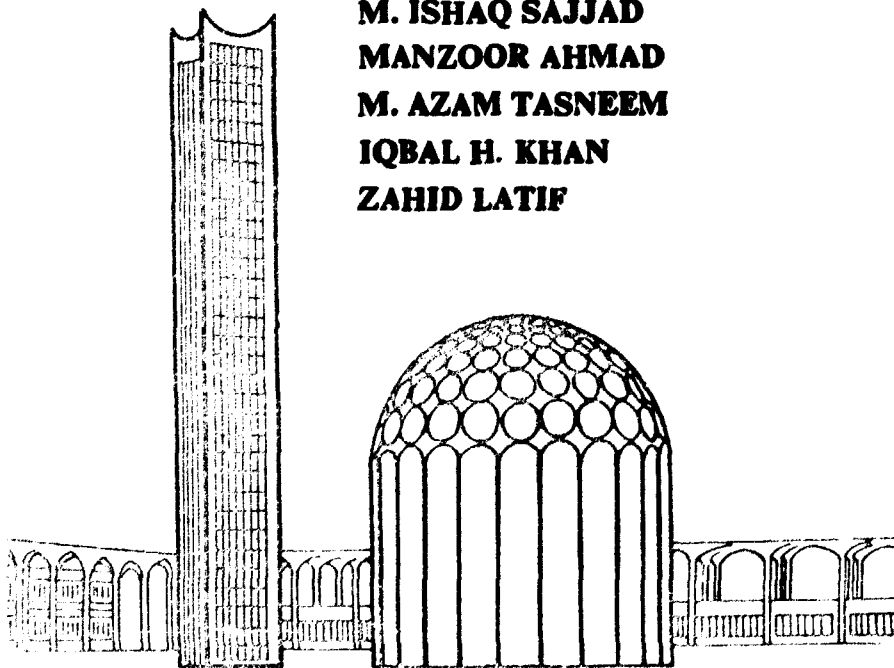


**MASS SPECTROMETRIC ANALYSIS
OF STABLE CARBON ISOTOPES IN ABIOGENIC
AND BIOGENIC NATURAL COMPOUNDS**

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A B S T R A C T

This report describes general methodology of $\delta^{13}\text{C}$ analysis on mass spectrometer and various preparation systems developed for conversion of samples into isotopically non-fractionated and purified CO_2 gas. Laboratory standards required for ^{13}C analysis have been calibrated against international standards.

The reproducibility/accuracy of sample preparation and analysis on mass spectrometer for $^{13}\text{C}/^{12}\text{C}$ measurement is well within the internationally acceptable limits.

**MASS SPECTROMETRIC ANALYSIS OF STABLE CARBON ISOTOPES
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1. Introduction

For understanding the carbon chemistry of an aquifer, it is helpful to determine different carbonate sources in that aquifer. The use of stable carbon isotopes analysis along with standard chemical analysis helps in estimating the sources of carbon in groundwater, as the carbon isotopic composition of various components of the carbon reservoir generally falls within distinct ranges [Keeling, 1958; Craig, 1953; Wickman, 1952; Galimov, 1966; Kunker, 1969]. $\delta^{13}\text{C}$ (‰) of atmospheric CO_2 as -7.0 ± 0.4 , sedimentary organic carbon and petroleum -28 ± 4 , land plants (wood & leaves) -25 ± 5 , soil CO_2 -24.7 (temperate climate); -18.2 (arid climate). In order to obtain a consistent pattern/information on the origin and geochemistry of various kinds of natural waters, all elements of the water cycle in nature are to be taken into account: sea water, precipitation, groundwater, and continental sea water.

The most commonly measured isotopes of dissolved constituents in groundwater are ^{13}C and ^{14}C in the inorganic carbon species (HCO_3 , CO_3 , H_2CO_3 , CO_2 (aqueous)). The age of groundwater is determined by measuring the decay rate and dilution factors of ^{14}C concentration where fractionations of ^{13}C provides information on carbonate solution that causes dilution of ^{14}C . In addition, photosynthesis bacterial activity and other chemical reactions cause fractionation of carbon isotopes that

help to identify the source of carbon.

Since the mid sixties, breath gas analysis (^{13}C measurement) has become an increasingly important tool for clinical diagnosis research. An isotopically labeled compound is administered to the patient or a volunteer. The metabolism of the food or drug taken is monitored by examining the carbon isotope ratio in expiratory CO_2 . Given appropriate instrumentation, this method reveals specific information about gastric disorders such as fat malabsorption, bacterial overgrowth, ileal malabsorption, lactose intolerance and biliary cirrhosis.

Contrary to the long-lived ^{14}C tracer, the ^{13}C method can be employed without any risk, thus offering an ideal alternative in metabolic studies. Recently, special attention is paid to ^{13}C breath tests as a diagnostic method for new-born infants. The sampling procedure causes almost no physical strain and makes stable isotope analysis suitable even for prematurely born or seriously ill babies.

New developments in isotope ratio mass spectrometers and their peripherals have opened the way towards routine clinical use, all the more since relatively cheap ^{13}C tracer substances have become available.

The samples for which $^{13}\text{C}/^{12}\text{C}$ ratio in CO_2 is to be measured mass spectrometrically, are first converted into CO_2 gas. This report describes various preparation systems developed, basic equations for processing the mass spectrometric data, mass spectrometric analysis of samples, calibration of standards and reproducibility of the results.

2. Sample Preparation Systems

Three types of preparation systems have been fabricated. First one is for the conversion of carbonates and dissolved-bicarbonates into CO₂ gas, second for burning plants, oil and gases etc. into pure CO₂ and the third is for the purification of breath CO₂.

2.1. Sample Preparation of Carbonates

Extraction of CO₂ from a carbonate is done by treating it with some acid. McCrea [1950] tried different acids and according to him the best way to prepare CO₂ from limestone is by treating the sample with orthophosphoric acid.

We have tried 95 % as well as 100 % H₃PO₄. Results are consistent with both types of concentrations. A mixture of commercially available 85 % H₃PO₄ and calculated quantity of P₂O₅ is stirred for few hours at a temperature of 150°C till all the oxide is dissolved and solution becomes clear.

About 100 mg powder of carbonate is evacuated in a special reaction vessel, as shown in figure 1 containing acid (5ml) in a side-arm. The acid (at 25.0°C) is tipped on the sample by tilting the vessel. This solution is put into constant temperature water bath at 25°C and allowed to react for overnight. The carbon dioxide is then expanded into vacuum line (figure 1) via a cold trap at -80°C to dry it and finally collected in sample tube (at -196°C) for analysis on mass spectrometer.

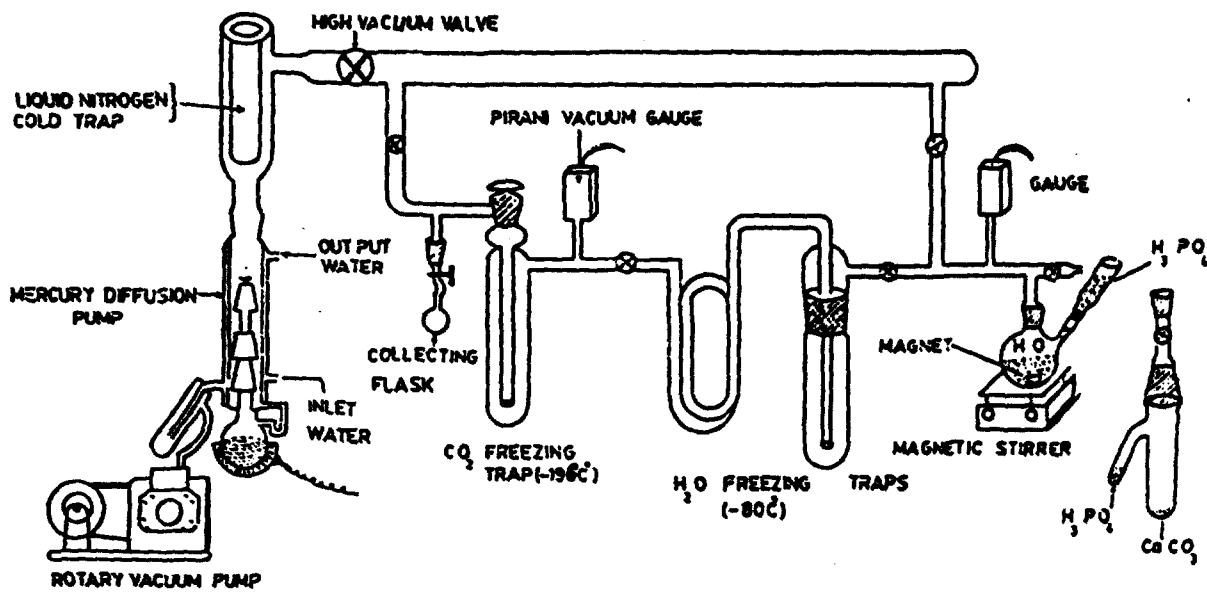


FIGURE 1. CO₂ PREPARATION FROM CARBONATES AND BICARBONATES.

2.2. Sample Preparation of Bicarbonates

Just like carbonates, all the inorganic carbon dissolved as bicarbonate, carbonate and CO_2 is evolved from the solution by its treatment with 100 % H_3PO_4 .

5 ml of H_3PO_4 is evacuated in a round bottom flask (figure 1). About 250 ml of water sample is allowed to enter through a side arm into the flask. It is then slowly stirred with a magnetic stirrer, while the evolved CO_2 is passed through two traps at -80°C to dry it and collected into sample tube at -196°C . At the final stage, CO_2 is purified by condensing it at -196°C then evolving & collecting it into sample tube while keeping the condensing trap at -80°C . In about 5 minutes, all the dissolved carbon is converted into CO_2 and 100% yield is obtained.

2.3. Sample Preparation of Plants, Gas and Oil

A sample combustion system has been fabricated to prepare carbon dioxide from plants, gas and oil and is shown in figure 2. The combustion of all three is performed in the same manner whereas the introduction of sample is slightly different for plant, gas and oil. A piece of plant sample is simply placed in a boat and introduced into system while the oil is frozen at -196°C in the boat and then placed into the system. In both cases after evacuation, the sample is burnt from outside with a gas burner whose temperature can be adjusted by providing a stream of oxygen or air. Whereas the gas is first filled into flask under vacuum and this flask is coupled with system. After evacuation,

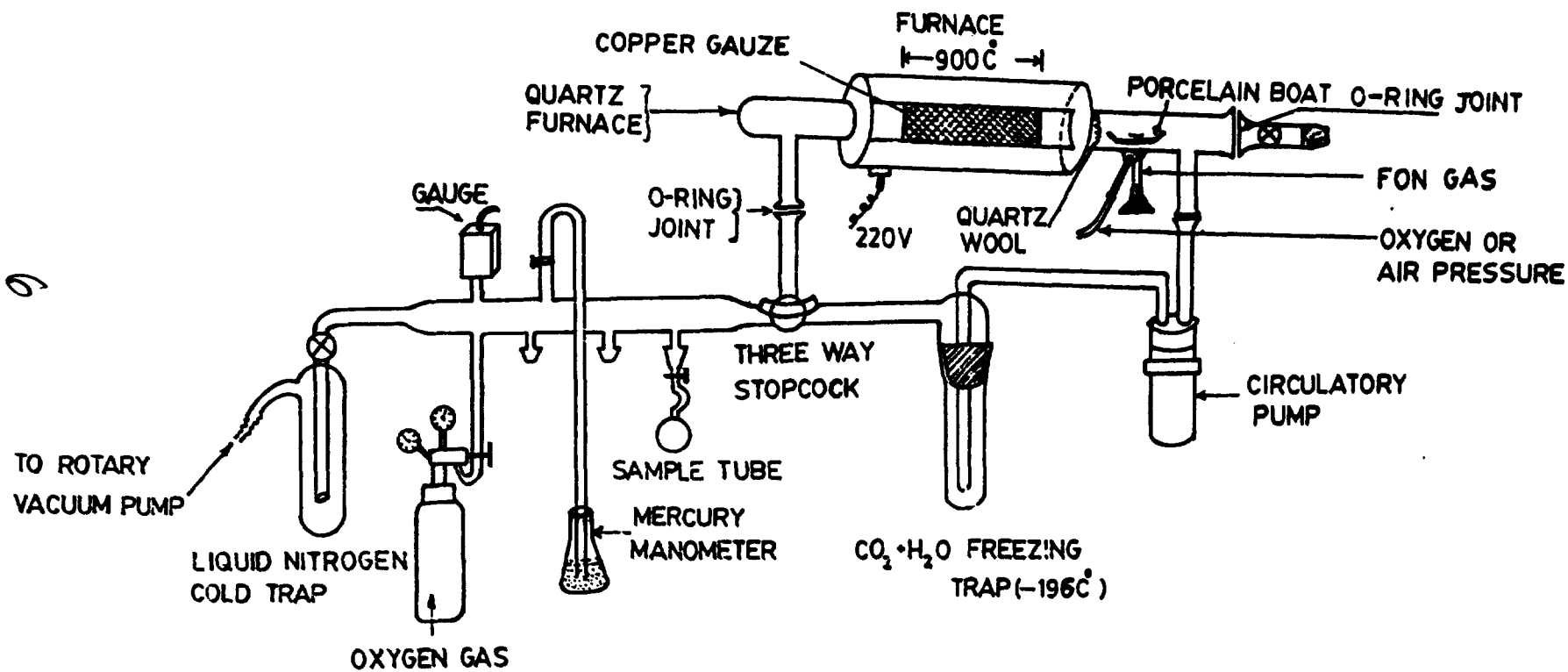


FIGURE 2. CO₂ PREPARATION SYSTEM FROM PLANTS, OIL AND GAS.

separating valve is opened and gas enters the combustion furnace.

In the second part of system, the evolved CO/CO₂ are passed over copper gauze heated with the help of specially designed heating furnace. All over the gauze length, the temperature is maintained at 900 °C. In the presence of oxygen provided from a tank, CO is converted into CO₂ and is condensed in a trap at -196°C, where water is also condensed. To accelerate the process of conversion of CO into CO₂, the gases (CO, O₂) are circulated with the help of a circulation pump and repeatedly passed over the heated copper gauze. Craig [1953], used a mercury toepler pump for such a circulation, we have replaced it with magnetically driven circulation pump as the operation of the toepler pump is complicated and sometimes unreliable. After 5 minutes of circulation, all CO is converted into CO₂ and is condensed in liquid nitrogen trap. This trap is then surrounded by liquid nitrogen-acetone mixture at -80°C and CO₂ is transferred into sample tube where it is again dried by condensing at -196°C and thawing at -80°C. This purified CO₂ is finally analyzed on a mass spectrometer.

2.4. Purification of expiratory CO₂

For breath tests, the patient is asked to blow into a balloon fitted with a glass stopcock. When sufficient expiratory CO₂ is filled into the balloon the stopcock is closed. This CO₂ contains water vapours, nitrogen, oxygen and some other gases. For its purification, the balloon is connected to CO₂ purifying system as shown in figure 3. The system is evacuated before the

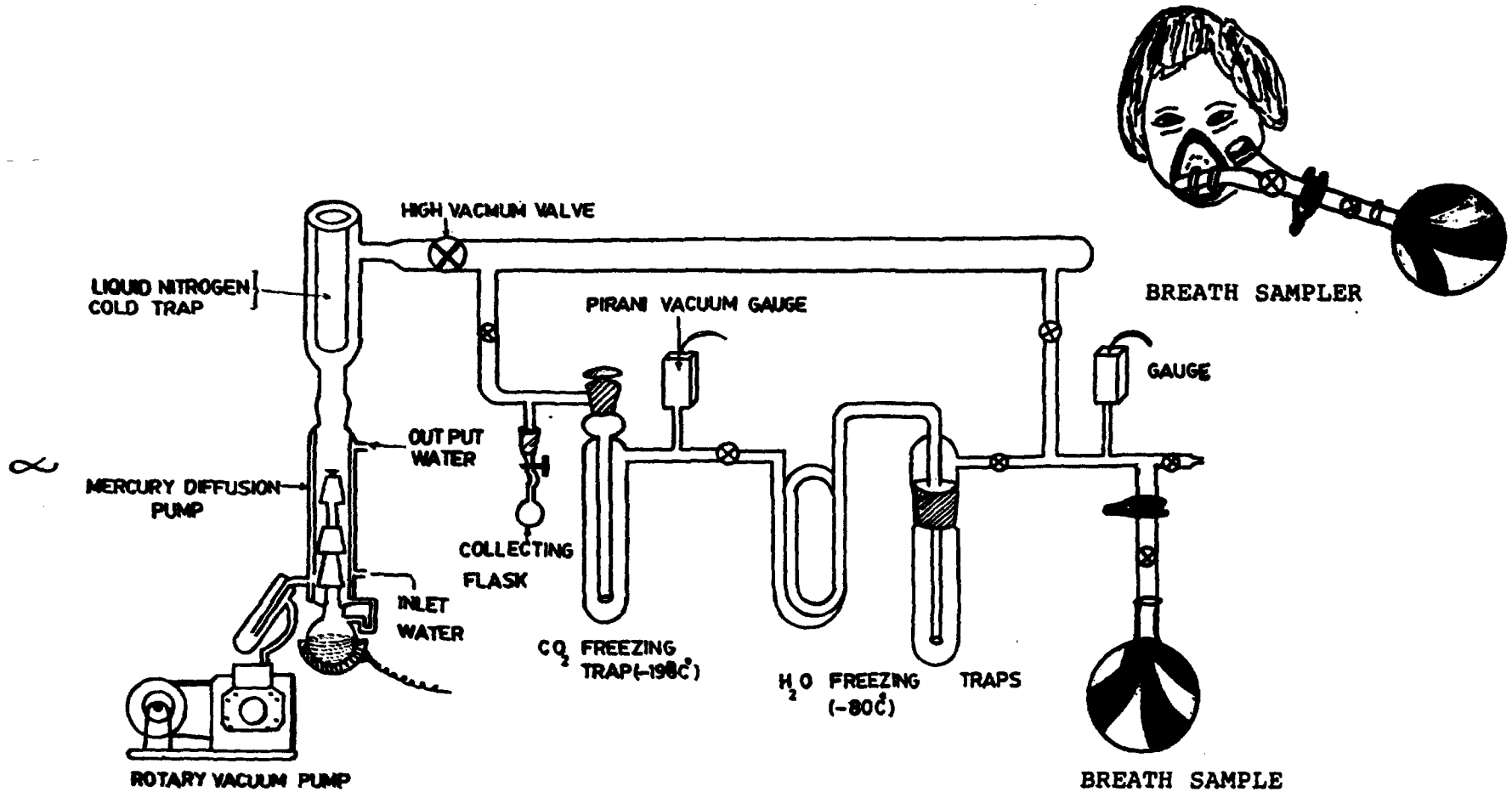


FIGURE 3. CO₂ PURIFICATION SYSTEM FOR BREATH SAMPLES.

sample in the balloon is expanded. The water vapours are trapped in water traps at -80°C , CO_2 is frozen in CO_2 freezing trap at -196°C and non condensable gases are pumped away. After a few minutes, all the CO_2 in the balloon is collected in LN_2 trap. For further purification, LN_2 trap is replaced by -80°C trap, CO_2 is expanded and collected in a flask at -196°C .

3. Basic Equations for Processing Mass Spectrometric Data

Mass spectrometric analysis of CO_2 is performed through isotopic masses: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$, $^{13}\text{C}^{16}\text{O}^{16}\text{O}$, $^{12}\text{C}^{16}\text{O}^{18}\text{O}$, $^{13}\text{C}^{16}\text{O}^{17}\text{O}$, $^{12}\text{C}^{17}\text{O}^{17}\text{O}$, $^{12}\text{C}^{16}\text{O}^{17}\text{O}$. For carbon-13 analysis, one measures mass 45 versus combined (mass 44 + mass 46) in CO_2 (figure 4). Similarly for the analysis of oxygen, one measures mass 46 versus the combined (mass 44 + mass 45) in CO_2 . The isotopic ratio actually measured corresponds to that of all species having mass 46 to all species having mass 44 and 45 which are collected together while measuring $^{18}\text{O}/^{16}\text{O}$ ratio. Similar is the case of ^{13}C analysis.

The mass spectrometer provides ratios of a samples 'A' and a sample 'B'. Their delta values, in terms of corresponding ratios (mass 45 to mass 44+46 or mass 46 to mass 44+45), with respect to the same working standard (W.S) are:

$$\delta_{ws}^A \text{ ‰} = \left\{ \left(R_A - R_{ws} \right) / R_{ws} \right\} \times 1000 \text{ ----- (1)}$$

$$\delta_{ws}^B \text{ ‰} = \left\{ \left(R_B - R_{ws} \right) / R_{ws} \right\} \times 1000 \text{ ----- (2)}$$

Where; δ_{ws}^A and δ_{ws}^B denote the delta values of sample 'A' and sample 'B' with respect to working standard respectively. From the above equations, the delta value of sample 'A' with

respect to sample 'B' (δ_B^A) can be shown as:

$$\delta_B^A = \delta_{ms}^A - \delta_{ms}^B \left\{ \frac{(1000 + \delta_{ms}^A)}{(1000 + \delta_{ms}^B)} \right\} \text{-----(3)}$$

This can also be written as:

$$\delta_B^A = 1000 \left\{ \left(\frac{\delta_{ms}^A - \delta_{ms}^B}{1000 + \delta_{ms}^B} \right) \right\} \text{-----(4)}$$

In order to transform (δ_B^A) into delta value of sample 'A' with respect to international standard 'PDB' (δ_{PDB}^A), the following relationship can be applied:

$$\delta_{PDB}^A = \delta_B^A + \delta_{PDB}^B + \delta_B^A \times \delta_{PDB}^B \times 10^{-3} \text{----- (5)}$$

where; the value of δ_{PDB}^B should be known.

Craig [1957] had reported the following equations for a collector system - major collector extending on both sides of minor as in our case:

$$\delta^{13}C = 1.0676 \delta^{45}S - 0.0294 \delta^{18}O \text{----- (6)}$$

$$\delta^{18}O = 1.0014 \delta^{46}S + 0.0091 \delta^{13}C \text{----- (7)}$$

$\delta^{18}O$ and $\delta^{13}C$ in the last correction terms of equation (6) & (7) can be replaced by $\delta^{46}S$ and $\delta^{45}S$ respectively. However, solving equations (6) and (7) simultaneously, the following equations, in terms of $\delta^{45}S$ and $\delta^{46}S$, can be obtained:

$$^{13}C = 1.0679 \delta^{45}S - 0.0294 \delta^{46}S \text{----- (8)}$$

$$^{18}O = 1.0017 \delta^{46}S + 0.0097 \delta^{45}S \text{----- (9)}$$

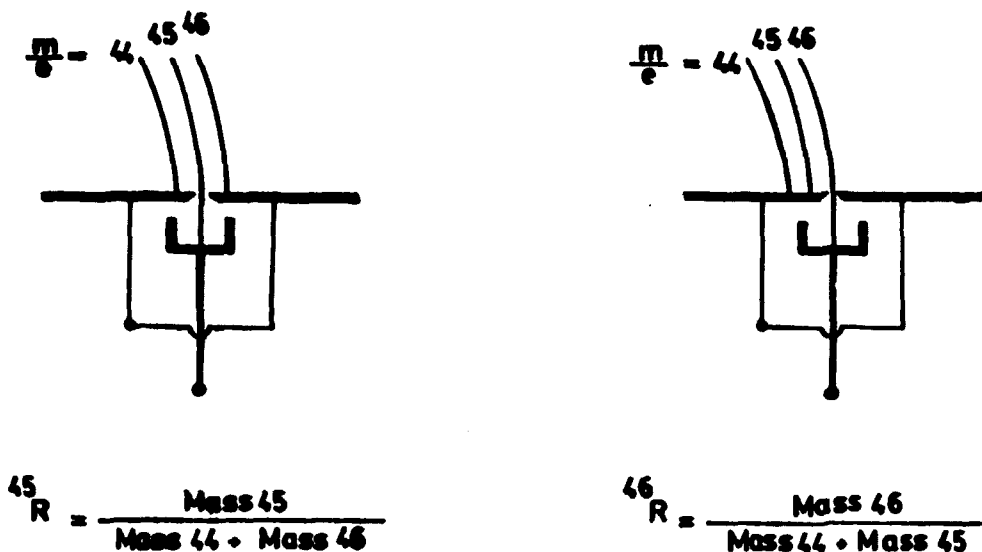


FIGURE 4. ION COLLECTOR SLITS CONFIGURATION.

3.1. On-line data acquisition

For highly precise ratio measurements on a mass spectrometer, a double sample inlet system is used. High precision is achieved using a working standard on one side and unknown sample on the other side. Sample and working standard are alternately introduced into the mass spectrometer and the ratios determined. Isotopic ratios thus obtained through the mass spectrometer are retrieved by the on-line computer and are used in the subsequent computations to obtain delta values of samples against working standard (δ_{ω}°) and finally against international standard. Standard deviation of the mass spectrometric measurement is also computed and each sample is checked for the standard deviation to be within permissible limits.

To handle all the above processes during the isotopic measurement, HP-41CX handheld computer has been coupled with the mass spectrometer through HP-JL/HP-JB interface. There are two

types of digital multimeters which are in use with the HP-41CX. The PREMA 6040S model with HP-IB interface which can be directly coupled with HP-41CX and the PREMA 5040 model with BCD interface which can be coupled with HP-41CX through PREMA 5023 IEC-BUS interface. The general procedure of isotopic analysis of Carbon-13 is same for any of the two digital multimeters. However, two different computer programmes have been developed for the two digital multimeters because of some characteristic differences in the mechanism of data retrieving system. Therefore a suitable computer programme is to be used for a particular digital multimeter connected with the computer(Table -1).

3.2. HP-41CX Computer.

HP-41CX handheld computer has a main memory of 2237 bytes (319 register) for programming and an extended memory of 2534 bytes (362 registers) for data/files storage. Total memory is expandable upto 6437 bytes (919 registers). HP-41CX has the facilities like printer (thermal,dot matrix), plotter, magnetic card reader & digital cassette drive for programme/data storage, HP-IL/HP-IB interface for communication with peripheral devices, (e.g. printer, plotter, digital multimeter, digital cassette drive etc). It has four plug-in ports for software modules, memory expansion module, peripherals, interfacing and added flexibility. In addition it has bar code reading capability, 12 character LCD, built-in time module providing time calendar alarm and stop watch functions, 200 separate operations in the function library with 58 functions right on the alphanumeric

keyboard, text-file editor, built-in extended functions/memory module and a broad range of available software pacs for scientific, engineering, and business applications. However, such pacs could not fullfil our particular requirements and we had to develop our own software.

3.3. Computer Programming.

Two separate computer programmes for mass spectrometric analysis of ^{13}C have been developed for two different digital multimeters (PREMA 6040S & PREMA 5040). Table-1 gives detailed information about the programmes which suit different sets of peripheral devices attached with the HP-41CX. A complete list of computer programmes for C^{13} analysis (with PREMA 5040 DMM and PREMA 5023 IEC-BUS interface) is given in elsewhere [Khan et al, 1987].

TABBL-1. COMPUTER PROGRAMMES FOR THE ANALYSIS OF C-13 FOR DIFFERENT SETS OF PERIPHERAL DEVICES ATTACHED WITH HP-41CX.

MEASUREMENT/ ANALYSIS	PROGRAMME TITLE	PERIPHERAL DEVICES WHICH SHOULD BE CONNECTED WITH HP-41CX COMPUTER.							
		HP-1L Module HP-82163A	HP-1L/HP-1B Interface. HP-82169A	PREMA 5023 IEC-BUS Interface	PREMA 5040 Digital Multimeter.	PREMA 6040S Digital Multimeter.	HP-82905B Printer.	HP-82143A Printer.	HP82161A Cassette Drive.
	INTERFACE-->	1L	1L-1B	BCD-1B	BCD	1B	1B	1L	1L
CARBON-13	C-13	YES	YES	YES	YES	NO	YES	NO	OPTIONAL
CARBON-13	CARBON	YES	YES	NO	NO	YES	YES	NO	OPTIONAL

3.4. Measurement of samples.

During the measurement of carbon-13 samples, three alternate ratios of sample and working standard (reference) are taken at the same CO₂ inlet pressure. Delta C-13, delta O-18, delta-45, delta-46 and standard deviation of the mass spectrometric analysis are computed as under:

Ratio of sample	Ratio of reference	Difference
(S)	(R)	(S-R)
S1	R1	D1 = (S1-R1)
S2	R2	D2 = (S2-R1)
		D3 = (S2-R2)
S3	R3	D4 = (S3-R2)
		D5 = (S3-R3)

Mean ratio of reference $\bar{R} = (R1+R2+R3)/3$ ----- (10)

Mean difference $(\overline{S-R}) = (D1+D2+D3+D4+D5)/5$ ----- (11)

a) Evaluation of δ^{45} and δ^{46}

Delta-45 and delta-46 can be computed as follows:

Delta-45 (‰) $\delta^{45} = \{(\overline{S-R}) / \bar{R}\} \times 1000$ ----- (12)
(when mass 45 selected)

Delta-46 (‰) $\delta^{46} = \{(\overline{S-R}) / \bar{R}\} \times 1000$ ----- (13)
(when mass 46 selected)

The per mil (‰) standard deviation (S.D) of the mass spectrometric analysis is computed as follows:

$$S.D \text{ (‰)} = \left[\left\{ \frac{(S-R)^2 - 5(\overline{S-R})^2}{4} \right\} / \bar{R} \right] \times 1000 \text{ ---(14)}$$

b) Evaluation of δ_{PDB}^{13C}

The delta value of C-13 of sample against international standard PDB (δ_{PDB}^{13C}) may be computed as follows:

From equation (8), (δ_{WS}^{13C}) is given by:

$$\delta_{WS}^{13C} = 1.0679 \delta_{WS}^{145} - 0.0294 \delta_{WS}^{146} \quad \text{----- (15)}$$

Using general relation (eq. 5), delta C-13 of a sample against PDB (δ_{PDB}^{13C}) is given by:

$$\delta_{PDB}^{13C} = \delta_{WS}^{13C} + \delta_{PDB}^{13C} + \delta_{WS}^{13C} \times \delta_{PDB}^{13C} \times 10^{-3} \quad \text{---- (16)}$$

Where; $\delta_{PDB}^{13C} = -35.79$

Substituting this value in equation (16) and solving equation (15) & (16) simultaneously, the delta value of sample against PDB becomes:

$$\delta_{PDB}^{13C} = [\{ (1.0679 \delta_{WS}^{145} - 0.0294 \delta_{WS}^{146}) \times (-35.79) \} / 1000] + (-35.79 + 1.0679 \delta_{WS}^{145} - 0.0294 \delta_{WS}^{146}) \quad \text{----- (17)}$$

c) Evaluation of δ_{PDB}^{18O}

From equation (9), delta O-18 of sample against working standard (δ_{WS}^{18O}) is given by the following relationship:

$$\delta_{WS}^{18O} = 0.0097 \delta_{WS}^{145} + 1.0017 \delta_{WS}^{146} \quad \text{----- (18)}$$

Using general relation (eq. 5), delta O-18 of sample against PDB (δ_{PDB}^{18O}) is given by:

$$\delta_{PDB}^{18O} = \delta_{WS}^{18O} + \delta_{PDB}^{18O} + \delta_{WS}^{18O} \times \delta_{PDB}^{18O} \times 10^{-3} \quad \text{----- (19)}$$

Where; the value of delta O-18 of working standard

against PDB (δ_{PDB}^{18O}) = -11.36. Substituting this value and solving equation (18) & (19) simultaneously, delta O-18 of sample against PDB standard becomes:

$$\delta_{PDB}^{18O} = \left[\left(0.0097 \delta_{45}^{18O} + 1.0017 \delta_{46}^{18O} \right) \times (-11.36) \right] / 1000 + \left(-11.36 + 0.0097 \delta_{45}^{18O} + 1.0017 \delta_{46}^{18O} \right) \quad \text{----- (20)}$$

3.5. Programming Philosophy.

In routine, CO₂ samples are prepared and measured against cylinder CO₂ gas referred to as working standard. Six values of ratio (three each of sample and standard) are taken and delta values of sample against working standard are computed for mass 45 and mass 46 (eq. 10 & 11). The standard deviation of mass spectrometric analysis is computed through equation (12). If the standard deviation is within permissible limits, these values of δ_{45}^{18O} and δ_{46}^{18O} are referred for the evaluation of δ_{45}^{13C} and δ_{46}^{18O} (eq. 15 & 18) otherwise the sample measurement is repeated. Finally the values of δ_{45}^{18O} , δ_{46}^{18O} , δ_{PDB}^{13C} and δ_{PDB}^{18O} along with sample numbers are printed.

4. Reproducibility of Results and Calibration of Standards

4.1. Carbonates

Two carbonate samples were repeatedly processed in sample preparation system and analyzed on modified GD-150 mass spectrometer [Sajjad et. al; 1980; 1981; 1982] . The results against a laboratory standard are given in tables 2 & 3.

a) **First Carbonate Sample**

Table 2. Results of repeated analyses of first carbonate sample.

Analysis No.	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
1	6.43	9.15
2	6.54	9.10
3	6.54	9.24
4	6.48	9.17
5	6.55	9.14
6	6.57	9.26
7	6.47	9.17
8	6.49	9.11
9	6.53	9.08
Mean δ	6.51 ‰	9.16 ‰
st.dev.	0.05 ‰	0.06 ‰

b) **Second Carbonate Sample**

Table 3. Results of repeated analyses of second carbonate sample.

Analysis No.	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
1	5.96	8.66
2	6.12	8.69
3	6.06	8.68
4	5.98	8.66
Mean δ	6.03 ‰	8.67 ‰
st.dev.	0.07 ‰	0.07 ‰

c) Calibration of Standards

The universally accepted carbon standard is PDB. It is carbonate standard derived from the rostrum of *Belemnitella americana* from the Pee Dee Formation of South California (USA). NBS-20 has relation with PDB [Craig, 1957] as:

$$\delta^{13}\text{C} (\text{NBS-20 vs PDB}) = -1.06 \text{ ‰}$$

At PINSTECH, we have prepared a carbonate standard namely Pinstech Carbonate Standard (PCS) and calibrated it against many other standards at PINSTECH as well as at Institute for Radiohydrometrie (IfR) Munich, F.R. Germany (during the visit of the author to that Institute). The results are given below:

Table 4. Results of PCS against PDB measured at IfR, Munich.

No.	$\delta^{13}\text{C}(\text{‰})$	$\delta^{18}\text{O}(\text{‰})$	Standard Used
1	+2.26	-2.04	HeidleBerg Std-1
2	+1.98	-2.16	Using Carrara Marble
3	+1.85	-2.44	Using Toil Seat Marble
4	+2.14	-3.01	IfR Standard
Mean δ	+2.06	-2.41	
st.dev.	0.18	0.43	

Results of PCS against PDB measured at PINSTECH using NBS-20 are :

$$\delta^{13}\text{C} (\text{PCS vs PDB}) = +1.96 \pm 0.07 \text{ ‰}$$

$$\delta^{18}\text{O} (\text{PCS vs PDB}) = -2.48 \pm 0.04 \text{ ‰}$$

The results are in good agreement with the results of IfR /GSF Munich laboratory as given above.

4.2. Bicarbonates

The reproducibility of bicarbonate samples (from tap water) on different days is given in table 5.

Table 5. Results of analyses of bicarbonates.

Date	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
01.04.1984	-7.72 ‰	-5.90 ‰
	-7.66 ‰	-6.28 ‰
	-7.54 ‰	-6.05 ‰
	-----	-----
	Mean δ	-7.64 ‰
st.dev.	0.09 ‰	0.19 ‰
02.04.1984	-7.87 ‰	-5.86 ‰
	-7.88 ‰	-6.00 ‰
	-8.03 ‰	-5.87 ‰
	-7.68 ‰	-5.52 ‰
	-7.71 ‰	-6.26 ‰
	-----	-----
	Mean δ	-7.83 ‰
st.dev.	0.14 ‰	0.27 ‰

The reproducibility of $\delta^{18}\text{O}$ of bicarbonates is not good and that, in turn, affects the reproducibility of $\delta^{13}\text{C}$ through the correction term. The possible reason for this could be the non-equilibration conditions between water and CO_2 evolved from water through reaction with acid.

4.3. CO₂ Gas

We have selected two CO₂ gaseous standards for our laboratory and have calibrated both of these against PDB.

WS-1 (working standard-1) is CO₂ gas (99.99 %) cylinder imported from U.S.A and is very much depleted both in ¹³C and ¹⁸O.

WS-2 (working standard-2) is locally produced CO₂ gas. Its $\delta^{13}\text{C}$ is similar to WS-1 but $\delta^{18}\text{O}$ value is much higher.

Their calibrated delta values against PDB are:

$$\text{WS1 } \delta^{13}\text{C} = -35.31 \pm 0.02 \text{ ‰}$$

$$\text{WS1 } \delta^{18}\text{O} = -35.93 \pm 0.12 \text{ ‰}$$

$$\begin{aligned} \text{WS2 } \delta^{13}\text{C} &= -35.97 \pm 0.07 && \text{using PCS} \\ &= -35.97 \pm 0.05 && \text{using NBS-20} \end{aligned}$$

$$\begin{aligned} \text{WS2 } \delta^{18}\text{O} &= -11.36 \pm 0.04 && \text{using PCS} \\ &= -11.36 \pm 0.05 && \text{using NBS-20} \end{aligned}$$

The above results clearly prove that the reproducibility of the preparation systems and the mass spectrometer is excellent.

4.4. Plant, Oil and Gas.

The known quantities of plants, oil and gas were repeatedly combusted in the preparation system and CO₂ produced analyzed on the mass spectrometer. Results against PDB are given in table 6.

Table 6. Results of analyses of plant, oil and gas samples.

S.No.	Sample	No. of Preparations	$\delta^{13}\text{C}$ (‰)
1	Wood-1	2	-29.88 ± 0.08
2	Wood-2	3	-26.94 ± 0.13
3	Petrol	2	-22.84 ± 0.12
4	Oil	3	-29.78 ± 0.09
5	Sui gas	5	-34.00 ± 0.11
6	Fon gas	3	-23.71 ± 0.10

The reproducibility is very good for $\delta^{13}\text{C}$.

5. Conclusion

The $\delta^{13}\text{C}$ analysis facility set up at PINSTECH can be employed for a variety of samples. The preparation systems developed, efficiently convert the samples into CO_2 gas with out any fractionation. One solid carbonate (CaCO_3) and two gaseus (CO_2) standards have been prepared and calibrated against international standard 'PDB' for internal use. The reproducibility of results and accuracy of mass spectrometric analysis is within the internationally accepted limits of 0.1 ‰ to 0.2 ‰ .

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