SPARK COMBUSTION OF ^{3}H AND ^{14}C LABELED SAMPLES SUITABLE FOR LIQUID SCINTILLATION COUNTING

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Abstract

A new method of combusting labelled ^{3}H and ^{14}C samples is accomplished through continuous spark ignition. Liquid, wet tissue and powdered samples are combusted in disposable steel planchets. Radioisotope collection is in liquid scintillation cocktails as $^{3}H_2O$ and $^{14}CO_2$. Quantitative collection and minimum memory is enhanced through controlled combustion rates. Comparison of the spark ignition method is made with presently available commercial combustion equipment.

Introduction

The liquid scintillation spectrometer is a versatile instrument for the measurement of low-energy beta emitters, especially tritium and carbon. The instrument, utilizing computers for data computation and presentation has proven especially valuable in biological and clinical application. On the other hand, biological materials are most difficult to prepare as true solutions for liquid scintillation counting and therefore present unique sample preparation problems.

Numerous methods and techniques have been developed in an attempt to solubilize diverse biological materials for scintillation counting. Generally these direct dissolution methods are limited by the small amount of material that can be dissolved in the more efficient cocktails. Furthermore, the severe quenching with highly colored biological materials results in decreased counting efficiency. Chemiluminescence is also a constant problem since many biological materials, when prepared for counting, generate light spontaneously even in the absence of radioactivity.

To overcome problems of sample solubility, color quenching and chemiluminescence, a more universal preparation technique for highly diverse biological materials can be achieved if the sample is completely oxidized, and the oxidation products are quantitatively dissolved in a scintillator to produce a minimum constant quenched sample. The most common example is the combustion and oxidation leading to collection of tritium as water and carbon-14 as carbon dioxide (1-3).

Recently there have been significant advances in the field of instrumental methods for sample combustion of biological materials as applied to liquid scintillation counting. Among the new developments are instruments that give greater precision and accuracy, increased ease of operation, and, most important, are completely automated, thereby removing operator error (4-9).

We presently would like to report on a new oxidation method that utilizes a continuous spark ignition for sample combustion. This technique offers certain advantages not existing in the presently available commercial units.

Experimental

A) INSTRUMENT DESIGN. The combustion chamber of the continuous spark ignition system is perhaps the most unique new aspect of this combustion method. The novel aspect is the employment of a continuous electrical spark to ignite the sample and sustain ignition throughout total sample combustion. Figure 1 shows the construction of the chambers in detail. The sample to be combusted is placed in a 3/32-inch low shouldered steel planchet of approximately 1¼-inch diameter. The planchet when placed in the combustion chamber rests on and makes electrical contact with a negative ground electrode. A stationary positive electrode is positioned about ¼ to ½ inches above the top most portion of the sample. This electrode is constructed of a hollow 1/8-inch steel tubing with a 1/32-inch wall thickness and extends to the outside of the chamber by a seal connector through the wall of the pyrex combustion chamber. The electrode is spiraled in a 1-inch diameter coil and insulated from the instrument by passing within a ¼-inch thick walled ceramic tube of 1½-inch I.D. The electrode is finally connected to a oxygen flow metering device and an electrical oscillating circuit. The ceramic tubing is attached to a heat gun in such a manner that hot air of >200°C can be blown through the ceramic tube, over the coiled steel electrode and exhausted on the surface of the pyrex combustion chamber. The pyrex combustion chamber

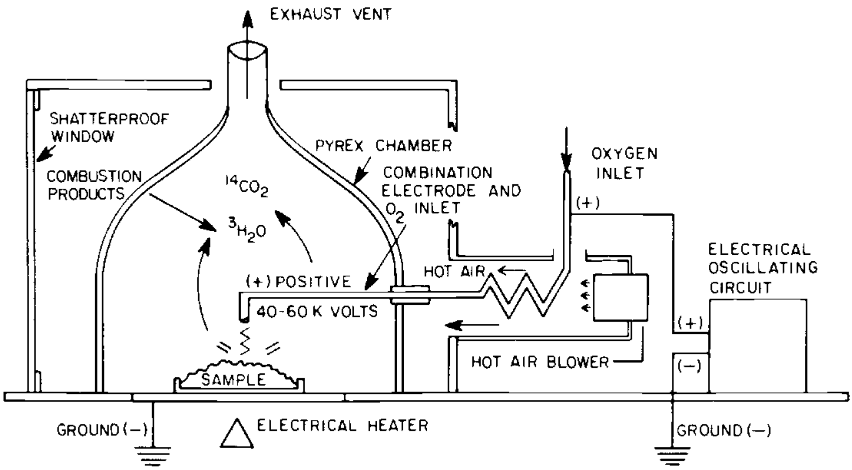

Figure 1. Spark Ignition Combustion Chamber

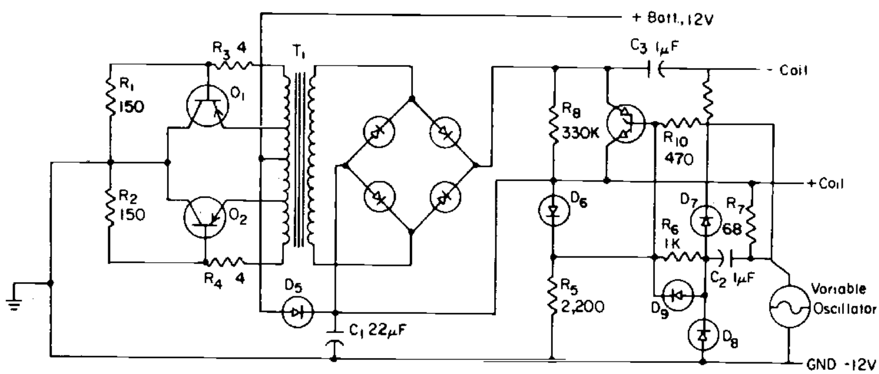

Figure 2. Spark Ignition Electrical Oscillator Circuit

is semi-hemispheric in shape with a gas exhaust portal at the top. It has a 3-inch O.D. at its maximum width with ¼-inch walls and a vyton "O" ring for airtight sealing at its base. A 1/16-inch thick sheet metal housing with a shatterproof window fits over the pyrex chamber for the purpose of containing the raised temperature of the chamber and as a safety consideration. An electrical heater is also placed in the chamber base in close proximity but electrically isolated from the negative electrode on which the steel sample planchet rests to enhance sample combustion rates.

Figure 2 is a schematic of the electrical oscillating circuit. A 12-volt D.C. source is fed into a capacitance discharge system such as is used in automotive ignition systems which generate an energetic spark of 40,000-60,000 volts and a current of low milliamp range. A variable oscillator is used to give a spark frequency discharge of 25,000 cps. The output of the electrical oscillator circuit is through a 12-volt coil which is directly connected to the combustion chamber.

Figure 3 shows the overall schematics of the equipment and procedures used to combust, transfer, separate and collect the ^{14}C carbon dioxide gas and ^{3}H water vapor originating from sample combustion. The combustion gases are transferred from the combustion chamber to the tritium water vapor trap through a heated metal transfer line to minimize the formation of water condensate. The tritium trap consists of spiraled tubing cooled to +5°C by a glycol refrigerant. The ^{14}C carbon dioxide gas leaving the tritium trap is further scrubbed of any residual H-3 water vapor by passing through an exchange column on its way to the carbon dioxide trap. The tritium exchange column consists of a 1/16-inch I.D. roughened steel walled tubing which has wetted walls of organic scintillator. To flush the water condensate from the exchange column and primary collecting column to the sample vial, two 8 ml aliquots of Searle-Analytic PCS (Phase Combining System) were used giving a sample volume of 16 ml of scintillator solution. The carbon dioxide gas next passes into a carbon dioxide collecting column consisting of a gas bubbler emersed in a solution consisting of a mixture of 5 ml of 2 methoxyethyl amine and 3 ml of toluene in which the carbon dioxide is absorbed as a soluble carbamate. A secondary exchange column of similar construction proper-

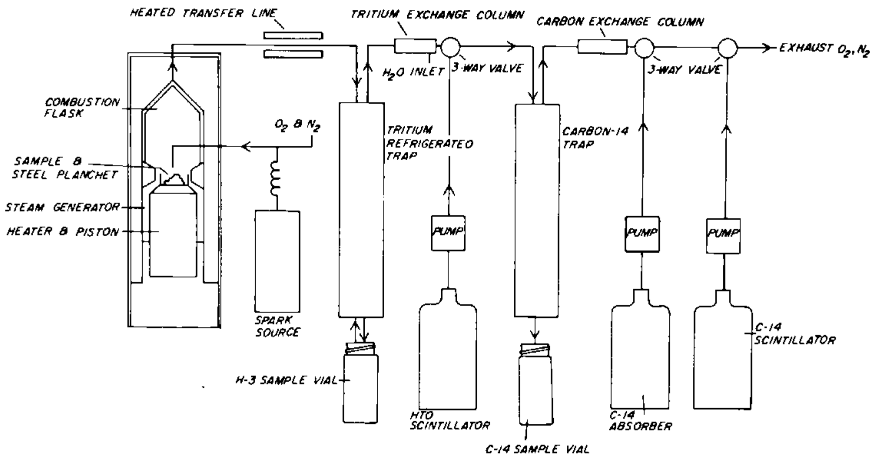

Figure 3. Spark Ignition Combustion System

ties as the tritium exchange column is used but its surfaces are wetted with the amine cocktail described above. The primary carbon collecting column is emptied after complete sample combustion by a valve at the bottom of the trap. A scintillator of toluene and PPO & POPOP concentrate is used in two 4 ml aliquots to clean the exchange column and primary collecting column of residual carbamate-amine solution. This cleaning solution along with the 8 ml of amine-toluene cocktail make up a total of 16 ml of cocktail used for the carbon sample vial.

B) INSTRUMENT OPERATION. Samples to be combusted in either a wet, dry or frozen condition are placed directly on a steel planchet. It is not necessary to wrap the sample in any combustible material to sustain ignition as the continuous spark ignition insures constant ignition conditions. The planchet is placed on a piston which is shown in Figure III. This piston raises and lowers from the bottom of the combustion chamber and serves as an entry port for the sample. The top of the piston is the negative electrode plate which makes electrical contact with the steel planchet. A heater within the piston is used for rapid elevation of sample temperature to enhance dehydration.

The initial sequence for sample combustion once the sample is within the combustion chamber and the combustion sequence initiated is a 10 second instrument activation time in which four functions are carried out. First, the heater within the piston elevator is activated. Second, the air-heat gun is turned on and hot air is directed over the coiled positive electrode and onto the surface of the pyrex combustion chamber. The third activity is that of hot nitrogen gas passing through the heated positive electrode and onto the surface of the sample for the purpose of surface drying the sample and elimination of any highly volatile combustible constituents which might be associated with the sample. The fourth step is that of loading the carbon dioxide collecting column with 8 ml of a mixture of 2-methoxyethyl amine and toluene. At the end of the ten second period, the hot nitrogen gas flow ceases, and the heated oxygen at a temperature >200°C and flow rate of 400 cc/minute is directed into the chamber through the positive electrode and over the sample to be combusted. The spark ignition system is activated and a continuous spark is visible passing down the same pathway as the heated oxygen onto the surface of the sample. Wherever the

spark discharge strikes the sample a visible flame is present. The spark strikes and moves about the surface of the sample, igniting it at the closest point of the sample to the tip of the positive electrode. Combustion takes place at a uniform rate over the topmost surface of the sample. When wet or frozen samples are combusted, the rate of combustion can be controlled by the applied heat from the heater within the base of the sample holder. During the final stages of sample combustion, the spark will seek out and ignite the last vestiges of the uncombusted sample on the planchet. The spark ignition system can either be manually operated or can be automatically programmed.
At the termination of the combustion step a nitrogen gas flow is reactivated to sweep out the last trace amounts of combusted gases to the appropriate collecting systems.

The ^{3}H-water vapor collecting column containing the condensed water vapor is emptied of its contents by two 8 ml aliquots of PCS (Phase Combining System) which empties into a sample vial. The ^{14}C carbon dioxide collecting column is emptied by first allowing the 8 ml of cocktail amine to drain into the carbon sample vial and then washing the collecting column with two 4 ml aliquots of concentrate scintillator into the sample vial. At this stage, the instrument's piston lowers automatically and the instrument is ready to receive another sample. A recent innovation to further reduce the instrument's memory and spillover has been the incorporation of a steam generator into the combustion chamber and a water inlet on the tritium exchange column. The two components have not been fully tested; therefore, the data presented in the section "Results and Discussion" do not include their use.

Analytical Results

The analytical capabilities of the spark ignition system were tested in the following manner. Radioactive standards of known activity of ^{14}C and ^{3}H labelled thymidine were pipetted onto #2 Whatman filter paper of 25 mm diameter having an average weight of approximately 70 mg. Filter paper was selected as the combustible sample as this material has been the chosen sample used by commercial combustion systems when reporting their recovery data. The amount of recovered radioactivity, after sample combustion, was determined by counting the sample vials containing the collecting cocktails used in the spark ignition system in a Mark II liquid scintillation counter. The liquid counter was calibrated for counting efficiency using the liquid scintillation cocktails of the spark system with known activity of ^{14}C and ^{3}H labelled toluene. Measurements were also made for instrument memory and spillover for each sample. Spillover measurements were carried out by collecting carryover radioactivity in the opposite collecting column. Memory measurements were made by burning a non-labelled filter paper after combustion of each radioactive standard and collecting the residual radioactivity in the designated collecting column. Data presented in Table I is an average value for three combusted ^{14}C and ^{3}H thymidine-filter paper standards.

Table I. Tritium and Radiocarbon Recovery from Combusted Radioactive Thymidine Filter Paper Samples.

Radioactive Standard	Cocktails	Average Recovery (%)	Spillover (%)	Memory (%)	Counting Efficiency (%)
^{14}C Thymidine	2-methoxy ethyl amine toluene PPO & POPOP	98.5±1.0	.07	.05	68
^{3}H Thymidine	PCS (Phase Combining System)	97.8±1.5	.8	.2	47

The ability of the spark ignition system to combust a wide spectrum of sample materials was tested in the following manner. Samples were selected representing typical materials that would be used in medical and biological studies and that would possess high quenching properties or would need to be combusted in order to be counted in a liquid scintillation counter. The samples were prepared in the dry, wet or frozen condition and in varying weights. Combustion times shown in Table II represent the actual time period for complete spark combustion and do not include the 10-second nitrogen flushing of the combustion chamber prior to ignition.

Table II. Combustion Times for Selected Samples in Frozen, Wet or Dry States.

Sample	Combustion Time	Sample	Combustion Time
paper (dry) 100 mg	8 sec.	crab meat (dry) 170 mg	19 sec.
paper (wet) 100 mg	12 sec.	crab meat (wet) 140 mg	13 sec.
wood (dry) 50 mg	10 sec.	crab meat (frozen) 110 mg	21 sec.
shell (crab) 80 mg	25 sec.	liver (dry) 200 mg	15 sec.
fat (dry) 210 mg	10 sec.	liver (wet) 60 mg	10 sec.
fat (wet) 160 mg	16 sec.	liver (frozen) 115 mg	31 sec.
fat (frozen) 185 mg	21 sec.	liver (wet) 730 mg	3 min.

A comparison of the capabilities of the spark ignition system to combust samples comparable to commercial combustion systems on the market today was carried out in the following manner. Rat fecal pellets were selected as the sample material to be used in the study as they fit the requirements of a typical biological sample that would require combustion prior to liquid scintillation counting. Fecal pellets were made up into two bulk samples, one labelled with ^3H and the other ^{14}C. The tritiated feces were fabricated by feeding one group of rats ^3H labelled steroids. The radiocarbon labelled fecal material was made by feeding a second group of rats ^{14}C labelled dipeptide. The fecal material from each group was separately collected, homogenized as an aqueous slurry and freeze

^{14}C RAT FECAL SAMPLES

PACKARD MODEL 305 DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm
1.	209327	617	0.30	339.6	644
2.	224610	1177	0.52	361.1	653
3.	222460	745	0.33	385.6	605
4.	199746	892	0.47	338.2	591
5.	187905	808	0.43	377.3	520
	AVERAGE % MEMORY =		0.41	AVERAGE D.P.M./mgm =	602.6

INTERTECHNIQUE OXYMAT

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm
1.	157183	487	0.310	252.3	623
2.	173559	1180	0.678	297.7	583
3.	209167	962	0.460	325.3	643
4.	196880	885	0.450	326.5	603
	AVERAGE % MEMORY =		0.475	AVERAGE D.P.M./mgm =	613

CONTINUOUS SPARK IGNITION PROTOTYPE DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm
1.	206939	99	0.047	349.2	593
2.	213440	102	0.048	392.7	544
3.	262217	168	0.064	422.2	621
4.	303243	118	0.039	390.0	778
5.	172604	133	0.077	368.9	468
	AVERAGE % MEMORY =		0.055	AVERAGE D.P.M./mgm	600.7

PACKARD MODEL 306 DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm
1.	179413	101	0.056	308.8	581
2.	180172	82	0.046	294.4	612
3.	206592	97	0.047	322.8	640
4.	190032	76	0.040	321.0	592
	AVERAGE % MEMORY =		0.047	AVERAGE D.P.M./mgm =	606

Table III. Combustion Results for ^{14}C Rat Fecal Samples dried. To assist in handling the samples, the material was pelletized.

Three commercial combustion instruments were selected for the comparison tests. These instruments were selected on the basis of present use in the liquid scintillation field today, performance and availability. The three instruments selected were the Packard 305, Packard 306, and the Intertechnique Oxymat. The procedure used to combust the ^{14}C and ^{3}H labelled pellets in the Packard 305 was to wrap the fecal pellets in filter paper and press the sample into a single, paper coated pellet. The sample preparation for burning pellets in the Packard 306 instrument was to wrap the pellets in tissue paper and place the wad in a Combusto-Cone paper basket specially designed to fit into a platinum heater coil within the instrument. Pellets combusted in the Oxymat instrument were placed into a polycarbonate plastic cup with a snap-on lid. Preparation for combustion of the pellets in the spark ignition system required only placement of the fecal pellets onto a steel planchet. Tables III and IV show the comparative combustion results of the ^{14}C and ^{3}H labelled fecal pellets as dpm/mg with the four instruments. Instrument memory, recorded as dpm, is with background subtracted.

A note should be made that the ^{3}H spillover rates as shown in Table IV for the Packard 306 are probably a factor higher than with normal operation. This was apparently due to a holdup in the water column collector that was not apparent at the time of operation.

Discussion and Summary

The prototype continuous spark ignition system reported in this paper offers a unique method for combusting either ^{14}C or ^{3}H labelled samples. Important features designed into the instrument which enhance its capabilities are as follows. The samples can be combusted in the dry, wet or frozen state, thereby reducing presample preparation time. Maximum sample specific activity is sustained by the lack of the need to add foreign combustible material to sustain ignition. Pre-nitrogen gas drying and heating of the sample, prior to ignition, is used to maximize combustion safety and to condition the sample for ignition. Cost per sample combustion is held to a minimum by using low-cost disposable steel planchets to contain the sample during combustion. Spark ignition over the upper dried surface of the sample enhances a uniform rate of combustion

^{3}H RAT FECAL SAMPLES

PACKARD MODEL 305 DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm.
1.	18726	172	0.92	258.8	74.1
2.	24034	174	0.72	322.3	76.2
3.	19691	124	0.63	273.7	73.4
4.	20267	155	0.76	270.8	76.5
5.	22228	110	0.50	309.0	73.3
	AVERAGE % MEMORY =		0.71	AVERAGE D.P.M./mgm. =	74.69

INTERTECHNIQUE OXYMAT

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm.
1.	18399	147	.80	251.2	73.2
2.	18507	207	1.12	263.6	70.2
3.	18822	139	.74	299.9	62.8
4.	19200	184	.96	258.6	74.2
	AVERAGE % MEMORY =		.91	AVERAGE D.P.M./mgm. =	70.1

CONTINUOUS SPARK IGNITION PROTOTYPE DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm.
1.	28707	266	0.93	334.3	86.7
2.	27898	180	0.65	305.7	91.8
3.	27282	22	0.08	295.9	92.3
4.	30580	60	0.20	334.3	91.7
5.	27820	3	0.01	314.7	88.4
	AVERAGE % MEMORY =		0.37	AVERAGE D.P.M./mgm =	90.18

PACKARD MODEL 306 DATA

SAMPLE #	TOTAL D.P.M.	MEMORY D.P.M.	MEMORY %	SAMPLE WEIGHT (mgm)	D.P.M./mgm.
1.	20028	86	.43	263.4	76.1
2.	27015	66	.24	337.2	80.1
3.	28675	98	.34	326.6	87.8
4.	21759	65	.30	276.7	78.6
	AVERAGE % MEMORY =		.33	AVERAGE D.P.M./mgm =	80.6

Table IV. Combustion Results for ^{3}H Rat Fecal Samples for the total sample. Combustion rates can be further controlled by the amount of external heat applied to the sample during combustion. Nuclide collection is in separate vials ready for counting, thereby allowing optimum counting channels to be used in the liquid counter.

Present capabilities of the instrument allow for the combustion of samples up to 40 millimoles of carbon dioxide for ^{14}C samples and up to 85 millimoles of water for ^{3}H samples. Analytical recovery of the ^{14}C and ^{3}H labelled thymidine filter paper samples (Table I) show results in the 97-98% range with a precision of ±1.5%. Memory and spillover values recorded for these standards were .05% and .07% for ^{14}C and .2% and .8% for ^{3}H. Counting efficiencies of the collecting cocktails for the combusted labelled thymidine samples showed 68% for ^{14}C and 47% for ^{3}H.

Further modifications in the instrument are being conducted to increase the collection efficiency and to lower the amount of memory and spillover, especially for ^{3}H. Paramount in this work is the use of a steam generator with the combustion system and a H_2O exchange column. Continuous repetitive sample combustion time is not feasible to estimate at this time, as the instrument is still in a prototype design. However, from the combustion results shown in Table II, it can be seen that the combustion times are rapid for a wide spectrum of samples, and would appear to be comparable to commercial combustion instruments sold on the market today.

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