlet system of IRMS utilizes a reference gas (WSTD) and sample gas for the measurement of isotope ratio. These two gases are flowed into the ionization chamber alternatively through capillary tubes connected to the changeover valve A, B, C and D (Fig. 3). The isotope ratio of sample is estimated by repeatedly measuring the reference gas and sample gas. The gas pressure at both sample side and reference side are kept same during the measurement (Wada et al., 2008). The WSTD gas is continuously flowing during the analytical session and therefore it is necessary to monitor the drift in isotope ratio. We have



**Fig. 9.** Long term (one year) drift in carbon and oxygen isotopic composition for CO<sub>2</sub> laboratory standard gas. Forty-four MSTD measurements carried out from April 2020 to March 2021 are presented here. A)  $\delta^{13}\text{C}_{\text{V-PDB}}$  vs.  $\delta^{18}\text{O}_{\text{V-SMOW}}$  plot. B) Drift in  $\delta^{13}\text{C}_{\text{V-PDB}}$  values of MSTD for a period of one year. C) Drift in  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values of MSTD for a period of one year.



**Fig. 10.** Fractionation of carbon and oxygen isotopes during capillary flow. MV represents micro volume trap and VSA represents IRMS reservoir.

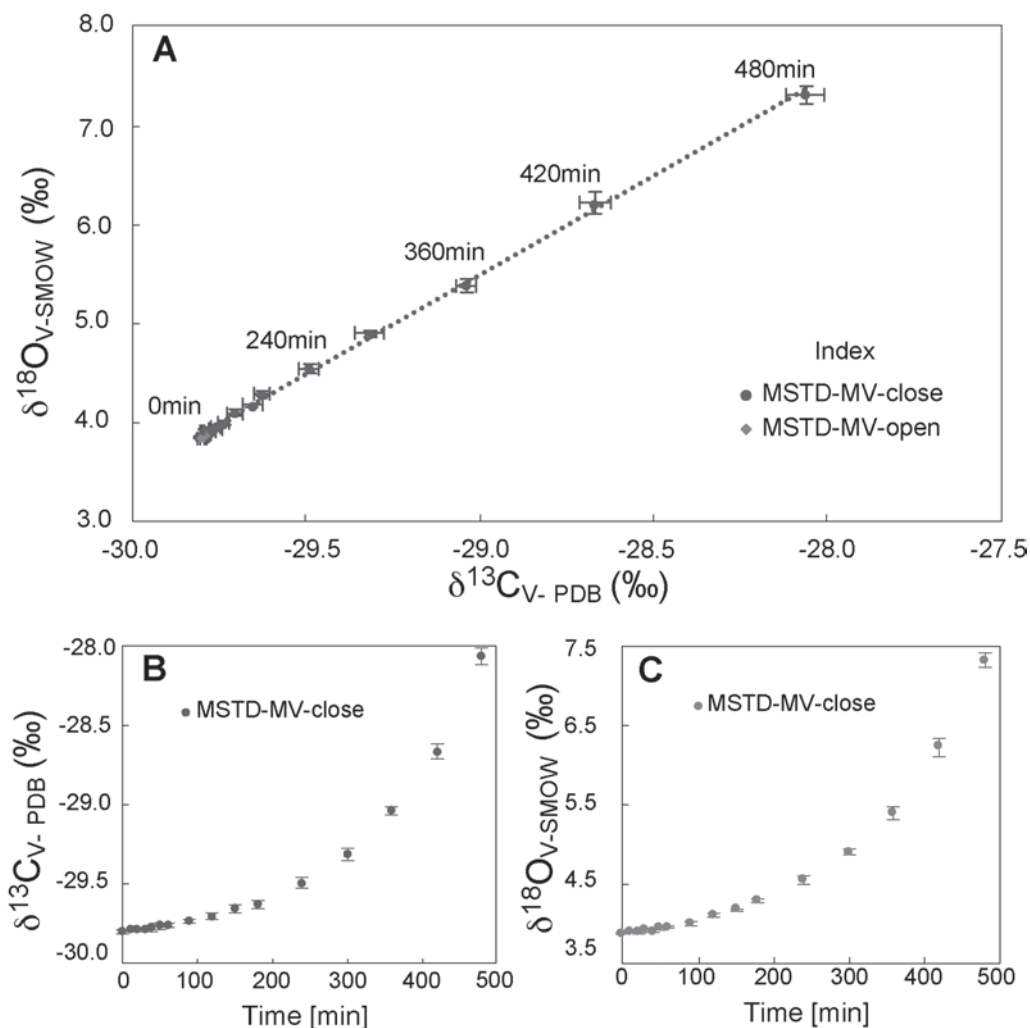
carried out experiments by continuously measuring the same MSTD gas to monitor the isotope fractionation during capillary flow as well as repeated measurements of MSTD gas. Fig. 10 shows the shift in isotope values of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  related to elapsed time for reference gas. Each analysis used a new aliquot of MSTD gas.

In the case of MSTD gas flowing continuously from  $V_{\text{SA}}$  through the capillary, repeated measurements for six hours showed only a drift of  $-0.17\%$  for  $\delta^{13}\text{C}_{\text{V-PDB}}$ , and  $-0.37\%$  for  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values. On the other hand, in the case of measurements using twice the volume of reference gas, the variation of  $\delta^{13}\text{C}_{\text{V-PDB}}$  was  $-0.04\%$  and for  $\delta^{18}\text{O}$  it was  $-0.32\%$ . The drift in  $\delta^{13}\text{C}_{\text{V-PDB}}$  values decreased considerably, although the  $\delta^{18}\text{O}$  values have some minor shifts even at larger gas flow.

### Preparation of phosphoric acid for carbonate digestion

The method for the preparation of phosphoric acid for carbonate acid digestion follows the description given in Bowen (1966) and Coplen et al. (1983). At first,  $\text{P}_2\text{O}_5$  is added to 85%  $\text{H}_3\text{PO}_4$  (orthophosphoric acid) in a weight ratio of 3:7. A small volume of ( $\sim 10$  mg; Coplen et al., 1983) of  $\text{CrO}_3$  is added, turning the solution yellow. The solution is heated at  $\sim 200$  °C for





**Fig. 11.** A) Variation related to analysis time between  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values in micro-volume trap. B) Variation of carbon isotope in micro-volume trap with time. C) Variation of oxygen isotope in micro-volume trap with time.

7 hours, turning green. A small volume ( $\sim 3$  ml) of  $\text{H}_2\text{O}_2$  is added and continued to heat at  $220^\circ\text{C}$  for an additional 4.5 hrs. The resulting acid has a chemical formula of  $\text{H}_4\text{P}_2\text{O}_7$  (pyrophosphoric acid), and a density of about 1.9 g/cc. This acid is bottled in vacuum to avoid contact with atmosphere, since pyrophosphoric acid is extremely hygroscopic and absorb moisture from the atmosphere if kept open.

### Experiments with micro-volume trap

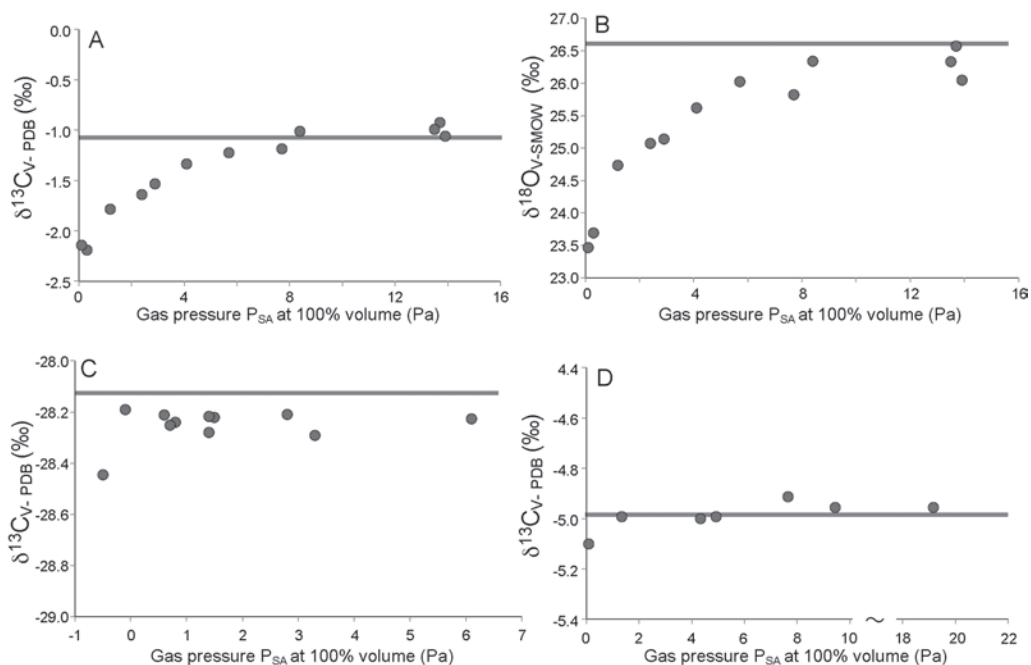
For the micro volume sample gas, we installed the new capillary tube with a micro-

volume trap (MV) directly to the changeover valve (Fig. 4). Using this MV-trap small volume of MSTD gas was measured. The value of MSTD gas filled between valve Nos. 15, 20 and 19 gave  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values of  $-29.78 \pm 0.01\%$  and  $3.96 \pm 0.01\%$ , respectively. After this analysis, valve No. 15 was closed and repeatedly analyzed at regular intervals. The value of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  indicated exponential fractionation related to time (Fig. 11A). The linear trend of  $\delta$  values between carbon and oxygen indicate a clear mass depended Rayleigh fractionation through the capillary tube as shown by the exponential trend with time for both carbon and oxygen isotopes (Fig. 11B, C). The fractionation of MSTD gas in the micro-volume trap shows a large variation because the fraction of gas remaining in the micro-volume trap and the flowed volume is large enough to cause fractionation.

High precision carbon and oxygen isotope ratios were obtained using this micro-volume trap by adopting the following improvements and optimization of preparation procedure for carbonate and elemental carbon samples following Wada and Itoh (1990). It is possible to analyze  $\sim 1$  micro-mole  $\text{CO}_2$  gas using this micro-volume trap at high precision ( $<0.1\%$  for both  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$ ). The small volume  $\text{CO}_2$  sample gas is purified using an *n*-pentane slush, which is maintained at a temperature c.  $-125^\circ\text{C}$ , as explained in the previous section. When small carbonate samples are reacted with phosphoric acid,  $\text{H}_2\text{O}$  is also produced in variable amounts depending upon the temperature of reaction. Previous studies have shown that  $\text{H}_2\text{O}$  cannot be completely separated from  $\text{CO}_2$  using dry ice or alcohol slush at low partial pressures and further lowering of temperature is essential to reduce the  $\text{H}_2\text{O}$  molecules being trapped in liquid nitrogen trap (Wada et al., 2008).

### Measurement of small volume calcite and graphite standard samples

NBS-20 calcite, NBS-21 graphite, laboratory graphite (SP1) standard and a laboratory diamond standard (artificial diamond crystal) were used to test the precision of small volume gas samples measurements. Measurements were carried out using  $\text{T}_3$  trap and capillary flow using the built-in-reservoir  $V_{\text{SA}}$ . The average of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values for NBS-20 calcite are  $1.03 \pm 0.09\%$ , and  $26.22 \pm 0.29\%$  ( $1\sigma$ ;  $n = 5$ ), respectively for samples weighing from 1.97 mg to 3 mg, which shows very good accuracy and precision (Fig. 12A, B). Samples with lower volume showed systematic lowering of both  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values (Fig. 12A, B). The international standard of carbon and oxygen isotope is NBS-20 (limestone, Solenhofen, Germany), and its recommended values are  $-1.06\%$  and  $26.64\%$  for  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^{18}\text{O}_{\text{V-SMOW}}$ , respectively. The minimum volume required for accurate measurements is c. 2 mg of calcite. In the case of graphite, the average of  $\delta^{13}\text{C}_{\text{V-PDB}}$  values for NBS-21 is  $-28.24 \pm 0.03\%$  ( $1\sigma$ ;  $n = 9$ ) and its recommended values is  $-28.1\%$ . The minimum volume required for accurate measurement is c. 20  $\mu\text{g}$  (Fig. 12C). The results for



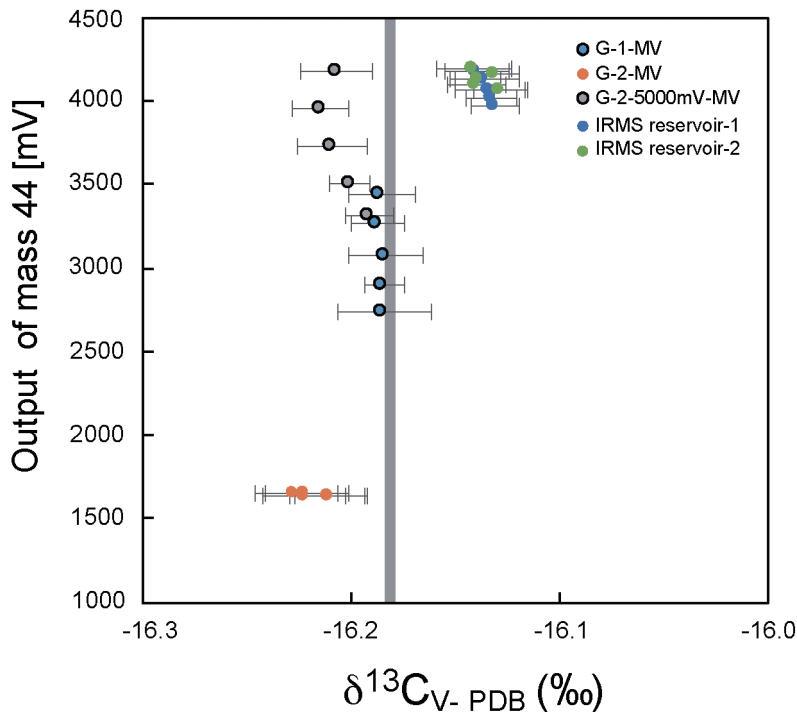
**Fig. 12.** The results of carbon and oxygen isotope measurements of international standards and a laboratory diamond standard. A)  $\delta^{13}\text{C}_{\text{V-PDB}}$  values of NBS-20 limestone plotted against gas pressure ( $P_{\text{SA}}$ ) at maximum volume of sample reservoir. B)  $\delta^{18}\text{O}_{\text{V-SMOW}}$  values of NBS-20 limestone plotted against gas pressure ( $P_{\text{SA}}$ ) at maximum volume of sample reservoir. C) The  $\delta^{13}\text{C}_{\text{V-PDB}}$  results of NBS-21 graphite powder plotted against gas pressure ( $P_{\text{SA}}$ ) at maximum volume of sample reservoir. D) The  $\delta^{13}\text{C}_{\text{V-PDB}}$  results of laboratory diamond standard.

diamond analysis were also similar, with  $\delta^{13}\text{C}_{\text{V-PDB}}$  values giving consistent results for both large volume (768  $\mu\text{g}$ ) and small volume samples (12.4  $\mu\text{g}$ )  $-4.98 \pm 0.06\text{‰}$  ( $1\sigma$ ;  $n = 7$ ) (Fig. 12D).

We also tested the carbon isotope measurements of laboratory standard graphite (SP1) using the micro-volume trap. The results show that carbon isotope measurements can be accurately done using MV trap (Fig. 13). However, measurements using MV-trap show considerable fractionation ( $>0.5$ ) for oxygen isotopes due to rapid pressure decrease in the sample side during the measurement and hence it was concluded that small volumes of  $\text{CO}_2$  gas can be measured accurately for both carbon and oxygen isotopes for the required precision using  $T_3$  at a lower output of c. 500 mV and further experiments are required to use the MV trap of simultaneous measurements of carbon and oxygen isotopes.

### Concluding remarks

A new inlet vacuum system was built and connected to the MAT-253 mass spectrometer



**Fig. 13.** The results of carbon isotope measurements of laboratory graphite standard (SP1) using the newly set up micro-volume trap. Note the vertical axis is the output of mass 44.

for the preparation and analyses of carbon and oxygen isotopes using  $\text{CO}_2$  gas. The vacuum line holds high vacuum for time intervals required for a single session of measurements spanning for >12 hours. A minimum volume c. 2 milligram of calcite is required for the precise measurement carbon and oxygen isotopes, whereas c. 12 micrograms of pure carbon (graphite or diamond) is required for the precise measurement of carbon isotopes.

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**Table I.** Carbon and oxygen isotope results of laboratory standard CO<sub>2</sub> gas for one year starting from April, 2020 to March 2021.

Measurement date	[44] signal (mV)	$\delta^{13}\text{C}$ (‰)	$1\sigma$	$\delta^{18}\text{O}$ (‰)	$1\sigma$
20200403-F	2074	-29.773	0.028	3.991	0.027
20200406-F	2246	-29.749	0.040	3.983	0.025
20200406-F	2202	-29.820	0.016	3.899	0.028
20200626-S	2127	-29.794	0.038	3.973	0.025
20200627-S	2134	-29.801	0.021	3.944	0.035
20200628-S	2105	-29.838	0.012	3.898	0.031
20200722-F	1378	-29.787	0.049	4.000	0.111
20200723-S	977	-29.814	0.054	3.987	0.108
20200723-F	796	-29.801	0.070	3.961	0.180
20200916-S	1793	-29.763	0.031	4.057	0.033
20200916-F	1890	-29.762	0.019	4.027	0.041
20200920-F	2193	-29.809	0.014	4.004	0.021
20201017-S	2162	-29.876	0.032	3.950	0.037
20201021-S	2069	-29.877	0.003	3.885	0.056
20201105-S	1514	-29.879	0.038	3.998	0.019
20201112-S	1453	-29.897	0.033	3.845	0.010
20201112-F	2157	-29.815	0.010	4.016	0.043
20201121-S	1411	-29.768	0.034	4.030	0.050
20201215-S	1997	-29.815	0.107	3.903	0.183
20201217-S	1178	-29.795	0.026	3.999	0.070
20201218-S	1060	-29.786	0.042	3.969	0.050
20210114-S	1606	-29.761	0.018	3.934	0.101
20210115-S	1037	-29.862	0.037	3.797	0.090
20210118-S	1538	-29.763	0.025	4.014	0.050
20210120-S	1544	-29.816	0.012	3.949	0.040
20210121-S	1518	-29.858	0.036	3.822	0.037
20210122-S	1524	-29.752	0.052	4.011	0.060
20210123-S	1504	-29.783	0.020	3.989	0.021
20210125-S	1462	-29.772	0.017	4.003	0.013
20210126-S	1465	-29.783	0.027	3.994	0.055
20210213-S	1520	-29.772	0.034	4.025	0.028
20210213-F	1416	-29.777	0.020	3.923	0.040
20210214-S	1503	-29.818	0.017	3.915	0.028
20210218-S	1453	-29.762	0.025	4.020	0.039
20210218-F	1347	-29.798	0.011	3.928	0.060
20210219-S	1232	-29.780	0.038	4.046	0.045
20210219-F	1376	-29.737	0.024	4.015	0.046
20210314-S	1694	-29.741	0.025	4.047	0.020
20210315-S	1622	-29.755	0.020	4.032	0.033
20210326-S	1356	-29.723	0.035	4.049	0.038
20210326-F	1371	-29.782	0.030	3.974	0.063
20210402-S	1193	-29.764	0.029	4.051	0.043
20210402-F	1154	-29.810	0.018	3.953	0.046
20210405-S	1163	-29.850	0.016	3.917	0.028
Average and standard deviation		-29.796	0.041	3.971	0.062

**Table 2.** Carbon and oxygen isotope results of NIST NBS-20 limestone, NBS-21 graphite and NU-Diamond.

Sample	Weight	PSA @ VSA100	$\delta^{13}\text{C}$ (‰)	$1\sigma$	$\delta^{18}\text{O}$ (‰)	$1\sigma$
NBS-20 limestone	(in mg)					
MSTD-S			-29.755	0.020	4.032	0.033
NBS-20-1		13.9	-1.060	0.010	26.050	0.010
NBS-20-2	2.9436	13.7	-0.924	0.011	26.574	0.020
NBS-20-3		13.5	-0.990	0.010	26.331	0.040
NBS-20-4	2.1572	7.7	-1.184	0.026	25.821	0.037
NBS-20-5	1.9664	8.4	-1.012	0.016	26.342	0.070
NBS-20-6	1.5356	5.7	-1.226	0.013	26.028	0.052
NBS-20-7	0.9903	4.1	-1.334	0.016	25.624	0.058
NBS-20-8	0.8171	2.9	-1.533	0.023	25.138	0.068
NBS-20-9	0.7681	2.4	-1.639	0.031	25.070	0.031
NBS-20-10	0.4901	1.2	-1.782	0.034	24.737	0.039
NBS-20-11	0.2357	0.3	-2.189	0.031	23.694	0.076
NBS-20-12	0.1640	0.1	-2.142	0.047	23.470	0.066
MSTD-F			-29.865	0.007	3.761	0.020
Average of 1-5 measurements			-1.034	0.097	26.224	0.292
Recommended values			-1.060		26.640	
NBS-21 graphite	(in $\mu\text{g}$ )	PSA @ VSA100				
MSTD-S			-29.741	0.025	4.047	0.020
NBS-21-1	120.9	6.1	-28.227	0.015		
NBS-21-2	66.5	3.3	-28.292	0.017		
NBS-21-3	58.2	2.8	-28.208	0.025		
NBS-21-4	47.1	1.5	-28.220	0.020		
NBS-21-5	45.5	1.4	-28.280	0.020		
NBS-21-6	44.7	0.8	-28.240	0.031		
NBS-21-7	43.4	0.6	-28.210	0.090		
NBS-21-8	33.1	1.4	-28.216	0.063		
NBS-21-9	24.7	0.7	-28.250	0.048		
NBS-21-10	9.4	0.1	-30.310	0.110		
NBS-21-11	7.0	-0.1	-28.190	0.014		
NBS-21-12	1.1	-0.5	-28.446	0.042		
MSTD-F			-29.814	0.018	3.729	0.027
Average of 1-9 measurement			-28.238	0.030		
Recommended value			-28.10			
NU-Diamond	(in $\mu\text{g}$ )	PSA @ VSA100				
MSTD-S			-29.764	0.031	4.040	0.020
NU-Dia-1	768.4	19.2	-4.935	0.010		
NU-Dia-2	132.3	9.7	-4.952	0.020		
NU-Dia-3	106.2	7.8	-4.904	0.009		
NU-Dia-4	68.4	4.9	-4.984	0.026		
NU-Dia-5	64.4	4.1	-5.012	0.016		
NU-Dia-6	26.1	1.5	-4.988	0.049		
NU-Dia-7	12.4	0.1	-5.101	0.046		
MSTD-F			-29.895	0.025	3.745	0.025
Average of 1-9 measurement			-4.982	0.064		

**Appendix-I.** Step-by-step procedure for carbon and oxygen isotope measurements using the new inlet system of the Thermo Fischer Scientific MAT-253 isotope ratio mass spectrometer at Niigata University. Procedure for start up, inlet of reference CO<sub>2</sub> gas (WSTD IN), laboratory standard measurement in the beginning (MSTD start), carbonate sample measurement, graphite/diamond measurement, laboratory standard measurement in the end (MSTD final), returning back of reference CO<sub>2</sub> gas to the storage reservoir (WSTD Back), close down of the inlet system and changing phosphoric acid in the carbonate reaction system are given in order.

1	Start Up Preperation
2	Date = ?
3	Room Temperature =?
4	HV=?
5	Vac=?
6	Magnet Steps=?
7	Box=?
8	Trap=?
9	Turbo Pumps RPM=?
10	PST at 100=?
11	PSA at 100=?
12	PR=?
13	PL=?

1	WSTD IN
2	PL=?
3	Close Valve 15
4	Close Valve 16
5	Close Valve 17
6	Close Valve 18
7	Close Valve 19
8	Close Valve 20
9	Close Valve 21
10	Close Valve 22
11	Close Valve 13
12	Close Valve 6
13	Close Valve 2
14	Close Valve 3
15	Close Valve 4
16	Close Valve 8
17	Close Valve 9
18	Close Valve 10
19	PL=?
20	VST to 20
21	Open Valve 14
22	Open valve 12
23	Open valve 25
24	PL=?
25	Wait 1 Minute
26	Close Valve 12
27	Set Liquid N2 on T5
28	Wait 5 minutes
29	PL=?
30	Close Valve 25

31	Open valve 21
32	PL=?
33	PST at 20=?
34	VST to 100
35	PST at 100=?
36	Open Valve 11
37	Open Valve B
38	[45]ST=?
39	Peak Centre check
40	Auto Focus
41	[45]ST=?
42	Pass to Gas Configuration

1	MSTD Start Measurement
2	PL=?
3	Open Valve 27
4	PL=?
5	Close Valve 27
6	Close Valve 21
7	PL=?
8	Open Valve 26
9	Wait 1 Minute
10	Close Valve 26
11	Open Valve 14
12	Open Valve 6
13	Open Valve 4
14	Open Valve 10
15	Open Valve 27
16	PL=?
17	Wait 1 Minute
18	Close Valve 14
19	Close Valve 6
20	Close Valve 4
21	PSA at 100=?
22	[45] SA = [45] ST ?
23	Measurement
24	Open Valve 21
25	PL=?



1	Carbonate Measurement
2	PR=?
3	Close Valve M20
4	Drop Thimble to Reaction Vessel
5	Wait 10 Minutes
6	T2> -126, Cool Pentane Trap
7	Open Valve 18
8	Open Valve 17
9	Close Valve 19
10	PR=?
11	Set Liquid N2 at T1
12	Open Valve M20
13	PR=?
14	Wait 10 Minutes
15	Close Valve M20
16	Open Valve 19
17	PR=?
18	Close Valve 17
19	Close Valve 18
20	Close Valve 19
21	Set Liquid N2 at T3
22	Open Valve 15
23	Open Valve 16
24	Open Valve 7
25	Open Valve 8
26	Remove Liquid N2 at T1
27	Defrost Trapped CO2 at T1
28	PR=?
29	Wait for 5 Minutes
30	Pr=?
31	Close Valve 7
32	Open Valve 9
33	Defrost Trapped CO2 at T3
34	PSA at 100=?
35	Close Valve 9
36	Adjust VSA
37	[45] SA = [45] ST ?
38	Measurement
39	After Measurement, VSA to 100
40	Open Valve 2
41	Open Valve 4
42	Open Valve 3
43	PSA at 100=?
44	Open Valve M20
45	Open Valve 19
46	Stir the Reaction Vessel
47	Wait 5 Minutes
48	Close Valve M20
49	Drop Next Thimble
50	Repeat the steps

1	Graphite/Diamod Measurement Start
2	PR=?
3	Open Valve 19
4	Set Tube Sample
5	Open Valve M21 Slowly
6	Wait 5 Minutes
7	Close Valve 19
8	Open Valve 18
9	Open Valve 17
10	Wait 1 Minute
11	PR=?
12	T2> -126, Cool Pentane Trap
13	Set Liquid N2 at T1
14	Crack Sample Tube
15	PR Maximum=?
16	PR Minimum=?
17	Wait 5 Minutes
18	PR=?
19	Close Valve M21
20	Open Valve 19
21	PR=?
22	Close Valve 17
23	Close Valve 18
24	Close Valve 19
25	Set Liquid N2 at T3
26	Open Valve 15
27	Open Valve 16
28	Open Valve 7
29	Open Valve 8
30	Remove Liquid N2 at T1
31	Defrost Trapped CO2 at T1
32	PR=?
33	Wait for 5 Minutes
34	Pr=?
35	Close Valve 7
36	Open Valve 9
37	Defrost Trapped CO2 at T3
38	PSA at 100=?
39	Close Valve 9
40	Adjust VSA
41	[45] SA = [45] ST ?
42	Measurement
43	After Measurement, VSA to 100
44	Open Valve 2
45	Open Valve 4
46	Open Valve 3
47	PSA at 100=?
48	Set Next Tube Sample

1	MSTD Final Measurement
2	PL=?
3	Open Valve 27
4	PL=?
5	Close Valve 27
6	Close Valve 21
7	PL=?
8	Open Valve 26
9	Wait 1 Minute
10	Close Valve 26
11	Open Valve 14
12	Open Valve 6
13	Open Valve 4
14	Open Valve 10
15	Open Valve 27
16	PL=?
17	Wait 1 Minute
18	Close Valve 14
19	Close Valve 6
20	Close Valve 4
21	PSA at 100=?
22	[45] SA = [45] ST ?
23	Measurement
24	Open Valve 21
25	PL=?

1	WSTD Back
2	PL=?
3	Close all Valves
4	PL=?
5	Set Liquid N2 on T5
6	Open Valve 25
7	PL=?
8	Open Valve 12
9	Open Valve 14
10	PL=?
11	Wait 5 Minutes
12	PL=?
13	Close Valve 25
14	Open Valve 21
15	PL=?
16	WSTD Back Finish

1	Close Down
2	Open Valve 21
3	Open Valve 20
4	Open Valve 19
5	Open Valve 18
6	Open Valve 17
7	Open Valve 16
8	Open Valve 15
9	Open Valve 8
10	Open Valve 9
11	Open Valve 10
12	Open Valve 11
13	Open Valve 12
14	Open Valve 13
15	Open Valve 2
16	Open Valve 3
17	Open Valve 4
18	Close Valve 7
19	Close Valve 14

1	Phosphoric Acid Exchange
2	Close Valve M22
3	Lower Stage of Reaction Chamber rotating Anti-clockwise
4	Remove Oil Inlet Tubes
5	Open Sample Holder Lid
6	Rotate Ring Clockwise Near Neck of Reaction Vessel
7	Replace Reaction Vessel
8	Rotate Ring Anti-Clockwise Near Neck of Reaction Vessel
9	Fit the Oil Inlet Tubes
10	Raise Stage of Reaction Chamber rotating Clockwise
11	Close Sample Holder Lid
12	Open Valve M22
13	Open Valve 19
14	Open Valve M20 Slowly Looking at Turbo Pump
15	Wait 15 Minutes
16	Close Valve 19
17	Open Valve 18
18	Open Valve 17
19	Wait 1 Minute
20	PR=?
21	Start Carbonate Measurement