

[54] COMBUSTION METHOD APPARATUS FOR PREPARING SAMPLES FOR LIQUID SCINTILLATION COUNTING

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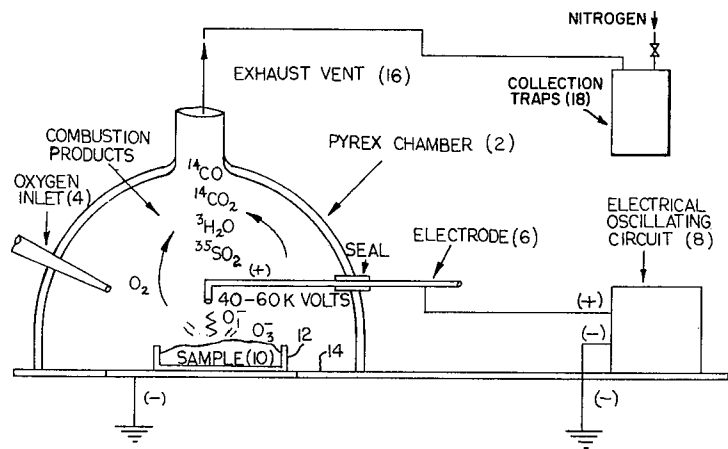
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[57] ABSTRACT

Many different methods of combustion have been used to produce oxidation products of C<sup>14</sup>, S<sup>35</sup>, or H<sup>3</sup> compounds. However the oxygen flask combustion technique developed by W. Schoniger is preferred. An improvement of the Schoniger technique is provided herein making lower combustion temperatures possible. Ignition of the sample is maintained without the addition of foreign combustible material of nonsample origin by means of a high intensity continuous spark and the resulting formation of reactive ozone and singlet oxygen.

6 Claims, 2 Drawing Figures



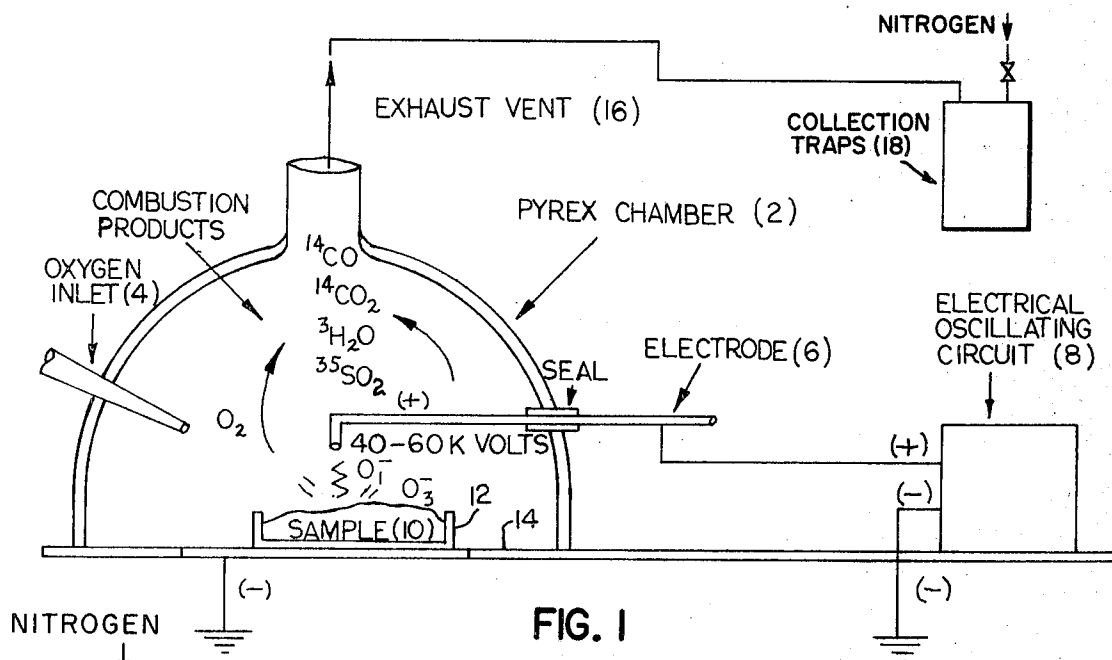


FIG. 1

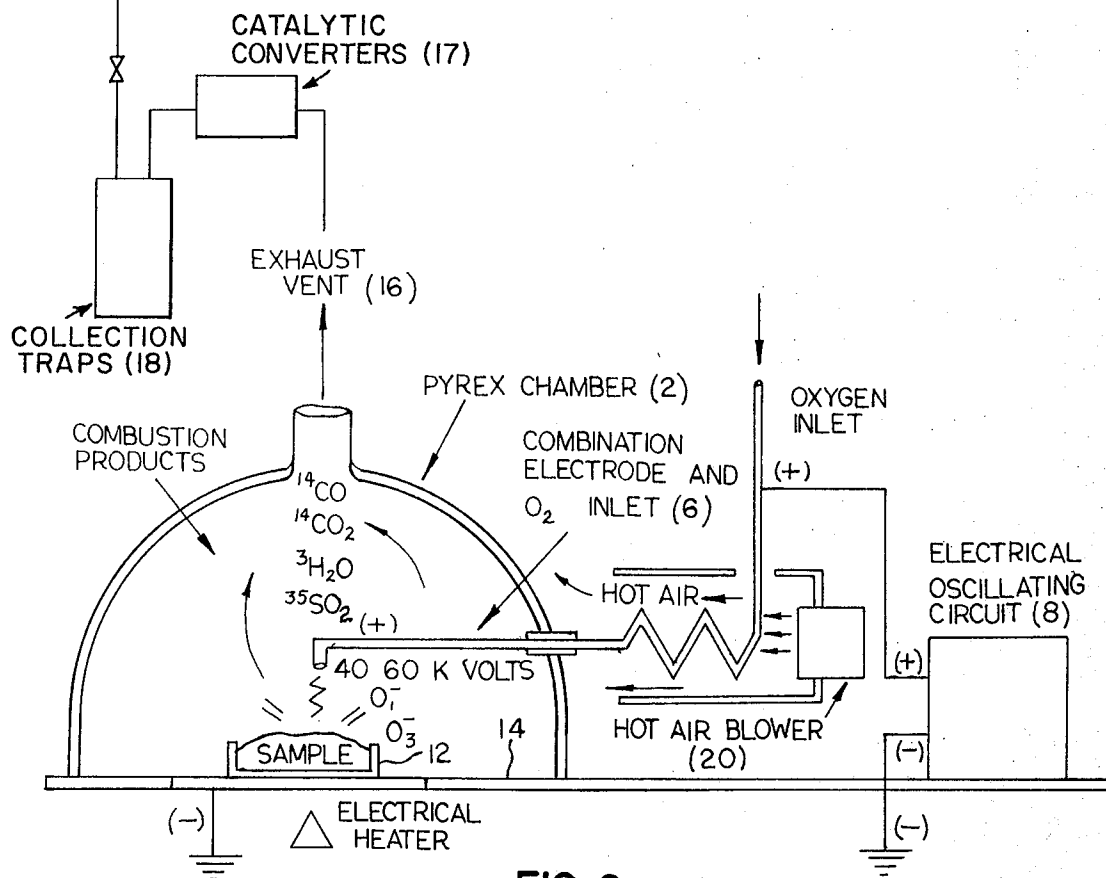


FIG. 2

## COMBUSTION METHOD APPARATUS FOR PREPARING SAMPLES FOR LIQUID SCINTILLATION COUNTING

### BACKGROUND OF THE INVENTION

This invention in one of its aspects pertains to an improvement in the method of preparing samples for radioactive isotope tracer studies. However, since such methods have quite naturally been borrowed from the known techniques of quantitative chemistry, the invention broadly relates to the preparation of samples for chemical analysis. In a more specific aspect this invention provides an improved combustion method for preparing samples of biological origin for liquid scintillation counting.

There are a number of reasons combustion techniques have been employed in the preparation of radioactive-labeled samples containing carbon 14, tritium 3 and sulfur 35 for liquid scintillation counting. Such techniques produce samples which are uniform and which readily dissolve in scintillation solvents. Combustion solves most problems of quenching due to the color or chemical nature of the original sample material. The process also leads to greater uniformity of results.

Many different methods of combustion have been used to produce oxidation products of  $C^{14}$ ,  $S^{35}$  or  $H^3$  compounds. However, such techniques as the wet combustion method, the sealed tube method, the oxygen train method and the oxygen bomb method are generally quite complicated and time consuming. Accordingly the process of choice for combusting samples involved in scintillation counting is the oxygen flask combustion technique developed by W. Schoniger. This sample preparation procedure broadly involves wrapping the sample to be ignited in a combustible material and placing it in a glass flask filled with oxygen and then igniting it. The sample is generally held in some kind of platinum holder. Following combustion the gases are collected by any one of several procedures, depending upon the particular counting system chosen, e.g., upon the scintillation solution, the phosphor and spectrum shifter. Ideally the optimum counting method would be to convert all the radioisotopes into substances soluble in the solvent used to dissolve the scintillation fluors.

A number of improvements on the early oxygen flask combustion technique have recently appeared. One arrangement is to suspend the dried sample material in the center of a confined quantity of oxygen and to provide for its ignition and its containment with minimal loss of heat until combustion is complete. There are, accordingly, aspects of the technique which warrant improvement. One such improvement is exemplified by U.S. Pat. No. 3,485,565 in which the patented flask is in the shape of a flame to minimize the volume of atmosphere around the flame. Other improvements have been made in methods of trapping combustion products, use of infrared ignition, use of subatmospheric pressures, refined cooling techniques and magnetic stirring.

There are several disadvantages inherent in all of the prior art oxygen flask combustion methods, such as the danger of explosion, the boiling and spattering of wet materials and rapid deterioration of the combustion chamber. Most of the disadvantages are due to the

combustion temperatures used. In accordance with this invention lower combustion temperatures are possible. This reduces condensation products in the combustion chamber and virtually eliminates spattering. A uniform and controlled rate of combustion can be accomplished which enhances complete sample combustion. The method is safer in that a rapid combustion does not take place, but rather a controlled reaction due to the low combustion temperature of the sample. In fact the combustion rate can be controlled so that only a selected site on the sample is at combustion temperature. The method allows selected sites of the sample to be combusted without total sample combustion. The combustion rate can, therefore, be controlled so as to permit quantitative collection of the combustion gases and water vapor.

### SUMMARY OF THE INVENTION

In accordance with this invention biological samples, hydrocarbons and other labeled organic products are prepared for analysis by rapid combustion. Moreover the system herein does not utilize a high temperature combustion technique, but relies on excited singlet and triplet oxygen to promote a low temperature combustion. Basically the Schoniger flask method is employed. However, the method directs a continuous high intensity spark to the surface of the sample. Since the sample is blanketed with oxygen, the high intensity spark forms ozone and singlet oxygen. The combustion reaction occurs only at the site of spark contact with the sample and since the spark is continuous it erodes the sample away with only a slight indication of any visible flame. Ignition of the sample is maintained without the addition of foreign combustible material of nonsample origin. The spark continues striking the sample until combustion is complete. The combustion temperature is lower than the normal combustion temperature for the material due to the formation by the spark of the reactive singlet oxygen and ozone at the surface site of the reaction.

### DETAILED DESCRIPTION OF THE INVENTION

The invention herein is applicable when chemical and biochemical analytical procedures require rapid quantitative conversion of samples to carbon dioxide, sulfur dioxide and water. It is uniquely suited to labeled samples wherein the combustion products are to be counted for their radioactivity by liquid scintillation counting, such as tissue, bone, wood, paper, urine, blood and the like. The chief distinction over known combustion techniques is that a continuous spark is directed to the sample surface herein, continuous insofar as a visual observer is concerned. This spark emanates from a current source, such as a capacitive discharge ignition system, capable of generating a DC current which oscillates in the frequency range of 20 cps to 25,000 cps with sufficient intensity to jump across two electrodes with the sample between. A spark has been used in oxygen flask combustion. Its use, however, has been limited to an initial impulse to ignite paper in which the sample is wrapped. A conventional oxygen-sustained combustion then follows. Herein the spark is moved from site to site across the sample until it is completely oxidized without the aid of combustible materials as fuel to maintain a combustion temperature.

As indicated, the technique employed herein is basically that of Schoniger, the oxygen flask being modified

by the addition of electrodes and the electrical circuit. Nevertheless some modification, such as the inclusion means for introducing oxygen, are desirable.

A preferred oxygen flask will accordingly be described in conjunction with a drawing of such a flask.

FIG. 1 is a sectional view of the apparatus generally used.

FIG. 2 is a sectional view illustrating a modification of the basic apparatus.

In the particular embodiment of FIG. 1, the reaction chamber 2 is a flat-bottomed pyrex glass vessel containing a combustion chamber within its lower portion. A side arm 4 is used to admit oxygen although a hollow electrode is preferred, as shown in FIG. 2. A positive charged electrode 6 is connected to an electrical oscillating circuit 8 and extends through the chamber as a side arm, terminating directly above the sample 10. The sample to be combusted is contained in the chamber inside a conductive metal boat 12 which rests on a base plate 14 which is connected to electrical oscillating circuit 8 by a common negative ground. The boat, therefore, is the other electrode. This arrangement places the sample between the two electrodes.

An exhaust vent 16 is located at the top of the vessel to allow exhaust gases to leave the combustion chamber where they are collected in absorption solvents or in a condensation chamber as is known in the art. Thus, the gaseous combustion products escape as indicated by the arrows through the tubing to be collected in flasks surrounded by freezing mixtures or solvents in which the emitted gases are highly soluble. The composition of the counting solvent will, of course, depend upon whether the samples contain H<sup>3</sup>, C<sup>14</sup> or S<sup>35</sup> and whether the sample is single or double labeled. For counting tritium as tritiated water a mixture of methyl alcohol and toluene with a PPO phosphor and a POPOP spectrum shifter is used. In the case of C<sup>14</sup> an amine such as ethylamine or phenylamine is employed as the scintillation solvent, any of the well known phosphors being included in the amine. Sodium hydroxide or similar basic chemical solutions can also be used to trap carbon dioxide formed by the combustion. The combustion products are swept out of the combustion chamber during sample ignition by the addition of excess oxygen. The gaseous combustion products are passed to collection traps 18 containing collection solvents for subsequent use in liquid scintillation counting. The combustion chamber and collection traps 18 are purged with nitrogen gas after the combustion step to remove any residual combustion products from their walls, whereby the samples are concentrated in the collection traps 18 and the collection solvents are purged of excess oxygen gas in a manner well known in the art. Nitrogen gas can also be used to purge the last of the combustion products from the chamber which eliminates excess oxygen being contained in the collecting solvents.

FIG. 2 illustrates a more desirable embodiment of the basic apparatus. The addition of sample heating means, and of a different method of admitting oxygen will be apparent from a description of the method of combustion using the apparatus shown in FIG. 2. The sample to be combusted in chamber 2 is placed in a boat 12 which rests on a negative charged plate 14 constituting one electrode in an electrical circuit. Through the passageway in the overhead positive electrode 6 chamber

2 is purged with oxygen. The electrode is positioned about one-fourth to one-half inch above the sample and a capacitive discharge electrical circuit creating an approximately 40,000 volt output across the electrodes is energized. The resulting electrical spark jumps from electrode to the sample, and combustion occurs at the point of contact of the sample. Ozone and singlet oxygen are formed by the high intensity spark. The reaction or combustion occurs only at the site of spark contact with the sample, eroding the sample away as it is moved across. In most reactions there is only a sign of any visible flame. The electrode can be physically moved to new combustion sites during the process or the spark may jump directly by itself to the nearest point of contact. Combustion of milligram samples is normally complete in a few seconds and seldom in over three or four minutes. The characteristic ozone odor is quite noticeable following combustion.

In the case of hydrated or chemically wet samples, combustion is enhanced by drying the sample with heated oxygen during ignition. This is accomplished by heater 20 preheating the oxygen to greater than 100° C. prior to admitting it to the combustion chamber. Even more important, this preheater can be employed prior to injecting oxygen to heat nitrogen gas to be used to drive off sample ingredients which volatilize at such temperatures and which in the presence of oxygen could form an explosive mixture within the combustion chamber. Advantage is found in admitting the oxygen into the combustion chamber through a hollow electrode. This process intensifies the formation of singlet and triplet oxygen since the oxygen stream is in close proximity to the high intensity spark. It also eliminates the need for construction of an additional oxygen orifice. The oxygen flow rate is regulated depending on the desired rate of combustion and the resistance of the sample to combustion. Generally oxygen will be admitted at a rate of 1 cc to 1 liter per minute or higher.

The efficiency of the oxygen flask combustion technique herein can be seen from combustion rates for a number of different samples.

TABLE

SAMPLE	TIME	SAMPLE	TIME
Paper 100 mg 5 × 10 mm	8 sec.	Wood 50 mg 2 cc	10 sec.
Fat (dry) 210 mg	10 sec.	Liver (dry) 200 mg 3mm	15 sec.
Shell - crab 80 mg 4 × 5 mm	25 sec.	Liver (wet) 60 mg	10 sec.
Crab meat 140 mg	13 sec.	Liver (wet) 730 mg	3 min.

It can be seen that an excellent method is provided for the combustion of products which are labeled with radioactive hydrogen, carbon, or sulfur. The system does not utilize high temperature combustion but relies on excited states of oxygen such as singlet and triplet oxygen to bring about a low temperature combustion. The reaction products are swept out by excess oxygen during combustion and by nitrogen thereafter. The process lends itself to the usual adaptations. The exhaust vent 16 may be constructed to contain catalytic converters 17 for converting CO to CO<sub>2</sub> and H<sub>2</sub> to H<sub>2</sub>O. Thus, if carbon monoxide is formed a heated copper

oxide converter can be provided in the combustion products outlet line. Means can be included for converting hydrogen to water using appropriate platinum catalyst. Sample placement into the flask can be accomplished either by lowering the base of the combustion chamber, such as in U.S. Pat. No. 3,485,565 or raising the upper combustion chamber flask or inserting the sample through a slide tray at the base of the chamber. It will also be appreciated that any type electrical conduction of sample boat or tub can be used. Indeed, the sample can be placed directly on the base plate if desired. A tub, however, is easier to insert into the combustion chamber, easier to clean and when necessary, ideally suited for rapid sequential multisample handling. Additional heating can be incorporated into the apparatus when wet samples are being combusted. This serves to hasten the dehydration of the sample and avoids condensation of oxygen products on the chamber walls. Heating is accomplished by several means: (a) an electric heater beneath the sample can be employed, (b) a hot air stream can be directed onto the outer flask walls or (c) the oxygen or nitrogen directed into the combustion chamber can be preheated. The output of the electrical circuit employed will generally be 40,000 to 80,000 volts derived from a 6 or 82 volt input DC circuit. However the circuit can be designed for a 110 or 220 volt AC input and/or a constant discharge rather than an intermittent spark of high frequency. The current can range from a few milliamps to multiampereage, but is preferably in a low (less than 1 amp) range. A DC circuit is preferred since the direction of spark travel is constant from the overhead electrode towards the sample, thereby forming a more even combustion reaction. Such electrical circuits will be apparent to those familiar with such equipment. These and other modifications will occur to those skilled in the art. Such ramifications are within the scope of this invention.

What is claimed is:

1. A method for the combustion of organic materials containing a radioactive isotope in an oxygen atmosphere to produce gaseous by-products for subsequent radioactivity measurement comprising the steps of providing a combustion chamber including an electrically conductive plate adapted to receive a sample of material to be burned and an electrode positioned near the sample, introducing oxygen into said combustion chamber from an oxygen source, energizing said electrode to produce a continuous high intensity spark therefrom to said electrically conductive plate to form singlet and triplet oxygen to react locally at the surface of the sample and to burn the sample at said surface, continuing the localized oxidation until combustion of

the total sample is complete, and collecting gaseous products of combustion produced thereby.

2. A process for the combustion of organic material containing a radioactive isotope in an oxygen atmosphere to produce gaseous by-products for subsequent radio-activity determinations comprising placing a sample of organic material between two ignition electrodes, blanketing the sample with oxygen, generating an electric spark between the electrodes from an oscillating electric current having a frequency of from 20 cps to 25,000 cps and of sufficient energy to excite oxygen to convert a portion of the oxygen to reactive singlet and triplet oxygen, directing said spark to the surface of the sample to generate additional quantities of singlet and triplet oxygen and to provide locally sufficient heat to effect oxidation and combustion at the surface of the sample, continuing localized combustion, and burning away portions of the sample thereby, until the sample is completely converted to its gaseous combustion products, and collecting said gaseous combustion products.

3. The process of claim 2 wherein the oxygen is preheated prior to introduction into the combustion chamber for the purpose of enhancing the formation of excited oxygen states and to dry the sample of excess water vapor.

4. The process of claim 2 wherein nitrogen gas is preheated and introduced into the combustion chamber prior to the oxygen for the purpose of volatilizing and driving off highly combustible sample ingredients which could form an explosive mixture in the presence of oxygen.

5. The process of claim 2 further comprising passing the gaseous combustion products to collection traps containing collection solvents for subsequent use in liquid scintillation counting, and wherein the combustion chamber and collection traps are purged with nitrogen gas after the combustion step to remove any residual combustion products from their walls.

6. In a method of oxidizing organic samples containing radioactive isotopes using oxygen supplied to an air-tight combustion chamber to produce gaseous oxides for subsequent radioactivity determinations, the improvement comprising positioning an organic sample between two spaced electrodes in said combustion chamber, and energizing said electrodes with an oscillating electrical potential to produce and sustain an electrical spark passing between the electrodes and through the aforesaid sample to ionize oxygen in said chamber and to produce localized heating in said sample, thereby effecting total oxidation of said sample into gaseous oxides, and collecting said gaseous oxides.

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