

# Stable Carbon Isotope Ratio Measurements With a Gas Density Meter

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A method is presented for measuring  $C^{13}/C^{12}$  isotope ratio of organic compounds by burning the material in a stream of normal  $CO_2$ , separating the combustion products by gas chromatography and sequentially measuring the density of the enriched  $CO_2$  peak with a gas density meter. Data are presented for the analysis of enriched ethyl acetate from 15 to 1 percent enrichment. The method is applicable for other stable isotope analysis.

Key Words: Carbon-13; carbon dioxide; ethyl acetate; gas chromatography; gas density meter; isotope ratios.

## 1. Introduction

Several years ago, Liberti, Conti, and Crescezzi [1]<sup>1</sup> and Phillips and Timms [2] made molecular weight determinations using a gas density meter [3]. The latter investigators found that with nitrogen as both the carrier and reference gases, molecular weights up to 150 could be determined to an accuracy of 1 percent. The present study can be considered to be an extension of these earlier investigations. Primary attention will be devoted to the measurement of the molecular weight of a substance, carbon dioxide, to the highest possible precision. This will permit the determination of the isotopic composition or  $C^{13}/C^{12}$  ratios of enriched samples of numerous organic compounds.

When a sample of gas density  $\rho$  and volume  $v$  is swept into the gas density meter, the integral response (peak area  $A$ ) of the detector can be written as

$$A = kv(\rho - \rho') \quad (1)$$

where  $k$  is a cell constant and  $\rho'$  is the density of the carrier and reference gases. Since the latter is known, a direct determination of the density of the sample can be made. Its molecular weight can then be determined from the perfect gas law or some other appropriate equation of state. In this study carbon dioxide is used as the carrier and reference gas. An enriched sample of a volatile compound is com-

pletely burned and the density of the newly formed carbon dioxide is compared to that of the normal gas. In this mode of operation full advantage is taken of the fact that the gas density meter is a differential detector. A signal will be generated only when enriched carbon dioxide is passing through the detector.

## 2. Experimental Procedure

A schematic drawing of the flow system and the gas density meter is given in figure 1. The procedure was as follows: (a) injection of enriched samples, (b) its combustion at 730 °C as it passed through a 6-in (0.20 in i.d.) long stainless steel column packed with 40–60 mesh copper oxide, (c) separation of the combustion products, principally carbon dioxide and water, by a 4 ft silica gel column (coated with 2 percent ethyl-hexasebacate), and (d) density determination in the gas density meter. The test substance used was enriched ethyl acetate. Analyses were carried out with the original mixture as well as various samples diluted with normal ethyl acetate. The sample size ranged from 10 to 25  $\mu$ l. Freshly ground wire copper oxide sieved to 40–60 mesh was found to give the optimum conditions for combustion. A finer mesh gave more efficient combustion but the resulting pressure drop across the columns was found to be too great. Under the present conditions combustion to carbon dioxide was always over 99 percent complete. Methane which was the principal minor product was completely eluted before the appearance of the  $C^{13}O_2$  peak. After about 70  $\mu$ l of the ethyl acetate

<sup>1</sup> Figures in brackets indicate the literature references at the end of this paper.

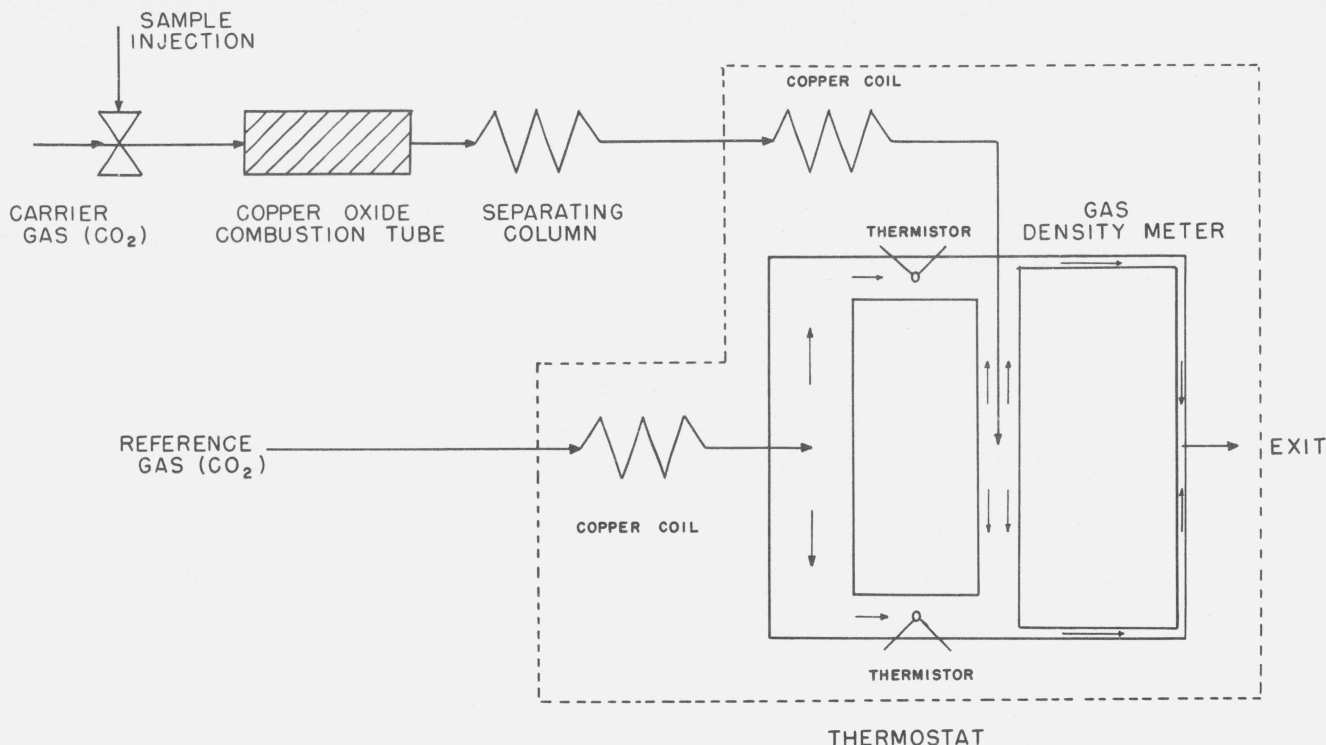


FIGURE 1. Flow system and gas density meter.

was burned ethane and ethylene appeared as combustion products and the copper oxide had to be renewed.

The gas density meter was essentially a copy of the one designed by Nerheim [4]. Two 10-ft 1/4-in o.d. copper coils immediately preceding the meter permitted the carrier and reference gases to equilibrate to thermostatic temperature. The density meter and thermostating coils were immersed in a 25-gal oil bath. Better results were obtained when the bath temperature was allowed to drift rather than attempting to regulate it. The temperature change was approximately  $0.01^\circ$  per hour. A one-liter tank down stream from the meter effectively isolated the detector from atmospheric disturbances. All experiments were carried out at two atmospheres pressure, with the detector at approximately  $25^\circ\text{C}$ . Two sets of matched thermistors were tried out as the sensing elements, Fenwal G 112 ( $8000\ \Omega$ )<sup>2</sup> and Fenwal G 128 ( $100,000\ \Omega$ ). The former when amplified (5 X) by a Honeywell deviation amplifier had about the same sensitivity as the  $100,000\ \Omega$  thermistors but with a somewhat lower noise level. The thermistors formed adjacent arms of a standard Wheatstone bridge circuit [5] with the operating current at 5 mA for the  $8000\ \Omega$  thermistors and 1 mA for the  $100,000\ \Omega$  thermistors.

Detector response was displayed on a  $1\mu\text{V}$  full scale potentiometer recorder (Leeds and Northrop Speedomax) with adjustable speed. The chart speed and attenuation were adjusted so that the area of the recorded peaks could be copied on a  $8 \times 17$ -in Xerox paper. The peak areas were determined as the average weight of three cutouts of the peak. The reproducibility of the weight of a cutout of the same peak was about 1.0 percent. The weight of each peak was approximately 13 g. The carbon dioxide used in these experiments was "Coleman Grade" from the Matheson Company. The flow rates were about  $50\ \text{cm}^3/\text{min}$  for the carrier gas and  $100\ \text{cm}^3/\text{min}$  for the reference gas.

The gas density meter was calibrated by recording the response of the detector to the passage of known volumes  $v_A$  (at 2 atm and 25 K) of argon. The latter was injected into the system immediately before and after a  $\text{C}^{13}\text{O}_2$  determination. The cell constant  $k'$  was determined using the equation

$$k' = A[v_A(M_A - M_{\text{CO}_2}/Z)]^{-1} \quad (2)$$

where  $M_A$  is the molecular weight of argon,  $M_{\text{CO}_2}$  the molecular weight of carbon dioxide (in natural abundance),  $A$  the average weight of the Xerox cutouts, and  $Z$  the compressibility of carbon dioxide at 2 atm and  $25^\circ\text{C}$ . This relationship is similar to that of eq (1) except that the molecular weight dependence is now explicitly given. It is a mark of the sen-

<sup>2</sup>Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

sitivity of the density meter that failure to take the compressibility factor (0.990) [6] into account would have resulted in an error of 10 percent in the isotope ratio determinations. It was not necessary to consider the compressibility of argon since by the time the 100–200  $\mu\text{l}$  sample reached the detector it was highly diluted and behaved as a perfect gas. This assumes no interaction between argon and carbon dioxide. From the cell constant and the peak area  $A'$  of the enriched  $\text{CO}_2$ , the molecular weight increment was calculated from the expression

$$\Delta M_{\text{CO}_2} = \frac{ZA'}{k'} \left[ \frac{4ZRTv_1d \left(1 + \frac{\Delta M}{M}\right)}{PM \left(1 + \frac{\Delta M}{M}\right)} \right]^{-1} = \frac{A'PM}{4k'RTv_1d}$$

where  $v_1$  is the volume of enriched ethyl acetate injected into the system,  $d \left(1 + \frac{\Delta M}{M}\right)$  the liquid density of the enriched ethyl acetate,  $M$  its molecular weight, and  $P$  and  $T$  the pressure and temperature at which experiments were carried out (2 atm and 25 °C). The factor of 4 takes into account the number of carbon atoms in ethyl acetate. The absolute ratio of carbon-13 to carbon-12 was calculated from the relation

$$\text{C}^{13}/\text{C}^{12} = \Delta M_{\text{CO}_2} + 0.0111.$$

The last number represented the natural abundance [7].

### 3. Results and Discussion

A typical chromatogram of a 1 percent enriched sample is shown in figure 2. This signal is a direct measure of the density difference between the enriched and normal carbon dioxide. On the basis of the observed peak height and base line noise, it can be concluded that the minimum detectable isotopic enrichment ( $S/N=2$ ) is about 0.02 percent. This is equivalent to a molecular weight difference of 0.0002 g/mol. Sensitivity can probably be improved with the injection of larger samples. The experimental results are summarized in table 1. The uncertainty limits for each individual run should not exceed 6 percent. This arises from estimated uncertainties in area measurement, in the volume of the injected liquid sample and the determination of the cell constant. It can be seen from table 1 that practically all the data fall within this limit. The larger error at the 3.17 percent enrichment is probably due to a dilution error. This is supported by the good reproducibility of the three determinations carried out at this enrichment. Peak height measurements also gave good values for isotope enrichment, providing the same sample size was used each time. A typical calibration plot is given in figure 3. The main deficiency of such a procedure is the necessity for calibration with known enriched samples.

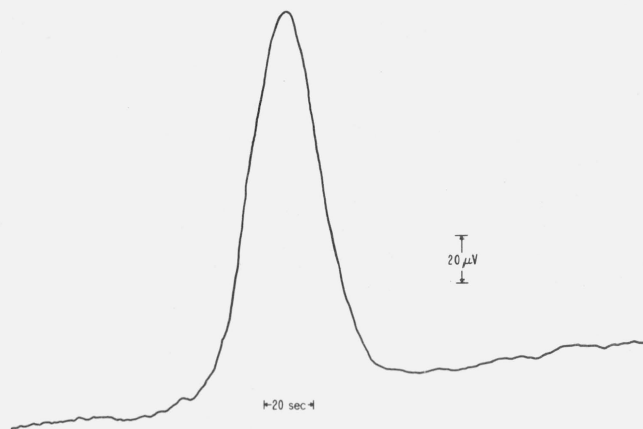


FIGURE 2. Chromatogram of 1 percent enriched  $\text{C}^{13}\text{O}_2$ .

Isotope ratios are usually determined using a mass spectrometer. The sensitivity limits are governed by the cracking pattern and the presence of the various natural occurring isotopes. It is estimated [8] that for carbon dioxide a typical commercial analytical instrument will have uncertainty limits of between 0.05 to 0.10 percent enrichment. On this basis, it is

TABLE 1. Gas density analysis of  $\text{C}^{13}$  content  $\text{CO}_2$  produced by burning enriched ethyl acetate

% $\text{C}^{13}$ (abs.) natural abundance	Volume of sample (liquid ethyl acetate) $\mu\text{l}$	Observed change in average molecular weight $\Delta M$	% $\text{C}^{13}$ cal. from $\Delta M$
16.08 <sup>a</sup>	7	0.1457	15.67 <sup>c</sup>
16.08	7	.1458	15.68
16.08	10	.1466	15.76
16.08	10	.1511	16.21
16.08	10	.1532	16.42
16.08	10	.1511	16.21
16.08	10	.1548	16.51
16.08	10	.1508	16.18
			16.08 $\pm$ 0.25 <sup>e</sup>
8.59 <sup>b</sup>	10	.0727	8.37
6.092 <sup>b</sup>	7	.04899	5.999
6.092	7	.04905	6.005
			6.002 $\pm$ 0.003
5.385 <sup>b</sup>	10	.04283	5.383
5.385	10	.04219	5.319
			5.351 $\pm$ 0.03
3.599 <sup>b</sup>	10	.0235	3.45
3.599	10	.0234	3.44
			3.445 $\pm$ 0.005
3.17 <sup>b</sup>	25	.0180	2.90 <sup>c,d</sup>
3.17	10	.0177	2.87
3.17	10	.0176	2.86
			2.88 $\pm$ .02
2.324 <sup>b</sup>	10	.01202	2.302
2.324	10	.0123	2.332
			2.317 $\pm$ .02
1.45 <sup>b</sup>	10	.00345	1.445

<sup>a</sup> Enrichment in material as reported by supplier.

<sup>b</sup> Enriched ethyl acetate (16.08%) diluted with normal ethyl acetate.

<sup>c</sup> The estimated uncertainty in the numbers in this column, based on the various parts of the experimental procedure is 6 percent.

<sup>d</sup> Dilution technique used here is believed to be an important source of error.

<sup>e</sup> Standard deviation.

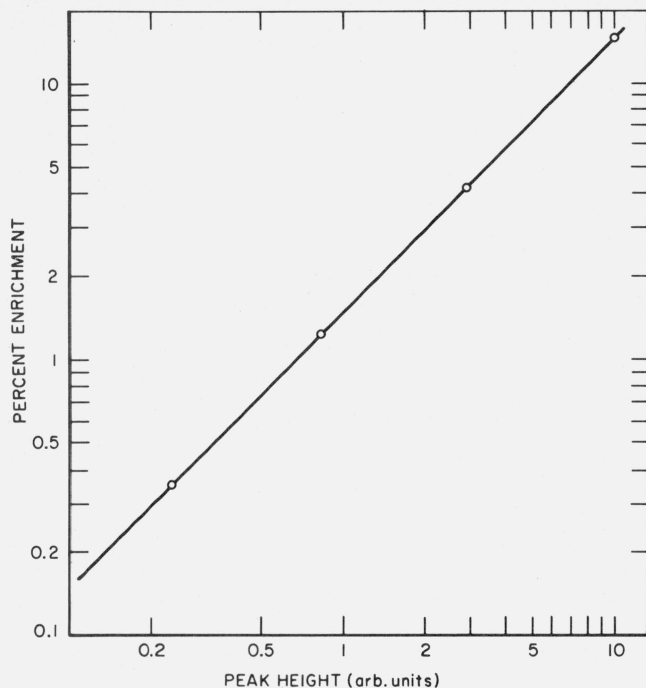


FIGURE 3. Calibration of percent enrichment versus peak heights.

clear that the sensitivity of the gas density meter is sufficient for it to be employed in routine determinations of isotope ratios. The chief attraction of such a procedure in comparison with mass spectrometric determinations is the low capital cost for gas density determinations. Practically all the equipment used in these studies is on hand in any gas chromatographic laboratory. The experimental procedure is exactly the same as that used in routine chromatographic analysis and thus extremely simple and straight forward.

Although the present study is only concerned with the determination of carbon-13/carbon-12 ratios in ethyl acetate, it is quite clear that with the present set-up practically any organic compound could have been used. Ethyl acetate was selected as the test substance solely for its ready availability and convenience of handling. Furthermore flow systems similar to that given here can probably also be set up to determine oxygen-18/oxygen-16 [9] and nitrogen-15/nitrogen-14 [10] ratios. Due to the great difference in their thermal conductivities deuterium/hydrogen

ratios can probably be determined most accurately using thermal conductivity detectors. With regard to the lower hydrocarbons, such as methane and ethane where there may be combustion problems or for that matter any gaseous compounds, the best procedure would be to discard the burner completely and to use as the carrier and reference gases the normal versions of the substance whose isotope ratio is to be determined. This should have the added benefit of increasing sensitivity.

Finally, the unique capabilities of the gas density meter as a chromatographic detector in experiments similar to the type described here deserve wider appreciation. When the carrier gas has physical and chemical properties similar to that of the solute or such cases where there are high concentration of detectable material ultrasensitive ionization detectors will respond mainly to the carrier gas and may approach saturation. This is not usually encountered in normal chromatography, but may occur in vacancy chromatography or when a volatile substrate is used. Thermal conductivity detectors which have about the same sensitivity as the gas density meter become unpredictable in mixtures of components having roughly the same conductivity. Calibration of the gas density on the other hand is independent of the molecular constitution of the species studied, which is, in sharp contrast to the behavior of other classes of detectors. It is foreseeable that there will be an increased use for the gas density meter in the future as a gas chromatographic detector and for other physical chemical experiments.

#### 4. References

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