The Combustion of Tritium-Labelled Organic Compounds

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The tritium (T) in solids can be determined directly only on small amounts of material, and then only with poor efficiency, owing to absorption by the sample of the low energy tritium β -rays. The tritium in water, however, can be estimated with high efficiency by reducing the water to hydrogen, and measuring the currents obtained through hydrogen-filled ion chambers (Henriques & Margnetti, 1946; Kamen, 1947); or, more conveniently, by preparing tritio-methane from the water and determining the tritium content in a proportional counter (White, Campbell & Payne, 1950). Thus the estimation of the tritium content of organic substances will usually necessitate their combustion to water; a technique suitable for the routine combustion of organic compounds for this purpose has been devised.

METHOD AND APPARATUS

Kamen (1947) has recommended the combustion techniques developed for deuterium assays as suitable for the combustion of tritiated metabolites, but states that contamination must be constantly guarded against by testing for radioactivity in water obtained from the combustion of samples of inactive material. If activity is found in the blanks the combustion tube has to be flushed several times by burning inactive hydrogen compounds until satisfactory blanks are obtained. Such a procedure will be too tedious for routine analysis.

Possible causes of contamination of the apparatus are retention of water in porous materials, and exchange of tritium with labile hydrogen of substances such as glass. The copper oxide filling and the quartz or glass tubes used in most combustion methods are porous, the porosity of the quartz increasing markedly after several analyses. Tritium is known to cling tenaciously to glass (Williams & Ronzio, 1950) and Geiger counters are difficult to decontaminate after they have contained THO (Pace, Kline, Schachman & Harfenist, 1947). By using deuterium, Rittenberg (1948) has found that a baked and evacuated Pyrex vessel appears to have a layer of water 3 molecules deep over its entire surface, although the method did not differentiate between absorbed water and OH groups of silicates. For these reasons it seemed advantageous to use an all-metal combustion apparatus.

The metal tube must have high resistance to oxidation at high temperatures, since part of the combustion tube is exposed to O_2 at 1000° . Kamen (1947) states that monel metal reaction vessels used for reduction of THO are easier to decontaminate than vessels made from stainless steel, suggesting that high nickel content alloys would be desirable. For these reasons inconel tubes were tried. These were obtained from Henry Wiggin and Co. Ltd., London. Some scale is formed inside these tubes after repeated use, but their life is very long and in this respect they are much superior to quartz tubes.

The copper oxide filling used in the deuterium techniques has been replaced by the Pt catalyst introduced by Kopfer (1878). Kopfer used platinum black. Dennstedt (1897) modified the method to use strips of platinum foil. The authors use a roll of 150 mesh platinum gauze 20 cm. long. In order to ensure complete oxidation and to limit poisoning of the catalyst the platinum is kept at 1000° .

The apparatus is shown in Fig. 1.

O_2 from a rubber bladder of 20 l. capacity is sucked through the apparatus by a small mechanical pump. A metal reservoir of 5 l. capacity between the pump and the rest of the apparatus prevents transient variations in O_2 flow. The rate of flow is controlled by a valve connecting the reservoir to air. This arrangement results in a very steady flow of O_2 through the apparatus without the necessity of refilling Mariotte bottles, and has been found to prevent the uneven burning of the sample which sometimes occurs in Pregl combustion trains.

Water and volatile impurities are removed from the O_2 by the spiral copper trap *A*, immersed in dry ice-propanol. The sample is inserted through the removable gas-tight breech. The boat containing the sample is mounted in the holder *D*, attached to the block *C*, which fits loosely in the combustion tube. The rapid flow of O_2 between *C* and the combustion tube limits back diffusion of volatile materials.

A combustion of 500–600 mg. of substance proceeds smoothly to completion if the sample-heating furnace is raised at a constant rate from room temperature to 700° in 30–45 min., and is maintained at this temperature for 15 min. Even volatile substances burn steadily from a silica boat

in these conditions. The time between successive analyses has been reduced to a few minutes by using an unlagged sample heating furnace which rapidly

combustion tube. An electric clock driving a 'Simmerstat' control automatically raises the temperature of the sample furnace.

Fig. 1. Apparatus for combustion of tritium-labelled organic compounds. *A*, spiral made from $\frac{3}{8}$ in. outside diam., $\frac{1}{4}$ in. inside diam. copper tube, 6 turns, 1 turn/in.; *B*, brass crush nipples; *C*, see text; *D*, boat holder 7 cm. long; *E*, sample heater; *F*, inconel tube $\frac{3}{8}$ in. inside diam., $\frac{1}{2}$ in. outside diam.; *G*, main furnace at 1000°; *H*, roll of platinum gauze, 20 cm. long; *J*, copper rod; *K*, copper tube; *L*, brass B29 cone joint; *M*, Pyrex vessel, 3.2 cm. inside diam., 15 cm. overall length; *N* and *P*, copper tubes for attachment of anhydrous trap.

cools. In the present apparatus the sample is heated by two unlagged heaters *E*, each consisting of a 700 W. electrical element supported by

Fig. 2. Adapter for connecting Pyrex vessel to apparatus for tritium estimation.

magnesia packing in an inconel sheath which was obtained from Hotpoint Ltd., London. Each heater is folded as shown in Fig. 1, and is clamped along the

The water from the combustion is collected in the Pyrex vessel *M* immersed in dry ice-propanol, and attached to the combustion tube by the cone joint *L*. *L* is lubricated with silicone high vacuum grease. The copper rod *J* and tube *K* conduct sufficient heat from the furnace zone to prevent condensation within *K*. It has been found that at the O_2 flow rates normally used (20–50 ml./minute) 90–95% of the water of combustion is collected in *M*. For specific activity measurements quantitative collection is unnecessary. For accurate determination of the efficiency of combustion a $CaCl_2$ and an anhydrous trap are connected between *P* and *N*.

After attachment to *M* of the adapter shown in Fig. 2, the specific activity of the water is determined by the method already described (White *et al.* 1950).

RESULTS

Completeness of combustion

Evidence for the completeness of combustion is contained in Table 1.

Table 1. *Percentage hydrogen content of test substances measured with the combustion apparatus*

Substance	Hydrogen content (%)	
	Calculated	Observed
Benzoic acid	4.92	4.96
		5.00
		4.92
		4.94
Resorcinol	5.46	5.53
		5.50
Vanillin	5.26	5.06
		5.24
β -Naphthol	5.56	5.45
		5.56
Dextrose	6.67	6.63
		6.59
Cholesterol	11.9	12.1
		11.8

Preparation of tritiated test substance

A tritiated test substance of unknown composition was prepared by heating 0.3 ml. of THO (activity 1.4×10^5 counts/sec.) with 2 g. β -naphthol in an evacuated Pyrex ampoule in the presence of active nickel for 16 hr. at 175° . The resulting substance was dissolved in ether and crystallized. It was then vigorously stirred with 200 ml. of boiling water, allowed to cool and then thoroughly shaken with 50 ml. of ether in a separating funnel. The ether phase was separated and the ether evaporated at room temperature. The crystals were again treated with 30 ml. of boiling water and the substance extracted with ether. The radioactivity in the water used for the second washing was only 19.2 counts/sec./ml., showing that most of the labile or semilabile tritium attached to or in the vicinity of oxygen atoms had been removed. After evaporation of the ether the specific activity of the substance was found to be 21.9 counts/sec./mg.

Test for contamination of apparatus

To test the contamination of the apparatus 123 mg. of the test substance (activity 2.93×10^3 counts/sec.) were burned. The apparatus was then flushed with oxygen at 30 ml./min. for 15 min., followed by combustion of a sample of inactive dextrose. The water from the dextrose had an activity of only 1.2 counts/sec. corresponding to a contamination of only 0.04%. In biological ex-

periments samples will rarely contain more than 200–300 counts/sec., so that the above contamination is negligible.

Substances containing nitrogen and sulphur

Oxides of nitrogen and sulphur, formed by the combustion of substances containing these elements, will combine with some of the hydrogen. It was necessary to prove that this combination does not result in differences in the specific activity of the water collected. The tritiated test substance (63.3 mg.) was dissolved in ether and 10 g. of β -naphthol added. The ether was evaporated. The ratio of test substance to β -naphthol is such that the percentage of hydrogen in the mixture can be taken as equal to that of β -naphthol. Glycine (343.5 mg.) and 1-amino-2-naphthol-4-sulphonic acid (122.3 mg.) were added to 857.7 mg. of the mixture, and the whole was carefully mixed by grinding in a small mortar. From the figures in Table 2 it can be seen that the presence of sulphur and nitrogen is without effect on the specific activity of the water obtained. It is thus unnecessary to introduce reagents for the retention of nitrogen and sulphur.

Table 2. *Specific activities of water from combustion of tritiated test substance + β -naphthol, with and without dilution by glycine and 1-amino-2-naphthol-4-sulphonic acid*

Substance burned	Specific activity of water (counts/sec./g.)	
	Calculated	Observed
Test substance + β -naphthol	—	276
	—	276
	—	269
Test substance + β -naphthol + glycine + 1-amino-2-naphthol-4-sulphonic acid	131	136
	131	128

SUMMARY

1. An apparatus has been developed for the routine combustion of tritiated metabolites. Combustion is complete, rapid and smooth.

2. The apparatus shows negligible contamination and the analyses are not influenced by the presence of nitrogen or sulphur.

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