ATOMIC ENERGY COMMISSION SUPPLEMENT

[CONTRIBUTION FROM THE DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH, ARGONNE NATIONAL LABORATORY]

A Method for Simultaneous Determination of Carbon-14 and Total Carbon

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An analytical method is described whereby a single sample of either organic or inorganic carbon-containing material may be assayed for total carbon content as well as carbon-14 content. The carbon analysis is carried out by wet oxidation with subsequent purification of CO_2 in a vacuum system. CO_2 is measured manometrically and then transferred to a proportional gas counter for carbon-14 determination. Reasonably accurate counts may be obtained with material having a specific activity of only one disintegration per minute per millimole of carbon. The versatility of the method with respect to size of samples, chemical nature of samples, and amount of radioactivity present makes it particularly suitable for biological investigation.

Carbon-14 has a specific radioactivity of 4.4 curies per gram when carrier-free and only 6.9×10^{-12} c./g. in the natural carbon of living things. Experimentation in biology often requires that samples with specific activities near both extremes of this trillion-fold range be accurately compared. This is especially true with *in vivo* experiments involving hormones, vitamins, etc., and with long-term retention–excretion studies of any type. In such work counting methods are needed which are routine and which not only approach the sensitivity of the one used by Libby and co-workers[1,2] but also permit the direct determination of very active samples. For this the radiation measuring equipment must be responsive in a nearly linear fashion over a wide range of activity, and the apparatus must accommodate samples of widely differing mass without sacrificing linearity of response. In much work, it is also desirable that the measurement of radioactivity be coupled with an analysis for total carbon, preferably one carried out on the same sample.

These considerations and others have prompted several groups of workers to develop carbon-14 measuring techniques that offer greater sensitivity and precision than is obtained with counting of solid $BaCO_3$. Libby first suggested counting $C^{14}O_2$ as a gas inside a Geiger–Müller counter.[3] Miller developed a method for counting $C^{14}O_2$ at pressures up to 50 cm. with CS_2 as an added filling gas. The characteristics of these counters were studied by Brown and Miller[4] and more recently by Eidinoff,[5] who was able to count $C^{14}O_2$ at pressures up to 175 cm. Skipper and associates have used this type of counter in biological experiments.[6] Engelkemeir, *et al.*,[7] have used CO_2 counters with an additional filling of argon–alcohol for the determination of the half-life of C^{14}, and recently Engelkemeir and Libby[8] have published an extensive study of end and wall losses in these tubes. All workers have commented upon the high coincidence loss of this type of G–M counter.

Bernstein and Ballentine[9] have developed and studied the properties of similarly designed tubes for use in the proportional region. Methane is used as a filling gas, making the tube self-quenching. The response is linear up to 200,000 counts per minute. The pressures of CO_2 used by these workers were not as great as those that have been described with G–M counting methods, but, since the voltage plateau does not shift with increasing CO_2 pressure as much as in G–M tubes, a common operating voltage may be used over a wide range of CO_2 pressures. All investigators working with gas counters hold the view that these tubes count at approximately the absolute disintegration rate of the sample contained within the sensitive volume of the counter. Good evidence to support this view has recently been published by Eidinoff.[10]

Several workers have performed simultaneous analyses of carbon and carbon-14. This has been done by weighing the purified samples of $BaCO_3$ that are to be used for the count.[11] In addition to being relatively insensitive as a counting method, this procedure has the additional disadvantage of

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(1) W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

(2) W. F. Libby, E. C. Anderson and J. R. Arnold, *Science*, **109**, 227 (1949).

(3) W. F. Libby, *Ind. Eng. Chem., Anal. Ed.*, **19**, 2 (1947).

(4) S. C. Brown and W. W. Miller, *Rev. Sci. Instruments*, **18**, 496 (1947).

(5) M. L. Eidinoff, *Anal. Chem.*, **22**, 529 (1950).

(6) H. E. Skipper, C. E. Bryan and L. White, Jr., *J. Biol. Chem.*, **173**, 371 (1948).

(7) A. G. Engelkemeir, W. H. Hamill, M. G. Inghram and W. F. Libby, *Phys. Rev.*, **75**, 1825 (1949).

(8) A. G. Engelkemeir and W. F. Libby, *Rev. Sci. Instruments*, **21**, 550 (1950).

(9) W. Bernstein and R. Ballentine, *ibid.*, **21**, 158 (1950).

(10) M. L. Eidinoff, *Anal. Chem.*, **23**, 632 (1951).

(11) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. T. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 86.

being subject to considerable error because of the solubility of $BaCO_3$, the tendency of the sample to become contaminated with air CO_2, the tendency of the precipitate to incorporate adventitious alkali, and the tenacious adherence of the substance to glassware.

Other workers[12,13] have measured the CO_2 obtained on combustion by volumetric and titrimetric procedures. Van Slyke, Steele and Plazin[14] have recently combined the manometric wet combustion method of Van Slyke and Folch[15] with the proportional gas counting method of Bernstein and Ballentine.[9]

In the method described here various features of many of the above-mentioned procedures are combined with certain innovations to give a method for carbon-14 analysis which, after a year of routine use, we believe to be more generally useful and versatile than any of the methods used separately. This paper will be divided into two sections: the first describes the carbon analysis; and the second the measurement of radioactivity.

I. The Carbon Analysis

Apparatus and Its Development.—The analysis is carried out in the apparatus diagrammed in Fig. 1. The carbon-containing sample is oxidized in flask a at a pressure of 60 cm. of N_2. The evolved gases pass through: c, a preliminary −78° cold trap; a packed Vycor tube heated at 900° in one section and 450° in another; then through trap h held at −78°; and finally through trap i held at −195°. When combustion is complete, the entire system is evacuated and the contents of i are transferred to the calibrated volume n for manometric measurement. At this point the sample is available as pure CO_2.

Successful development of the method required that CO_2 and only CO_2 be quantitatively trapped in i. It was found that at any reasonable flow rate of the gas mixture during the evacuation, considerable quantities of water were escaping h and similar quantities of CO_2 were escaping i when standard concentric type traps (as k) were used. Lengthening and narrowing the trap dimensions failed to remedy this defect, and it appeared that CO_2 and water were condensing in the gas phase to form smokes. The present type traps provide for revaporization of these smokes during passage through the upper portion of successive loops. The gases may then condense on the glass surface of the next cooled loop segment. Several loops in each trap ensure quantitative recovery. The present traps consist of 12 such loops. The initial loop in each trap is made of larger-diameter tubing than the remaining loops since the bulk of the condensing material is caught during its first passage through the cold trap. Multiple concentric traps should be avoided because of the excessive time required for the heat conduction necessary to redistil the CO_2 condensed on the central tube.

The gases present in the apparatus during an analysis may be divided into three groups: (1) Gases which at low pressures do not condense at the temperature of liquid nitrogen (O_2, N_2, NO, etc.). (2) Gases which quantitatively condense at the temperature of Dry Ice (I_2, H_2O, SO_3, etc.). (3) Gases which at low pressures do not condense at the temperature of Dry Ice but do so quantitatively at the temperature of liquid nitrogen.

Gases of group (1) are pumped off; gases of group (2) are removed by traps c and h; but gases of group (3) other than CO_2 must be removed by some other method. Fortunately, the contaminating gases are not numerous. Inorganic gases that might be present and have these properties are HCl, Cl_2 and N_2O. Inorganic chlorides present in the sample will appear partially as HCl (this comes off before heating begins). The remaining inorganic and organic chloride appears as Cl_2. Silver wool at 450° in g is apparently effective in the removal of both gases, negative blanks having been obtained with NaCl samples.

N_2O was found to be produced in yields of 80–90% of the theoretical amount on a nitrogen basis when amino acids were oxidized. This gas is quantitatively decomposed to N_2 and O_2 during passage through the 900° tube.

Reagents.—The combustion mixture of Van Slyke and Folch containing sulfuric, phosphoric, chromic and iodic acids is prepared as described by these authors.[15] KIO_3 is added in the dry state. CrO_3 may also be added dry, thus obviating the necessity of periodic checking of the mixture.[16]

Phosphoric acid for lubrication of stopcocks A and B and the ground joints of a and d is prepared by heating reagent concentrated orthophosphoric acid to approximately 300° and then adding enough P_2O_5 to double the volume. A few crystals of CrO_3 are added to oxidize traces of organic material, and the mixture is allowed to cool. A very viscous fluid results which is superior to untreated concentrated phosphoric acid when used in a vacuum system. Stopcocks and joints lubricated with this mixture are vacuum tight for hours, and if reservoir cups such as in A, B and d are filled with the mixture, leaks are impossible. On standing, the mixture may partially crystallize, causing the stopcocks to freeze. They can readily be loosened by heating with a small bunsen flame.

Silver wool is packed into the portion of the Vycor tube in g (450°), and platinum foil is used to fill the portion of the tube contained in f. The platinum provides a heated surface for catalysis and, in addition, prevents the complete collapse of the tube when heated to 900° under vacuum. After a few days, the tube does become quite flattened but has never been stopped up even after months of use.

Method.—A single complete analysis will be described in detail with the assumption that a carbon analysis has just been completed and the sample is being counted in tube s. The apparatus is in the following state: All stopcocks except D, E, I, G and H are closed. The microfurnaces, Phillips gage, oil diffusion pump and mechanical forepump (not shown) are operating. Leveling bulb v may be at any position on its range depending upon the size of the previous sample. The mercury meniscus of manometer r is one atmosphere higher than the bulb meniscus. The meniscus in capillary tube o is somewhere between its zero mark and the meniscus in r. Bulb n, which may contain residual CO_2 of the previous sample, is immersed in a dewar flask containing water. Traps c and h are surrounded by Dry Ice–trichloroethylene-baths in dewar flasks. Traps k and l are in liquid nitrogen. Flask a contains the residue of the previous combustion. The lower, uncalibrated portion of b contains unused combustion fluid.

(1) Open A, allowing the residual combustion fluid to drain into a. Remove a, rinse (caution), and place in an oven. The contained porcelain combustion boat may conveniently be kept in the flask during drying and storage.

(2) Weigh the next sample in its porcelain combustion boat on a balance of sufficient sensitivity to give four significant figures to the sample weight. Slide the boat into its flask so that no spillage occurs until the boat reaches the bulb. Place 300–400 mg. of KIO_3 into the flask for each estimated millimole of carbon in the sample. Place the sample flask upright in a covered container. Several samples may be weighed at once if desired; however, sufficient time will be available during one analysis to weigh the succeeding sample.

(3) Open J to pump out residual CO_2 of the previous sample, if any, and then close J. Close G and H and open F to shunt out the diffusion pump.

(4) Place a ring of viscous phosphoric acid on the ground glass joint of a (new sample) and place on apparatus with enough twisting to give a uniform film for the seal and then add additional phosphoric acid around the rim of a. Close A and open B and C. Usually a drop of acid or combustion mixture will flow down the neck of the flask. The bend in a prevents this from reaching the sample suddenly. If any fluid threatens to reach the bulb of a, close B.

(5) Place liquid nitrogen around i, close E, and open P until manometer j reads 15 cm. below atmospheric pressure.

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(12) R. B. Loftfield, private communication in ref. 11, p. 76.

(13) R. Steele and T. Sfortunato, Brookhaven National Laboratory Report T-6 (1949).

(14) D. D. Van Slyke, R. Steele and J. Plazin, *Federation Proc.*, **10**, 264 (1951).

(15) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(16) D. D. Van Slyke, private communication.

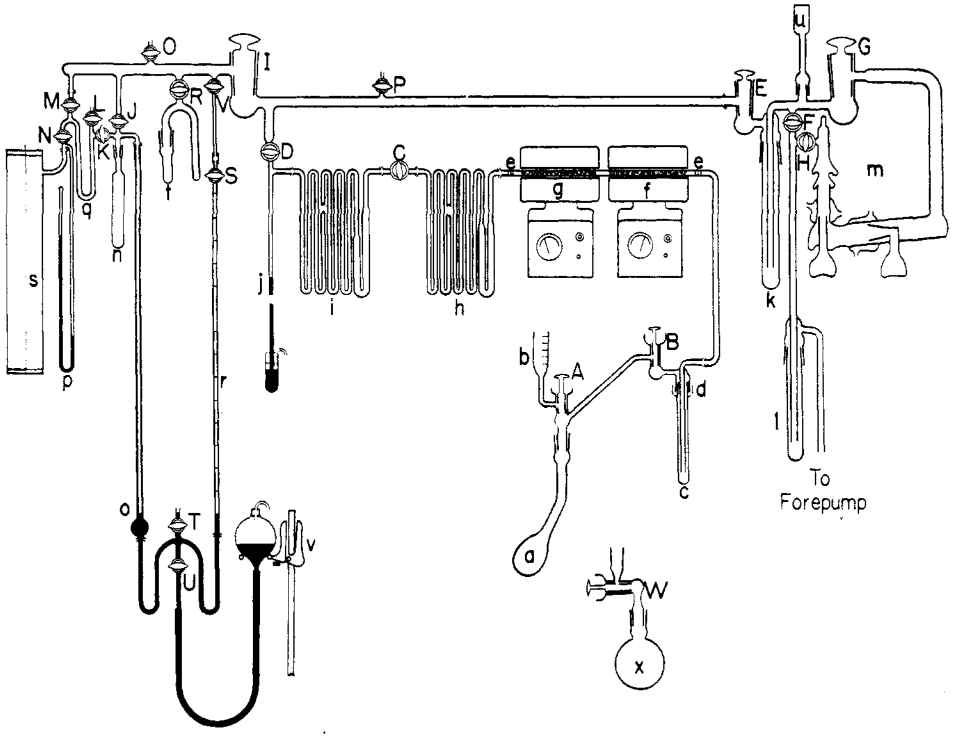

Fig. 1.—Apparatus for analysis of carbon-14 and total carbon: A and B, mercury seal type vacuum stopcocks (2 mm.), cups filled with phosphoric acid; C, D, F, H and R, vacuum stopcocks (6 mm.); E, vacuum stopcock (10 mm.); I and G, vacuum stopcocks (15 mm.); J to P and V, vacuum stopcocks (2 mm.); L, to tank methane; O, to tank CO_2; P, to tank N_2; S, T and U, stopcocks of Van Slyke manometer; W, mercury seal type vacuum stopcock with 24/40 ₸ joints; a, digestion flask (100-ml. Kjeldahl) fitted with 24/40 ₸ outer joint; b, calibrated funnel for addition of combustion fluid; c, removable Dry Ice–trichloroethylene cold trap; d, phosphoric acid cup for sealing c; e, pyrex to quartz graded seals; f, microfurnace (Sargent #49090) at 900°. This section of Vycor tube is packed with platinum foil; g, microfurnace (same) at 450°, this section is packed with silver wool; h, Dry Ice cold trap. First loop 20 mm. (o.d.): remaining 11 loops 10 mm. loops 10 inches long; i, liquid nitrogen cold trap (same design as h); j, mercury manometer 0–1 atm.; k and l, liquid nitrogen cold traps for pump and gage protection; m, two-stage oil diffusion pump (Distillation Products Inc. #GF-20W, drawing abbreviated); n, calibrated bulb, interchangeable. Volume calibrated by weight of mercury needed to fill bulb and associated volume to the zero mark of o; o, capillary tubing (3 mm.) with semi-ball joints and 10-ml. bulb. Zero mark set with manometer at zero and system pumped out. A small strip of scotch tape used as marker; p, mercury manometer for measuring pressure in counter; q, freeze-out trap for filling counter; r, Van Slyke manometer; s, proportional gas counter; t, side arm trap for collecting samples as carbonate, or for temporary storage of CO_2; u, Phillips gage (Distillation Products Inc. #PHG-1); v, leveling bulb (tubulature covered with a dust filter) and micrometer leveling bulb support (Burrell #A40-555) moving on $^{7}/_{16}''$ rod; x, auxiliary flask.

Tank nitrogen is attached to P with an Ascarite and Drierite absorber in the line. Air may not be substituted for nitrogen because oxygen will condense in i. The pressure regulator of the nitrogen tank may be conveniently adjusted to deliver the proper pressure into the apparatus. Close P and *open B if it was closed.*

(6) Allow combustion fluid in b (5 ml. for each estimated millimole of carbon) to flow into a through A and then place a micro-burner under a, heating until iodine fumes are first visible. If, during heating, the mensicus in j gets to within 5 cm. of its mercury reservoir, begin step (7).

(7) Open E so that the system is gradually evacuated over a 3–5 minute period. At no time should the meniscus in j move suddenly and rapidly. As the pressure in the system diminishes, E may be opened wider. Very rapid evacuation, even with multiple traps, results in some loss of CO_2. The next sample may be weighed during this step.

(8) When manometer j shows essentially zero pressure, open E wide and cut in the diffusion pump. Allow the pressure to descend until it reaches 1–2 microns on the Phillips gage. Close B and C. The pressure on the ion gage will decrease more rapidly. Open J.

(9) Remove the water from around n, dry the bulb with a towel, and place in liquid nitrogen. By this time the pressure on the gage should be below 0.02 micron.

(10) Open U and lower v until the meniscus in r is at the zero mark. The meniscus in o will also read zero. This has been initially marked with a narrow strip of scotch tape. A small lens mounted in front of both zero marks allows a precise setting. If the menisci do not come to zero, check for bubbles in the mercury and for leakage at S. The manometer is evacuated in this case by pumping through V and S until the vacuum gage is at a low pressure. Then raise v to allow mercury through S, after which S and V are closed.

(11) Close E and remove liquid nitrogen from h. Transfer of CO_2 may be accelerated by blowing a stream of compressed air on the cold portion of i.

(12) When the mercury in o has again risen to the zero mark, all CO_2 will have been trapped in n. Close J; after v is raised to an upper position, remove the liquid nitrogen from n. Surround n with warm water until all contained solid has disappeared and then with a dewar flask containing a thermometer and water at room temperature.

(13) Bring the meniscus in o to zero and close U when the pressure has stabilized. Read the manometer, the temperature in the dewar flask, and the temperature adjacent to the manometer.

(14) The previous sample, which was being counted during steps (1) to (13), is now evacuated through M and N after E is opened. Shunting out the oil diffusion pump and switching off the voltage to tube s are necessary before M and N are opened. When the vacuum gage comes on scale, cut in the diffusion pump and allow the pressure to descend into the submicron region before the next step.

(15) If all of the sample is to be counted, place liquid nitrogen around q, close M, and open K. When the manometer can be placed at zero on both limbs, the transfer is complete. Close K and remove liquid nitrogen from q. When all CO_2 has vaporized, open L and allow methane to enter until manometer p reads the desired pressure. Close L and N. Turn on voltage and count after 4 minutes. If only a portion of the sample is to be counted, it may be released through K without freezing into q, but a second reading of pressure must be made as in (13). The apparatus is now in the state first described.

With extremely active samples a very small aliquot may be necessary in order to prevent jamming. In such cases a more accurate measurement may be had by use of an alternate method. The ratio of the calibrated volume to the sum of the calibrated volume and the section of the manifold volume above J may be previously determined by isolating and measuring a gas sample in n (nitrogen may be used), then exhausting the manifold section through I, closing I, and opening J. The pressure is again measured. The ratio of the two pressures is the inverse of the volume ratios since deviations from the ideal gas laws are negligible under these conditions. For the aliquoting, an empty tube is placed on t, R is opened, and the manifold is evacuated. Liquid nitrogen is placed around t, R is closed, and J is opened. The manometer is set at zero, J is closed, and R is again opened, allowing the CO_2 in the manifold to be frozen into t. This has reduced the sample in n by a factor equal to the ratio of the small volume to the total volume. Each repetition of these steps reduces the sample size by the same factor until the desired order of magnitude of sample activity has been reached. If the process is to be repeated more than twice, a small amount of pure inactive CO_2 should be added through O and mixed with the sample by freezing into n. This reduces possible surface errors and allows quantitative transfers. The added CO_2 need not be measured accurately. The final sample is frozen into the counter as before.

Auxiliary Methods.—By substituting acid (dilute perchloric is most generally useful) for the combustion mixture, carbonate in samples may be measured. This procedure is more rapid since it may be carried out in a vacuum. The apparatus is equally useful for the quantitative collection of the CO_2 liberated in other reactions. It has been used to collect the carbon of urea after decomposition by urease. For such slow reactions a number of auxiliary flasks, x, are needed. Solid reagents are placed in x, the stopcock assembly, W, is attached, and the flask evacuated. Liquid reagents may be added through W as desired. Temporary separation of two solid reagents may be achieved by placing one in the stopcock of W. When the CO_2 yielding reaction is complete the assembly is attached as described for flask a and CO_2 is trapped as before.

Accurate analyses of CO_2 in gas samples have also been carried out. The volume of a bulb such as x is calibrated, and the sample is collected through W after previous evacuation. For very low CO_2 concentrations the sample may be collected in a large vacuum desiccator.

Routine carbon analyses may be completed in 20 minutes. A technician working alone requires about 30 minutes per sample when a radioactivity determination is coupled with the chemical procedure, except in instances of very low radioactivity where statistical considerations govern the duration of counting.

A series of interchangeable bulbs, n, with different volumes has been calibrated to allow the measurement of samples differing widely in mass. Initial calibrations were performed by weighing the mercury necessary to fill the bulb and associated volume. Subsequent calibrations have been made by performing a series of analyses on standard calcite samples.

Calculation.—After the manometer reading is corrected for thermal expansion of glass and mercury, the pressure is again corrected for deviations from the ideal gas law by applying the van der Waals equation. The latter correction is slight (0.005%/cm. is added to the pressure reading). A graph of both correction factors is useful and easily prepared. The final calculation is then made using the ideal gas equation.

Results.—Table I gives the results of a series of carbon analyses carried out on three organic compounds and on calcite. The amino acids, alanine and cystine, were "shelf" reagents. Phthalate and calcite were prepared as primary standards. It is evident that the precision of this technique compares well with that attained in other carbon procedures.

TABLE I

RESULTS OF CARBON ANALYSES

Substance	Carbon, mM. Theoretical	Carbon, mM. Found	Recovery. %
Potassium acid	2.039	2.041	100.1
phthalate	2.043	2.041	99.9
	1.840	1.838	99.9
	1.967	1.975	100.4
	1.654	1.661	100.4
	1.875	1.872	99.8
	2.010	2.003	99.7
		Av.	100.0
Alanine	2.052	2.073	101.1
	2.406	2.421	100.6
	1.727	1.742	100.9
	2.387	2.380	99.7
	2.447	2.450	100.1
		Av.	100.5
Cystine	2.985	2.963	99.3
	2.079	2.080	100.0
	1.875	1.886	100.6
	2.436	2.440	100.2
	1.319	1.328	100.7
	1.312	1.316	100.3
		Av.	100.2
Calcite	2.293	2.289	99.8
	1.914	1.908	99.7
(Acidified only)	1.606	1.599	99.6
	1.378	1.375	99.8
	1.773	1.780	100.4
	2.008	2.009	100.0
		Av.	99.9

II. The Radioactivity Measurement

The proportional counting method of Bernstein and Ballentine[9] has been modified for the carbon-14 measurement. Several counters were constructed and tested. The most satisfactory performance was obtained from the one diagrammed in Fig. 2. It consists of a stainless steel cathode (6.35 cm. i.d. and 50.8 cm. in length) with flat lucite ends and a 2-mil tungsten anode. Insulators are soldered into brass fittings which are in turn connected as guard rings and carry the same potential as the anode. The advantages of this tube over glass envelope tubes are: sturdiness and permanence, low end loss, and the fact that it is easily disassembled for cleaning and repair. The counter is permanently mounted on the vacuum frame with a cathode follower preamplifier (gain of 2) attached to its upper end. A small housing (not shown) similar in design to the preamplifier chassis holds the lower guard ring connector. The preamplifier is connected by cables (approximately 10 feet in length) to the high-voltage supply and the amplifier scaler. The components used were the Model 1090 5000-volt power supply and the Model 162 amplifier-scaler, both from the Nuclear Instrument and Chemical Corporation, Chicago, Ill. A crystal diode connected between the two amplifier loops after the manner of Bernstein and Ballentine[9] reduced positive overshoots. The output pulse from the amplifier which is differentiated and fed into the first trigger pair is 40 volts negative when the amplifier is overloaded. These maximum pulses overshoot 20 volts in a positive direction and return smoothly to the axis with a 40-microsecond time constant but this relatively slow recovery does not seem to increase the dead time. Examination of the trigger pair output on the servoscope (Tektronix, type 511-AD) showed no double pulsing. When a Cyclotron Specialities, Inc., impulse register (type 401-A) is used with a scaling factor of 128, 700,000 counts per minute may be recorded without jamming.

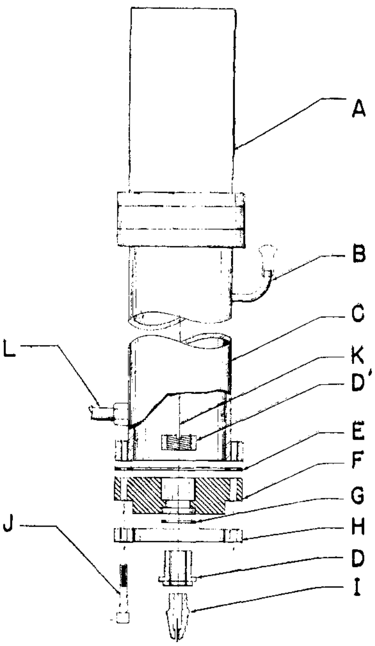

Fig. 2.—Proportional counter (drawn to scale with one end exploded): A, preamplifier chassis; B, gas inlet with kovar to Pyrex seal and ball joint; C, stainless steel cathode (20 inches long); D and D′, brass insulator mount with nut; E, Teflon gasket; F, lucite end piece; G, rubber gasket; H, brass ring for supporting lucite; I, insulator (Hermetic Seal Products Co., #CR-2); J, Allen head bolt; K, 2-mil tungsten anode with supports; L, brass hexagonal nut silver-soldered to cathode and threaded rod for spot-heating tube (soldering iron attached as heater but not shown).

When the counter was first put into use, it was noted that a period of about 30 minutes was required for the counting to reach a final constant rate. This troublesome phenomenon was apparently the result of stratification of sample gas in the tube dead space. After a spot near the bottom of the tube barrel was warmed with an electric soldering iron attached as shown in Fig. 2, the waiting time was found to be reduced through convection to about 4 minutes.

Background and Memory Effects.—The unshielded counter has a background counting rate of 700 counts per minute. When the portion of the cathode between the flanges was surrounded by 0.6 cm. of lead, the background rate was reduced to 450 counts per minute.

After prolonged use with very active samples, the background count does increase. The counting behavior in this respect suggests that $C^{14}O_2$ is slowly adsorbing or diffusing into the lucite, the Teflon, or the insulators since the background count slowly increases when the tube is filled with pure methane and allowed to stand. This effect is not bothersome unless extremely weak samples are being assayed. After prolonged use with highly active samples, the background increased approximately 100 counts per minute.

Counting Efficiency.—The measured volume of the tube, including the connecting tubing and manometer, p, is 1653 cc. The total "dead space" volume including the connecting tubing, manometer, and the volume in both ends not surrounded by the cathode is 29 cc. or 1.8% of the total volume. Experiments have shown that 0.5% of a sample remains in the volume of q after filling the tube by the described procedure. Applying the calculations of Engelkemeir and Libby, the end and wall losses of a tube having this size and shape should be, respectively, 3.5 and 1.2%. The latter values depend on the validity of applying data obtained with argon–alcohol-filled G–M counters to this type of tube. The total of all the above losses is 7.0%, so the counting rate of a sample in this tube should be about 93% of the disintegration rate.

Eight analyses were carried out on aliquots of National Bureau of Standards carbon-14 standard #123. The absolute activity of this material as determined by the NBS is 1280 d./sec./cc. In assaying the same sample, two other laboratories found higher disintegration rates, one as high as 1510 d./sec./cc. However, a calculation of the half-life of carbon-14 using the disintegration rate found in this standard by the NBS and the measured C^{14} ratio (mean value of mass spectrometric determinations in four laboratories) shows the half-life to be 5,740 years, which is in excellent agreement with the value recently reported by Engelkemeir, *et al.*[7] When counting was carried out at the upper limit of the voltage plateau (at the potential just below where anomalous pulses are seen on the oscilloscope) we found the corrected disintegration rate of this standard to be 99.0 ± 0.5% (standard error) of the NBS-determined rate, a difference which is not significant.

Resolving Time.—Figure 3 is a graph of the counting rate of a series of aliquots of a homogeneous sample of $C^{14}O_2$. In agreement with Bernstein and Ballentine[9] the rate is essentially linear up to 200,000 counts per minute. The resolving time of the equipment calculated from these data by either of two standard formulas[17] is 5 μs. This is also the measured width of the amplified pulse at operating potential. The broken curve of Fig. 3 represents the corrected counting rate when a dead time of 5 μs. is used in either of the two standard equations.

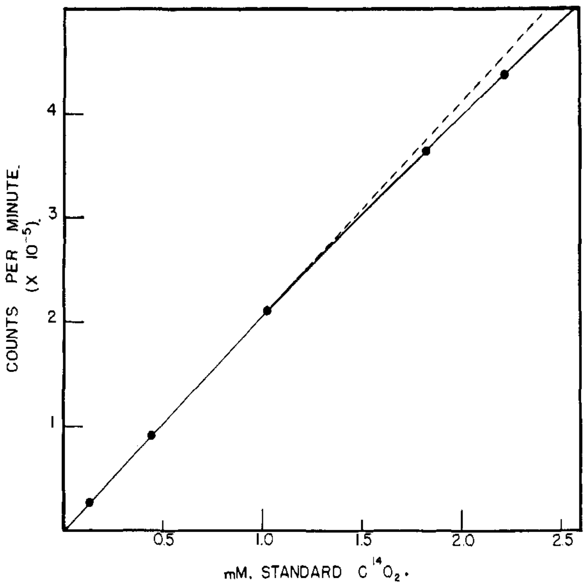

Fig. 3.—Counting rate *versus* sample activity.

Counting Characteristics.—The effect of several variables on the count *vs.* voltage response was studied.

Using a negligible partial pressure of quite active $C^{14}O_2$, the effect of methane pressure was determined. Figure 4

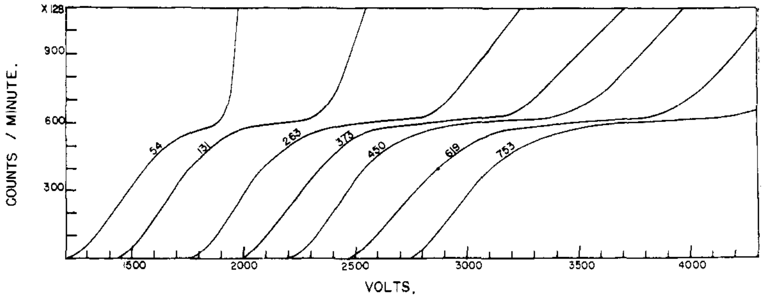

Fig. 4.—Shift of voltage plateau with methane pressure (CO_2 pressure negligible and equal with each curve). Pressure indicated on each curve in mm.

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[17] T. P. Kohman, *Anal. Chem.*, **21**, 352 (1949).

gives a series of plateau curves. The graph shows that as the methane pressure is increased the plateau shifts to higher voltages and becomes longer. However, the observed counting rate at the upper end of each plateau remains constant.

Figure 5 gives the effect of pulse amplification on the counting of a single sample. For all practical purposes amplification of the pulse by increased amplifier gain is equivalent to amplification within the tube by increased voltage. At lower amplifier gain settings the count increases more slowly above the plateau, making the latter appear longer. However, the length of the plateau below the point where anomalous pulses appear on the oscilloscope was found equal with all gain factors used, and at this point on each plateau the counting rate is equal.

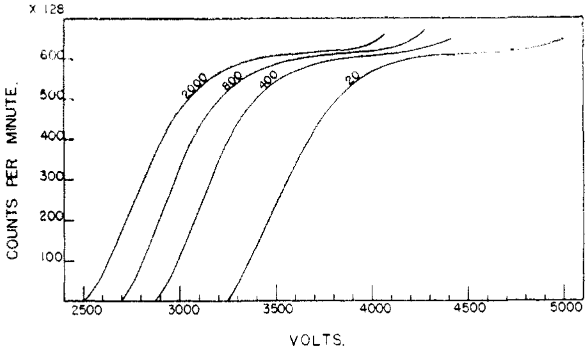

Fig. 5.—Shift in voltage plateau with pulse amplification. Nominal gain factors above each curve.

When the total pressure of CO_2 plus methane is held at one atmosphere, the effect of the partial pressure of CO_2 upon the apparent specific activity of successive aliquots of a homogeneous sample of low-activity $C^{14}O_2$ is shown in Fig. 6. These results confirm the observations of Bernstein and Ballentine,[9] who found that CO_2 partial pressures below 10 cm. do not appreciably affect the counting properties of these tubes. As CO_2 pressures increase above 10 cm., the observed pulse becomes ragged in appearance. This is associated with "overshooting" of the counting rate at the lower end of the plateau. However, the rate becomes essentially normal again at higher voltages. It is seen that by counting at 3800 volts the measured specific activity is equal at all CO_2 partial pressures below one-third of an atmosphere.

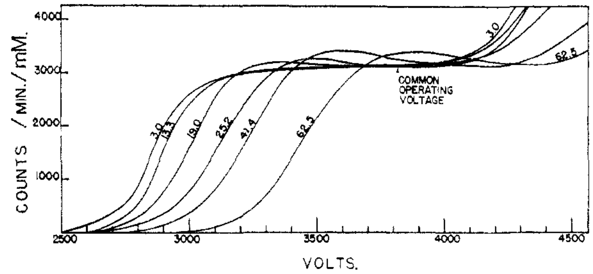

Fig. 6.—Shift of voltage plateau with CO_2 partial pressure (indicated in cm. on each curve). Total pressure was made up to 1 atmosphere with methane.

Pure CO_2 without added methane may be successfully counted without external quenching, as displayed in Fig. 7. The pulses obtained are quite jagged, and the "overshooting" of the counting rate is more pronounced than when methane is added. However, if counts are always taken at voltages on the plateau where the counting rate reaches a minimum, the resulting specific activities are essentially equivalent at all CO_2 pressures above 12 cm. and are also equivalent to activities obtained at lower pressures with added methane. Hence samples of CO_2 which vary in size between 0 and 66 mM. may be directly measured for C^{14} content.

Sensitivity.—It is of interest to compare the sensitivity of this counting method with the counting of solid $BaCO_3$ samples in an internal gas flow counter. In our experience several types of the latter counter have counted at approximately the rate expected when the assumption is made that each β-particle leaving the sample surface registers a count.

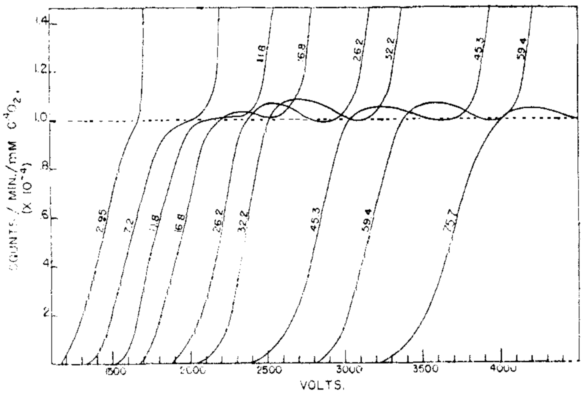

Fig. 7.—Count *versus* voltage reponse for a series of samples of pure CO_2. Pressures in cm. on each curve.

In order to make a comparison between two counters it is necessary to establish a criterion of sensitivity. This is arbitrarily chosen here as the lowest specific activity which may be determined with a theoretical accuracy of 5% (coefficient of variation) in one hour of counting. In the computation it is assumed that the background count of the typical gas flow counter is 25 counts per minute and that the background rate of the CO_2 counter is 450 counts per minute. It is further assumed that there is an abundance of available sample for the determination since this is usually the case with samples of very low activity.

The equation

$$r = [1 + (1 + 8v^2tb)^{1/2}]/2v^2t$$

where r is the actual counting rate of the sample, b is the background rate, v is the desired coefficient of the variation of the determination, and t is the duration of the count, may be easily derived from accepted statistical formulas used in the analysis of counting data.[17]

In the specific case selected the equation simplifies to

$$r = [1 + (1 + 1.2b)^{1/2}]/0.3$$

Thus for the above precision the CO_2 sample must register 80.9 counts per minute over background, whereas the $BaCO_3$ sample needs register only 21.9 counts per minute in excess of background. A $BaCO_3$ sample, one inch in diameter and of infinite thickness (20 mg./cm.^{2}), contains about 0.5 mM. of carbon, of which only about 10% counts. To satisfy the selected criteria it is thus necessary that the sample have a specific activity of 440 disintegrations/min./mM. In the CO_2 counter it is possible to count 92% of the total disintegrations in 66 mM. of carbon when the tube is filled to one atmosphere with pure CO_2. To attain the counting rate necessary for 5% precision in one hour, the carbon need have an activity of only 1.33 disintegrations/min./mM. On this basis the CO_2 counter is 340 times more sensitive than the internal gas flow counter and about 1000 times more sensitive than the thin mica window G–M tube.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF OREGON STATE COLLEGE]

Preparation of S^{35}-Labeled Sulfur Dioxide and Sulfuric Acid[1]

BY B. J. MASTERS AND T. H. NORRIS

A simple and convenient technique for the preparation of S^{35}-labeled sulfur dioxide and concentrated sulfuric acid is described. The method depends on the occurrence of an exchange reaction between these two compounds and leads to high specific activities and good percentage yields.

Sulfuric acid labeled with S^{35} has been shown by one of the present authors[2] to undergo isotopic exchange with sulfur dioxide at elevated temperatures, the observed half-time being *ca.* 7.5 hours at 211°. Extending this work, we have devised a simple and expedient method for preparing both labeled sulfur dioxide and concentrated sulfuric acid, with neutron-irradiated potassium chloride as starting material. The technique yields high specific activities with comparatively small activity waste. The heterogeneous, gas–liquid nature of the system makes separations and purification easy.

One evident advantage of the technique is that one may activate only a portion of a sulfuric acid solution, the remainder, of similar chemical composition, then being available for analysis, and further activation as necessary. The procedure also suggests a possible method of "counting" sulfuric acid in a gas counter. One could effect complete exchange between a little sulfur dioxide and excess acid. The gas, then of the same specific activity as the acid, could be counted by the technique described by Norris.[3]

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Experimental

The success of the procedure depends upon minimizing dilution of activity at each step. High specific activity barium sulfate, precipitated from a highly concentrated solution of irradiated potassium chloride, is dissolved in a minimum amount of concentrated sulfuric acid; a small excess of sulfur dioxide is then activated by complete exchange with this solution. This gas can thereupon be used to label a somewhat larger excess of concentrated acid in a second exchange. All operations are conducted by standard high vacuum techniques.

The following actual preparation of labeled acid illustrates the method. Irradiated potassium chloride was obtained from the U. S. Atomic Energy Commission (Item No. 17, Isotopes Catalog No. 3). Since the material used in this work was about fourteen months or almost five half-lives old, all activities quoted hereafter would, for fresh material, be greater by a factor of approximately 25. In a small centrifuge tube 1.79 g. of this salt, having *ca.* 0.25 mc. S^{35}, was dissolved in water containing 0.018 millimole of sulfuric acid carrier[4] and the volume was made up to 10 ml. After heating the solution, a small excess, 0.2 ml., of 0.2 *M* $BaCl_2$ was added and the resulting precipitate was digested for a time. Precipitation of activity was *ca.* 90% complete after one hour, as shown by rough checks of the activity of the clear solution before and after precipitation. Further digestion appeared to increase the completeness of precipitation somewhat.

After centrifugation, the supernatant was decanted for recovery of Cl^{36}, and the active barium sulfate (*ca.* 4 mg.) was washed with several small portions of water and finally with acetone, being centrifuged after each wash. Then, after drying in an oven, the sulfate was dissolved in 1.22 millimoles of 96% sulfuric acid. This solution, still in the original centrifuge tube, was sealed into a bomb tube (net volume 159 ml.) with 302 mm. of sulfur dioxide (2.57 millimoles). With the sealed tube in a furnace at 350° for six days complete exchange was obtained. The long period is presumably necessary because of the small amount of acid present; we are currently making a detailed study of the kinetics of this reaction.

The labeled sulfur dioxide thus prepared, recovered by opening the bomb *via* a break-off tip, was condensed and sealed into a second, similar bomb containing 35.7 millimoles of 96% sulfuric acid to be activated. Heating of this bomb at 270° for 20 hours yielded complete exchange; use of the lower temperature avoided excessive acid vapor pressure. For rapid exchange, it is apparently essential that the acid be exposed in the form of a thin layer. Finally the sulfur dioxide was again removed *via* a break-off tip and the preparation was complete.

The 3.65 g. of sulfuric acid thus prepared, counted as barium sulfate with an end-window counter, according to technique described by Norris,[2] showed a specific activity (corrected to zero thickness) of 1.4×10^6 c./m./mmole, corresponding to 60% of total starting activity. This acid was diluted fivefold with inactive acid before use in our experiments. With freshly irradiated potassium chloride, dilution by a factor of 125 would have been possible. The labeled sulfur dioxide fresh from the first exchange had a specific activity (calculated from the measured activity of the final sulfur dioxde) of 2.04×10^7 c./m./mmole counted as above, corresponding to 64% of starting activity. The use of a larger excess of sulfur dioxide in the first exchange would, naturally, lead to a higher percentage yield, with corresponding decrease in resulting specific activity.

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[1] Published with the approval of the Oregon State College Monographs Committee, Research Paper No. 190, Department of Chemistry, School of Science.

[2] T. H. Norris, THIS JOURNAL, **72**, 1220 (1950).

[3] T. H. Norris, *ibid.*, **74**, 2396 (1952).

[4] The presence of sulfate carrier *before* dissolution of the potassium chloride was found essential for obtaining any degree of completeness of activity precipitation. W. S. Koski, *ibid.*, **71**, 4042 (1949), has shown that the radiosulfur exists in such potassium chloride crystals almost exclusively in the +6 oxidation state, being carried by sulfate.