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AN IMPROVED PROCEDURE FOR WET OXIDATION OF THE ^{14}C NBS OXALIC ACID STANDARD

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ABSTRACT. An improved procedure for wet combustion of ^{14}C NBS oxalic acid standard has been devised which gives consistent high carbon yields and average $\delta^{13}C$ values of —19.1‰. The principal cause of fractionation in earlier attempts to prepare CO_2 by the wet oxidation method was the inexact nature of the end-point. The new procedure employs a chocolate-brown end-point by adding 5ml more of the sulfuric acid-potassium permanganate solution after the initial reddish brown end-point is reached. The sulfuric acid-potassium permanganate solution is added to the NBS oxalic acid in a steady drop-wise flow, heat is applied to the generating apparatus, and a cycling technique is utilized to collect the CO_2.

Fifteen samples of the NBS oxalic acid were processed. The per cent carbon yields range from 98.8 to 100.9% with an average of 99.7% and an average $\delta^{13}C$ of —19.12‰. The results obtained by this procedure are much more consistent than previous results obtained in several laboratories by direct combustion.

WET OXIDATION OF OXALIC ACID

Wet oxidation of the ^{14}C NBS oxalic acid standard to carbon dioxide is an accepted technique used by most radiocarbon laboratories. The procedure is simple and straightforward. After some years of use, however, investigators have discovered that fractionation does occur during CO_2 evolution because of difficulty in determining the proper end-point of the reaction.

Fractionation during this chemical reaction has been documented by several investigators. Lindsay (1949) reported as much as 3.5% change in the ^{13}C concentration in the CO_2 during the decomposition of oxalic acid, and Bernestein (1957), working with formic acid decomposition by sulfuric acid, stated that as much as 53% variations occurred. Grey *et al* (1969), in preparing CO_2 from the ^{14}C oxalic acid standard, reported a $\delta^{13}C$ value of –25.5‰ relative to PDB due to incomplete reaction.

Fractionation during oxidation of the NBS oxalic acid standard is of concern because it may offset counting rates, resulting in systematic errors in age determinations.

Craig (1961) determined the $^{13}C/^{12}C$ ratio of the standard gas samples from several laboratories, prepared by both wet oxidation and direct combustion in oxygen. $\delta^{13}C$ values by direct combustion with oxygen ranged from –17.15 to –22.72‰ with an average of –19.3‰ (10 samples), while the wet oxidation values ranged from –18.37 to –31.37‰. Rejection of the four most depleted values gave an average of –19.6‰ (6 samples). According to Craig, depleted values are to be expected if the oxidation has not gone to completion.

Since the primary ^{14}C standard for the University of Texas Radiocarbon Laboratory is NBS oxalic acid (as it should be for all laboratories), the personnel of the laboratory were compelled to improve the wet oxidation procedure so that no significant fractionation would occur.

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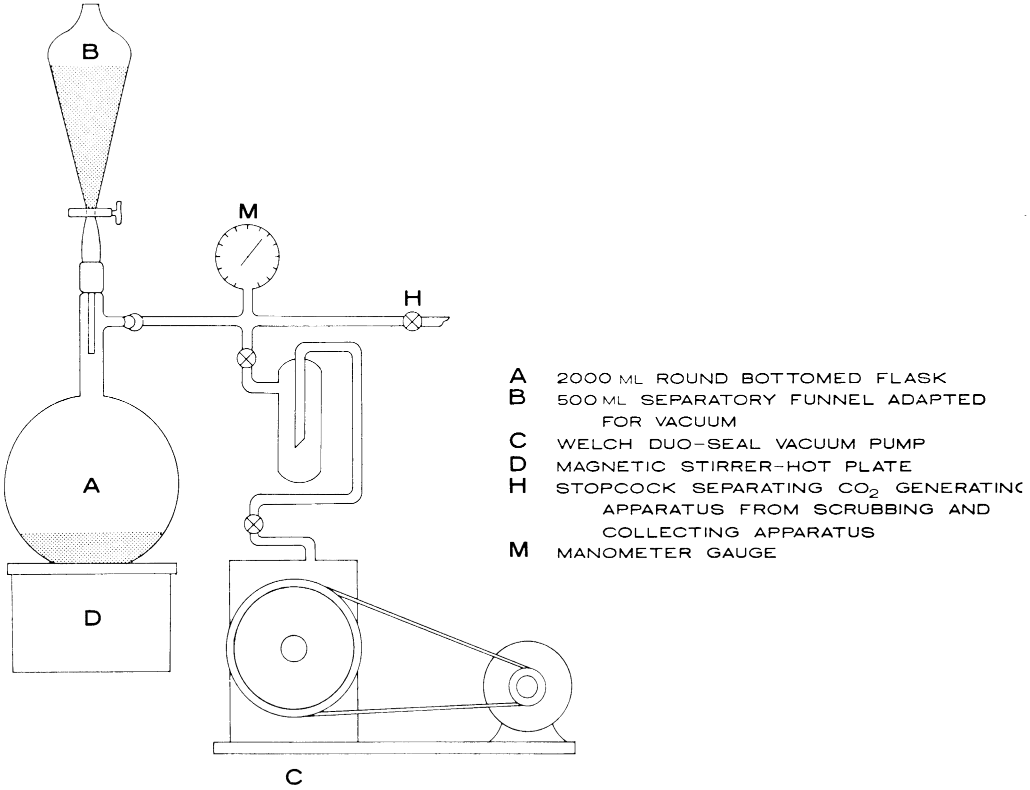

Fig 1. Schematic of the CO_2 generating apparatus for the preparation of NBS oxalic acid standard.

Thus, a new technique, which gives high yields and consistent $\delta^{13}C$ values that are close to the average value obtained by Craig (1961), is described.

PROCEDURE

For the generation of approximately 6.77L of CO_2 at STP from 3.31gm carbon, 17.35gm ^{14}C NBS oxalic acid standard $(CO_2H)_2 \cdot 2H_2O$, is weighed on an analytic balance and transferred to a 2000ml round-bottomed flask adapted for vacuum conditions. Degassed distilled H_2O is added to the sample to immerse the contents. A separatory funnel with a 24/40 joint adapted for vacuum is attached to the flask (fig 1). Vacuum is applied by a Welch Duo-Seal mechanical pump. All pumps used in the system are trapped, and the traps are refrigerated by a dry ice/acetone bath. A pressure of 0.01 Torr is normally achieved in approximately 15 min. The separatory funnel is filled with sulfuric acid-potassium permanganate solution containing 16.25gm $KMnO_4$, 40ml concentrated H_2SO_4, and 250ml degassed distilled H_2O. This solution is prepared by stirring and gently heating the contents until the $KMnO_4$ dissolves.

Vacuum is also applied to the scrubbing and collecting apparatus, which consists of three gas wash bottles in series containing potassium-permanganate ($KMnO_4$), silver nitrate ($AgNO_3$) and potassium dicromate-sulfuric acid $[(K_2Cr_2O_7) + (H_2SO_4)]$ solutions respectively, a double water trap (E) cooled by dry ice/acetone mixture, and two CO_2 collecting traps (F & G) (fig 2). One of the CO_2 collecting traps is a 10-ft glass coil (G) placed purposely at the end of the collection train to insure total CO_2 removal. The gas wash-bottles are used to scrub the CO_2 free of impurities. Gas scrubbing is applied to all samples, whether background, unknown, or NBS standard.

When both the generating apparatus and the collecting apparatus are evacuted to 0.01 Torr pressure, the sulfuric acid-potassium permanganate solution is added to the NBS oxalic acid sample in a steady drop-wise flow, while the contents of the flask are continuously agitated by a magnetic stirrer. When enough CO_2 is generated to register approximately 38cm Hg pressure on a monometer, the stopcok (H), which separates the generating apparatus from the scrubbing and collecting apparatus, is opened and the CO_2 is collected and frozen at liquid nitrogen temperature in the straight (F) and coiled (G) traps while the system is closed to the vacuum pump (I). The steady drop-wise flow of the sulfuric acid-potassium permanganate solution and the continuous stirring of the contents are maintained throughout the entire procedure.

The "apparent" end-point of the reaction between the sulfuric acid-potassium permanganate solution and the NBS oxalic acid sample is reached when the solution in the flask changes from clear to reddish-brown. This particular end-point apparently does not satisfactorily insure total evolution of CO_2 from the sample according to Grey *et al* (1969). Thus, to the contents of the flask, 5ml more sulfuric acid-potassium permanganate solution is added until the color of the solution changes to a chocolate-brown tint. At this point, heat from a stirrer-hot plate is applied and the solution is gently warmed which serves to drive out additional CO_2 that may be trapped in the solution.

After the chocolate-brown end-point is reached a cycling procedure is started. The stopcock valve (J) separating the scrubbing from the collecting apparatus is closed and the collecting apparatus is then evacuated for five min through the spiral trap (G) by pump (I). After five min, the system is closed to the pump (I) and the stopcock (J) separating the collecting apparatus from the generating and scrubbing apparatus is re-opened for five min, allowing the remaining CO_2 to expand from the round bottomed flask (A) to the traps (F & G) where it is frozen out at liquid nitrogen temperature. This cycling is continued for 30 min to insure that all the CO_2 generated from the NBS sample is trapped. At the end of the cycling procedure, when the pressure in the entire system is typically close to 0.02 Torr, the stopcock (J) is closed, isolating the collecting apparatus from the scrubbing apparatus, and the collecting system is evacuated through the spiral trap (G) to a pressure

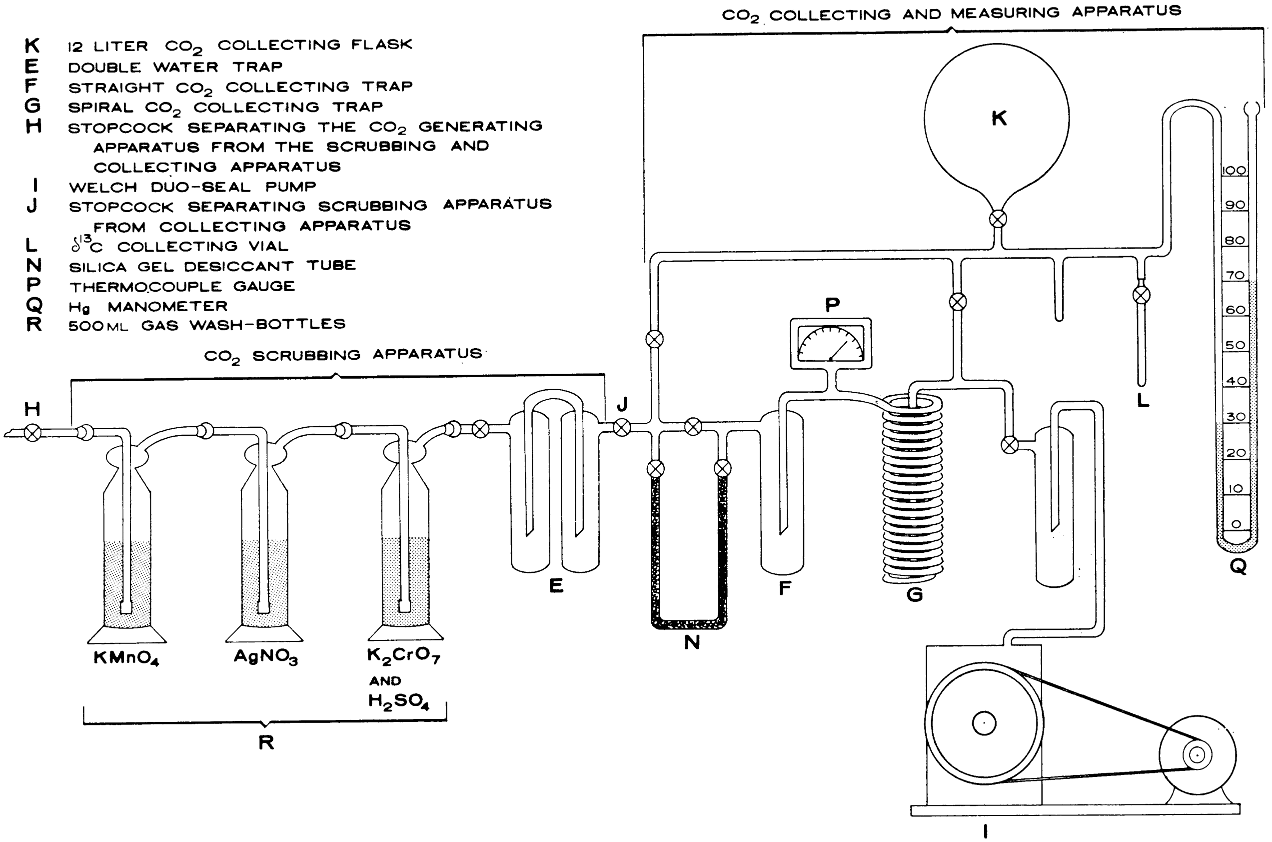

Fig. 2. Schematic of the chemical line for scrubbing, collecting, and measuring CO_2 during the preparation of NBS oxalic acid standard.

of 0.01 Torr. Finally, the CO_2 collected is expanded into a measuring flask (K) and into a collecting vial (L) for the measurement of the $\delta^{13}C$.

RESULTS

Fifteen consecutive NBS oxalic acid samples were processed by the method above, and then analyzed for $\delta^{13}C$ by a dual collector mass-spectrometer. The results of these samples are presented in Table 1.

TABLE 1

Yield in grams of carbon, per cent carbon, and $\delta^{13}C$ relative to PDB obtained by wet oxidation.

Sample No.	Yield in gm Carbon	% Carbon Yield	$\delta^{13}C$ in ‰ relative to PDB
NBS-1	3.34	100.9	−19.11
NBS-3	3.32	100.3	−19.51
NBS-4	3.28	99.1	−19.05
NBS-5	3.27	98.8	−18.65
NBS-6	3.29	99.4	−19.01
NBS-7	3.29	99.4	−19.15
NBS-8	3.28	99.1	−18.71
NBS-9	3.34	100.9	−19.34
NBS-10	3.31	100.0	−19.31
NBS-11	3.33	100.6	−19.13
NBS-12	3.30	99.7	−19.30
NBS-13	3.28	99.1	−18.93
NBS-14	3.33	100.6	−19.35
NBS-15	3.27	98.8	−19.14
NBS-16	3.28	99.1	−19.08
	Av = 3.30	Av = 99.7 ± .8	Av = −19.12 ± .23

The average of −19.12‰ for the 15 NBS oxalic acid samples processed by wet oxidation is close to the average value of −19.3‰ obtained by Craig (1961) by direct combustion.

To test the yields obtained by our wet oxidation procedure *vs* those obtained by direct combustion, we prepared six samples by direct combustion, utilizing a micro-combustion system with a moderate vacuum, and a micro-furnace containing CuO maintained between 800-900°C. During combustion, the generated gases are recycled with excess oxygen via a Toepler pump to insure total conversion to CO_2. The products are collected in a liquid nitrogen cooled trap, and transferred through dry-ice traps prior to analysis.

The average of −18.69‰ obtained from the six samples combusted in a stream of oxygen is significantly enriched relative to the average value of −19.12‰ obtained by the new wet oxidation procedure (Table 2).

TABLE 2

CO_2 and $\delta^{13}C$ in ‰ relative to PDB obtained by combustion in oxygen

Sample No.	CO_2 yields in mm Hg/mg oxalic acid	$\delta^{13}C$ in ‰ relative to PDB
5234	16.00	−18.65
5231	17.00	−18.87
5727	15.50	−18.45
5728	15.90	−18.71
5729	16.10	−18.84
5731	15.30	−18.59
		Av = −18.69 ± .16

A plot of the CO_2 yield *vs* the $\delta^{13}C$ ratio of the six samples processed by direct combustion, suggests that the depleted $\delta^{13}C$ values correlate with the larger CO_2 yields (fig 3). This observation suggests that during the evacuation of the system prior to combustion, depleted oxalic acid preferentially sublimates. For this reason, our $\delta^{13}C$ values for oxalic acid obtained by combustion are enriched relative to those obtained by wet oxidation. Thus, combustion of large samples of oxalic acid causes sublimation and isotopic fractionation.

The new wet oxidation technique differs from the normal accepted wet oxidation technique (Kim, 1970; Kolthoff & Sandell, 1952; Polach, 1972; Polach & Krueger, 1972) in the following ways: 1) The sulfuric acid-potassium permanganate solution is added to the NBS oxalic acid in a steady drop-wise flow while the contents of the flask are continuously stirred and the system is closed to the vacuum pump. 2) The chocolate-brown end-point is employed after adding 5ml more sulfuric acid-potassium permanganate when the initial "apparent" reddish-brown end-point is reached. 3) Heat is applied to gently warm the sample to drive off all the CO_2 that may be trapped in solution or trapped in the flask or generating apparatus. 4) A cycling technique is used by pumping

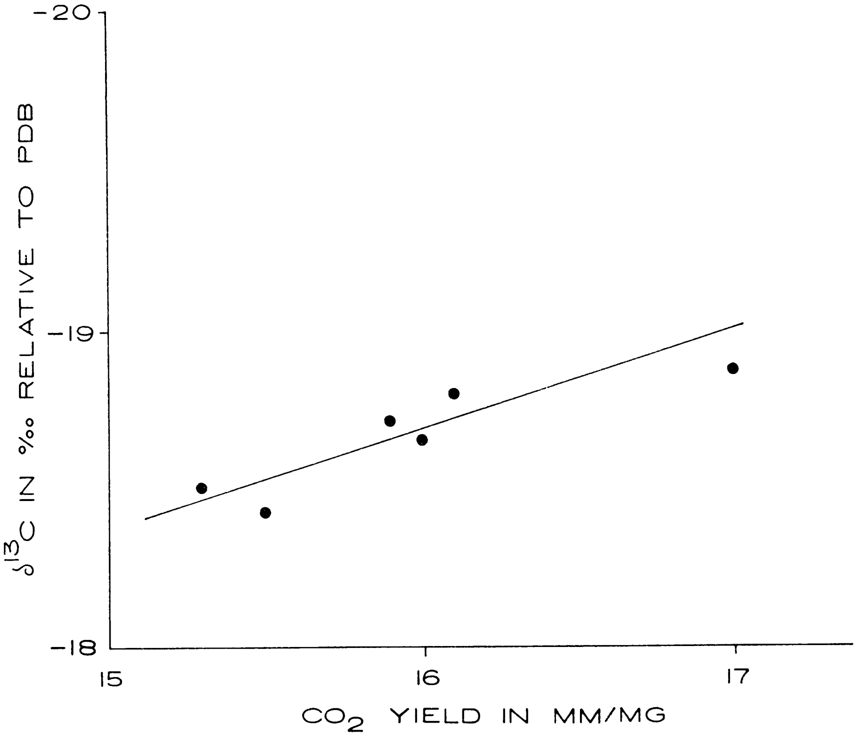

Fig 3. CO_2 yield in MM/MG vs $\delta^{13}C$ direct combustion of NBS oxalic acid standard.

the collecting apparatus while the generating apparatus is sealed for 5 min, and then closing the system to the vacuum pump and re-opening the generating apparatus.

This action allows the remaining CO_2 to expand from the flask into the liquid nitrogen traps where it is frozen out. The process is carried out for 30 min on an alternating basis.

Although this investigation is directly concerned with fractionation in the preparation of CO_2 by the wet oxidation procedure of ^{14}C NBS oxalic acid, it is also of interest to investigate whether further fractionation occurs when the CO_2 obtained by wet oxidation is converted to the counting liquid, benzene, via lithium-carbide and acetylene trimerization (Tamers, 1965).

Therefore, six NBS oxalic acid samples were prepared and converted to benzene and the product analyzed for $\delta^{13}C$ (Table 3). The average $\delta^{13}C$ of $-19.42 \pm .12‰$ is slightly different from our average value of $-19.12 \pm .23‰$ obtained by analyzing the CO_2 prepared from oxalic acid by wet oxidation. Correction for the activity of the counting liquid utilizing the formula proposed by Broecker and Olson (1961) accounts for a correction of approximately five radiocarbon years. These analyses substantiate that carbon isotope fractionation during the acetylene and benzene syntheses is negligible (Noakes *et al*, 1967; Hubbs *et al*, 1967).

TABLE 3
Per cent yield and $\delta^{13}C$ in ‰ relative to PDB of benzene obtained by wet oxidation

Sample No.	Over-all % yield from CO_2	$\delta^{13}C$ of benzene in ‰ relative to PDB
NBS-1	98.3	−19.59
NBS-2	96.4	−19.48
NBS-3	96.0	−19.40
NBS-4	99.8	−19.44
NBS-5	99.7	−19.35
NBS-7	99.5	−19.23
		Av = −19.42 ± .12

CONCLUSIONS

The new oxidation technique is easily carried out and minimizes the chances of sublimation while oxalic acid is under vacuum to expel air from the system.

Since ^{13}C fractionation by the wet method is insignificant, ^{14}C counting errors must also be insignificant.

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